Advances in
PARASITOLOGY

VOLUME 21

Advances in
PARASITOLOGY

VOLUME 21

Editorial Board

Advances in
PARASITOLOGY

Edited by

J. R. BAKER

Culture Centre of Algae and Protozoa, Cambridge

and

R. MULLER

Commonwealth Institute of Parasitology, St. Albans

VOLUME 21

1982

ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

London New York
Paris San Diego San Francisco São Paulo
Sydney Tokyo Toronto

ACADEMIC PRESS INC. (LONDON) LTD.
24/28 Oval Road,
London NW1

United States Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue,
New York, New York 10003

British Library Cataloguing in Publication Data

Advances in parasitology.
Vol. 21
1. Veterinary parasitology
591.2'3'05 SF810.H3

ISBN 0-12-031721-4
LCCCN 62-22124

Typeset by Bath Typesetting Ltd., Bath
and printed in Great Britain by
Thomson Litho Ltd., East Kilbride, Scotland

CONTRIBUTORS TO VOLUME 21

Y. W. LOKE, *Division of Experimental and Cellular Pathology, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, England*

W. I. MORRISON, *International Laboratory for Research on Animal Diseases, P.O. Box 30709, Nairobi, Kenya*

MAX MURRAY, *International Laboratory for Research on Animal Diseases, P.O. Box 30709, Nairobi, Kenya*

M. D. RICKARD, *University ofMelbourne, Veterinary Clinical Centre, Princes Highway, Werribee, Victoria 3030, Australia*

M. J. TURNER, *MRC Biochemical Parasitology Unit, Molteno Institute, University of Cambridge, Downing Street, Cambridge CB2 3EE, England*

D. D. WHITELAW, *International Laboratory for Research on Animal Diseases, P.O. Box 30709, Nairobi, Kenya*

J. F. WILLIAMS, *Department of Microbiology and Public Health, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824, USA*

This Page Intentionally Left Blank

PREFACE

Astute readers will already have noticed that there are now only two editors of "Advances in Parasitology". On the retirement of Professor W. H. R. Lumsden from the senior editorship, the editors and the publishers decided to broaden the range of expertise available to advise upon policy and propose or criticize contributions. An international editorial board composed of experts in as many fields of parasitology as possible has been invited to advise the editors generally and to help on specific topics when required.

We were delighted when the distinguished scientists whose names are listed on p. ii agreed to serve as board members. Their wide experience and knowledge will help us considerably, and cannot fail to improve the coverage and raise the standard of the material included in future volumes. We were particularly glad that Professor Lumsden agreed to serve. The experience he acquired on taking over the major responsibility for "Advances" after Professor Dawes's death was considerable; this experience he willingly passed on to us during our years of friendly and, we believe, fruitful collaboration as joint editors, and we welcome its continuation. Professor Lumsden shares with us a deep interest in this publication, and it is good that this will be actively continued.

We are always happy to receive suggestions for future contributions, either general or specific, and we solicit such proposals from readers. All will be considered sympathetically by us, in the light of comments by appropriate board members when necessary. However, we prefer not to receive unsolicited completed typescripts. We state again our intention to maintain a broad interpretation of parasitology, both applied (medical, veterinary and agricultural) and non-applied (conceptual or theoretical), and to emphasize as far as possible the first word of the publication's title.

1982

J. R. BAKER
R. MULLER

This Page Intentionally Left Blank

CONTENTS

Hydatidosis/Cysticercosis: Immune Mechanisms and Immunization Against Infection

M. D. RICKARD AND J. F. WILLIAMS

Host Susceptibility to African Trypanosomiasis: Trypanotolerance

MAX MURRAY, W. I. MORRISON AND D. D. WHITELAW

International Laboratory for Research on Animal Diseases, Nairobi, Kenya

I. Introduction

The exploitation of genetic resistance to disease is becoming an increasingly important consideration in livestock development programmes, particularly where conventional disease control measures are not effective or are too costly. Such an approach may be directly applicable to African animal trypanosomiasis.

In African trypanosomiasis, the control measures currently in use include diagnosis and treatment, chemoprophylaxis, and control or eradication of tsetse with insecticides. Unfortunately, many years of these strategies have had little effect on the problem at the continental level. There are several factors responsible for this. Firstly, there are 22 species of *Glossina* (tsetse) capable of transmitting infection; these are adapted to a wide range of habitats, thereby contributing to the widespread nature of the disease. Secondly, the three trypanosome species pathogenic for domestic livestock, *Trypanosoma congolense*, *T. vivax* and *T. brucei*, exhibit a wide host range for both domestic and wild animals. Thirdly, the phenomenon of antigenic variation which leads to persistent parasitaemia provides an excellent opportunity for transmission of infection by tsetse. At the same time, the implementation of current control measures poses several problems. The use of drugs, both therapeutically and prophylactically, can be costly because repeated treatments are required and diagnostic facilities are necessary if the drugs are to be used properly. Furthermore, frequent use or misuse can lead to the development of chemoresistance, a risk compounded by the fact that the number of drugs commercially available at present is extremely limited. Tsetse control, followed in some cases by eradication, has been successful in certain regions; e.g. Nigeria, Zambia, Botswana and in South Africa (reviewed by MacLennan, 1981). However, as with drug strategies, the cost is high and it is essential that the eradicated area is kept under rigorous surveillance for several years and protected by natural or man-made tsetse barriers to prevent reinvasion. In addition, the emotive question of environmental hazards created by the use of insecticides arises. At present, there is no effective field vaccine available against African trypanosomiasis. The major constraints to the development of a vaccine include the existence of different species of trypanosomes and of different serodemes within the same species, all with the capability of producing different repertoires of variable antigen types (VATs).

In the face of these problems, increasing attention has been focused on the potential use of genetically resistant or trypanotolerant livestock. There is no clear definition of trypanotolerance. At one extreme, Pagot (1974) defined trypanotolerance as a "racial aptitude [of cattle] to maintain themselves in good condition and to reproduce while harbouring trypanosomes without showing clinical signs of the disease." He recommended that such cattle be

introduced widely into high tsetse challenge areas throughout Africa. At the other end of the scale, Stephen (1966) stated that "tolerance is far from absolute" and concluded that propagation of trypanotolerant breeds was not to be recommended as a satisfactory means of improving the supply of protein in densely populated areas of West Africa. In our experience, both these statements are true for the circumstance under which each author made his observations. The major fact by which trypanotolerance may be defined is based on the field observation that certain breeds of cattle, sheep, and goats, as well as some species of wild animals, can survive in endemic tsetse fly-infested areas without the aid of chemotherapy where other breeds cannot. The term trypanotolerance may be misleading because infection of animals considered to be trypanotolerant can, in some instances, cause severe clinical disease. Thus, such breeds are not truly tolerant but could be described more correctly as exhibiting a greater degree of resistance to the disease; it might be better, as discussed by Wakelin (1978), to use the term reduced susceptibility.

With regard to the economic potential of trypanotolerance, we agree with the views of Stewart (1951) and Chandler (1952) that more information is required on several aspects of this trait before widespread exploitation of trypanotolerant breeds can be recommended. It is important to know the extent of the differences in resistance between different breeds and also within the same breed living at different levels of trypanosomiasis risk, under different management systems in different ecological zones. At the same time, an understanding is required of the mechanism(s) underlying trypanotolerance, the genetics of heritability, and the stability of the trait, i.e. how it is affected by environmental factors.

In the current review, we present and discuss the available information on each of these aspects of trypanotolerance, with regard to cattle, sheep, goats, wildlife and man. We also evaluate experimental results derived from mouse models and consider their relevance to trypanosomiasis of livestock.

II. Origin of Trypanotolerant Livestock

The majority of published information suggests that trypanotolerant livestock are confined to West and Central Africa. While this is broadly the case, there is no obvious reason why they should not have evolved in other regions of Africa infested by the tsetse fly.

Domestic cattle were probably introduced into Africa from the near East around 5000 BC (Payne, 1964; Epstein, 1971). Goats arrived at approximately the same time but sheep did not appear until early in the third millenium BC. These estimations are based on rock paintings and engravings.

Three major breeds of cattle were imported or migrated with nomadic people into north-east Africa. These were the humpless Hamitic longhorn, the humpless Shorthorn and the humped Zebu. The Hamitic longhorn and the Shorthorn would be classified as ancestral *Bos taurus* types while the Zebu would be the *Bos indicus* type. Sanga breeds have resulted from a mixture of Zebu and Hamitic longhorn and/or Shorthorn. The time of origin and the subsequent migration routes of domestic cattle in Africa are shown in Fig. 1.

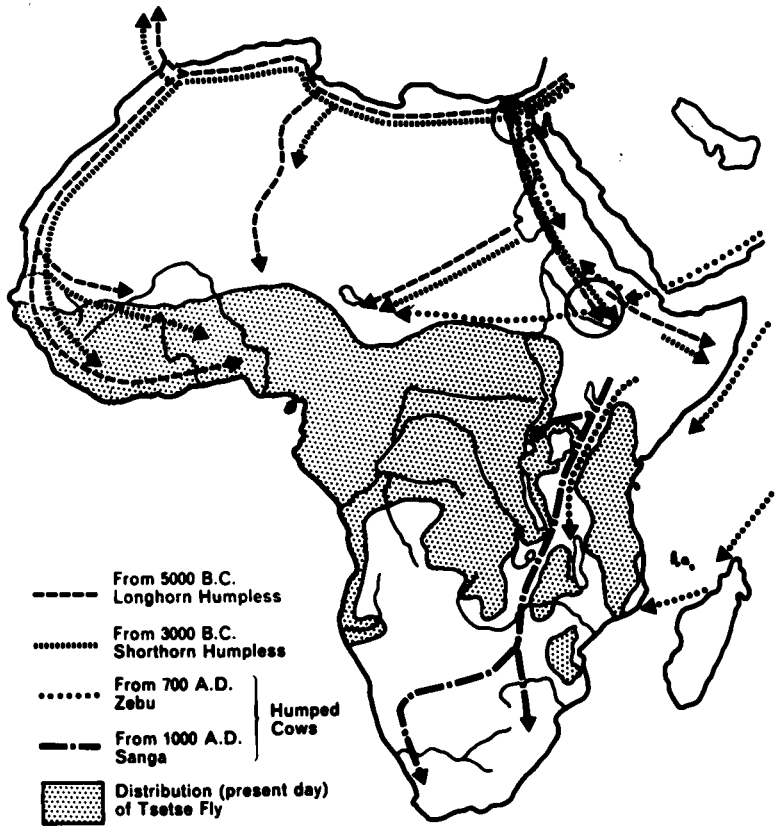

FIG. 1. Map of Africa showing the foci of origin and time of subsequent spread of indigenous breeds of African cattle. Data are based on Payne (1964) and Epstein (1971).

The Hamitic longhorn arrived in Egypt about 5000 BC, while the Shorthorn were first introduced into the same area about 2750 to 2500 BC. Zebu arrived in Egypt between 2000 and 1800 BC and Sanga were recognized between 2000–1500 BC. However, it was not until the Arab invasion of Africa after 669 AD that the number of Zebu imported into Africa rose sharply and the large-scale spread of Zebu and Sanga followed.

TABLE 1

Classification of trypanotolerant cattle[a]

Group	Breed	Estimated no. in millions
N'DAMA	N'Dama	3·4
WEST AFRICAN SHORTHORN (MUTURU)		
Dwarf West African Shorthorn	Lagune/Dahomey Forest Muturu Liberian Dwarf	0·1
Savanna West African Shorthorn	Baoule Ghana Shorthorn Somba Savanna Muturu Doayo Bakozi Kapsiki	1·7
ZEBU × HUMPLESS		2·4

[a] Data from ILCA (1979).

At present, the breeds descended from the Hamitic longhorn, the N'Dama, and from the Shorthorn, the West African Shorthorn, are concentrated in West and Central Africa (Table 1). It is these breeds which are considered trypanotolerant, and they are found largely in tsetse-infested areas. As the tsetse challenge becomes lighter and with northern extension beyond the tsetse belt, Zebu crosses and Zebu become more common and eventually predominate. Thus, it would appear that the presence of the tsetse fly has had a major influence on the eventual distribution of different breeds of cattle. It is then interesting to speculate on the reason for the lack of taurine cattle in East Africa, where *Bos indicus* types are the main indigenous breeds, despite the tsetse. One possible explanation is that rinderpest pandemics curtailed the movement of the trypanotolerant breeds such as the N'Dama which are thought to be less resistant to rinderpest than West African Zebu (Cornell and Evans, 1937).

Another interesting aspect of the migration of the Hamitic longhorn and the West African Shorthorn is that, in addition to moving west and then south into West Africa from the Nile delta (Fig. 1), they crossed into the Iberian peninsula and from there to the rest of Europe and the Americas; e.g. the Hamitic longhorn is represented in Scotland by the West Highland Cattle, in Brazil by the Franquiro, and in Mexico and the southern U.S.A. by the Texas Longhorn. The Shorthorn is partly responsible for the Jersey, Guernsey and Kerry breeds in the British Isles.

III. Evidence for Genetic Resistance to Trypanosomiasis

A. CATTLE

In one of the first accounts of West African livestock, Pierre (1906) recorded the ability of certain cattle to survive in tsetse-infested areas. Subsequently, the resistance of the taurine animals was recognized increasingly in West Africa (reviewed by Godfrey *et al.*, 1964 and ILCA, 1979), as well as in the Sudan (Archibald, 1927). In 1951, Stewart described his experience with what he termed "West African Shorthorn Cattle" in Ghana (Gold Coast) over a period of 20 years from 1929 to 1948. These animals were genetically heterogeneous and comprised Hamitic longhorn, Shorthorns and Zebu. The contribution made by each "breed" appeared to depend on the level of tsetse challenge; the greater the tsetse risk, the smaller the percentage Zebu in each animal. His overall conclusion was that these animals possessed very high resistance to the disease. He pointed out that they were rather small for beef, for work and for milk, but these features had blinded people to their basic value. namely, the fact that the West African Shorthorn lives, breeds and thrives in areas were Zebu and other exotic cattle die of trypanosomiasis. Furthermore, he emphasized that a vast area of West Africa depended on these small cattle and that, although they might be deficient as a market animal compared with Zebu, the development of mixed farming would be impossible without them as the West African Shorthorn cattle provided the peasant with his draught oxen, his manure and his milk.

Stewart (1937, 1951) examined the susceptibility of these cattle to needle challenge with both *T. vivax* and *T. congolense* (including a proven pathogenic strain from Tanzania). He showed that, despite becoming infected, the animals were able to control their parasitaemia and did not develop any overt clinical signs. Similar results ensued when they were exposed to natural tsetse challenge, including *Glossina palpalis*, *G. morsitans* and *G. longipalpis*. While it was found that most animals showed little evidence of clinical disease and some recovered spontaneously, their resistance broke down if the tsetse challenge was high enough or as a result of stress, e.g. lack of grass or repeated bleeding during experimental studies. Furthermore, in a series of crossbreeding studies Stewart (1951) was able to produce much larger cattle than "West African Shorthorn" that still retained a significant measure of trypanotolerance. As a result of 20 years experience, he concluded that, "the creation of trypanosomiasis-resistant cattle is a more practical and natural procedure than mass inoculation of cattle with drugs in attempts to maintain these doped cattle in tsetse areas." In his investigations, Stewart established the basis for recognizing the importance of trypanotolerance and pointed the way for future studies of the resistance of several West African taurine breeds.

1. *N'Dama*

Most studies of trypanotolerance in cattle have focused on the N'Dama which is the main descendant of the Hamitic longhorn in Africa. It is thought that this breed spread in West Africa from the Fouta Djallon plateau in Guinea (Epstein, 1971). It now is the most common trypanotolerant breed in West Africa with an estimated population of 3·4 million (Table 1). Its popularity is probably based on the fact that it is the largest of the trypanotolerant breeds (Fig. 2a; Table 2) and is thought to be the most productive.

Some of the first experimental studies on the extent of trypanotolerance exhibited by the N'Dama were carried out in Nigeria. The superior resistance of the N'Dama to trypanosomiasis was consistently confirmed by comparisons of weight loss, anaemia and survival. This was true regardless of the nature of the challenge. In some experiments, animals were exposed to natural field challenge of *G. palpalis* or *G. morsitans* (Chandler, 1952; van Hoeve, 1972); in others, the animals were challenged with *G. palpalis* infected in the laboratory with *T. vivax* (Chandler, 1958; Desowitz, 1959); and other investigations involved the inoculation of bloodstream forms of *T. congolense* (Chandler, 1958). These studies emphasized the potential of trypanotolerant livestock and presented evidence that trypanotolerance was an innate characteristic (Chandler, 1952, 1958).

TABLE 2

Weights (kg) of some indigenous African breeds of cattle[a]

	1 year old	Mature cows
N'Dama	114 ± 14	248 ± 20
West African Shorthorn	79 ± 14	162 ± 20
Keteku–Borgou (Zebu × Shorthorn)	130 ± 10	260 ± 30
Zebu	180 ± 20	300 ± 30

[a] These figures (means ± S.D.) are presented to give some idea of the size of the animals under discussion but vary considerably with management systems. (From ILCA, 1979.)

While there is no question of the general validity of the foregoing results, some of the conclusions might be criticized for the following reasons: the clinical history of many of the animals was not precisely known, the antigenic characteristics of the various trypanosomes to which the animals were exposed was not investigated and, lastly, only small numbers of cattle were available for study. Without these data, it is impossible to define the relative contributions of innate and acquired resistance.

(a)

(b)

(c)

FIG. 2. N'Dama bull (a); West African Shorthorn (Muturu, b); East African Zebu (c): (a) and (b) are reproduced by permission of the International Livestock Centre for Africa, Addis Ababa.

As a result, Stephen (1966) and Roberts and Gray (1972a) evaluated the resistance to trypanosomiasis of N'Dama and Zebu which had been reared free from tsetse exposure. The Zebu dams had never been exposed to tsetse although the N'Dama dams had. Later, Roberts and Gray (1973b) extended these investigations to include N'Dama born of dams that had never been exposed to tsetse. In all these experiments the animals were challenged under laboratory conditions with wild caught *G. morsitans submorsitans* which were found to be infected with *T. vivax*, *T. congolense* and *T. brucei*. Although once again the number of animals available was small and in some experiments there was a considerable age range (Roberts and Gray, 1972a), the greater resistance of the N'Dama was obvious. This result, assessed on the basis of weight loss, anaemia and survival, confirmed that the trait was an innate characteristic.

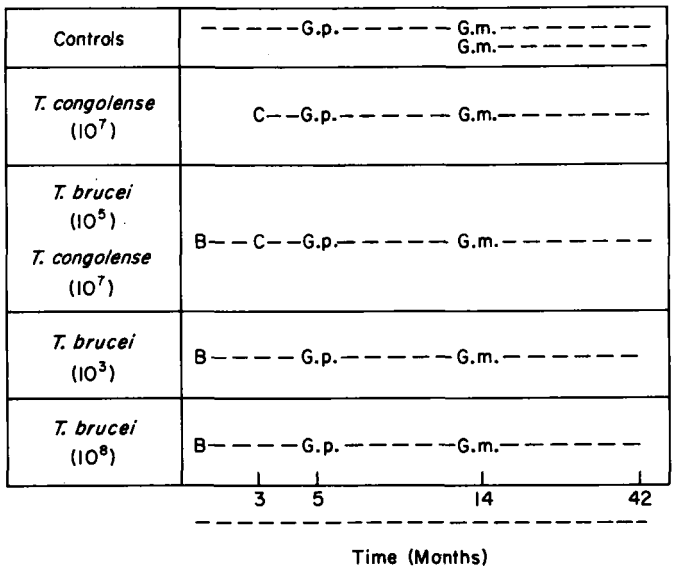

FIG. 3. Experimental design. Equivalent numbers of N'Dama and Zebu were exposed to a range of challenge regimes over a period of 42 months. B = needle challenge with *T. brucei;* C = needle challenge with *T. congolense;* G.p. = natural challenge with *Glossina palpalis gambiensis*; G.m. = natural challenge with *Glossina morsitans submorsitans.*

More recently, a series of large-scale experiments (Murray *et al.*, 1979c, d, e; the final results are summarized in this review) has been carried out in The Gambia, West Africa to evaluate further the extent of the difference in resistance to trypanosomiasis of N'Dama and Zebu cattle. Groups of N'Dama and Zebu were initially subjected to needle inoculation with bloodstream forms of *T. brucei* and *T. congolense* and monitored for parasitaemia and

anaemia for 2–5 months. The survivors were then exposed to a natural field challenge of *G. palpalis gambiensis* for 9 months. Subsequently, all animals and additional challenge controls were moved to another area where they came under challenge with *G. morsitans submorsitans* for 28 months. The experimental plan is shown in Fig. 3. Perhaps the most important aspect of these studies was the background of the cattle used. The Zebu were purchased from a ranch in Northern Senegal, well beyond the northern limits of the tsetse fly belt. N'Dama were obtained from the Government Agricultural Experimental Station at Yundum, The Gambia, a location at possible risk from *G. palpalis gambiensis* but where trypanosomiasis had never been reported. All animals were serologically negative for trypanosomiasis. Animals of both sexes were studied and at the start of the experiment were aged 3 to 4 years. When live calves were born (25 in all), they were included in the investigation; the Zebu produced no live calves.

The outcome of these investigations confirmed the marked difference in susceptibility to trypanosomiasis between N'Dama and Zebu as judged by mortality, parasitaemia, anaemia and productivity. The first part of the study involved subcutaneous challenge of cattle (Murray *et al.*, 1979d, e) with bloodstream forms of *T. brucei* and *T. congolense*. In order to study the effect of intensity of challenge, groups of N'Dama and Zebu were inoculated with 10^8 *T. brucei* ($n = 17$ for each group), 10^5 *T. brucei* ($n = 13$) and 10^3 *T. brucei* ($n = 11$). Ninety-six days later, to evaluate the susceptibility of trypanosome-infected animals, the groups infected with 10^5 *T. brucei* were superinfected with 10^7 *T. congolense*. At the same time, to serve as challenge controls, 11 animals of each breed which had not been previously exposed were inoculated with 10^7 *T. congolense* (Fig. 3).

TABLE 3

Numbers of cattle dying from trypanosomiasis

Nature of challenge	Zebu	N'Dama
Needle challenge[a]	9/40	0/37
G. palpalis gambiensis[b]	21/31	0/37
G. morsitans submorsitans[c]	31/31	15/73

[a] *T. brucei* and/or *T. congolense*.
[b] Geometric mean number of flies per trap per day = 0·17.
[c] Geometric mean number of flies per trap per day = 40.

All 52 N'Dama and 52 Zebu became infected and during the course of the experiment 15 N'Dama and 12 Zebu were selected for necropsy. In the remainder, clear differences in resistance emerged between the two breeds. None of the 37 N'Dama died, whereas 9 of the 40 Zebu (22%) died between

56 and 157 days after inoculation. Three died following challenge with *T. brucei*, three after superimposing *T. congolense* upon *T. brucei* and three following challenge with *T. congolense* (Table 3).

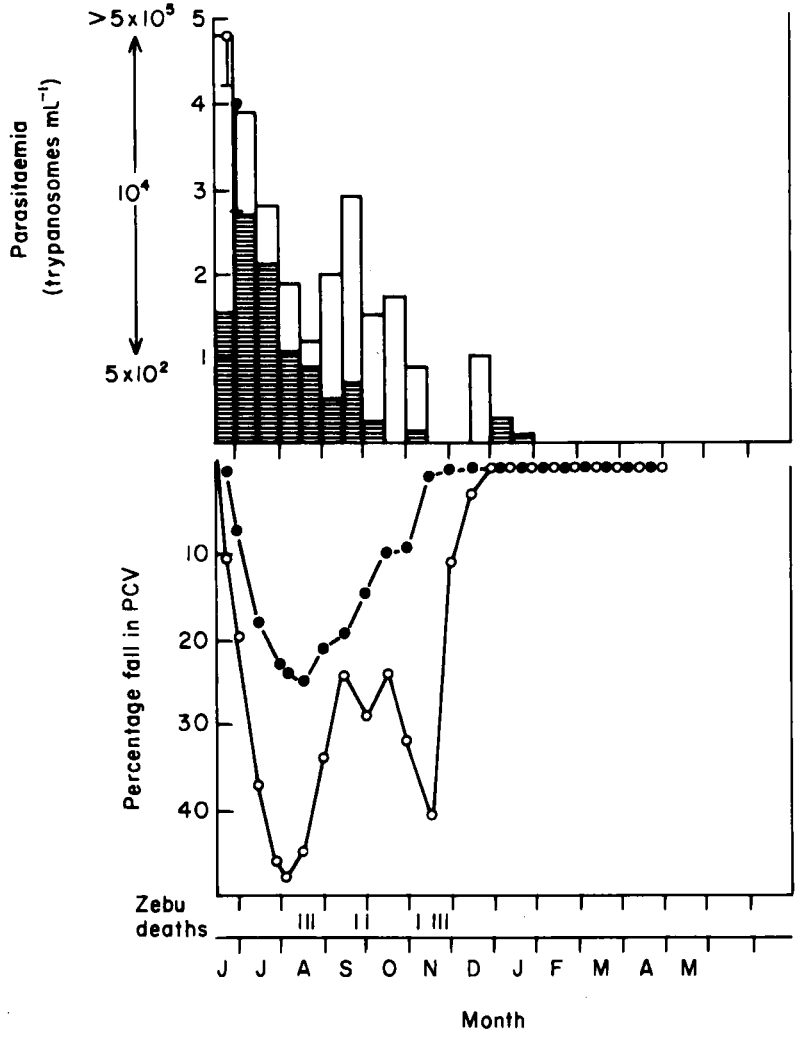

FIG. 4. Average bi-monthly parasitaemia score in N'Dama and Zebu inoculated with 10^7 *T. congolense*, percentage fall in packed cell volume (PCV) and Zebu mortalities. Hatched areas of the histogram represent N'Dama. The level of the first peak of parasitaemia plus 1 S.D. is shown for N'Dama (●) and Zebu (○). (Scoring system and estimated level of parasitaemia described by Murray *et al.*, 1979a; data reproduced from Murray *et al.*, 1979c.)

This result was reflected by marked differences in the degree of anaemia found in the two breeds. The rate of development and severity of the anaemia was significantly greater in the Zebu. This was true for both *T. congolense* (Fig. 4) and for *T. brucei*, although the severity of the anaemia and the extent of the difference between breeds was much greater in *T. congolense*-infected animals. Previous infection with *T. brucei* did not appear to have any influence on susceptibility to *T. congolense*, although, as before, the anaemia was more severe in the Zebu (Murray *et al.*, 1979c). Although the prepatent period was similar between breeds, the level, prevalence and duration of parasitaemia was consistently less in the N'Dama (Fig. 4).

After the initial needle challenge, the cattle were exposed to what was considered a light challenge with *G. palpalis gambiensis* (Table 3). During the ensuing 9 months, only 13 of the 37 N'Dama became detectably infected and these infections were only transient. None died and the 27 females produced nine calves. In contrast, of the 31 Zebu exposed, all became infected and 21 died of trypanosomiasis. Only 10 (32%) of the original group survived (Table 3). At the end of this period, all of the 37 N'Dama and the 10 surviving Zebu had apparently "self cured". Thus, the packed red blood cell volumes (PCV) in most animals had returned to normal and parasites could no longer be detected in the blood. Attempts to demonstrate parasites by subinoculation of blood and tissues into laboratory animals also failed.

The remaining cattle, their calves and additional susceptible controls were then transferred to an area where *G. morsitans submorsitans* was prevalent at a level of challenge considered to be the highest in The Gambia. During the period of study, of the 31 Zebu exposed, all became infected and died between 6 weeks and 22 months later (Table 3). The group of N'Dama under challenge included 48 adults and 25 calves. Of these, 13 adults and two calves died of trypanosomiasis (Table 3). Although all adult N'Dama became infected, nine of the calves never became detectably infected, suggesting the possibility of age-related resistance.

To emphasize further the extent of the difference in resistance between N'Dama and Zebu challenged by *G. morsitans submorsitans*, the results of one aspect of our investigation, in which 10 N'Dama and 10 Zebu were subjected to intensive surveillance, are described here in some detail (Murray *et al.*, 1981a). These animals were all 3-year-old females which had never been previously exposed to trypanosomiasis. All Zebu died of trypanosomiasis within 8 months of first exposure (Fig. 5). In contrast, only three N'Dama died of trypanosomiasis and all had been suckling calves before they succumbed 11 to 14 months after initial exposure. The prevalence, level and duration of parasitaemia were significantly less in the N'Dama, differences largely attributable to *T. vivax* and to a much lesser extent *T. brucei* (Table 4; Fig. 5). No significant difference was found for *T. congolense*.

Thus, during the first 47 days of challenge, 55% of all Zebu blood samples were positive for trypanosomes. Of these, 53% (or 96% of all positive samples) contained *T. vivax*. In contrast, 12% of the N'Dama samples contained parasites and only 2% (or 17% of all positive samples) were positive for *T. vivax*.

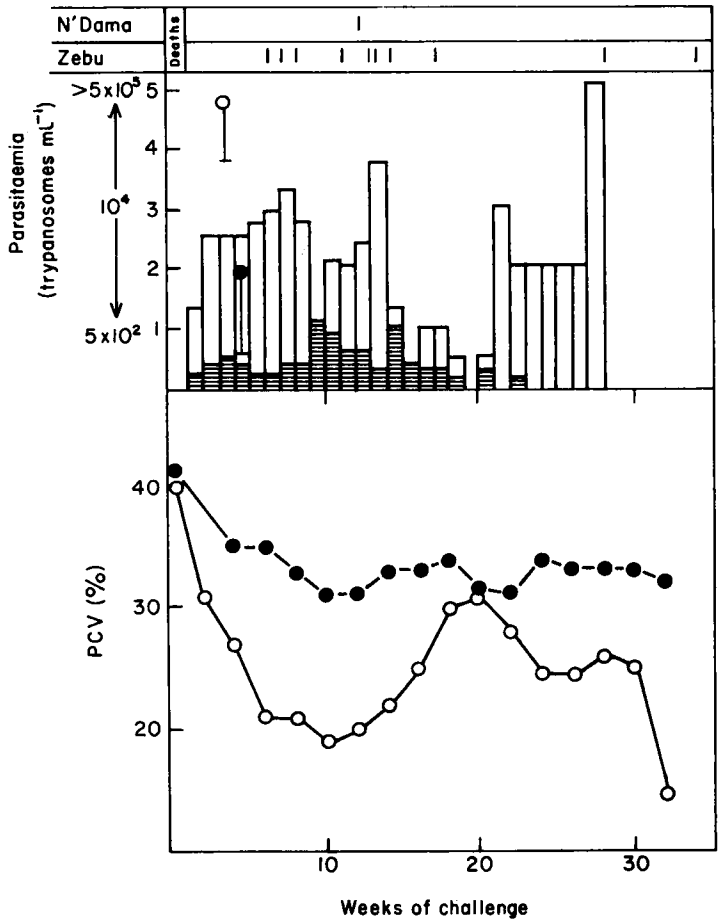

FIG. 5. Average weekly parasitaemia score in N'Dama and Zebu exposed to natural challenge of *G. morsitans submorsitans*, PCV values and mortalities. Hatched areas of the histogram represent N'Dama. The level of the first peak of parasitaemia plus one standard deviation is shown for N'Dama (●) and Zebu (○). (The one N'Dama death was due to anthrax.)

The N'Dama also developed much less severe anaemia than the Zebu (Fig. 5). In fact, the PCV of the N'Dama did not fall between 8 months after exposure (32, S.D. = standard deviation = 3), when all Zebu were dead,

and 21 months (PCV = 31, S.D. = 2) when the experiment was terminated. Perhaps the most impressive aspect of the study was the relative productivity of the N'Dama. In addition to lower mortality, the N'Dama experienced no abortions and produced five calves, three of which were alive at the end of the experiment; at this time three of the surviving N'Dama were pregnant. By contrast, the Zebu aborted in early and late pregnancy and produced no live calves.

TABLE 4

Prevalence of trypanosomes in N'Dama and Zebu cattle during the first 47 days of G. morsitans submorsitans *challenge*

	Number of samples				
	T. vivax	*T. congolense*	*T. brucei*	Total positive	Total examined
N'Dama[a]	10(2%)	41(10%)	14(3%)	51(12%)[b]	423
Zebu[a]	247(53%)	27(6%)	47(10%)	254(55%)[b]	465

[a] All animals were examined daily.
[b] Several infections were mixed.

Studies in Senegal have also compared the susceptibility of N'Dama and Zebu exposed to natural tsetse challenge (Touré *et al.*, 1978; Touré and Séyé, 1980). The N'Dama were more resistant than the Zebu, although there was some overlap. Furthermore, both Zebu and N'Dama, which had been exposed to trypanosomiasis previously, showed greater resistance than animals that had not. The greater resistance of the N'Dama and the animals with previous exposure was reflected by less severe anaemia and lower levels of parasitaemia. As in The Gambia, there was some indication that the higher levels of parasitaemia in the Zebu group were attributable mainly to *T. vivax*, at least in the initial phases of parasitaemia.

Further confirmation of the superior resistance of N'Dama to trypanosomiasis has been obtained in recent studies of N'Dama and Zebu inoculated with bloodstream forms of *T. vivax* (Saror *et al.*, 1981). Three groups of six animals were investigated. One group of Zebu and one group of N'Dama had been born and raised in tsetse-free areas while the remaining group of N'Dama were born and reared in an endemic trypanosomiasis area. All cattle were males, aged 2 to 3 years. The N'Dama were more resistant than the Zebu as judged by their less severe anaemia and, significantly, the N'Dama reared in an endemic trypanosomiasis area were even more resistant than the N'Dama not previously exposed. Once again, these results were reflected by lower parasitaemias in the most resistant animals.

2. *West African Shorthorn*

The other main group of trypanotolerant cattle in West Africa are broadly classified as West African Shorthorn. These animals are smaller in stature than the N'Dama (Table 2). A large number of breeds with a wide range of phenotypes makes up this group of about 1·8 million cattle (Table 1; Fig. 2b). There have been very few controlled studies of the different degrees of trypanotolerance exhibited by these breeds.

We have already described Stewart's (1937, 1951) observations on the resistance of Ghanaian cattle, which he described as West African Shorthorn; these animals were really a mixture of Hamitic longhorn (N'Dama), Shorthorn and Zebu. The trypanotolerant characteristics of the Muturu (a West African Shorthorn breed) were emphasized by Ferguson (1967). However, it was not until the studies of Roberts and Gray (1972a, 1973b) that attempts were made to evaluate the extent of trypanotolerance in Muturu and compare them with N'Dama and Zebu. The Muturu were born either of dams that had been exposed or, in the later studies, of dams that had not; all the animals studied were reared in a tsetse-free environment. These animals were challenged with wild-caught trypanosome-infected *G. morsitans* and their level of resistance was found to be intermediate between that of the N'Dama and Zebu, as judged by clinical condition, anaemia and survival. This result was confirmed by van Hoeve (1972), when animals of the same breeds were exposed to a natural tsetse challenge in the field. Similarly, Esuruoso (1977) also showed that Muturu were more resistant than Zebu to needle challenge with *T. vivax*. The genetic nature of the resistance was emphasized by the fact that these cattle were the second generation of a group reared in a tsetse-free area of Nigeria.

Many criticisms can be levelled at the foregoing studies of trypanotolerance, especially with respect to the questionable trypanosomiasis history of many of the cattle examined and to the small numbers used. This situation reflects one of the current constraints on basic studies of this subject, namely that trypanotolerant breeds of cattle which have not been exposed to trypanosomiasis are not readily available. Despite this, we feel that it is now established that trypanotolerant breeds such as the N'Dama and the West African Shorthorn are innately more resistant than the Zebu.

3. *Cattle in East Africa*

The possibility that genetic resistance to trypanosomiasis might have developed in cattle in East Africa has largely been ignored and the terms *Bos indicus* and Zebu are used synonymously with trypanosusceptibility. However, there is epidemiological evidence that, in some areas, Zebu have developed a degree of tolerance. For example, Cunningham (1966) has pointed out that thousands of Zebu cattle survive around the shores of Lake

Victoria even though they are continuously exposed to tsetse (Fig. 2c). He reported a 30% prevalence of trypanosomiasis and the presence of neutralizing antibodies in 90%. By definition, these animals must be considered trypanotolerant. More recently, we have evaluated genetic resistance to trypanosomiasis on a dairy ranch exposed to tsetse in the coastal region of Kenya (Trail *et al.*, in press). On this ranch, the adult breeding animals consist of two types, a 2/3 Sahiwal–1/3 Ayrshire, and a 1/3 Sahiwal–2/3 Ayrshire. Trypanosomiasis is controlled by treatment of natural infection. Animals with a PCV of less than 30% are treated with Berenil (diminazene aceturate, Hoechst). Each animal in the breeding herd of about 800 cows is bled and examined four or five times a year. Data analyses over a period of 6 years, using treatment as an indication of infection, showed that the 2/3 Sahiwal required less than half the number of treatments needed by the 1/3 Sahiwal. This was established at a very high level of significance (Fig. 6). Thus, genetic resistance does exist in cattle in East Africa although its extent in comparison with West African breeds awaits evaluation.

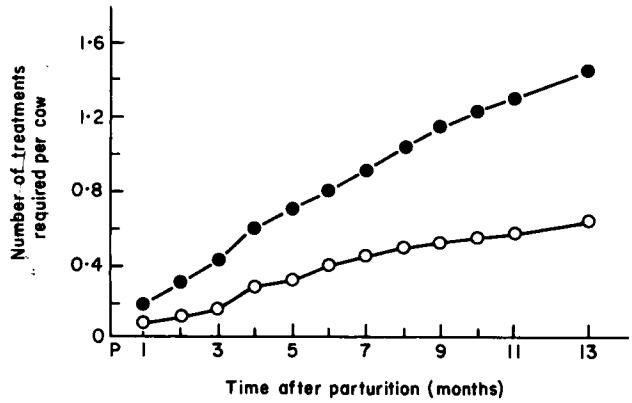

FIG. 6. Least-squares breed group means for number of current drug treatments (cumulative) for trypanosomiasis required by cattle at monthly intervals after parturition (P); (by permission of Trail *et al.*, in press). ●, 1/3 Sahiwal–2/3 Ayrshire breed; ○, 2/3 Sahiwal–1/3 Ayrshire.

B. SHEEP AND GOATS

In contrast to the increasing amount of information that has become available on the susceptibility of breeds of cattle to trypanosomiasis, the status of small ruminants is far less clear. There is little experimental work on the susceptibility of sheep and goats to infection either within or between breeds, but the fact that they are able to live in tsetse-infested zones without

any veterinary attention, and yet show little or no sign of disease, is taken as evidence of their trypanotolerance. Sheep and goats are present throughout Africa, despite the fact that there are large areas devoid of cattle, and can readily adapt to a variety of environments.

The main characteristic of the breeds that survive under threat of trypanosomiasis is their small size. The trypanotolerant sheep of West and Central Africa are sufficiently homogeneous to be considered as a single group known as the Djallonké or Fouta Djallon sheep. These "West African Dwarf" sheep (Mason, 1951) are ubiquitous south of the 14th parallel, and while difficult to subdivide systematically comprise two subgroups, the Dwarf Forest type and the Savannah type. In northern areas, crossbreeds between trypanotolerant sheep and the larger Sahelian breeds (Maure, Tuareg, Fulani) can be found.

A similar variation in size exists with goats, and the Dwarf goat or Fouta Djalla goat corresponds very closely in name, size, and distribution to the Dwarf sheep. It is smaller in the south (the true Dwarf) and larger in the northern part of its range (Mason, 1951). Djallonké sheep and Dwarf goats are found throughout the tsetse areas of West and Central Africa where there is no other breed of small ruminant (ILCA, 1979).

The situation in East Africa is even less well documented. However, a recent study (Griffin and Allonby, 1979a, b) in Kenya has indicated a trend similar to that in West Africa. Indigenous breeds of goat, the Galla and small East African, and sheep, the Red Masai and Black Persian, appear less susceptible to trypanosomiasis than exotic breeds. Both breeds of goat are smaller than imported varieties, but are not true dwarfs.

The epidemiology of trypanosomiasis in sheep and goats in West Africa is similar to that of cattle. There are breed differences in susceptibility and the dwarf types are generally more resistant. These smaller, more tolerant types may be maintained either as village livestock, or in the main savannah areas, where they are capable of withstanding slight to moderate tsetse risks. However, when the intensity of challenge increases, it is common for these animals to die (MacLennan, 1970). Similarly, in areas of high challenge in Zimbabwe, Blackhead Persian sheep, which are more resistant than cattle to *T. congolense*, become infected and suffer unthriftiness and abortion (Boyt, 1971; MacKenzie and Cruickshank, 1973).

Numerous surveys have described the incidence of trypanosomiasis in various regions of Africa, but give little useful information on the persistence or pathogenicity of the parasite in the host. The small ruminant population may act as a reservoir for human infections (Robson and Rickman, 1973; Rickman, 1974), and Mahmoud and Elmalik (1977) pointed out a possible role for goats in maintaining *T. congolense* as a source of infection through

mechanical transmission to calves in Sudan. Goats of an unspecified breed experienced a mild chronic infection in which trypanosomes were still demonstrable after 6 months, and some recovered spontaneously after more than 2 years of infection. In contrast, no calves survived similar infection for more than 7 weeks. Prolonged symptomless infections of goats suggested that they might be a reservoir of infection for cattle grazing side by side with them, and also demonstrated their reduced susceptibility to the disease. An earlier survey of sheep and goats in Upper Nile Province had shown many apparently healthy animals infected with *T. vivax* (Karib, 1961).

Reports indicate that the most widespread disease of small ruminants in East Africa is caused by *T. congolense*, whereas *T. vivax* is a more prominent cause of trypanosomiasis in the sheep and goats of West Africa. However, there have been few experimental studies of sheep and goats, and their use has hitherto been more as a model for bovine trypanosomiasis. Investigations have been carried out on Dwarf breeds in West Africa to investigate the pathological changes that accompany infections. Bungener and Mehlitz (1976) showed that *T. brucei* caused fatal infections of Cameroon Dwarf goats, whereas few clinical signs accompanied *T. vivax* or *T. congolense* infections. *T. brucei* was also pathogenic for West African Dwarf sheep (Ikede, 1979), and Red Sokoto Dwarf goats succumbed within 70 days to *T. vivax* infection (Saror, 1980). Earlier experimental studies with needle inoculation of trypanosomes also showed that Dwarf breeds not only became infected, but also developed anaemia and emaciation (Edwards *et al.*, 1956). Thus, while the Dwarf breeds are generally accepted to be more trypanotolerant, it is apparent that they can be severely affected following experimental infections as well as under certain circumstances in the field (MacLennan, 1970).

The introduction of exotic breeds for sheep and goat improvement programmes in many developing countries has precipitated the requirement for detailed comparative studies under controlled conditions of animals of known history to determine the extent of trypanotolerance among small ruminants both in East and West Africa.

The study of Griffin and Allonby (1979a, b) was the first to compare the susceptibility of indigenous and exotic breeds of sheep and goats to both needle challenge and fly challenge. They compared East African and Galla goats and Red Masai and Black Persian sheep, with exotic Saanen goats and Merino sheep (10 animals per group). The indigenous breeds fared better than the imported breeds after needle challenge with *T. congolense*. The exotic indigenous crossbreed goats (Saanen/Galla) were intermediate between the two extremes. By the time the experiment was terminated at 14 weeks, all the Saanen goats and 50% of the Merino sheep had died. No indigenous sheep died, while two, one, and two animals died from the East African, Galla and Saanen/Galla groups respectively. Field studies (Griffin

and Allonby, 1979b) also indicated lower infection rates and mortality in indigenous breeds of sheep and goats exposed to natural challenge. We have confirmed the superior resistance of Red Masai over Merino sheep to needle challenge with *T. congolense*. Merino sheep experienced a sharp drop in PCV during the first 3 weeks of infection to a level 15% below normal, and displayed high parasitaemia. However, over this initial period, despite equally severe parasitaemia, the PCV of the Red Masai fell only 5–7% below pre-infection levels. Moderate anaemia developed in the Red Masai after 7 weeks of infection, by which time most of the Merinos were dead. Thereafter, parasites became less numerous in the circulation and the haematocrit slowly returned to near normal levels.

Thus, as with cattle, there is now evidence for significant genetic resistance in sheep and goats. It will be of considerable importance to carry out comparative studies of the extent of trypanotolerance in cattle and small ruminants exposed to similar levels of trypanosomiasis risk.

C. WILDLIFE

The susceptibility of indigenous breeds of domestic livestock to trypanosomiasis is generally considered to be intermediate between exotic breeds at the susceptible end of the spectrum and the wildlife population at the opposite extreme.

Wild animals have a reputation for being highly resistant and in some cases completely refractory to trypanosome infection. Such conclusions are usually based on the fact that wildlife survives in areas heavily infested with tsetse, and also on surveys involving the examination of blood samples for trypanosomes using a variety of light microscope parasitological techniques. While the results of these kinds of studies give an indication of the incidence of trypanosomes in particular species of wildlife, they yield little information on susceptibility to the disease. Scientific names of animals mentioned in this section are given in the Appendix (p. 56).

1. *Prevalence of trypanosomiasis in wildlife*

During post-mortem examinations, early workers found trypanosomes in the blood of wild animals on numerous occasions. Natural infections of oribi (Bruce, 1914) and waterbuck (Bruce, 1915; Kinghorn and Yorke, 1912) with *T. congolense* were reported early this century. Ashcroft (1959) reviewed the results of several parasitological surveys, which showed that of 1242 wild animals examined in various locations at various times, 19·5% were infected with trypanosomes. *T. brucei* accounted for 4·2%, *T. vivax* for 6·3% and *T. congolense* for 10·4%. Because the majority of cases were diagnosed

by blood slides, it is certain that these results were underestimated. In his own study attempting to isolate human-infective *T. brucei* from game animals, Ashcroft (1958) found trypanosomes in only 11 of 74 animals in Tanzania; seven were positive by blood film and four by rat subinoculation.

The early surveys showed that the animals with the greatest incidence of all forms of trypanosomiasis were waterbuck (52%), kudu (45%), reedbuck (44%), giraffe (37%), bushbuck (31%), and eland (29%). Buffalo, bushpig, duiker, hartebeest, impala, oribi, puku, roan antelope, topi and warthog had a similar incidence (between 10% and 16%). The incidence in zebra was low (6%) and trypanosomes were not found in sable antelope, rhinoceros, Thomson's gazelle or wildebeest.

Improvements in techniques of parasite detection and identification over the last two decades have extended the list of infected animals and indicated a different distribution. Nevertheless, it has been stated that "there are indications that the number of animals harbouring trypanosomes is far in excess of those showing detectable parasitaemias even when using a combination of diagnostic methods" (Dillmann and Townsend, 1979).

Baker *et al.* (1967) demonstrated a relatively high incidence of trypanosomes in wildebeest (27%) and also found infected Thomson's gazelle and lion. No trypanosomes were detected in zebra. *T. brucei* was identified in the blood of two lions in the Serengeti (Sachs *et al.*, 1967) and the overall incidence of *Trypanosoma* species in lions in this region was very high (68·8%). Reedbuck were the most frequently infected of eight species examined in southern Tanzania (Geigy *et al.*, 1967), two infected zebra were reported by McCulloch (1967) and in Baker's (1968) summary of surveys in the Serengeti, only buffalo, leopard and jackal were free from infection.

Geigy *et al.* (1971) surveyed 13 species of wild mammal for possible reservoirs of human trypanosomiasis in Tanzania. Unlike previous surveys (Baker, 1968), no *T. brucei* sub-group or *T. congolense* were found in the ten wildebeest examined. One zebra was found to be infected with *T. vivax*. Of the *T. brucei* strains isolated, human-infective parasites were found in hyaena, lion and hartebeest (Geigy *et al.*, 1975). Bushbuck, the first wild animal species shown to harbour human-infective trypanosomes (Heisch *et al.*, 1958), waterbuck and warthog (Dillmann and Townsend, 1979) and giraffe (Awan, 1979) are also potential reservoirs for human infections.

2. *Persistence of natural infections*

While the above surveys provided no information about the period of time that an animal had been infected, there is evidence on this point from other sources. Hornby (1952) reported the case of an antelope which died in London Zoo in 1945, 11 years after its capture in Nigeria. Although it had been in apparent good health during its captivity, *T. congolense* was

found in its blood at necropsy. Similarly, *T. brucei* was found in a hyaena after it died in London Zoo in 1942. This animal had been captured 6 years previously in Uganda (Hoare, cited by Baker, 1968). Obviously, both of these animals must have been infected before leaving Africa and maintained symptomless infections for many years. The long-term transmission experiments conducted at the Tinde Laboratory in Tanzania also demonstrated that trypanosomes could be found in the blood of wild animals for long periods after fly infection (Willett, 1970).

3. *The effects of infection in wild animals*

In all the reports summarized by Baker (1968), only three animals were observed to be clinically ill. Following necropsy, McCulloch (1967) attributed the lethargy and poor condition of two zebra to trypanosomiasis, but the cause of illness in one lion was not determined (Sachs *et al.*, 1967). Burridge *et al.* (1970) stressed that there was no sign of disease in bushbuck, waterbuck or elephant, in which they detected trypanosomes.

As part of the study by Geigy *et al.* (1971), post-mortem examinations of warthog, impala, Thomson's gazelle, hartebeest, topi, zebra and lion were carried out (Losos and Gwamaka, 1973). Significant histological lesions were found in two impalas, one gazelle, three hartebeest and two lions, but they were mild and suggested that the infections were probably asymptomatic. Dillmann and Townsend (1979) observed young lions, in poor condition, whose blood showed unusually high parasitaemias. They noted that animals of this age were exposed to social stress within the pride, which in turn resulted in nutritional stress, but were unable to determine whether the lions' poor condition was the cause of the higher parasitaemias or vice-versa. High parasitaemias in wounded buffaloes, buffaloes in poor condition, and bushbuck rams injured in the course of territorial conflicts also emphasized the importance of stress (Awan and Dillmann, 1973). In this context, Lumsden (cited by Baker, 1968) concluded that stress contributed to the death of a rhinoceros which showed a high parasitaemia when captured. An account of an outbreak among cattle in Kenya (Wijers, 1969) indicated that the wild animal population also appeared to be affected. Dead or very thin, weak bushpigs were found in the forests after advances of tsetse.

Thus, while surveys have shown only limited clinical illness in infected wild animals, it is true to say that they can be affected by trypanosomiasis, as emphasized by Ford (1971). Moreover, susceptible individuals which become clinically ill may die and will certainly be at greater risk from predators. In either event, the effects of infection in such animals are unlikely to be detected and recorded.

4. *Wild animals as hosts of* Glossina

Weitz (1963) showed that the genus *Glossina* has a wide range of hosts, of

which pigs of different species are generally favoured. There are also hosts which are attractive to only one or two species of tsetse, e.g. rhinoceros, elephant (*G. longipennis*), reptiles (*G. palpalis* and *G. tachinoides*), hippopotamus (*G. fuscipleuris* and *G. brevipalpis*), porcupine (*G. tabaniformis*), kudu and elephant (*G. morsitans* in Central and East Africa). Certain animals accounted for 8 or 9% of the identified feeds of only one species of tsetse: duiker (*G. austeni*), giraffe (*G. swynnertoni*) aardvark (*G. fusca*), and ostrich (*G. longipennis*). In addition, there are some animals on which flies rarely, if ever, feed. Although the zebra occupies large areas of *G. morsitans* country in East Africa, its blood has never been found in any meal. Impala, wildebeest, waterbuck, gazelle, dikdik, monkey, baboon, oribi, eland, dogs, cats and other carnivores are also of negligible importance as hosts for the tsetse.

In view of these results, it is apparent that there is a discrepancy between the species of wildlife which are preferred hosts of the tsetse and those shown to be naturally infected with trypanosomes in the field. Because many animals that are rarely bitten by tsetse are frequently infected with trypanosomes, one must conclude that transmission occurs by other means. For example, trypanosomes may be non-cyclically (mechanically) transmitted by biting flies and through the ingestion of infected meat by carnivores. In addition, if sufficient pressure is exerted by the absence of preferred hosts, the fly is sufficiently adaptable in its feeding habits to find an alternative host.

This last point may explain the discrepancies between the data of Ashcroft (1958), who found a high incidence of infection in waterbuck and impala, and Weitz (1963), who concluded that these species were ignored by all tsetse flies as a source of food except on isolated occasions. The question of non-cyclical transmission was discussed by Baker (1968), who suggested that the high proportion of infected herbivores among species which are rarely if ever bitten by tsetse flies, was due to non-cyclical transmission by insects such as *Tabanus* and *Stomoxys*. Furthermore, the high incidence of infection in lions, which are rarely used by tsetse as a source of food, could be due to killing and eating infected herbivores. Cats (Duke *et al.*, 1934) and bushbabies (Heisch, 1952) have been experimentally infected by eating infected animals. It is likely that trypanosomes penetrate small lesions of the buccal mucosa which must often be damaged by the bones of the prey.

5. *Experimental studies in wild animals*

In view of the body of opinion which favours farming of wild animals, there is an urgent requirement for investigations on the effects of trypanosomiasis on wildlife. The major constraint is the availability of sufficient numbers of suitable animals, especially animals which have not been previously exposed, and the experiments that have been done in the past reflect this problem.

In a limited early study (Carmichael, 1934), several wildlife species were either not infectible with *T. congolense* or developed only transient infections and then recovered. However, there was a spectrum of susceptibility to *T. brucei* and some fatalities occurred. Needle challenge with *T. brucei* and *T. rhodesiense** was examined by Ashcroft *et al.* (1959), who recognized two broad categories of response to infection. Gazelle, dikdik, blue forest duiker, jackal, bat-eared fox, aardvark, hyrax, serval and monkey usually died, whereas species in the other category showed a spectrum of tolerance to infection. Bush duiker, eland, Bohor reedbuck, hyaena, oribi, bushbuck and impala were all infectible and remained parasitaemic for a considerable time; very slight parasitaemias were exhibited by warthog, bushpig and porcupine, while baboons were apparently refractory to infection.

Assuming a similar relative susceptibility of species of wildlife to trypanosomes other than *T. brucei* and *T. rhodesiense*, Ashcroft (1959) correlated these results with bloodmeal data (Weitz and Jackson, 1955; Weitz and Glasgow, 1956; Glasgow *et al.*, 1958) and found that none of the species which experience fatal infections was a common host for the fly. However, reedbuck, which are fed on frequently by tsetse, were relatively susceptible to infection, whereas baboon, which tsetse rarely bite, were completely resistant. Therefore, a species which is bitten frequently by tsetse is not always resistant to trypanosomes, nor are species that are rarely bitten necessarily susceptible. Moreover, the results bore little relation to the taxonomic position of the species: the bush duiker and blue forest duiker belong to the same sub-family, yet one is tolerant to an infection that kills the other.

A study of a small number of duiker and gazelle (Roberts and Gray, 1972b) showed that duiker were very resistant to *T. vivax* and developed only sporadic parasitaemia with no anaemia or signs of disease. The parasitaemia in gazelle was similar to that of cattle. The animals were prostrate and febrile at peak parasitaemia, but this effect was transient: parasitaemia became slight and recovery followed. *T. congolense* caused a similar syndrome in gazelle. While this parasite was more easily detected in duiker than *T. vivax*, it was sporadic and transient. The authors suggested that the duiker possessed immune mechanisms superior to the gazelle and cattle.

We have recently had a unique opportunity to investigate the susceptibility to trypanosomiasis of several species of wild animals that had never been exposed to trypanosomes (Murray *et al.*, 1981b). Preliminary studies indicated that transmission rates from *G. morsitans* infected with *T. congolense* were lower in eland and waterbuck than in domestic animals. The superior innate resistance of these species of wildlife was further confirmed when they developed lower levels of parasitaemia and less severe anaemia.

Thus, there is a wide spectrum of susceptibility to trypanosomiasis, but the evidence that many species are very resistant is overwhelming. Future studies

* Usage of the names "*rhodesiense*" and "*gambiense*" here and elsewhere follows that of the original authors.

should provide more information on the nature of the disease in wild animals, more data on their role in the epidemiology of the disease in man and his domestic animals, and a more complete understanding of the important mechanisms that determine host resistance or tolerance to trypanosomes.

D. MAN

In considering the epidemiology of human sleeping sickness, Buyst (1977) argued that natural selection of people with an innate ability to develop a more efficient immune response to the infection might play a significant role in the disease syndrome that evolved. Thus, many of the Bantu speaking people are well adapted to an ecology of light woodlands with medium humidity. Such groups have a history extending over several thousand years of almost uninterrupted contact with tsetse and presumably with man-infective trypanosomes. These people tend to develop a less acute form of the disease and there are even some cases in which spontaneous self-cure has occurred (Buyst, 1974).

In contrast, the disease syndrome reported in Caucasians is almost invariably very acute (Davis *et al.*, 1974). Also, Bantu speaking people whose ancestors have had little or no contact with man-infective trypanosomes for several centuries may experience a more acute form of the disease, as occurred in Zambia (Buyst, 1974). Similarly, Nilotic groups, who are adjusted to a hot and dry environment, have had little contact with tsetse or man-infective trypanosomes until relatively recently. These groups have exhibited very acute types of sleeping sickness both in Ethiopia and around Lake Victoria. Fairbairn and Burtt (1946) have also observed a wide range in susceptibility to trypanosomiasis in man.

As with livestock, it is difficult to assess from field studies the role played by acquired as opposed to genetic resistance. Nevertheless, we feel that Buyst (1977) has presented an excellent case for the role of genetic resistance in the susceptibility of man to trypanosomiasis.

E. MICE

The lack of suitable herds for study and the genetic heterogeneity of domestic livestock have made it difficult to analyse the mechanisms and inheritance of trypanotolerance. Accordingly, we initiated a series of experiments on the susceptibility of different inbred strains of mice to *T. congolense*, *T. brucei* and *T. vivax* (Morrison *et al.*, 1978; Jennings *et al.*, 1978; Morrison and Murray, 1979; Whitelaw *et al.*, 1980). It was found that strains differed

markedly in their susceptibility, as judged by survival. In a comparison of eight strains of mice to infection with *T. congolense*, Morrison *et al.* (1978) showed that the C57BL/6 was least susceptible, the A/J was the most susceptible, while strains such as the C3H/He were intermediate (Table 5). Jennings *et al.* (1978) also demonstrated that the C57BL was the least susceptible strain. A similar pattern of susceptibility of a range of mouse strains was found using different isolates of *T. congolense* (Morrison *et al.*, 1978); the degree of difference in susceptibility was related to the virulence of the infecting organisms. Thus, the most virulent isolates produced rapidly lethal infections in all mouse strains with minimal differences in survival time. All mouse strains survived the first peak of parasitaemia when challenged with a few isolates of low virulence.

The susceptibility of different strains of mice to *T. congolense*, at least during the early stages of infection, appears to be closely related to the capacity to control and reduce the numbers of trypanosomes in the circulation (Fig. 7) (Morrison *et al.*, 1978; Whitelaw *et al.*, 1980). Both found that the parasitaemia in the least susceptible strains underwent remission while in the most susceptible strains it did not and remained at levels greater than $10^5\ \mu l^{-1}$. While Morrison *et al.* (1978) also observed marked differences between mouse strains in the level of the initial peak of parasitaemia which correlated with survival, Whitelaw *et al.* (1980) did not. These results probably reflect variations in the virulence of the organisms used in the two studies.

TABLE 5

Duration of survival of inbred strains of mice infected with T. congolense

Mouse strain	Percentage deaths					Mean survival time (days)
	Days after infection:					
	20	40	80	120	160	
A/J	85·7	100				15·8
SWR/J	88·0	100				16·9
129/J	58·5	90·2	100			22·6
BALB/c/A	20·0	28·8	100			49·5
DBA/1J	0	62·2	100			36·3
C3H/HeJ	0	12·5	100			59·0
AKR/A	0	7·9	45·4	97·7	100	81·7
C57BL/6J	0	2·8	13·8	61·1	100	110·2

The possibility that differences in parasitaemia between mouse strains infected with *T. congolense* might be related to the infectivity of the trypanosomes and to their rate of replication could not be confirmed. Titrations

showed that infectivity of the *T. congolense* used was the same in mice of high (A/J) and low susceptibility (C57BL). Furthermore, the finding that the prepatent period was similar in the different mouse strains indicated that there was no significant difference in the replication rate, at least in the initial phases of infection (Morrison *et al.*, 1978).

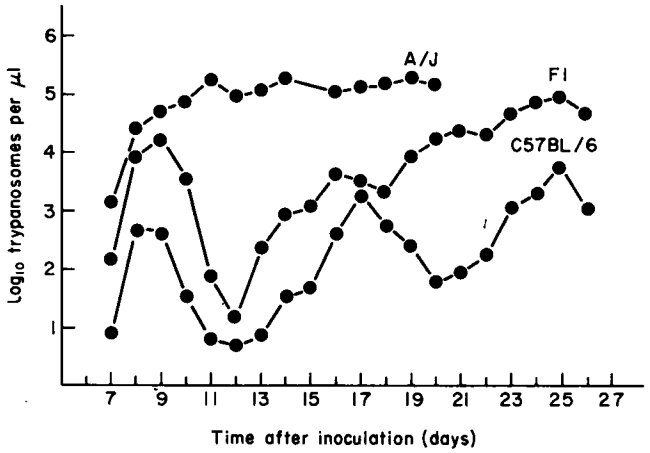

FIG. 7. Parasitaemia in A/J, C57BL/6 and F_1 hybrids ((B6A) F_1) infected with *T. congolense*. Daily mean values of parasitaemia are given for 12 mice of each strain monitored over the first 26 days after infection. Only small numbers of A/J mice survived beyond day 20. Data are reproduced from Morrison *et al.* (1978).

TABLE 6

Duration of survival of inbred strains of mice infected with T. brucei

Mouse strain	Percentage deaths					Mean survival time (days)
	Days after infection:					
	20	40	80	120	160	
C3H/He	8	100				29
A/J	0	67	100			39
C57BL/6J	0	0	67	100		58

Similar but less extensive studies on susceptibility of inbred strains of mice have been carried out with *T. brucei* and *T. vivax* (Clayton, 1978; De Gee *et al.*, in press). As with *T. congolense*, the differences in susceptibility were found to be dependent on the virulence of the trypanosomes used but the magnitude of the differences in survival was always less than that observed with *T. congolense*. Furthermore, the rank order of susceptibility within the

mouse strains showed some differences from *T. congolense*. Thus, A/J and C57BL strains were again of highest and lowest susceptibility, respectively, to *T. vivax*. With SWR/J and BALB/c, however, which showed high and moderate susceptibility to *T. congolense* respectively (Table 5), the converse was true for *T. vivax* (De Gee *et al.*, in press). With *T. brucei*, the C3H/He strain, which exhibited intermediate susceptibility to *T. congolense*, was consistently the most susceptible (Clayton, 1978; see Section VII B 2, p. 49); the A/J was of intermediate susceptibility (Table 6). A consistent feature of these studies was that the C57BL was always the least susceptible strain regardless of the trypanosome species.

IV. Productivity of Trypanotolerant Livestock

A variety of opinions exists as to just how resistant and how productive trypanotolerant breeds are, under various ecological and management regimes and at different levels of trypanosomiasis risk. The productivity of trypanotolerant livestock is especially called into question because of their small size in comparison with more susceptible breeds (Table 2). As pointed out by Roberts and Gray (1973a), many misconceptions about trypanotolerant breeds have arisen because productivity indices generated in herds living under poor conditions and exposed to tsetse have been compared with data collected from a variety of breeds of cattle kept under good conditions. There is also the belief that trypanotolerant breeds are poor breeders. The results of Ferguson (1967) and Roberts and Gray (1973a) contradict these observations; they reported that Muturu and N'Dama were able to produce their first calf by 24 months and had mean calving intervals of less than one year. Other workers (Touchberry, 1967; Doppler *et al.*, 1980) have reported less favourable findings, indicating that all results must be interpreted within the context of the circumstances under which they were made.

In a recent major survey of the status of trypanotolerant livestock in West and Central Africa (ILCA, 1979), indices of productivity were examined using all the basic production data that could be found for each region, each management system and for different levels of challenge. The findings of this survey illustrated that there is a lack of comparative data on different breeds living under each of these circumstances. However, the results strongly suggested that in areas of no or low tsetse challenge the productivity of trypanotolerant cattle relative to other indigenous breeds is much higher than previously assumed. Furthermore, comparative data could not be obtained in some areas because the level of trypanosomiasis risk was such that breeds other than trypanotolerant ones could not survive. This was emphasized

many years ago by Stewart (1951). Similarly, as described earlier, we lost 21 of 31 Zebu from trypanosomiasis in an area of *G. palpalis gambiensis* challenge where only one-third of 37 N'Dama became transiently infected and showed no evidence of disease. Furthermore, of 31 Zebu maintained in a *G. morsitans submorsitans* region, all died of trypanosomiasis, whereas only 15 of 73 N'Dama succumbed.

Even under circumstances where Zebu survive, the differences from N'Dama in terms of bodyweight are striking. Roberts and Gray (1973a, b) found that on unimproved but tsetse-free pastures the Zebu (average weight of 243 kg at 18 months) out-performed the N'Dama (average weight of 180 kg). However, following tsetse challenge, the order of performance was reversed, with Zebu losing 37 kg over 26 weeks, when they might have been expected to gain 58 kg, i.e. an effective weight deficit of 95 kg. N'Dama gained 16 kg compared to an expected gain of 35 kg, i.e. an effective deficit of 19 kg. Because of the range in ages of the animals exposed to tsetse, absolute figures for body weights were not supplied. However, it is interesting to speculate on the outcome on the basis of the results quoted above. By the end of the experiment, an 18-month-old Zebu would have weighed 301 kg if left unexposed, whereas an N'Dama of the same age would weigh only 215 kg, a difference of 86 kg. Exposure to tsetse removed these differences almost completely; the Zebu would be expected to weigh 206 kg, the N'Dama 196 kg.

That genetic resistance to trypanosomiasis is not necessarily associated with low productivity was confirmed by our recent findings that on a dairy ranch in Kenya, Sahiwal crosses were as productive as Ayrshire crosses, although they required only half the number of treatments for trypanosomiasis (Trail *et al.*, in press).

While much more information is required, these observations indicate that genetically-resistant breeds can be productive within the limitations of their environment.

V. Genetics of Trypanotolerance

It is important to obtain information on the heritability of trypanotolerance for two reasons: first, to determine the feasibility of further selection of stock that are even more resistant to the disease and, second, to obtain the necessary information to improve the productivity of trypanotolerant stock by cross-breeding without loss of resistance. In many of the experiments already described, there was a considerable range of resistance within the breeds. This indicated that the factors governing susceptibility may have a complex genetic basis and offered the hope that it might be possible to increase trypanotolerance by selective breeding.

There have been relatively few studies of the heritability of trypanotolerance in cattle. Stewart (1951) reported cross-breeding studies involving N'Dama, Zebu and Ghanaian Shorthorn (a trypanotolerant genetic mix) in which he produced a larger, more productive animal that retained its resistance to trypanosomiasis. Similarly, Chandler (1952) found that N'Dama/Zebu crossbreeds retained a significant degree of trypanotolerance when exposed to natural tsetse challenge. Nine of 12 Zebu exposed died of trypanosomiasis, but only four of 12 crosses died; all eight N'Dama were alive and in good condition at the end of the experiment. In breeding experiments in the Ivory Coast involving large numbers of N'Dama and Jersey, Letenneur (1978) found that the F1 cross produced an excellent animal as regards growth and milk production. It was stated that such crosses retained their tolerance, although no information was given on the level of fly challenge or on the prevalence of trypanosomes. However, crossbreeds with greater than 50% Jersey background appeared to be less hardy and gave equivocal results.

While these results suggest that trypanotolerance is a dominant trait, wide differences in results must be expected with outbred populations. Indeed, it is likely that the degree of trypanotolerance observed in first generation crosses will vary widely depending on the parental combination. Thus, future studies will require the use of large groups of animals with known trypanotolerant status of both parental breeds. The offspring should be tested over several generations. Only then will it be possible to determine the feasibility of selection for maximum trypanotolerance along with the desired characteristics of the other breed. Such a programme would be greatly aided by the availability of genetic markers that would permit monitoring of resistance without having to infect all of the animals. It has been proposed that resistant animals could be detected by haemoglobin type because N'Dama show almost 100% gene frequency for haemoglobin type A (HbA) and Zebu are a mixture of A and B. However, certain exotic breeds of cattle such as the Friesian are also predominantly HbA (Bangham and Blumberg, 1958) and are highly susceptible to trypanosomiasis, as are Zebu with Hb AA.

With current evidence that trypanotolerant breeds of sheep and goats exist, it will also be necessary to investigate heritability in these species, a task possibly less forbidding because of their shorter gestation period.

Because it is difficult to study heritability of trypanotolerance in domestic livestock, we carried out a series of experiments to investigate the underlying genetic basis of susceptibility to trypanosomiasis in inbred strains of mice (Morrison and Murray, 1979). The observation that a range of inbred strains of mice exhibited a spectrum of susceptibility to *T. congolense* (Morrison *et al.*, 1978), rather than discrete categories of high, low and intermediate susceptibility, suggested the involvement of several genes. Further studies

examined the susceptibility of F1 hybrids and backcrosses between strains of high (A/J), low (C57BL/6), and intermediate (C3H/He) susceptibility. Low susceptibility was found to be incompletely dominant in F1 hybrids of A/J and C57BL, in that the hybrids survived as long as the more resistant parent (C57BL/6) but developed intermediate levels of parasitaemia (Fig. 7). The survival and parasitaemia data obtained with backcrosses of these F1 hybrids with the parental strains suggested that two genes might control susceptibility to infection. However, the F1 hybrids of C3H/He with A/J and C57BL/6 did not conform to this two gene model. On the basis of these results and because the different strains of mice showed a spectrum of susceptibility, it was concluded that susceptibility to *T. congolense* is under complex genetic control (Morrison and Murray, 1979).

With the demonstration in mice that immune response (Ir) genes, located within the major histocompatibility complex (MHC), control antibody response to specific antigens (McDevitt and Benacerraf, 1969), considerable interest was generated in the role of the MHC in determining susceptibility to disease. Several studies demonstrated that susceptibility to certain virus infections in mice and the prevalence of certain diseases in man were at least partially linked to H-2 haplotype (Lilly and Pincus, 1973; McDevitt *et al.*, 1974) and with particular HLA antigens (McDevitt and Bodmer, 1974; Vladutiu and Rose, 1974) respectively. More recently, the knowledge that the MHC codes for genes which are involved in antigen recognition (Doherty and Zinkernagel, 1974) and in antigen presentation (Niederhuber, 1978), has also highlighted its potential role in influencing susceptibility to infections. In the mouse, the availability of H-2 congenic resistant strains, i.e. mice whose genotype differs only at the H-2 complex, readily permits investigations to determine the association of H-2 haplotype with susceptibility to infection. We have examined susceptibility to infection with *T. congolense* in H-2 congenic mice on a C57BL/10 genetic background (Morrison and Murray, 1979). Congenic mice of H-2 haplotypes a and k showed no significant difference in parasitaemia from C57BL/10 (haplotype b) mice. While the duration of survival was significantly longer in the C57BL/10, all three strains survived beyond 100 days. These results suggested that H-2 haplotype does not exert a major influence on susceptibility to infection with *T. congolense*. Similar results were obtained for *T. vivax* (De Gee *et al.*, in press).

Differences in the susceptibility of inbred strains of mice have also been reported for other parasites of the order Kinetoplastida, including *Leishmania donovani* (Bradley, 1977) and *T. cruzi* (Trischmann *et al.*, 1978). While a spectrum of susceptibility to *T. cruzi* was found in a range of mouse strains, strains of mice segregated into high and low susceptibility to *L. donovani*. On the basis of cross-breeding studies, it was concluded that susceptibility to this parasite was controlled by a single gene (Bradley, 1977).

Susceptibility to *T. cruzi* and *L. donovani* was also found to be unrelated to H-2 haplotype, but patterns of recovery from *L. donovani*, as judged by parasite load, have recently been shown to be controlled by a gene or genes within or closely adjacent to the H-2 complex (Blackwell *et al.*, 1980).

VI. Factors Affecting Genetic Resistance

Trypanotolerance is not an entirely stable characteristic and there is considerable evidence that suggests it can be supplemented or reduced by a number of factors affecting the host and its environment.

A. AGE AND SEX

Age appears to play a significant role in resistance to trypanosomiasis. A number of workers have confirmed that young calves are more resistant to trypanosomiasis (reviewed by Fiennes, 1970; Wellde *et al.*, 1981; see Section III A 1, p. 12). We have also found this to occur with *T. congolense*, when a comparison was made of experimental infections in 6-week-old Boran calves and 18-month-old animals. On the other hand, 1-week-old calves appeared to be more susceptible than adults to *T. brucei*. In both experiments, the dams had never been infected with trypanosomes.

Drager and Mehlitz (1978) noted significant age-related resistance in 416 buffalo in Botswana. In 74 calves of less than one year of age, trypanosomes were found in only one and antibody levels were low. On the other hand, the prevalence of parasites and antibodies in adult animals was much higher, e.g. 34% (31 of 90) animals aged between 1 and 3 years were infected with trypanosomes. Maternally derived immunity may have been an influencing factor in these findings (see Section VI B, p. 32).

We have also noted remarkable age-related resistance of C57BL mice to *T. congolense*. Five groups of mice aged 1 to 12 weeks were tested. While all mice became infected, there was a remarkable difference in survival of the mice challenged at 1 week of age. In this group, there were no deaths by day 50, one by day 100 and six by day 150 (Table 7). In all other groups, some mice had died by day 50, especially in the older age groups, and by day 150 all mice were dead. This difference in susceptibility was reflected by differences in parasitaemia. The initial parasitaemias of the 1-week-old mice were barely detectable, while peaks of around $10^5\ \mu l^{-1}$ developed in the other groups (Table 7). By the fourth week of infection, the parasitaemia in the group infected at 1 week of age had increased to levels comparable with

other groups. In contrast to the findings with *T. congolense*, we have found neonatal mice to be more susceptible than adults to infection with *T. brucei*, an observation also made by Clayton (1978). Thus, the results in mice parallel the situation in cattle.

TABLE 7

Effect of age on survival of C57BL mice challenged with T. congolense

Age (weeks) at challenge:		1	3	6	12	24
Number of mice (all female):		10	11	9	12	12
Percentage survival on day:	10	100	100	100	83	58
	15	100	100	89	58	42
	20	100	100	89	50	42
	30	100	91	89	50	42
	40	100	82	89	50	42
	50	100	82	89	50	42
	100	90	27	67	17	25
	150	40	0	0	0	0
Level of first peak of parasitaemia ($\log_{10}$ trypanosomes μl^{-1}; mean ± 1 S.D.)		3·7[a]	5·3 ±0·2	5·4 ±0·2	4·9 ±0·5	5·0 ±0·5

[a] Estimated.

There is no report of differences in susceptibility between the sexes in cattle, apart from increased susceptibility of cows during advanced pregnancy, at parturition (Chandler, 1952) and while suckling calves (see Section III A1, p. 12). However, C57BL/6 males are slightly but consistently and significantly more susceptible to *T. congolense* than females. They develop higher levels of parasitaemia, and more males than females die if the trypanosome population is sufficiently virulent to cause some deaths at the first peak of parasitaemia. Similar observations have been made in mice infected with *T. brucei* (Clayton, 1978) and *T. cruzi* (Hauschka, 1947).

B. MATERNALLY-DERIVED IMMUNITY

In the foregoing reports of age-related resistance, the importance of colostrum was frequently mentioned. While this is likely, hard experimental evidence is still lacking. The importance of understanding the role of colostrum is emphasized by the belief that the transfer of colostrum from an immune dam supplemented by early exposure to infection creates a highly resistant animal, providing it is not moved to another location (Desowitz, 1970).

The role of colostrum in resistance to trypanosomiasis has been more thoroughly investigated in laboratory rodents. Maternally-derived protection against *T. gambiense* has been demonstrated in neonatal mice but only if the offspring were both born of immune mothers and received colostrum from their mothers, a result that also suggests a role for placental transfer of immunity (Takayanagi *et al.*, 1978). However, in a comprehensive checkerboard study, D. D. Whitelaw and G. M. Urquhart (unpublished observations) demonstrated that immunity to *T. brucei* and *T. congolense* is conferred to newborn mice whose mothers were either experiencing an active infection or had been cured by Berenil before parturition (Fig. 8). The drug was not detectable in the colostrum. It appeared that protection was conferred only by colostrum. There was no evidence of placental transfer, since offspring of infected mothers were susceptible to challenge if suckled by non-immune mothers. On the other hand, litters from non-immune mothers were protected if they were suckled by an infected mother (Fig. 8). The protection conveyed by colostrum continued into the third week after birth and disappeared after weaning.

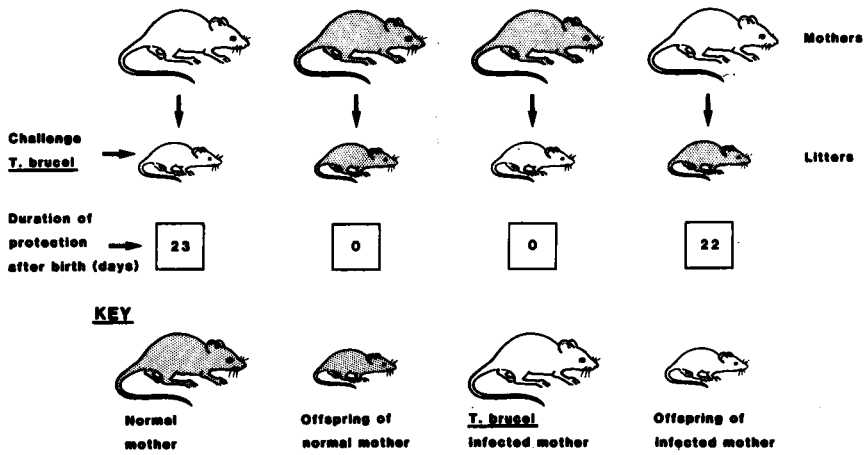

FIG. 8. Maternally-derived immunity in young mice to infection with *T. brucei*. Only mice that received colostrum from an infected mother (or a mother cured of infection by chemotherapy) were immune to homologous challenge. Young mice that received colostrum from an uninfected mother were susceptible to challenge, irrespective of whether or not they were the offspring of an infected, treated or normal parent.

C. INTENSITY OF CHALLENGE

Several reports indicate that trypanotolerance is relative rather than absolute and that trypanotolerant breeds can be severely affected if the level of risk is high enough (Stewart, 1937). The term trypanosomiasis risk is

preferred to tsetse challenge as many different factors influence whether or not an animal becomes infected. These include not only tsetse density, but also the tsetse species, trypanosome infection rate of tsetse, density and species of host under attack, species and virulence of trypanosomes. As yet, there is no formula in which all these factors can be incorporated to produce an accurate, quantified forecast of trypanosomiasis risk, or what is usually termed empirically as "low", "medium" or "high" tsetse challenge. Despite these qualifications, there is little doubt that trypanotolerant cattle including the N'Dama can be severely affected if the tsetse challenge is high enough (Godfrey *et al.*, 1964; Stephen, 1966; Murray *et al.*, 1979b; Clifford and Sanyang, 1979). In such areas, N'Dama may die and some are found in a poor productive state with a high prevalence of wasting, stunting, abortion and calf mortality. Many of these animals exhibit persistent low-grade anaemia. On the other hand, as evidence of the wide range of susceptibility within the N'Dama, many other animals in the same herds are in an excellent condition.

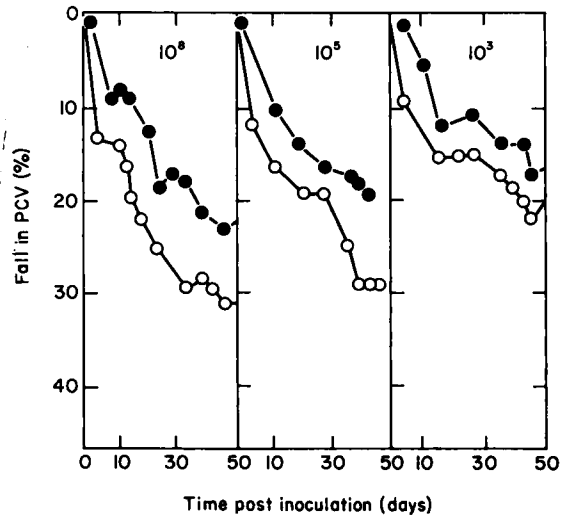

FIG. 9. Percentage fall in PCV from preinfection values in N'Dama (●) and Zebu (○) cattle infected with various doses of *T. brucei*. There is a stepwise increase in severity of anaemia as the dose increases. Preinoculation N'Dama PCV = 34 ± 4%; preinoculation Zebu PCV = 35 ± 4% (means ± 1 S.D.).

In an attempt to demonstrate and quantify the influence of the level of challenge on the degree of susceptibility to trypanosomiasis, we subjected groups of N'Dama and Zebu to subcutaneous challenge with graded doses of *T. brucei* bloodstream forms (Fig. 3) and found that the dose had a significant effect on the development of the disease (Murray *et al.*, 1979e). Both

the N'Dama and Zebu that received the heaviest challenge developed the highest parasitaemia and the most severe anaemia (Fig. 9). In confirmation of the trypanotolerant nature of the N'Dama breed, but reflecting the quantitative rather than the absolute nature of trypanotolerance, it was necessary to challenge the N'Dama with 10^5 times more organisms to induce anaemia of the same severity as that in the Zebu (10^8 compared with 10^3, Fig. 9).

These results indicate the potential significance of level of challenge and emphasize that the productivity potential of trypanotolerant livestock in the field will depend on the extent of their resistance to different levels of trypanosomiasis risk, especially in the medium to high range.

In contrast to the results in cattle, we have found that dose does not influence susceptibility in mice. While the prepatent period and the time to the first peak of parasitaemia were longer at lower doses, the level of the first peak and the mortality were similar regardless of dose.

D. VIRULENCE

The virulence of the parasite must play a significant role in host susceptibility to trypanosomiasis. The factors involved depend on the characteristics of the species of trypanosome as well as the strain of trypanosome itself.

Following needle challenge with *T. brucei*, *T. congolense* or *T. vivax*, N'Dama were as susceptible as Zebu to infection. It was only in development of parasitaemia that differences emerged (Murray *et al.*, 1979c; Saror *et al.*, 1981). However, under natural field conditions, N'Dama proved very resistant to tsetse transmission of *T. vivax*, whereas Zebu were highly susceptible; a small, but significant, difference was found with *T. brucei*. In contrast, there was no significant difference in the prevalence of *T. congolense* (Table 4; Murray *et al.*, 1981a). These field results indicate a relationship between trypanosome species and the resistance of breeds of cattle to tsetse-transmitted infection.

Factors that determine the virulence of individual strains of trypanosomes are being increasingly defined. On the basis of the proposal that virulence was related to VAT (McNeillage and Herbert, 1968; Van Meirvenne *et al.*, 1975), Barry *et al.* (1979) examined a set of cloned populations of *T. brucei*, all of which bore the same variable antigen. The clones made close to the time of isolation were found to be moderately virulent for mice, whereas those prepared from the same stock following repeated passage were extremely virulent. It appeared that virulence and VAT were not necessarily related. Instead, it was concluded that virulence was limited by the capacity of the clone to differentiate morphologically, i.e. to become pleomorphic. This

conclusion confirmed a well-recognized situation in laboratory animals, namely, that trypanosome strains subjected to repeated passages become increasingly monomorphic and virulent. When the parasite differentiates it switches from the rapidly dividing slender form to the non-dividing stumpy. The rapidly dividing parasites produce high parasitaemias and kill the host quickly, whereas recent pleomorphic isolates produce lower parasitaemias and are less virulent. Furthermore, there is evidence that the stumpy forms, not the slender parasites, are responsible for induction of the immune response (Sendashonga and Black, in press). Thus, pleomorphism in *T. brucei* appears to reduce virulence by limiting the peaks of parasitaemia by affecting the rate of division and/or by stimulating the immune response.

Another aspect of trypanosome virulence was defined by Sacks *et al.* (1980), who found that differences in the virulence of strains of *T. brucei* were directly associated with the capacity of subcellular membrane fractions to induce immunosuppression. The possibility that the host, as well as the parasite, possesses inherent capabilities to stimulate pleomorphism or to affect immunosuppression is discussed later (Section VII B 2 and VII C, p. 48 and p. 50).

Trypanosomes might also differ in virulence because of a greater or lesser capacity to generate toxic substances. Studies *in vitro* have shown that trypanosome fractions or substances released from dead or dying parasites contain a wide range of biologically active factors. These include haemolysins, platelet aggregating factors, inflammatory factors, hepatotoxins, proteolytic and lipolytic enzymes, complement-activating factors associated with variable surface glycoprotein, a lipopolysaccharide and toxic aromatic amino acid metabolites such as tryptophol (reviewed by Tizard *et al.*, 1978). The production and spectrum of activity of these factors within and between species of trypanosomes is not known; e.g., so far the generation of tryptophol has been demonstrated only for *Trypanozoon* parasites, whereas haemolysins (Murray, 1979) and platelet aggregating factors (C. E. Davis, personal communication) have been shown to occur in all species of African salivarian trypanosomes. If these factors are active *in vivo*, and there is evidence that this is so with trypanosome haemolysins (Murray, 1979), another aspect of host resistance might be related to the capacity to block their activity by, for example, generating antibodies.

E. PREVIOUS EXPOSURE

There are several reports indicating that cattle which survive trypanosomiasis, with or without the aid of chemotherapy, are subsequently more resistant to rechallenge. This has been confirmed many times in N'Dama in

West Africa by Chandler (1958), Desowitz (1959), Touré *et al.* (1978) and by Saror *et al.* (1981).

Similarly, evidence has been obtained that *Bos indicus* breeds in East Africa, usually maintained by a chemotherapeutic strategy, acquire resistance (Bevan, 1928, 1936; Whiteside, 1962; Fiennes, 1970; Wilson *et al.*, 1976; Bourn and Scott, 1978; Trail *et al.*, in press). These workers found that not only did the interval between infections increase, but also, when the decision to treat was made on the basis of detectable parasitaemia, animals were frequently parasitaemic without showing severe clinical effects. Emphasizing the potential practical importance of this type of control strategy, these results have been obtained sometimes with large numbers of animals, e.g. 450 oxen in Ethiopia (Bourn and Scott, 1978) and 800 adult milking animals on the Kenya coast (Trail *et al.*, in press). The details of management in the latter location have already been described in this review (see Section III A 3, p. 16). On this ranch it was found that the more times an animal had been treated, the fewer treatments it required in the future (Fig. 10). This was confirmed at a highly significant level and shown to be independent of age. Because of the extended intervals between treatments, any residual prophylactic effect of the drug (Berenil) was discounted. Thus, it would appear that these animals were becoming more resistant to challenge.

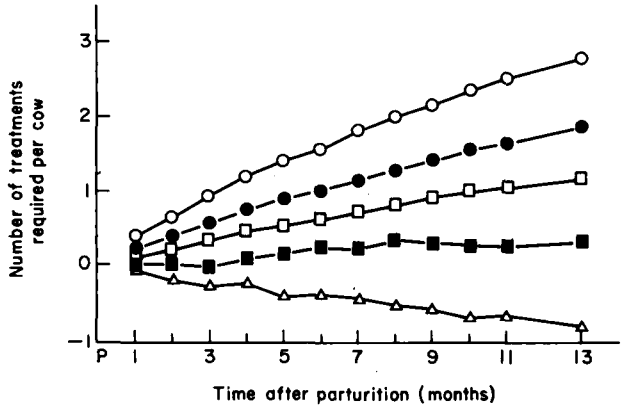

FIG. 10. Least-squares past treatment group means for number of current drug treatments (cumulative) against trypanosomiasis required by cattle at monthly intervals after parturition (by permission of Trail *et al.*, in press). ○, 0–1 past treatments; ●, 2–3 past treatments; □, 4–5 past treatments; ■, 6–7 past treatments; △, 8+ past treatments.

There are a number of possible explanations for the acquisition of resistance in the field. Firstly, it is possible that cattle gradually develop specific immunity to all or most of the trypanosome serodemes in a particular location. Although each serodeme is capable of elaborating a large number of VATs

during an infection (Capbern *et al.*, 1977), there is now evidence that the antigenic composition of the metacyclic trypanosomes extruded by the tsetse fly is always similar for a given serodeme (Jenni, 1977; Nantulya *et al.*, 1979), even though it probably consists of a mixture of VATs (Barry and Hajduk, 1979). Further studies, using monoclonal antibodies to characterize metacyclic populations, have suggested that the number of antigenic types may be limited and consistent for each serodeme. This was confirmed for both *T. brucei* (V. M. Nantulya and A. J. Musoke, personal communication) and *T. rhodesiense* (Esser *et al.*, 1981). Complete neutralization of the metacyclic population of *T. congolense* can be achieved by serum taken from cattle 14–16 days after tsetse challenge (W. I. Morrison, unpublished observations). Furthermore, animals treated with Berenil shortly after the establishment of infection are immune for up to 6 months to rechallenge with tsetse infected with the same serodeme. They are still susceptible to other serodemes. This has been confirmed in cattle with *T. congolense* and goats with *T. brucei* (Emery *et al.*, 1980), although goats similarly treated after tsetse-transmitted *T. vivax* infection showed no evidence of immunity (De Gee, 1980). Thus, at least with *T. congolense* and *T. brucei*, if animals in endemic areas survive a primary infection with a particular serodeme, they should be able to resist subsequent tsetse challenge with that serodeme for at least 6 months. The possibility that the number of serodemes may be limited in some locations offers the hope that immunization with metacyclic trypanosome antigens may be feasible

Another factor that may contribute to acquired resistance in the field is antigenic cross-reactivity of VATs from different serodemes. Evidence of cross-reactivity between different VATs, as they are presented on the surface of the live trypanosome, has been obtained in recent studies with different repertoires of the subspecies *Trypanozoon* (Van Meirvenne *et al.*, 1975, 1977; Vervoort *et al.*, 1981). Some VATs derived from widely differing field isolates of *T. b. brucei*, *T. b. rhodesiense*, *T. b. gambiense*, *T. evansi* and *T. equiperdum* show strong cross-reactions in immunofluoresence and immune lysis tests with infection antisera (Van Meirvenne *et al.*, 1977). They also cross-protect (T. Vervoort, personal communication). These cross-reactive VATs have been termed iso-VATs. Following purification, their variable surface glycoproteins (VSGs) were shown to have similar molecular weights (60 000 daltons) but different isoelectric points. Although they are not identical molecules, peptide mapping showed extensive amino acid sequence homology between the molecules (Vervoort *et al.*, 1981). At present, the frequency of these iso-VATs and their existence in *T. congolense* and *T. vivax* is uncertain. If iso-VATS are common, they might be expected to confer partial protection between different serodemes. (see pp. 139–141.)

There is now evidence from serological studies that during a trypanosome

infection certain VATs may recur within a few weeks of one another. This has been reported in cattle infected with a stabilate of *T. congolense* (Wilson and Cunningham, 1972) and a clone of *T. brucei* (Nantulya *et al.*, 1980). Whether these represent identical VATs or iso-VATs is not known.

In addition to the cross-reactivity exhibited by iso-VATs, a glycopeptide at the *C*-terminal region of the VSG cross-reacts in all isolates and species of trypanosome so far tested (Barbet and McGuire, 1978; Cross, 1979; Barbet *et al.*, 1979). However, unlike iso-VAT determinants, this common region is not accessible on the surface of live trypanosomes and its role, if any, in acquired resistance is unknown.

During the course of trypanosomal infections in cattle, other common antigens in the trypanosome have also been shown to elicit an antibody response. Thus, Shapiro and Murray (1982) found that the clinical course of the disease in trypanosome-infected cattle was associated with the ability to recognize at least one of three common trypanosome antigens of molecular weights 110 000, 150 000 and 300 000 daltons. Antibody to these antigens was detected only in animals that controlled the parasitaemia and made a clinical recovery. Whether these antibodies play a role in controlling the infection remains to be determined.

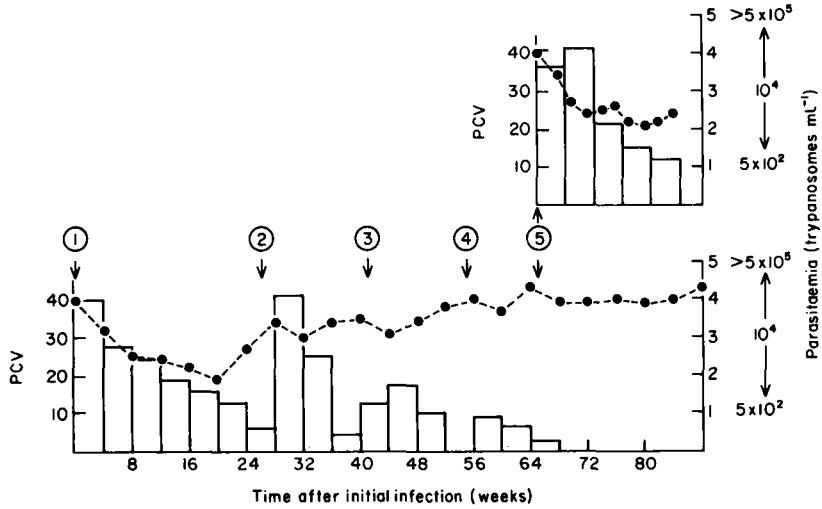

FIG. 11. Average monthly parasitaemia (histogram) and PCV (● - - - - - ●) of adult Hereford cattle sequentially challenged with unrelated *T. congolense* stocks (1, 2, 3, 4, 5). The upper graph represents the effect of the final challenge on previously uninfected animals.

We have observed the development of significant resistance in three adult Herefords subjected to several needle challenges with unrelated stocks of *T. congolense* (Fig. 11). Following successive challenges, the level of para-

sitaemia and the degree of anaemia that occurred were significantly less than in correspondingly challenged control animals. Preliminary studies yielded no evidence of serological cross-reactivity between the stocks of *T. congolense* used. An explanation for these findings, and possibly also a factor influencing acquired resistance in the field, emerged when we were investigating the effect of an established infection upon the susceptibility of the host to subsequent challenge (Morrison *et al.*, in press: see p. 68). Cattle were subjected to two challenges 5 to 6 weeks apart with unrelated isolates of *T. congolense*; the first challenge was by needle inoculation with bloodstream forms and the superinfection was introduced either by needle inoculation of bloodstream forms or by tsetse challenge. The results indicated that the initial infection interfered with establishment of the second infection despite the absence of detectable antibodies against the trypanosomes used for the second challenge. This interference required the presence of an active infection; animals were completely susceptible to the second challenge following treatment with Berenil. The basis of this apparently antibody-independent phenomenon is not known. It may reflect some form of competition between trypanosome populations or a non-specific response of the host that limits the growth of trypanosomes. Similarly, the extent to which it occurs should be defined because we have preliminary evidence that it is not a universal occurrence and that with some other combinations of trypanosomes there is little evidence of interference or, at least, a less marked effect. On the other hand, interference with the establishment of a second infection by tsetse challenge was always accompanied by the development of neutralizing antibody to the metacyclic trypanosomes. This antibody was synthesized despite the fact that there was no appreciable change in parasitaemia and in most animals no chancre. If the presence of this antibody corresponds to immunity to the homologous metacyclics, it would suggest that immunity could develop to metacyclic populations of the same serodemes without significant growth of trypanosomes in the bloodstream. Such a phenomenon might be relevant to trypanotolerance or to differences in susceptibility if its expression was host-dependent.

In conclusion there is sound evidence that, for reasons yet to be understood, resistance to trypanosomiasis does occur in the field in both trypanosusceptible and trypanotolerant breeds; there is some indication that the resistance might be more readily acquired by trypanotolerant animals (Desowitz, 1959; Roberts and Gray, 1973b).

F. STRESS

A range of stress factors has been incriminated in increasing susceptibility to trypanosomiasis. These include nutrition, overwork, intercurrent disease

(MacLennan, 1970) and repeated bleeding (Stewart, 1951). Other factors such as parturition and lactation have already been discussed. Perhaps the most important of these factors is the nutritional status of the host. Thus, in arid conditions when fodder is in short supply, animals may have to trek many miles in order to obtain sufficient food. Under these circumstances, it is likely that infected animals suffering from anaemia and myocardial lesions will be less able to cope and that their poor nutritional status will exacerbate the disease.

It is often believed that the resistance of trypanotolerant breeds is largely the result of acquired immunity to local trypanosomes and that tolerance diminishes if cattle are moved to distant locations (Mason, 1951). While there is good evidence that resistance is acquired if animals are kept in one area (Section VI E, p. 37), there are also several reports which describe the movement of trypanotolerant cattle over large distances (Stewart, 1951; Ferguson, 1967) and their successful introduction into tsetse areas where other breeds cannot survive. For example, the Dahomey (Lagune) breed was first established in Zaire from West Africa in 1904 and the N'Dama in 1920 (Mortelmans and Kageruka, 1976), and much more recently N'Dama were introduced into the Central African Republic, Gabon and Congo (ILCA, 1979).

G. SUSCEPTIBILITY TO OTHER DISEASES

Several reports suggest that trypanotolerant breeds of cattle and sheep may also be resistant to other important infectious diseases of West and Central Africa. N'Dama appear to be more resistant to tick-borne diseases, including heartwater (*Cowdria ruminantium*), anaplasmosis and babesiosis (Epstein, 1971; Linda Logan, personal communication). The fact that this observation includes several tick-borne infections might indicate a greater resistance to ticks *per se*. In addition, N'Dama and West African Shorthorn are resistant to streptothricosis (Stewart, 1937; Coleman, 1967; Oduye and Okunaiya, 1971) and helminthiasis (A. A. Ilemobade, personal communication).

Similarly, the Red Masai sheep of Kenya have been shown to be significantly more resistant than other breeds not only to trypanosomiasis (Griffin and Allonby, 1979a, b), but also to haemonchosis (Preston and Allonby, 1979).

On the other hand, N'Dama and West African Shorthorn are reputed to be much more susceptible to rinderpest than Zebu (Stewart, 1937, 1951; Cornell and Evans, 1937; Ferguson, 1967), and van Hoeve (1972) noted that Muturu, but not N'Dama, were highly susceptible to footrot.

While it is important to obtain critical experimental evidence to confirm

at least some of these reports, our discussions with workers in the field have firmly convinced us of the validity of the observations.

VII. Mechanisms of Trypanotolerance

This review presents considerable evidence that increased resistance to trypanosomiasis depends on an inherent capacity to limit parasitaemia. The mechanisms responsible for this are likely to be operative during the initial host–parasite–tsetse interaction in the skin, and/or in the circulation once the infection is established. At either of these locations, physiological or immunological factors might act against the trypanosomes.

Trypanotolerance may also be associated with reduced susceptibility to the effects of the disease because of a number of physiological factors which aid survival of trypanotolerant animals.

A. THE LOCAL REACTION IN THE SKIN

Following the successful feed of a trypanosome-infected tsetse, a raised indurated lesion as large as several centimetres in diameter develops in the skin of susceptible cattle, sheep and goats (reviewed by Emery *et al.*, 1980). This lesion is called the chancre. Its development precedes the onset of parasitaemia and, therefore, predicts imminent infection. The site of the reaction represents the point at which the trypanosome first becomes established in the host and it is here that the parasites multiply before their dissemination to the bloodstream (Emery *et al.*, 1980).

In resistant wild animals such as eland and waterbuck that develop low parasitaemias and little clinical evidence of disease, chancres appear less frequently and are smaller than in susceptible domestic animal controls (Murray *et al.*, 1981b). This suggests that the skin may play a role in trypanotolerance by affecting the transmission of infection. It is possible that the trypanotolerance of wild animals is related to the number of trypanosomes that become established in the skin. That numbers are important has been shown by the fact that the size and intensity of the skin reaction and the level of parasitaemia may be dose-dependent (Emery *et al.*, 1980; Murray *et al.*, 1979e).

The ability of the skin to influence susceptibility to trypanosomes may reflect either mechanisms at the local skin level or some systemic capacity to control the parasite. The physiological, structural or immunological factors which might be operative are unknown, but several are worthy of consideration.

For example, there are well established differences in skin structure and physiology in different breeds of cattle (reviewed by Epstein, 1971). Skin vasculature and the number and size of sweat glands and sebaceous glands (Amakiri, 1974, 1976) of indigenous African breeds of cattle differ from imported breeds. At the same time the skin of tropical breeds is more pigmented, as judged by melanocyte counts in the epidermis (Amakiri, 1979). The significance of these differences is uncertain. However, Carr *et al.* (1974) established an association between skin thickness and the prevalence of *T. congolense*. Within herds of East African Zebu, infections were more common in thinner skinned animals.

Because the chancre is the site of the first interaction between host and parasite, it is likely to be important in the induction of the immune response. Furthermore, there is also evidence that the immune response is effective at this level; animals rechallenged by tsetse infected with the homologous serodeme are immune and no detectable skin reaction develops (Emery *et al.*, 1980). As trypanosomes cannot be detected in the efferent lymph of these animals (Emery *et al.*, 1980), it can be assumed that they are killed in the skin or in the draining lymph node. Therefore, the immune response can control the parasite at the level of the skin, or at least at the local lymph node. Thus, trypanotolerant animals may generate a superior or more rapid local or general immune response which operates locally to reduce the number of parasites which become established or to localize the trypanosomes in the skin.

Another fascinating aspect of this subject is that certain animals which survive in tsetse endemic areas may do so only because they are rarely bitten. Nash (1969) suggested that this may reflect the reliability of an animal species as a continuing food source, i.e. one that will still be available when the fly again becomes active after digesting its last meal. Thus, the habits of the non-migratory warthog, bushbuck and rhinoceros, for example, which either live in selected forest thickets in close contact with the tsetse, or deposit dung in the same spot day after day, make them reliable targets for the fly. Conversely, animals like the zebra and wildebeest, migratory by nature, spend the noonday hours beneath the flimsiest of shade, an inhospitable habitat for the tsetse. This suggestion is supported by the results of tsetse blood meal analyses, which show that the fly feeds only very rarely on zebra and wildebeest.

It is also evident that some species of tsetse exhibit definite host feeding preferences (Weitz, 1963), although these traits are by no means stable and can be influenced by several environmental factors (Moloo, 1973). Smell, colour and size have been incriminated as some of the factors that determine the attractiveness of the host. The potential significance of tsetse preferences was shown by Roberts *et al.* (1980) when they compared the attractiveness

to tsetse of cattle and oryx under critical experimental conditions. They found that 5 times as many tsetse were attracted to cattle and were able to count full engorgement on cattle of 279 tsetse during the period of observation. Only four tsetse were seen to obtain partial blood meals from the oryx during the same time; this difference was partly the result of the oryx killing tsetse with its horns. A similar retaliatory capacity was observed over a period of 2 years in two monkeys (Nash, 1969) which, even when apparently asleep, seldom failed to catch and eat, or at least drive away, any tsetse which settled and attempted to feed. A defence mechanism, employed by impala, is neuromuscular twitching. The constant rippling of the skin prevents the tsetse from settling to feed, but if the animal is anaesthetized, twitching does not occur and the tsetse will then feed quite readily (Bursell, 1980).

B. THE IMMUNE RESPONSE

The fact that there is no significant difference in infection rates and prepatent periods following needle challenge of strains of mice or breeds of cattle with different susceptibilities suggests that differences in parasitaemia may be the result of innate differences in the immune response. In this respect there is considerable evidence that antibody is the most important effector mechanism in immunity to trypanosomiasis (reviewed by Murray and Urquhart, 1977). Several studies in laboratory animals have indicated that IgM is the important immunoglobulin (Ig) class in controlling the parasitaemia and in protection (Campbell *et al.*, 1978) and there is also evidence that this is so in cattle (Luckins, 1976; Musoke *et al.*, 1981). Whether antibody alone is sufficient to kill parasites *in vivo* or whether complement and/or macrophages are also required is not known.

TABLE 8

The survival of congenitally athymic (nude) C57BL mice challenged with T. congolense

		Nude infected	Intact infected	Nude uninfected
Percentage survival on day:	10	90	100	100
	15	36	91	100
	20	3	86	100
	30	0	86	100
Level of parasitaemia ($\log_{10}$ trypanosomes μl^{-1}): mean ± 1 S.D.		5·3 ± 0·2	5·4 ± 0·2	1st peak
		5·6 ± 0·1[a]	4·9 ± 0·3	2nd peak

[a] The level of parasitaemia is significantly different between nude and intact mice at the 2nd peak.

Indirect evidence that the immune response is involved in susceptibility to trypanosomiasis is provided by the fact that immunologically compromised mice, e.g. splenectomized (Morrison *et al.*, 1978) and irradiated mice lose their capacity to control parasitaemia and die more quickly of trypanosomiasis. It is interesting that the outcome with athymic nude mice depends on the species of trypanosome. For example, following challenge with *T. congolense*, C57BL and BALB/c nudes are not able to control the resulting parasitaemia as well as intact mice and die much earlier (Morrison *et al.*, 1978; Table 8). On the other hand, BALB/c nudes have a greater capacity to control parasitaemia with *T. rhodesiense* and live longer than control mice (Campbell *et al.*, 1978). Similarly, nude BALB/c (Jayawardena and Waksman, 1977) and outbred nude mice (Clayton *et al.*, 1979) control parasitaemia with *T. brucei* better than controls, although there is little difference in survival.

1. *Antibody response*

(a) *Cattle.* The superior capacity of trypanotolerant breeds to control parasitaemia is generally attributed to differences in the immune response. Because trypanotolerant animals control the first as well as subsequent peaks of parasitaemia, it is thought that they may be able to mount a better primary response. However, there are few data for this supposition. Our own preliminary investigations on N'Dama and Zebu challenged with bloodstream forms of *T. brucei* revealed no significant difference between these two breeds in the antibody levels to the stabilate used for challenge, although there was some indication that antibody persisted for longer in the N'Dama, as judged by immunofluorescence, agglutination, immune clearance and immune lysis. Before definite conclusions can be made, it is essential to compare not only the quantity but also the quality and class of antibody response.

Some workers have concluded that the trypanotolerant nature of the N'Dama lies in their capacity to mount a superior secondary immune response to the trypanosome. Thus, Desowitz (1959) found that N'Dama with previous experience of trypanosomiasis were able to eliminate trypanosomes more rapidly than Zebu following renewed challenge. Using a respiratory inhibition test, he demonstrated that the activity in N'Dama sera was greater than that in Zebu sera. However, as the precise antigenic nature of the trypanosomes used in the study was not known, further investigations are required for confirmation. The experiments of Roberts and Gray (1973b) provide circumstantial evidence to support Desowitz (1959). They found little difference in susceptibility of N'Dama and Zebu to primary challenge with infected wild tsetse. The greater resistance of the N'Dama became obvious only after the second challenge. The only other report on cattle that we are aware of was made by Chandler (1958), who stated that the neutralizing antibody response to trypanosomes was greater in N'Dama than in Zebu.

As described in Section VI E, an association has been demonstrated between the capacity of cattle to control *T. brucei* infections and their ability to recognize at least one of three common trypanosome antigens of molecular weights 110 000, 150 000 and 300 000 daltons (Shapiro and Murray, 1982: see p. 68). The ability of N'Dama to recognize these antigens was much greater than that of Zebu. All N'Dama recovered, eight of the nine having recognized two or more antigens. Five of nine Zebu died, only one having identified any of these proteins. Of the four Zebu that recovered, not one recognized more than two antigens. The role of these antibodies is not known but they may reflect an overall superior innate immune response in more resistant animals like the N'Dama.

(b) *Mice.* Much more information is available on the immune response of different strains of mice with varying susceptibility to trypanosomiasis.

The humoral responses, both in terms of changes in total serum immunoglobulins and in production of specific anti-trypanosomal antibody, have been examined in A/J and C57BL mice infected with *T. congolense* (W. I. Morrison, unpublished observations). The population of *T. congolense* was a cloned derivative of the stock used in initial studies to define susceptibility and its inheritance in different mouse strains (Morrison and Murray, 1979). This clone caused a chronic relapsing infection in C57BL mice but 60% of A/J mice died by the end of the first wave of parasitaemia (Table 5). There was a marked increase (approximately 4–8-fold) in the level of serum IgM of C57BL mice during and following the first peak of parasitaemia. At the same time, smaller increases of about 2·5-fold for IgG_1 and 1·5-fold for IgG_2 were observed. By contrast, fewer than 25% of the A/J mice showed significant increases in IgM. However, all the A/J mice showed a massive increase in serum IgG, particularly of the IgG_2 subclass which was increased by up to 10-fold. Trypanolysis tests showed that the sera of all C57BL mice contained lytic antibody for the infecting organisms, whereas antibody was detected only in about one-third of A/J mice (Fig. 12). Positive sera from both strains exhibited similar titres of antibody. Corroborative results were obtained by a solid phase radioimmunoassay. As before, specific antibody was not detected in A/J mice which died at the first peak of parasitaemia. A/J mice which showed remission in parasitaemia synthesized increased serum IgM and detectable specific antibody. Thus, despite the fact that large amounts of immunoglobulin, particularly of the IgG_2 class, are produced by A/J mice, many show no detectable anti-trypanosomal antibody response. This suggests an association between the capacity to respond to the trypanosome and the ability to control the infection. However, we have found that when each mouse strain is inoculated with a single dose of irradiated *T. congolense*, the antibody response of the A/Js is at least equal to that of the C57BLs. This suggests that the infection in A/J mice in some way diverts the humoral immune response towards the production of non-specific antibody.

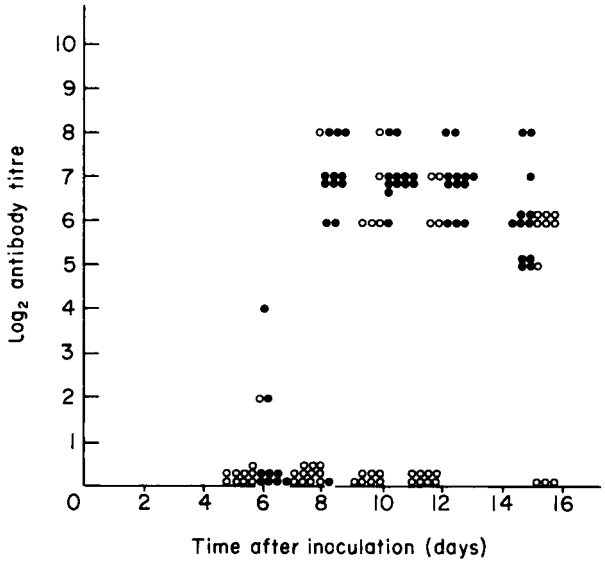

FIG. 12. Antibody titres, measured by immune lysis, in individual A/J (○) and C57BL/6J (●) mice infected with *T. congolense*.

In another study of the same type, outbred CFLP mice were highly susceptible to a different strain of *T. congolense* (D. D. Whitelaw, unpublished observations). Death occurred between 8 and 10 days, while C57BL mice developed a chronic infection. Total IgM levels were raised in the latter, but by the time of death in the CFLP, there was little increase in total Ig levels. Antibody specific for the infecting trypanosome, detectable by agglutination, neutralization, trypanolysis and immune clearance, was found only in C57BL mice. The absence of immunoglobulin increase in the susceptible strain contrasted with the previous study and possibly reflected the very early deaths of the CFLP strain.

Significant differences in the quality and quantity of antibody to trypanosomes, and possibly to other host antigens, could play a significant role in host susceptibility to trypanosomiasis by predisposing to hypersensitivity reactions, especially those favouring immune complex formation. Immune complexes are a well recognized feature of experimental trypanosomiasis (Murray *et al.*, 1975). More recently, direct evidence has been presented that hypersensitivity reactions, probably antigen–antibody complexes, play an important role in tissue damage of mice infected with *T. brucei* (Galvao-Castro *et al.*, 1978). It is well recognized that a range of susceptibility to immune complex disease occurs in different strains of mice (Morrison and

Wright, 1977). It is also known that several factors are involved in the formation, persistence and subsequent localization of these complexes, including the class of antibody and its affinity (reviewed by Steward, 1979). The severity of immune complex glomerulonephritis in five different inbred strains of rats infected with *T. rhodesiense* (Lindsley *et al.*, 1980) was found to depend on the quantity of antibody and immunoglobulin produced. The strain with the highest levels of IgM antibodies and total IgM developed the most severe glomerulonephritis; only moderate damage occurred when IgG was increased and there was no difference between different strains of rats.

While we believe that immune complex disease is important in the pathogenesis of *Trypanozoon* infections in experimental animal models, there is little histological evidence for its occurrence in cattle.

2. *Immunodepression*

Perhaps the question that should be asked in relation to trypanotolerance is not whether resistant animals have the innate capacity to mount a superior immune response but whether they are less liable to immunodepression during the course of a trypanosome infection. Immunodepression is a widely recognized result of trypanosome infections. It is particularly severe in laboratory animals (Goodwin *et al.*, 1972; Urquhart *et al.*, 1973; Murray *et al.*, 1974a, b) and in man (Greenwood *et al.*, 1973). In domestic animals, suppression of the antibody response to several viral and bacterial vaccines has been described (reviewed by Murray *et al.*, 1980).

TABLE 9

Response to mitogens and allogeneic cells and suppressor activity on a normal MLR of spleen cells from A/J and C57BL/10 mice infected with T. congolense[a]

Mouse strain	Percentage suppression on: Day 9		Day 24			
	Con A	LPS	Con A	LPS	MLR	Suppression of MLR
A/J	95	90	100	95	100	100
C57BL/10	13	4	91	82	52	38

[a] Con A = concanavalin A; LPS = lipopolysaccharide; MLR = mixed leucocyte reaction.

Differences in the degree of immunodepression have been observed in the splenic lymphocyte responses to mitogens and allogeneic cells and in suppressor cell activity in mouse strains with different susceptibilities to *T. congolense* (Morrison *et al.*, 1979). Responses to concanavalin (Con A) and

lipopolysaccharide (LPS) were severely depressed in the susceptible A/J mice by day 9 but less so in the more resistant C57BL/10 (Table 9). By day 24, there was no difference in mitogen suppression; however, the depression of the response of infected spleen cells in the mixed leucocyte reaction (MLR) and their suppressor activity on normal MLR at day 24 still correlated with mouse strain susceptibility (Table 9). That is, spleen cells from the more susceptible strains showed greater depression and suppressor activity. We obtained similar results following infection of C3H/He and C57BL/6 mice with *T. brucei*. The more susceptible C3H/He strain showed more profound depression as well as severe depletion of the lymphoid system as early as the second and third weeks after infection (S. K. Kar and M. Murray, unpublished observations).

It is possible that differences in susceptibility between different strains of mice might be related to their sensitivity to polyclonal activation. A number of studies have demonstrated polyclonal activation of B cells in the spleens of trypanosome-infected mice and an association between polyclonal activation and the induction of immunosuppression has been proposed (reviewed by Terry *et al.*, 1980). When spleen cell populations were studied in C57BL/6 and A/J strains of mice infected with *T. congolense*, both strains exhibited an increase in B lymphocytes. However, the increase in B cells was less marked in C57BL/6 mice than in the highly susceptible A/J. These results raise the possibility of differences in sensitivity to polyclonal activation or to immunosuppression between mouse strains of varying susceptibility to trypanosomiasis. Conversely, the findings might merely reflect differences in the levels of parasitaemia in the various strains of mice as suggested by Morrison *et al.* (1978).

Recent evidence suggests a direct relationship between immunosuppression and susceptibility of mice. The more depressed IgM responses become, the more susceptible the mouse (Sacks *et al.*, 1980; Selkirk and Sacks, 1980). Furthermore, the degree of immunosuppression was found to be both trypanosome strain (Sacks *et al.*, 1980) and mouse strain dependent (Selkirk and Sacks, 1980). Sacks *et al.* (1980) found that membrane fractions of the most virulent strains of *T. brucei* had the most marked immunosuppressive effect, particularly on IgM responses to both heterologous antigens and trypanosomes (Selkirk and Sacks, 1980, quoting Sacks and Askonas). Similarly, strains of mice with different susceptibilities to *T. brucei* showed corresponding differences in depression to heterologous antigens, with IgM responses depressed as early as 3 to 5 days after infection. These differences could not be attributed to a difference in the level of the first peak of parasitaemia, as there was none; differences in parasitaemia became obvious only at a later stage. These workers concluded that the extent and rapidity of the onset of suppression of the IgM response appeared to determine the

course of infection and proposed that differences in these quantities might explain trypanotolerance. The results of the foregoing studies are unique because most of the other studies with *T. brucei* infections in mice (Clayton, 1978; Murray and Morrison, 1979a; see Section VII C, p. 51) and in cattle (Murray and Morrison, 1979b) have shown a strong correlation between reduced susceptibility and the capacity to control parasitaemia, including the first peak. Also, MacAskill *et al.* (1981) failed to find depression of antibody responses to sheep red blood cells or to trypanosomes on day 5 in highly susceptible mice challenged with *T. brucei.*

While these observations on the association between immunosuppression and susceptibility offer one of the most interesting approaches to the understanding of trypanotolerance, it should be emphasized that in cattle the kinetics of infection are different. Cattle not infrequently undergo what appears to be self-cure and eliminate the parasites, suggesting an unimpaired capacity for dealing with the trypanosome. Self-cure is particularly common in trypanotolerant breeds such as N'Dama (Ige and Amodu, 1977; Murray and Morrison, 1979b). It should also be recognized that there is little evidence of immunosuppression of responses to mitogens and mixed leucocyte reactions of cells from different lymphoid organs in trypanosome-infected cattle (Masake and Morrison, 1981). Furthermore, there is little disorganization or derangement of the lymphoid organs of cattle (Murray *et al.*, 1980). Depression of antibody responses to viral and bacterial vaccines (reviewed by Murray *et al.*, 1980) might merely reflect the enormous increase in Ig catabolism that occurs in trypanosome-infected cattle (Nielsen *et al.*, 1978). Therefore, the evidence for immunosuppression of cattle with trypanosomiasis is equivocal. In fact, cattle retain a high degree of immunological competence, at least in dealing with the parasite.

C. CAPACITY TO STIMULATE PLEOMORPHISM

There is evidence that the capacity of *T. brucei* to differentiate from rapidly dividing long slender forms, which predominate during the rising phase of parasitaemia, to the non-dividing short, stumpy forms found just before remission (Ashcroft, 1957), may be important in determining the virulence of a particular strain. Monomorphic slender parasites are highly virulent, whereas pleomorphic parasites of the same variable antigen type cause chronic infections in mice (Barry *et al.*, 1979).

Further studies in mice have shown that slender forms did not stimulate any detectable antibody response, whereas, short lived stumpy forms or irradiated slenders did, as judged by radio-immune assay (Sendashonga and Black, in press). This suggested that dead or dying trypanosomes were essential for induction of immunity and that immune responses would be expected to be induced by late differentiating forms.

Confirming the relationship between virulence and monomorphism, it was found that when mice of any strain were infected with a monomorphic clone of *T. brucei*, they all died during the first wave of parasitaemia. Death resulted from massive parasitaemia with little parasite differentiation or detectable host humoral response (Sendashonga and Black, in press). When the same parasite was inoculated into cattle, it readily differentiated; remission followed the development of pleomorphism and the onset of a detectable antibody response (C. Sendashonga and S. J. Black, unpublished observations).

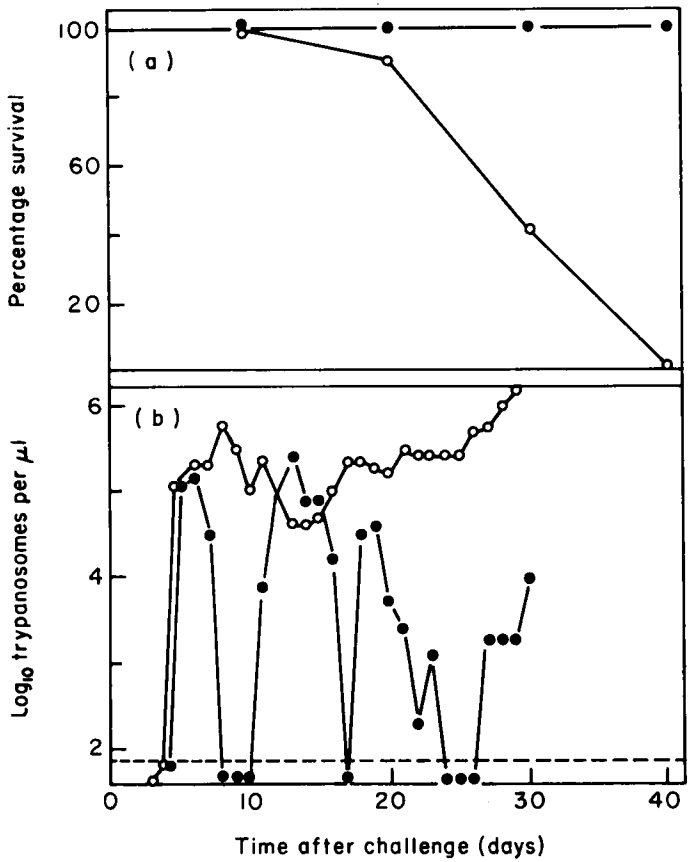

FIG. 13. (a) Percentage survival of 12 C3H/He (○) and 12 C57BL/6 (●) mice infected with *T. brucei*. (b) Typical parasitaemias of a C3H/He (○) and a C57BL (●) mouse.

These results led us to speculate that the host determines the level of susceptibility by influencing parasite differentiation. We found that, following inoculation with a pleomorphic strain of *T. brucei*, C57BL/6 mice could control parasitaemia and produce remission to a significantly greater extent

than more susceptible C3H/He mice (Fig. 13). Differentiation and the appearance of antibody (Fig. 14) also occurred earlier in C57BL/6 mice.

Similarly, the capacity of cattle to induce differentiation and control parasitaemia was associated with susceptibility. Following infection with *T. brucei*, 1-week-old calves developed severe anaemia and died 3 weeks later, whereas 20-month-old animals, after similar time to patency, maintained normal haematological values until the end of week 3 (Max Murray and S. J. Black, unpublished observations). Differentiation occurred earlier and the levels of parasitaemia were lower in the adults.

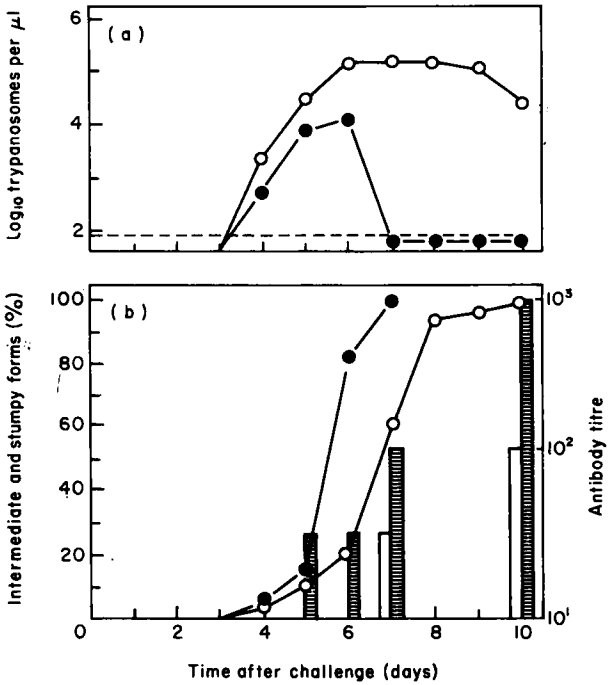

FIG. 14. (a) Mean parasitaemia of 12 C3H/He (○) and 12 C57BL/6 (●) mice infected with *T. brucei*. - - - - - - - - - represents level of detectability. (b) The rate of trypanosome differentiation in C3H/He (○) and C57BL/6 (●) and the titre of antibody by radioimmune assay (histogram: hatched areas represent C57BL/6).

In both mice and cattle, the capacity to control parasitaemia is related to susceptibility. There is evidence that parasite differentiation and the development of the immune response are associated. Further studies should determine whether differences in parasite differentiation and in the immune response are related to the inherent capacity of certain hosts to stimulate differentiation or to mount a more effective immune response.

D. PHYSIOLOGICAL FACTORS

1. *Factors affecting trypanosome metabolism*

Trypanotolerant animals might also be poor hosts for trypanosomes for non-immunological reasons such as the presence of harmful factors in their bloodstream. Some agents that influence host susceptibility have already been identified. It has been shown that cotton rats (*Sigmodon hispidus*) are completely refractory to *T. vivax* because of a trypanocidal factor identified as a serum macroglobulin (Terry, 1957). A high density lipoprotein in the serum of man lyses *T. brucei* but not *T. rhodesiense*; this forms the basis of the only method by which these two trypanosomes can be distinguished *in vitro* (Rifkin, 1978). It should be emphasized, however, that both these factors are trypanocidal and create a refractory state in the cotton rat and man. Trypanotolerance, on the other hand, describes a state of increased resistance or reduced susceptibility. If non-specific factors are involved, it is much more likely that they act by affecting trypanosome differentiation or growth rate (see Section VII C, p. 50). Alternatively, trypanotolerance may depend on more efficient effector mechanisms for eliminating the trypanosomes. For example, differences in complement reactivity may exist because, at least *in vitro*, immune lysis of trypanosomes is complement dependent. Different levels of conglutinin or immunoconglutinin might facilitate clearance of trypanosome aggregates from the circulation. Polyamine oxidase activity, which is known to be present in ruminant serum, may operate more effectively in trypanotolerant animals; the enzymic oxidation of spermidine, which is known to be present in trypanosomes, by polyamine oxidases has been shown to kill the parasite, probably by the production of aldehydes and other cytotoxic intermediates (H. Hirumi, personal communication).

Lastly, there may be differences in the activity of the mononuclear phagocytic system in removing trypanosomes from the circulation, which influence susceptibility. The activity of the mononuclear phagocytic system is known to differ among strains of mice (Passwell *et al.*, 1974).

2. *Erythropoietic response*

One of the major features of trypanotolerance is that resistant groups of animals, such as N'Dama cattle, Red Masai sheep and wildlife, develop less severe anaemia than more susceptible animals. A series of erythrokinetic and ferrokinetic studies of N'Dama and Zebu infected with *T. congolense* or *T. brucei* showed that the anaemia and its underlying processes broadly reflected the numbers of parasites in the blood (Dargie *et al.*, 1979a, b). Thus, it appeared that the differences in anaemia between N'Dama and Zebu were due to their capacity to control parasitaemia and could not be attributed to differences in innate erythropoietic responsiveness.

In contrast to cattle, we have found major differences between Red Masai sheep and the more susceptible Merino in the severity of anaemia despite similar levels of parasitaemia (D. D. Whitelaw and Max Murray, unpublished observations). Also, in wild animals significant anaemia rarely occurs, despite fairly high levels of parasitaemia in some species, e.g. 10^5 trypanosomes ml^{-1} may be present in the blood of eland infected with *T. congolense* (Murray *et al.*, 1981b). It could be speculated that the red blood cells of these animals have a greater capacity to resist the pathogenic effects of trypanosomes or that they are able to mount a faster and more efficient erythropoietic response.

3. *Food utilization and heat tolerance*

Another aspect of trypanotolerance which could be important and must be considered is that trypanosome-infected N'Dama are more resistant to constraints in production because of superior physiological adaptation to factors such as food utilization and heat tolerance. Unfortunately, critical observations concerning these qualities are not available at present. It is known from work on other breeds of cattle that genetically determined differences in voluntary food intake do exist. These have been ascribed to variations in fasting metabolism and maintenance energy requirements (Vercoe, 1974; Frisch and Vercoe, 1978). Where food intake is marginal, as it is in many areas of Africa, breeds with an inherently high maintenance requirement will suffer most, as demonstrated by weight loss or reduced weight gains. Thus, it is possible that trypanotolerant breeds such as N'Dama, especially when infected, are better adapted to remain productive in the savannah conditions of West and Central Africa.

Little is known about water conservation and heat tolerance in trypanotolerant breeds. Pagot (1974) has pointed out that N'Dama can withstand higher levels of humidity than Zebu. In addition, there is a considerable variation in the rectal temperature of N'Dama during the course of the day, with temperatures ranging from 34·4°C at dawn to 41·1°C in late afternoon in The Gambia (Greig and McIntyre, 1979). The teleological argument for such a phenomenon is that thermoregulation under cold conditions, i.e. below 20°C, for tropical breeds of cattle requires the use of body energy stores. Thus, when caloric intake is low, energy is conserved by allowing the body temperature to fall (D. Robertshaw, personal communication). We have also found in The Gambia that trypanosome-infected N'Dama, in contrast to Zebu, do not become febrile even during waves of parasitaemia (Murray *et al.*, 1981a). This might be due to the lower levels of parasitaemia encountered in N'Dama or it might be the result of differences in their thermoregulatory system.

More is known about the Zebu type breeds. Zebu are more heat tolerant than imported *Bos taurus*, either because their simple sac-like sweat glands

and shallow hair follicle depth help promote sweating (Jenkinson and Nay, 1973) or because their lower maintenance requirements produce less heat load. Amakiri (1974) examined the histological skin structure of Nigerian breeds of cattle including N'Dama, Muturu and White Fulani and found that on the basis of sweat gland size, the N'Dama were more akin to the exotic *Bos taurus* and by inference were less well-adapted to the tropical Nigerian environment. However, until sweating rate is actually measured, this conclusion is only speculative (D. Robertshaw, personal communication). The other characteristics of the skin, namely follicle depth and hair length, suggest that all three of these indigenous African breeds have the skin type associated with successful habitation of warm climates (Amakiri, 1974).

Studies in East Africa, where the indigenous breed is the East African Zebu, have shown that its water requirement is about half that of the Hereford and is similar to that of several species of wild animals (EAVRO, 1967). Zebu were also better able to conserve evaporative and faecal water than Hereford. Zebu deprived of water stopped eating and metabolized fat, with a resultant reduction of urinary and faecal water losses. Zebu could form faeces as dry as 110 g water to 100 g dry matter, whereas Hereford were unable to form faeces containing less than 300 g water to 100 g dry matter. As a result, Zebu were able to live comfortably without water at an environmental temperature of 22°C for 2 months or until their fat supplies were depleted, a fact confirmed for Turkana cattle living under drought conditions. This capacity for conserving water was inherited as a dominant trait in Zebu–Hereford crosses. It is likely that N'Dama have adapted to an even greater extent than Zebu because trypanosome-free N'Dama turn over less water in relation to their metabolic size (Dargie, 1980).

VIII. Conclusions

The range of host susceptibility to African salivarian trypanosomes, both within and between species, is extensive. Considering, firstly, that three species of trypanosomes are involved, secondly, that these species comprise an as yet undefined number of distinct families or serodemes all with the capacity to undergo antigenic variation, and lastly, that a large number of host species are at risk, trypanosomiasis represents one of the most fascinating areas in biology as well as one of the most dreadful and economically damaging disease problems of domestic livestock. That nature has been dealing with the problem in her own way over several thousand years by rigorous selection of characteristics that permit survival in the face of tsetse challenge has now been recognized. Thus, the exploitation of genetically resistant or trypanotolerant breeds of livestock is now widely advocated as one of the

most promising solutions for the control of the disease. This view has been encouraged by recent evidence largely refuting the dogma that the relatively small size of trypanotolerant livestock is equated with low productivity.

It has now been confirmed that trypanotolerance exists as an innate characteristic and that it is probably inherited as a dominant trait. While the level of trypanotolerance can be reduced under certain circumstances, it can also be enhanced, for example by previous exposure. Therefore, there is hope that it may be possible in the future to supplement the level of trypanotolerance both by genetic selection and by immunological or therapeutic procedures.

Investigations of trypanotolerance also represent an exciting approach to the important interactions between host and parasite. Most important, however, the exploitation of trypanotolerant livestock in the vast savannah regions could provide enormous socio-economic benefit to the people of Africa.

Appendix

Scientific names of wild animals mentioned in Section III C, pp. 19–24.

Aardvark	*Orycteropus afer*	Hyrax	*Dendrohyrax* sp.
Antelope, roan	*Hippotragus equinus*	Impala	*Aepyceros melampus*
Antelope, sable	*Hippotragus niger*	Jackal	*Canis* sp.
Baboon	*Papio* sp.	Kudu	*Strepsiceros* sp.
Buffalo	*Syncerus caffer*	Leopard	*Panthera pardus*
Bushbaby	*Galago crassicaudatus*	Lion	*Panthera leo*
Bushbuck	*Tragelaphus scriptus*	Monkey	*Cercopithecus* sp.
Bushpig	*Potamochoerus porcus*	Oribi	*Ourebia ourebi*
Dikdik	*Rhynchotragus* sp.	Ostrich	*Struthio camelus*
Duiker, blue forest	*Cephalophus monticola*	Porcupine	*Hystrix galeata*
		Puku	*Kobus vardoni*
Duiker, bush	*Sylvicapra grimmia*	Reedbuck	*Redunca* sp.
Eland	*Taurotragus oryx*	Reedbuck, Bohor	*Redunca redunca*
Elephant	*Loxodonta africana*		
Fox, bat-eared	*Otocyon megalotis*	Rhinoceros	*Diceros bicornis*
Gazelle, Thomson's	*Gazella thomsoni*	Serval	*Felis serval*
		Topi	*Damaliscus korrigum*
Giraffe	*Giraffa camelopardalis*	Warthog	*Phacochoerus aethiopicus*
Hartebeest	*Alcelaphus buselaphus*		
Hippopotamus	*Hippopotamus amphibius*	Waterbuck	*Kodus* sp.
		Wildebeest	*Connochaetes taurinus*
Hyaena	*Crocuta crocuta*	Zebra	*Equus burchelli*

Acknowledgements

We are grateful to Drs Sam Black and J. Trail in Nairobi and Professors G. M. Urquhart and W. I. M. McIntyre in Glasgow for their interest in

our studies in this field. Particular thanks are extended to Professor C. E. Davis for much helpful discussion and encouragement during the preparation of the manuscript, and to Mrs Leah Njuguna, Grace Maloba and Regina Ndonyo for typing. The support of Mr. Maxwell Murray is especially acknowledged. D. D. W. was supported by the Wellcome Trust, London.

References

Amakiri, S. F. (1974). Sweat gland measurements in some tropical and temperate breeds of cattle in Nigeria. *Animal Production* **18,** 285–291.

Amakiri, S. F. (1976). Arteriovenous anastomoses in the skin of tropical cattle. *Acta Anatomica* **96,** 285–300.

Amakiri, S. F. (1979). Melanin and dopa-positive cells in the skin of tropical cattle. *Acta Anatomica* **103,** 433–444.

Archibald, R. G. (1927). The tsetse fly-belt area in the Nuba Mountains province of the Sudan. *Annals of Tropical Medicine and Parasitology* **21,** 39–44.

Ashcroft, M. T. (1957). The polymorphism of *Trypanosoma brucei* and *T. rhodesiense*, its relation to relapses and remissions of infections in white rats, and the effect of cortisone. *Annals of Tropical Medicine and Parasitology* **51,** 301–312.

Ashcroft, M. T. (1958). An attempt to isolate *T. rhodesiense* from wild animals. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **52,** 276–282.

Ashcroft, M. T. (1959). The importance of African wild animals as reservoirs of trypanosomiasis. *East African Medical Journal* **36,** 289–297.

Ashcroft, M. T., Burtt, E. and Fairbairn, H. (1959). The experimental infection of some African wild animals with *Trypanosoma rhodesiense*, *T. brucei* and *T. congolense*. *Annals of Tropical Medicine and Parasitology* **53,** 147–161.

Awan, M. A. Q. (1979). Identification by the blood incubation infectivity test of *Trypanosoma brucei* subspecies isolated from game animals in the Luangwa Valley, Zambia. *Acta Tropica* **36,** 343–347.

Awan, M. A. Q. and Dillmann, J. S. S. (1973). *Trypanosoma brucei* infection in a lion (*Panthera leo*) in Zambia. *Tropical Animal Health and Production* **42,** 75–78.

Baker, J. R. (1968). Trypanosomes of wild mammals in the neighbourhood of the Serengeti National Park. *Symposium of the Zoological Society of London* **24,** 147–158.

Baker, J. R., Sachs, R. and Laufer, I. (1967). Trypanosomes of wild mammals in an area northwest of the Serengeti National Park, Tanzania. *Tropenmedizin und Parasitologie* **18,** 280–284.

Bangham, A. D. and Blumberg, B. S. (1958). Distribution of electrophoretically different haemoglobins among some cattle breeds of Europe and Africa. *Nature* (*London*) **181,** 1551–1552.

Barbet, A. F. and McGuire, T. C. (1978). Crossreacting determinants in variant-specific surface antigens of African trypanosomes. *Proceedings of the National Academy of Sciences of the U.S.A.* **75,** 1989–1993.

Barbet, A. F., McGuire, T. C., Musoke, A. J. and Hirumi, H. (1979). Cross-reacting determinants in trypanosome surface antigens. *In* "Pathogenicity of Trypanosomes" (G. Losos and A. Chouinard, eds), pp. 38–43. International Development and Research Council, Ottawa. (IDRC-132e.)

Barry, J. D. and Hajduk, S. L. (1979). Antigenic heterogeneity of bloodstream and metacyclic forms of *T. brucei*. *In* "Pathogenicity of Trypanosomes" (G. Losos and A. Chouinard, eds), pp. 51–56. International Development and Research Council, Ottawa (IDRC-132e).

Barry, J. D., Le Ray, D. and Herbert, W. J. (1979). Infectivity and virulence of *Trypanosoma* (*Trypanozoon*) *brucei* for mice. IV. Dissociation of virulence and variable antigen type in relation to pleomorphism. *Journal of Comparative Pathology* **89**, 465–470.

Bevan, E. W. (1928). A method of inoculating cattle against trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **22**, 147–156.

Bevan, E. W. (1936). Notes on immunity in trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **30**, 199–206.

Blackwell, J., Freeman, J. and Bradley, D. (1980). Influence of H-2 complex on acquired resistance to *Leishmania donovani* infection in mice. *Nature* (*London*) **283**, 72–74.

Bourn, D. and Scott, M. (1978). The successful use of work oxen in agricultural development of tsetse infested land in Ethiopia. *Tropical Animal Health and Production* **10**, 191–203.

Boyt, W. P. (1971). Trypanosomiasis control in Rhodesia. *Bulletin of the Office of Internal Epizootiology* **76**, 301–306.

Bradley, D. J. (1977). Regulation of *Leishmania* populations within the host, II. Genetic control of acute susceptiblity of mice to *Leishmania donovani* infection. *Clinical and Experimental Immunology* **30**, 130–140.

Bruce, D. and collaborators (1914). Reports of the Sleeping Sickness Commission of the Royal Society, No. 15. [*Proceedings of the Royal Society, London, B* **85–87** passim, 1912–1914.]

Bruce, D. and collaborators (1915). Reports of the Sleeping Sickness Commission of the Royal Society, No. 16. [*Proceedings of the Royal Society, London, B* **88** passim, 1914–1915.]

Bungener, W. and Mehlitz, D. (1976). Experimental *Trypanosoma* infections on Cameroon Dwarf goats: histopathological observations. *Tropenmedizin und Parasitologie* **27**, 405–410.

Burridge, M. J., Reid, H. W., Pullan, N. B., Sutherst, R. W. and Wain, E. B. (1970). Survey for trypanosome infections in domestic cattle and wild animals in areas of East Africa. II. Salivarian trypanosome infections in wild animals in Busoga District, Uganda. *British Veterinary Journal* **126**, 627–633.

Bursell, E. (1980). The future of tsetse biology. *In* "Insect Biology in the Future" (M. Locke and D. Smith, eds), pp. 905–924. Academic Press, London.

Buyst, H. (1974). The epidemiology, clinical features, treatment and history of sleeping sickness on the northern edge of the Luangwa fly belt. *Medical Journal of Zambia* **8**, 2–12.

Buyst, H. (1977). The epidemiology of sleeping sickness in the historical Luangwa Valley. *Annales de la Société belge de Médecine tropicale* **57**, 349–359.

Campbell, G. H., Esser, K. M. and Phillips, S. M. (1978). *Trypanosoma rhodesiense* infection in congenitally athymic (nude) mice. *Infection and Immunity* **20**, 714–720.

Capbern, A., Giroud, C., Baltz, T. and Mattern, P. (1977). *Trypanosoma equiperdum:* étude des variations antigèniques au cours de la trypanosomiase expérimentale du lapin. *Experimental Parasitoloy* **42**, 6–13.

Carmichael, J. (1934). Trypanosomes pathogenic to domestic stock and their effect in certain species of wild fauna in Uganda. *Annals of Tropical Medicine and Parasitology* **28**, 41–45.

Carr, W. R., MacLeod, J., Woolf, B. and Spooner, R. L. (1974). A survey of the relationship of genetic markers, tick infestation level and parasitic diseases in Zebu cattle in Zambia. *Tropical Animal Health and Production* **6**, 203–214.

Chandler, R. L. (1952). Comparative tolerance of West African N'Dama cattle to trypanosomiasis. *Annals of Tropical Medicine and Parasitology* **46**, 127–134.

Chandler, R. L. (1958). Studies on the tolerance of N'Dama cattle to trypanosomiasis. *Journal of Comparative Pathology* **68**, 253–260.

Clayton, C. E. (1978). *Trypanosoma brucei:* influence of host strain and parasite antigenic type on infections in mice. *Experimental Parasitology* **44**, 202–208.

Clayton, C. E., Ogilvie, B. M. and Askonas, B. A. (1979). *Trypanosoma brucei* infection in nude mice: B lymphocyte function is suppressed in the absence of T lymphocytes. *Parasite Immunology* **1**, 39–48.

Clifford, D. J. and Sanyang, B. J. (1979). An epidemiological study of trypanosomiasis in N'Dama cattle in The Gambia. *In* "International Scientific Council for Trypanosomiasis Research and Control, 15th Meeting, Banjul, The Gambia, 1977", pp. 152–154. Organization for African Unity (OAU/STRC No. 110). Scientific Publications Division of Eleza Services, Nairobi.

Coleman, C. H. (1967). Cutaneous streptothricosis of cattle in West Africa. *Veterinary Record* **81**, 251–254.

Cornell, R. L. and Evans, S. A. (1937). On the value and limitations of tissue vaccines against rinderpest. *Journal of Comparative Pathology* **50**, 122–135.

Cross, G. A. M. (1979). Biochemistry of variant antigens. *In* "Pathogenicity of Trypanosomes" (G. Losos and A. Chouinard, eds), pp. 32–37. International Research and Development Council, Ottawa (IDRC-132e).

Cunningham, M. P. (1966) Immunity in bovine trypanosomiasis. *East African Medical Journal* **43**, 394–397.

Dargie, J. D. (1980). Pathophysiology of trypanosomiasis in the bovine. *In* "Isotope and Radiation Research on Animal Diseases and their Vectors", pp. 121–131. International Atomic Energy Agency, Vienna (IAEA-SM-240/28).

Dargie, J. D., Murray, P. K., Murray, Max, Grimshaw, W. T. R. and McIntyre, W. I. M. (1979a). Bovine trypanosomiasis: the red cell kinetics of N'Dama and Zebu cattle infected with *Trypanosoma congolense*. *Parasitology* **78**, 271–286.

Dargie, J. D., Murray, P. K., Murray, Max and McIntyre, W. I. M. (1979b). Blood volumes and erythrokinetics of N'Dama and Zebu cattle experimentally infected with *Trypanosoma brucei*. *Research in Veterinary Science* **226**, 245–247.

Davis, C. E., Robbins, R. S., Weller, R. D. and Braude, A. I. (1974). Thrombocytopenia in experimental trypanosomiasis. *Journal of Clinical Investigation* **53**, 1359–1367.

De Gee, A. L. W. (1980). Host–parasite relationships in *Trypanosoma* (*Duttonella*) *vivax* with special reference to the influence on antigenic variation. Thesis, University of Utrecht, pp. 113–116.

De Gee, A. L. W., Shah, S. D. and Doyle, J. J. (in press). *Trypanosoma vivax:* the courses of infection in inbred mouse strains. *Experimental Parasitology.*

Desowitz, R. S. (1959). Studies on immunity and host parasite relationships. I. The immunological response of resistant and susceptible breeds of cattle to trypanosomal challenge. *Annals of Tropical Medicine and Parasitology* **53**, 293–313.

Desowitz, R. S. (1970). African trypanosomes. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds), vol. 2, pp. 551–596. Appleton-Century-Crofts, New York.

Dillmann, J. S. S. and Townsend, A. J. (1979). A trypanosomiasis survey of wild animals in the Luangwa Valley, Zambia. *Acta Tropica* **36**, 349–356.

Doherty, P. C. and Zinkernagel, R. W. (1974). T-cell-mediated immunopathology in viral infections. *Transplantation Reviews* **19**, 89–120.

Doppler, W., Sere, C. and Schreiber, J. (1980). The economics of beef production in various breeds and cross-breeds in Avetonou/Togo. *In* "Trypanotolerance and Animal Production" (E. Karbe and E. K. Freitas, eds), pp. 30–38. Centre de Recherche et d'Elevage Avetonou, Togo.

Drager, N. and Mehlitz, D. (1978). Investigations on the prevalence of trypanosome carriers and the antibody response in wildlife in Northern Botswana. *Tropenmedizin und Parasitologie* **29**, 223–233.

Duke, H. L., Mettam, R. W. M. and Wallace, J. M. (1934). Observations on the direct passage from vertebrate to vertebrate of recently isolated strains of *Trypanosoma brucei* and *Trypanosoma rhodesiense*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **28**, 77–84.

EAVRO (1967). "Annual Report: Physiology Section, Animal Production Division, East African Veterinary Research Organization", pp. 56–65. East African Community Services Organization, Nairobi. The Government Printer, Entebbe, Uganda.

Edwards, E. E., Judd, J. M. and Squire, F. A. (1956). Observations on trypanosomiasis in domestic animals in West Africa. I. The daily index of infection and the weekly haematological values in goats and sheep infected with *Trypanosoma vivax*, *T. congolense*, and *T. brucei*. *Annals of Tropical Medicine and Parasitology* **50**, 222–241.

Emery, D. L., Akol, G. W. O., Murray, Max, Morrison, W. I. and Moloo, S. K. (1980). Chancre—early events in the pathogenesis of African trypanosomiasis in domestic livestock. *In* "The Host-Invader Interplay" (H. Van Den Bossche, ed.), pp. 345–356. Elsevier/North Holland Biomedical Press, Amsterdam.

Epstein, H. (1971). "The origin of the domestic animals of Africa", volumes 1 and 2. Africana, New York.

Esser K. M., Schoenbechler, M. J., Gingrich, J. B. and Diggs, C. L. (1981). Monoclonal antibody analysis of *Trypanosoma rhodesiense* metacyclic antigen types. *Federation Proceedings* **40**, 1011.

Esuruoso, G. O. (1977). A comparative study of the course of *Trypanosoma vivax* infection in Zebu and Muturu cattle. *Bulletin of Epizootiological Diseases of Africa* **25**, 233–244.

Fairbairn, H. and Burtt, E. (1946). The infectivity to man of a strain of *Trypanosoma rhodesiense* transmitted cyclically by *Glossina morsitans* through sheep and antelope: evidence that man requires a minimum infective dose of metacyclic trypanosomes. *Annals of Tropical Medicine and Parasitology* **40**, 270–313.

Ferguson, W. (1967). Muturu cattle of Western Nigeria: II. Survivability, reproductive and growth performances in an area of light *Glossina palpalis* density. *Journal of the West African Science Association* **12**, 37–44.

Fiennes, R. N. T.-W. (1970). Pathogenesis and pathology of animal trypanosomiases. *In* "The African Trypanosomiases" (H. W. Mulligan, ed.), pp. 729–750. Allen and Unwin, London.

Ford, J. (1971). "The role of the Trypanosomiases in African Ecology. A study of the Tsetse Fly problem". Clarendon Press, Oxford.

Frisch, J. E. and Vercoe, J. E. (1978). Utilising breed differences in growth of cattle in the tropics. *World Animal Review* **25**, 8–12.

Galvao-Castro, B., Hochmann, A. and Lambert, P. H. (1978). The role of the host immune response in the development of tissue lesions associated with African trypanosomiasis in mice. *Clinical and Experimental Immunology* **33,** 12–24.

Geigy, R., Kauffmann, M. and Beglinger, R. (1967). A survey of wild animals as potential reservoirs of trypanosomiasis in the Ulanga District (Tanzania). *Acta Tropica* **24,** 97–108.

Geigy, R., Mwambu, P. M. and Kauffmann, M. (1971). Sleeping sickness survey in Musoma District, Tanzania. IV. Examination of wild mammals as a potential reservoir for *T. rhodesiense*. *Acta Tropica* **28,** 211–220.

Geigy, R., Jenni, L., Kauffmann, M., Onyango, R. J. and Weiss, N. (1975). Identification of *T. brucei*-subgroup strains isolated from game. *Acta Tropica* **32,** 190–205.

Glasgow, J. P., Isherwood, F., Lee-Jones, F. and Weitz, B. (1958). Factors influencing the staple food of tsetse flies. *Journal of Animal Ecology* **27,** 59–69.

Godfrey, D. G., Leach, T. M. and Killick-Kendrick, R. (1964). Bovine trypanosomiasis in Nigeria. III. A high incidence in a group of West African humpless cattle. *Annals of Tropical Medicine and Parasitology* **58,** 204–215.

Goodwin, L. G., Green, D. G., Guy, M. W. and Voller, A. (1972). Immunosuppression during trypanosomiasis. *British Journal of Experimental Pathology* **53,** 40–43.

Greenwood, B. M., Whittle, H. C. and Molyneux, D. H. (1973). Immunosuppression in Gambian trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **67,** 846–850.

Greig, W. A. and McIntyre, W. I. M. (1979). Diurnal variation in rectal temperature of N'Dama cattle in The Gambia. *British Veterinary Journal* **135,** 113–118.

Griffin, L., and Allonby, E. W. (1979a). Trypanotolerance in breeds of sheep and goat with an experimental infection of *Trypanosoma congolense*. *Veterinary Parasitology* **5,** 97–105.

Griffin, L. and Allonby, E. W. (1979b). Studies on the epidemiology of trypanosomiasis in sheep and goats in Kenya. *Tropical Animal Health and Production* **11,** 133–142.

Hauschka, T. S. (1947). Sex of host as a factor in Chagas' disease. *Journal of Parasitology* **33,** 399–404.

Heisch, R. B. (1952). Presence of trypanosomes in bush babies after eating infected rats. *Nature* (*London*) **169,** 118.

Heisch, R. B., McMahon, J. P. and Manson-Bahr, P. E. C. (1958). The isolation of *Trypanosoma rhodesiense* from a bushbuck. *British Medical Journal* (**ii**), 1203–1204.

Hornby, H. E. (1952). "Animal trypanosomiasis in Eastern Africa, 1949". His Majesty's Stationery Office, London.

Ige, K. and Amodu, A. A. (1977). Studies on the pathogenesis of trypanosomiasis for N'Dama cattle. *In* "International Scientific Council for Trypanosomiasis Research and Control; 14th Meeting, Dakar, Senegal, 1975", pp. 151–156. Organization for African Unity (OAU/STRC No. 109). Scientific Publications Division of Eleza Services, Nairobi.

Ikede, B. O. (1979). Genital lesions in experimental chronic *Trypanosoma brucei* infection in rams. *Research in Veterinary Science* **26,** 145–151.

ILCA (1979). "Trypanotolerant Livestock in West and Central Africa." International Livestock Centre for Africa, Addis Ababa (Monograph No. 2).

Jayawardena, A. N. and Waksman, B. H. (1977). Suppressor cells in experimental trypanosomiasis. *Nature* (*London*) **265,** 539–541.

Jenkinson, D. McE. and Nay, T. (1973). The sweat glands and hair follicles of Asian, African and South American cattle. *Australian Journal of Biological Science* **26**, 259–275.

Jenni, L. (1977). Comparisons of antigenic types of *Trypanosoma* (*T.*) *brucei* strains transmitted by *Glossina m. morsitans*. *Acta Tropica* **34**, 35–41.

Jennings, F. W., Whitelaw, D. D., Holmes, P. H. and Urquhart, G. M. (1978). The susceptibility of strains of mice to infection with *Trypanosoma congolense*. *Research in Veterinary Science* **25**, 399–400.

Karib, A. E. (1961). Animal trypanosomiasis in the Sudan. *The Sudan Journal of Veterinary Science and Animal Husbandry* **2**, 39–46.

Kinghorn, A. and Yorke, W. (1912). Trypanosomes infecting game and domestic stock in the Luangwa Valley, North Eastern Rhodesia. *Annals of Tropical Medicine and Parasitology* **6**, 301.

Letenneur, L. (1978). Crossbreeding N'Dama and Jersey cattle in Ivory Coast. *World Animal Review* **27**, 36–42.

Lilly, F. and Pincus, T. (1973). Genetic control of murine viral leukaemogenesis. *Advances in Cancer Research* **17**, 231–277.

Lindsley, H. B., Nagle, R. B., Werner, P. A. and Stechschulte, D .J. (1980). Variable severity of glomerulonephritis in inbred rats infected with *Trypanosoma rhodesiense*. Correlation with immunoglobulin class-specific antibody responses to trypanosomal antigens and total IgM levels. *American Journal of Tropical Medicine and Hygiene* **29**, 348–357.

Losos, G. J. and Gwamaka, G. (1973). Histological examination of wild animals naturally infected with pathogenic African trypanosomes. *Acta Tropica* **30**, 57–63.

Luckins, A. G. (1976). The immune response of Zebu cattle to infection with *Trypanosoma congolense* and *T. vivax*. *Annals of Tropical Medicine and Parasitology* **70**, 133–145.

MacAskill, J. A., Holmes, P. H., Jennings, F. W. and Urquhart, G. M. (1981). Immunological clearance of ^{75}Se-labelled *Trypanosoma brucei* in mice. III. Studies in animals with acute infections. *Immunology* **43**, 691–698.

MacKenzie, P. K. I. and Cruickshank, J. G. (1973). Phagocytosis of erythrocytes and leucocytes in sheep infected with *Trypanosoma congolense* (Broden 1904). *Research in Veterinary Science* **15**, 256–262.

MacLennan, K. J. R. (1970). The epizootiology of trypanosomiasis in livestock in West Africa. *In* "The African Trypanosomiases" (H. W. Mulligan, ed.), pp. 751–765. Allen and Unwin, London.

MacLennan, K. J. R. (1981). A general perspective of tsetse transmitted trypanosomiasis in relation to the rural economy in Africa—Part I. Tsetse infestation. *World Animal Review* **36**, 2–17.

Mahmoud, M. M. and Elmalik, K. H. (1977). Trypanosomiasis: goats as a possible reservoir of *Trypanosoma congolense* in the Republic of the Sudan. *Tropical Animal Health and Production* **9**, 167–170.

Masake, R. A. and Morrison, W. I. (1981). An evaluation of the structural and functional changes in the lymphoid organs of Boran cattle infected with *Trypanosoma vivax*. *American Journal of Veterinary Research* **42**, 1738–1746.

Mason, I. L. (1951). The classification of West African livestock. *Technical Communication No. 7 of the Commonwealth Bureau of Animal Breeding and Genetics, Edinburgh*. Commonwealth Agricultural Bureaux, Slough.

McCulloch, B. (1967). Trypanosomes of the *brucei* subgroup as a probable cause of disease in wild zebra (*Equus burchelli*). *Annals of Tropical Medicine and Parasitology* **61**, 261–264.

McDevitt, H. O. and Benacerraf, B. (1969). Genetic control of specific responses. *Advances in Immunology* **11**, 31–74.

McDevitt, H. O. and Bodmer, W. F. (1974). HL-A immune response genes and disease. *Lancet*, June 22, 1269–1275.

McDevitt, H. O., Oldstone, M. B. A. and Pincus, T. (1974). Histocompatibility linked genetic control of specific immune responses to viral infections. *Transplantation Reviews* **19**, 209–225.

McNeillage, G. J. C. and Herbert, W. J. (1968). Infectivity and virulence of *Trypanosoma* (*Trypanozoon*) *brucei* for mice. II. Comparison of closely related trypanosome antigenic types. *Journal of Comparative Pathology* **78**, 345–349.

Moloo, S. K. (1973). Relationship between hosts and trypanosome infection rates of *Glossina swynnertoni* Aust. in the Serengeti National Park, Tanzania. *Annals of Tropical Medicine and Parasitology* **67**, 205–211.

Morrison, W. I. and Murray, Max (1979). *Trypanosoma congolense:* inheritance of susceptibility to infection in inbred strains of mice. *Experimental Parasitology* **48**, 364–374.

Morrison, W. I. and Wright, N. G. (1977). Viruses associated with renal disease in man and animals. *Progress in Medical Virology* **23**, 22–50.

Morrison, W. I., Roelants, G. E., Mayor-Withey, K. S. and Murray, Max (1978). Susceptibility of inbred strains of mice to *Trypanosoma congolense:* correlation with changes in spleen lymphocyte populations. *Clinical and Experimental Immunology* **32**, 25–40.

Morrison, W. I., Roelants, G. E., Pearson, T. W. and Murray, Max (1979). Genetic control of susceptibility to *Trypanosoma congolense*. *In* "Function and Structure of the Immune System" (W. Müller-Rucholtz and H. K. Müller-Hermelink, eds). *Advances in Experimental Medicine and Biology*, vol. 69, pp. 709–714. Plenum Press, New York and London.

Mortelmans, J. and Kageruka, P. (1976). Trypanotolerant cattle breeds in Zaire. *World Animal Review* **19**, 14–17.

Murray, Max (1979). The anaemia of bovine African trypanosomiasis: an overview. *In* "Pathogenicity of Trypanosomes" (G. Losos and A. Chouinard, eds), pp. 121–127. International Research and Development Council, Ottawa (IDRC-132e).

Murray, Max and Morrison, W. I. (1979a). Non-specific induction of increased resistance in mice to *Trypanosoma congolense* and *T. brucei* by immunostimulants. *Parasitology* **79**, 349–366.

Murray, Max and Morrison, W. I. (1979b). Parasitaemia and host susceptibility to African trypanosomiasis. *In* "Pathogenicity of Trypanosomes" (G. Losos and A. Chouinard, eds), pp. 71–81. International Research and Development Council, Ottawa (IDRC-132e).

Murray, Max and Urquhart, G. M. (1977). Immunoprophylaxis against African trypanosomiasis. *In* "Immunity to blood parasites of animals and man" (L. H. Miller, J. A. Pino and J. J. McKelvey, Jr, eds), pp. 209–241. Plenum Publishing Corporation, New York.

Murray, P. K., Jennings, F. W., Murray, Max and Urquhart, G. M. (1974a). The nature of immunosuppression in *Trypanosoma brucei* infections in mice. I. The role of the macrophage. *Immunology* **27**, 815–824.

Murray, P. K., Jennings, F. W., Murray, Max and Urquhart, G. M. (1974b). The nature of immunosuppression in *Trypanosoma brucei* infections in mice. II. The role of the T and B lymphocyte. *Immunology* **27**, 825–840.

Murray, Max, Lambert, P. H. and Morrison, W. I. (1975). Renal lesions in experimental trypanosomiasis. *In* "Les Glomérulonéphrites de la Zone Intertropicale. Médecine et Maladies infectieuses. Leurs Rapport avec les Endémies Parasitaires", vol. 12, pp. 638–641.

Murray, Christine M., Murray, Max, Murray, P. K., Morrison, W. I., Pyne, C. and McIntyre, W. I. M. (1979a). Diagnosis of African trypanosomiasis in cattle—improved parasitological and serological techniques. *In* "International Scientific Council for Trypanosomiasis Research and Control, 15th Meeting, Banjul, The Gambia, 1977" pp. 247–254. Organization for African Unity (OAU/STRC No. 110). Scientific Publications Division of Eleza Services, Nairobi

Murray, Max, McIntyre, W. I. M., Murray, P. K., Urquhart, G. M., Jennings, F.W., Greig, W. A., Clifford, D. J., N'Dow, W. S. M., Touray, B., Sanyang, B. T. and Bray, R. S. (1979b). Cattle diseases and trypanosomiasis in The Gambia. I. Clinical studies. *In* "International Scientific Council for Trypanosomiasis Research and Control, 15th Meeting, Banjul, The Gambia, 1977", pp. 83–91. Organization for African Unity (OAU/STRC No. 110). Scientific Publications Division of Eleza Services, Nairobi.

Murray, Max, Morrison, W. I., Murray, P. K., Clifford, D. J. and Trail, J. C. M. (1979c). Trypanotolerance: a review. *World Animal Review* **31**, 2–12.

Murray, P. K., Murray, Max, Wallace, M., Morrison, W. I. and McIntyre, W. I. M. (1979d). Trypanosomiasis in N'Dama and Zebu cattle. I. An experimental investigation of susceptibility to *Trypanosoma brucei*, *T. congolense* and mixed infections. *In* "International Scientific Council for Trypanosomiasis Research and Control, 15th Meeting, Banjul, The Gambia, 1977", pp. 470–481. Organization for African Unity (OAU/STRC No. 110). Scientific Publications Division of Eleza Services, Nairobi.

Murray, P. K., Murray, Max, Wallace, M., Morrison, W. I. and McIntyre, W. I. M. (1979e). Trypanosomiasis in N'Dama and Zebu cattle. II. The influence of weight of infection on the severity of the disease. *In* "International Scientific Council for Trypanosomiasis Research and Control, 15th Meeting, Banjul, The Gambia, 1977", pp. 482–487. Organization for African Unity (OAU/STRC No. 110). Scientific Publications Division of Eleza Services, Nairobi.

Murray, Max, Morrison, W. I., Emery, D. L., Akol, G. W. O., Masake, R. A. and Moloo, S. K. (1980). Pathogenesis of trypanosome infection in cattle. *In* "Isotope and Radiation Research on Animal Diseases and their Vectors", pp. 15–32. International Atomic Energy Agency, Vienna (IAEA-SM-240/19).

Murray, Max, Clifford, D. J., Gettinby, G., Snow, W. F. and McIntyre, W. I. M. (1981a). A study of the susceptibility to African trypanosomiasis of N'Dama and Zebu cattle in an area of *Glossina morsitans submorsitans* challenge. *Veterinary Record* **109**, 503–510.

Murray, Max, Grootenhuis, J. G., Akol, G. W. O., Emery, D. L., Shapiro, S. Z., Moloo, S. K., Dar, F., Bovell, D. L. and Paris, J. (1981b). Potential application of research on African trypanosomiasis in wildlife and preliminary studies on animals exposed to tsetse infected with *Trypanosoma congolense*. *In* "Workshop: Wildlife Disease Research and Economic Development, 1980, Nairobi" (L. Karstad, B. Nestel and M. Graham, eds), pp. 40–45. International Research and Development Council, Ottawa.

Musoke, A. J., Nantulya, V. M., Barbet, A. F., Kironde, F. and McGuire, T. C. (1981). Bovine immune response to African trypanosomes: specific antibodies to variable surface glycoproteins of *Trypanosoma brucei*. *Parasite Immunology* **3**, 97–106.

Nantulya, V. M., Musoke, A. J., Barbet, A. F. and Roelants, G. E. (1979). Evidence for reappearance of *Trypanosoma brucei* variable antigen types in relapse populations. *Journal of Parasitology* **65**, 673–679.

Nantulya, V. M., Doyle, J. J. and Jenni, L. (1980). Studies on *Trypanosoma* (*Nannomonas*) *congolense*. III. Antigenic variation in cyclically transmitted strains. *Parasitology* **80**, 123–131.

Nash, T. A. M. (1969). "Africa's Bane: the Tsetse Fly". Collins, London.

Niederhuber, J. E. (1978). The role of I region gene products in macrophage-T lymphocyte interaction. *Immunological Reviews* **40**, 28–52.

Nielsen, K. H., Sheppard, J., Tizard, I. R. and Holmes, W. L. (1978). Experimental bovine trypanosomiasis: changes in the catabolism of serum immunoglobulins and complement components in infected cattle. *Immunology* **35**, 811–816.

Oduye, O. O. and Okunaiya, O. A. (1971). Haematological studies on the White Fulani and N'Dama breeds of cattle. *Bulletin of Epizootiological Diseases of Africa* **19**, 213–218.

Pagot, J. (1974). Les races trypanotolerantes. *In* "Les Moyens de Lutte contre les Trypanosomes et leurs Vecteurs: Actes du Colloque, Paris 12–15 Mars 1974", pp. 235–248. Institut d'Élévage et de Médecine Vétérinaire des Pays Tropicaux, Paris.

Passwell, J. H., Steward, M. W. and Soothill, J. F. (1974). Inter-mouse strain differences in macrophage function and its relationship to antibody responses. *Clinical and Experimental Immunology* **17**, 159–167.

Payne, W. J. A. (1964). The origin of domestic cattle in Africa. *Empire Journal of Experimental Agriculture* **32**, 97–113.

Pierre, C. (1906). "L'élévage dans l'Afrique Occidentale Française." Gouvernement General de l'Afrique Occidentale Française, Paris.

Preston, J. M. and Allonby, E. W. (1979). The influence of breed on the susceptibility of sheep to *Haemonchus contortus* infection in Kenya. *Research in Veterinary Science* **26**, 134–139.

Rickman, L. R. (1974). Investigations into an outbreak of human trypanosomiasis in the lower Luangwa Valley, Eastern Province, Zambia. *East African Medical Journal* **51**, 467–487.

Rifkin, M. R. (1978). Identification of the trypanocidal factor in normal human serum: high density lipoprotein. *Proceedings of the National Academy of Sciences of the U.S.A.* **75**, 3450–3454.

Roberts, C. J. and Gray, A. R. (1972a). The resistance of N'Dama, Muturu and Zebu cattle to trypanosomiasis. *In* "International Scientific Council for Trypanosomiasis Research, 13th Meeting, Lagos, Nigeria, 1971", pp. 97–101. Organization for African Unity (OAU/STRC No. 105). Scientific Publications Division of Eleza Services, Nairobi.

Roberts, C. J. and Gray, A. R. (1972b). Trypanosome infections in captive antelope. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **66**, 335.

Roberts, C. J. and Gray, A. R. (1973a). Studies on trypanosome-resistant cattle. I. The breeding and growth performance of N'Dama, Muturu and Zebu cattle maintained under the same conditions of husbandry. *Tropical Animal Health and Production* **5**, 211–219.

Roberts, C. J. and Gray, A. R. (1973b). Studies on trypanosome-resistant cattle. II. The effect of trypanosomiasis on N'Dama, Muturu and Zebu cattle. *Tropical Animal Health and Production* **5**, 220–233.

Roberts, L. W., Bhogal, M. S. and Karstad, L. (1980). I. Trypanosomiasis. A. Resistance of oryx to tsetse and trypanosomes. *In* "Wildlife Disease Research, Semi-Annual Progress Report, January–June 1980, Wildlife Disease Section, Veterinary Research Laboratory," pp. 2–3. Government of Kenya, Nairobi.

Robson, J. and Rickman, L. R. (1973). Blood incubation infectivity test results for *Trypanosoma brucei* subgroup isolates in the Lambwe Valley, South Nyanza, Kenya. *Tropical Animal Health and Production* **5**, 187–191.

Sachs, R., Schaller, G. B. and Baker, J. R. (1967). Isolation of trypanosomes of the *T. brucei* group from lion. *Acta Tropica* **24**, 109–112.

Sacks, D. L., Selkirk, M., Ogilvie, B. M. and Askonas, B. A. (1980). Instrinsic immunosuppressive activity of different trypanosome strains varies with parasite virulence. *Nature (London)* **283,** 476–478.

Saror, D. I. (1980). Observations on the course and pathology of *Trypanosoma vivax* in Red Sokoto goats. *Research in Veterinary Science* **28,** 36–38.

Saror, D. I., Ilemobade, A. A. and Nuru, S. (1981). The haematology of N'Dama and Zebu cattle experimentally infected with *Trypanosoma vivax*. *In* "International Scientific Council for Trypanosomiasis Research and Control, 16th Meeting, Yaounde, Cameroon, 1979" pp. 287–294 Organization for African Unity (OAU/STRC No. 111). Scientific Publications Division of Eleza Services, Nairobi.

Selkirk, M. E. and Sacks, D. L. (1980). Trypanotolerance in inbred mice: an immunological basis for variation in susceptibility to infection with *Trypanosoma brucei*. *Tropenmedizin und Parasitologie* **31,** 435–438.

Sendashonga, C. and Black, S. J. (in press). Induction and regulation of anti-VSSA humoral immune responses by slender and stumpy form *T. brucei* parasites. *Parasite Immunology*.

Stephen, L. E. (1966). Observations on the resistance of West African N'Dama and Zebu cattle to trypanosomiasis following challenge by wild *Glossina morsitans* from an early age. *Annals of Tropical Medicine and Parasitology* **60,** 230–246.

Steward, M. W. (1979). Chronic immune complex disease in mice: the role of antibody affinity. *Clinical and Experimental Immunology* **38,** 414–423.

Stewart, J. L. (1937). The cattle of the Gold Coast. *Veterinary Record* **49,** 1289–1297.

Stewart, J. L. (1951). The West African Shorthorn cattle. Their value to Africa as trypanosomiasis resistant animals. *Veterinary Record* **63,** 454–457.

Takayanagi, T., Takayanagi, M., Yabu, Y. and Kato, H. (1978). *Trypanosoma gambiense:* immune responses of neonatal rats receiving antibodies from the female. *Experimental Parasitology* **44,** 82–91.

Terry, R. J. (1957). Antibody against *Trypanosoma vivax* present in normal cotton rat serum. *Experimental Parasitology* **6,** 404–411.

Terry, R. J., Hudson, K. M., Faghihi Shirazi, M. and May, D. (1980). Secondary immunodeficiencies associated with African trypanosomiasis. *In* "Isotope and Radiation Research on Animal Diseases and their Vectors," pp. 133–145. International Atomic Energy Agency, Vienna (IAEA-SM-240/19).

Tizard, I., Nielsen, K. H., Seed, J. R. and Hall, J. E. (1978). Biologically active products from African trypanosomes. *Microbiological Reviews* **42,** 661–681.

Touchberry, R. W. (1967). A study of the N'Dama cattle at the Musaia animal husbandry station in Sierra Leone. *Bulletin of the University of Illinois Agricultural Experimental Station*, No. 724.

Touré, S. M. and Séyé, M. (1980). Techniques for the epizootiological study of African trypanosomiases. Results obtained in Senegal in 1979. *In* " A Report of the Expert Consultation on Research on Trypanosomiasis," Appendix V, pp. 51–60. Food and Agriculture Organization, Rome (AGA-801, 1979).

Touré, S. M., Gueyé, A., Séyé, M., Ba, M. A. and Mané, A. (1978). A comparison between the pathology of a natural trypanosome infection in Zebu and N'Dama cattle. *Revue d'Elévage et de Médecine Vétérinaire des Pays Tropicaux*, **31,** 293–313.

Trail, J. C. M., Murray, Max, Sandford, Jennifer, Snow, W. F., Durkin, J., Wilson, A. D. and Wissocq, Y. (in press). "Factors Affecting the Productivity of Dairy Cattle under Trypanosomiasis Risk". International Livestock Centre for Africa, Addis Ababa.

Trischmann, T., Tanowitz, H., Wittner, M. and Bloom B. (1978). *Trypanosoma cruzi:* role of the immune response in the natural resistance of inbred strains of mice. *Experimental Parasitology* **45**, 160–168.

Urquhart, G. M., Murray, Max, Murray, P. K., Jennings, F. W. and Bate, E. (1973). Immunosuppression in *Trypanosoma brucei* infections in rats and mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **67**, 528–535.

Van Hoeve, K. (1972). Some observations on the performance of N'Dama and Muturu cattle under natural conditions in Northern Nigeria. *In* "Report of the International Scientific Council for Trypanosomiasis Research, 13th Meeting, Lagos", pp. 103–106. Organization for African Unity (OAU/STRC No. 105). Scientific Publications Division of Eleza Services, Nairobi.

Van Meirvenne, N., Janssens, P. G., Magnus, E., Lumsden, W. H. R. and Herbert, W. J. (1975). Antigenic variation in syringe passaged populations of *Trypanosoma* (*Trypanozoon*) *brucei*. II. Comparative studies on two antigenic type collections. *Annales de la Société belge de Médecine tropicale* **55**, 25–30.

Van Meirvenne, N. Magnus, E. and Vervoort, T. (1977). Comparison of variable antigenic types produced by trypanosome strains of the subgenus *Trypanozoon*. *Annales de la Société belge de Médecine tropicale* **57**, 409–423.

Vercoe, J. E. (1974). Studies on adaptation of cattle to tropical environments and the role of radioisotopes. *In* "Tracer Techniques in Tropical Animal Production", pp. 73–87. International Atomic Energy Agency, Vienna.

Vervoort, T., Barbet, A. F., Musoke, A. J., Magnus, E., Mpimbaza, G. and Van Meirvenne, N. (1981). Isotypic surface glycoproteins of trypanosomes. *Immunology* **44**, 223–232.

Vladutiu, A. O. and Rose, M. R. (1974). HL-A antigens: association with disease. *Immunogenetics* **1**, 305–328.

Wakelin, D. (1978). Genetic control of susceptibility to parasite infection. *Advances in Parasitology* **16**, 219–308.

Weitz, B. (1963). The feeding habits of *Glossina*. *Bulletin of the World Health Organization* **28**, 711–729.

Weitz, B. and Jackson, C. H. N. (1955). The host animals of *Glossina morsitans* at Daga-Iloi. *Bulletin of Entomological Research* **46**, 531–538.

Weitz, B. and Glasgow, J. P. (1956). The natural hosts of some species of *Glossina* in East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **50**, 593–612.

Wellde, B. T., Hockmeyer, W. T., Kovatch, R. M., Boghal, M. S. and Diggs, C. L. (1981). *Trypanosoma congolense:* natural and acquired resistance in the bovine. *Experimental Parasitology* **52**, 219–232.

Whitelaw, D. D., MacAskill, J. A., Holmes, P. H., Jennings, F. W. and Urquhart, G. M. (1980). Genetic resistance to *Trypanosoma congolense* infections in mice. *Infection and Immunity* **27**, 707–713.

Whiteside, E. F. (1962). Interactions between drugs, trypanosomes and cattle in the field. *In* "Drugs, Parasites and Hosts" (L. G. Goodwin and R. H. Nimmo-Smith, eds), pp. 116–141. Churchill, London.

Wijers, D. J. B. (1969). The history of sleeping sickness in Yimbo location (Central Nyanza, Kenya). *Tropical Geographical Medicine* **21**, 323.

Willett, K. C. (1970). Epizootiology of trypanosomiasis in livestock in East and Central Africa. *In* "The African Trypanosomiases" (H. W. Mulligan, ed.), pp. 766–773. Allen and Unwin, London.

Wilson, A. J. and Cunningham, M. P. (1972). Immunological aspects of bovine trypanosomiasis. I. Immune response of cattle to infection with *Trypanosoma congolense* and the antigenic variation of the infecting organism. *Experimental Parasitology* **32**, 165–173.

Wilson, A. J., Paris, J., Luckins, A. G., Dar, F. K. and Gray, A. R. (1976). Observations on a herd of beef cattle maintained in a tsetse area. II. Assessment of the development of immunity in association with trypanocidal drug treatment. *Tropical Animal Health and Production* **8**, 1–12.

References added in proof

Morrison, W. I., Wells, P. W., Moloo, S. K., Paris, J. and Murray, Max (in press). Interference in the establishment of superinfections with *Trypanosoma congolense* in cattle. *Journal of Parasitology*.

Shapiro, S. Z. and Murray, Max (1982). African trypanosome antigens recognized during the course of infection in N'Dama and Zebu cattle. *Infection and Immunity* **35**, 410–416

Biochemistry of the Variant Surface Glycoproteins of Salivarian Trypanosomes

M. J. TURNER

MRC Biochemical Parasitology Unit, The Molteno Institute, University of Cambridge, Downing Street, Cambridge, CB2 3EE

I. Introduction

The last 6 years have seen an explosive growth of interest in the surface antigens of the salivarian trypanosomes. The reasons for this are 3-fold. Firstly, the medical and economic importance of these trypanosomes, as pathogens, remains as great, or greater, than ever. It is estimated that in Africa some 35 million people and 25 million cattle are exposed to the risk of infection within an area larger than that of the continental U.S.A. (WHO, 1979). Some 3 million cattle each year are believed to die of trypanosomiasis (WHO, 1979), and although only about 10 000 cases of human sleeping sickness are reported annually (de Raadt and Seed, 1977), the difficulties of accurate diagnosis are such that this is certainly a gross underestimate. All the old foci of human infection are active, and when the traditional methods of surveillance break down, as has happened in northern Uganda, outbreaks rapidly assume epidemic proportions. The situation in Uganda is extremely serious, and may be comparable in magnitude with the great epidemic originating on the northern shores of Lake Victoria at the turn of the century (Gashumba, 1981). Hence, sleeping sickness is still considered as a major health problem in many African countries, and for these reasons sleeping sickness was incorporated into the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, and the FAO has initiated a long-term programme for the prevention of animal trypanosomiasis. This initiative represents a commitment to the view that basic research on trypanosomes will, in the medium to long term, lead to new methods of cure and control. Secondly, the development of more sophisticated tools of molecular biochemistry, including gene cloning technology and monoclonal antibody production, has provided a tremendous impetus to the investigation of problems in parasitology previously held to be experimentally inaccessible, or "too messy". With these tools, the definition of antigens which may induce protective immunity has become possible, and their mass production for use in vaccines can be considered to be economically feasible. Detection of such antigens, and also of antigens which may otherwise be of no value in immunoprophylaxis, has meant that standardized reagents for serodiagnosis should also become commonplace within the next decade. Thirdly, the recognition that some parasites provide experimentally amenable systems which yield valuable information about processes of general interest to researchers into the biochemistry of eukaryotic organisms, has attracted a fresh influx of workers into this field. Nowhere is this more evident than in research on that trend-setting parasite, *Trypanosoma brucei*. In many respects, this organism seems almost to have been created to cater for the special interests of this new wave of biochemists and molecular biologists. So great is the trypanosome's potential use for research into topics as diverse as

mitochondrial gene expression, control of glycolysis, structure and function of membrane proteins, and control of gene activity, that recently a special workshop was dedicated solely to introducing it to research workers as a model eukaryotic cell*. While this "celebrity status" is deplored by some, I believe that this trend should be encouraged. The long term consequence of introducing more young scientists to the virtues of the parasitic protozoa as subjects worthy of intense research can only be good, scientifically, medically and economically.

The purpose of this review, therefore, is to describe the progress made over the past few years in characterizing antigens of the principal pathogenic salivarian trypanosomes. Although it will quickly be seen that the great bulk of information comes from work on the variant antigens of *T. b. brucei* in particular, much interesting work has been done on the variant antigens of other species, which will be discussed. Future perspectives for immunoprophylaxis, serodiagnosis, or chemotherapy of trypanosome infections will be presented.

II. Classification

The mammalian trypanosomes have been divided by Hoare (1964) into seven subgenera, which are grouped into two sections based on the site of production of infective metacyclic trypanosomes in the invertebrate host. The Stercoraria are those in which the developmental cycle in the vector is completed in the hind-gut; metacyclics are present in the faeces and transmission is contaminative. The Salivaria are those in which the developmental cycle in the vector is completed in the mouth parts or salivary glands, so that metacyclics are present in the saliva and transmission is by inoculation.

The systematic position of the genus *Trypanosoma*, and the seven subgenera which infect mammals, are shown in Table 1, and Table 2 summarizes the main features of each subgenus. Within each subgenus of the Salivaria the classification is somewhat complex, frequently involving several subspecies within each species. As trypanosomes are apparently asexual (but see Tait, 1980) any diversifications are immediately fixed in the clone derived from a mutant organism, and thus numerous biological races have evolved. Some of these are sufficiently distinct to form obvious species, but other diversifications have resulted in continuous ranges of characteristics. Groups have been separated as species if the characteristics distinguishing them do not

* European Molecular Biology Organization practical course on "The Molecular and Cell Biology of Trypanosomes". Organized by Professor F. R. Opperdoes (Brussels) and Professor P. Borst (Amsterdam), and held at the International Institute of Cellular and Molecular Pathology, Brussels, 18–29 August, 1980.

intergrade, but if they do intergrade, they are regarded as subspecies. This has the result that subspecies are not clearly defined. The best example of this is the subspecific classification of *T. brucei* into *T. b. brucei*, *T. b. gambiense*, and *T. b. rhodesiense*. The three subspecies are morphologically identical, and are defined in terms of differences in epidemiology and host range.

TABLE 1

Systematic position of the genus Trypanosoma *and the seven subgenera that infect mammals*

Phylum	Protozoa		
Subphylum	Sarcomastigophora		
Superclass	Mastigophora		
Class	Zoomastigophorea		
Order	Kinetoplastida		
Suborder	Trypanosomatina		
Family	Trypanosomatidae		
Genus	*Trypanosoma*		
Subgenera		*Herpetesoma*	Stercoraria
		Megatrypanum	
		Schizotrypanum	
		Duttonella	Salivaria
		Nannomonas	
		Trypanozoon	
		Pycnomonas	

TABLE 2

Some features of the subgenera of mammalian trypanosomes

Subgenus	Type species	Disease produced	Invertebrate host	Occurrence of antigenic variation
Megatrypanum	*T. theileri*	None	Tabanids and hippoboscids	Not found
Herpetosoma	*T. lewisi*	None	Fleas (and triatomid bugs for *T. rangeli*)	Two antigenic types only
Schizotrypanum	*T. cruzi*	Chagas's disease in man	Triatomid bugs	Not found
Duttonella	*T. vivax*	Souma in cattle	Tsetse	Yes
Nannomonas	*T. congolense*	Nagana in cattle	Tsetse	Yes
Trypanozoon	*T. brucei*	Nagana in cattle and sleeping sickness in man	Tsetse	Yes
Pycnomonas	*T. suis*	Chronic porcine trypanosomiasis	Tsetse	Not known

However, there are isolates of *T. brucei* which are difficult to classify according to this system and *T. b. gambiense* and *T. b. rhodesiense* should probably be regarded as nosodemes of *T. b. brucei*. *Trypanosoma evansi* is believed to have evolved from *T. brucei* by adaptation to non-cyclical transmission by biting Diptera, beyond the range of the tsetse fly (*Glossina* spp.). *T. evansi equiperdum* is morphologically indistinguishable from *T. evansi*, but has dispensed with the need for an intermediate host altogether and has adopted venereal transmission, whereas *T. equinum* probably evolved from *T. evansi* through loss of the kinetoplast. Within the subgenus *Duttonella*, a form of *T. vivax* has developed which is non-cyclically transmitted, again by biting Diptera, and this is usually regarded as a separate subspecies, *T. vivax viennei*. The specific and subspecific classification of the salivarian trypanosomes is shown in Table 3.

TABLE 3

Specific and subspecific classification of salivarian trypanosomes

Trypanozoon	Duttonella	Nannomonas	Pycnomonas
T. brucei brucei	*T. vivax vivax*	*T. congolense*	*T. suis*
T. b. rhodesiense	*T. v. viennei*		
T. b. gambiense		*T. simiae*	
	T. uniforme		
T. evansi evansi			
T. e. equiperdum			
T. equinum			

III. VARIANT ANTIGENS OF SALIVARIAN TRYPANOSOMES

A. INTRODUCTION

Antigenic variation in the mammalian host is the most striking characteristic of salivarian trypanosomes. Since this phenomenon has been most competently reviewed several times (Vickerman, 1974; Gray and Luckins, 1976; Doyle, 1977), only the essential outlines of the biology of antigenic variation will be presented here.

Whether infection is initiated cyclically, by tsetse fly, or non-cyclically, by biting Diptera or by syringe, infection of the bloodstream is characterized by relapsing parasitemia. At 5–7 day intervals during the course of infection, antibodies capable of both lysing and agglutinating the trypanosomes are

detectable in the serum, the host enters remission, and the parasitemia drops rapidly to a low, even subpatent level. Trypanosomes surviving this crisis are of a different antigenic type, unaffected by the circulating antibody, and multiply until they in turn are eliminated by the host's immune system. The cycle can continue, apparently indefinitely, usually leading to the death of the host. As long ago as 1916, Ritz demonstrated that infections initiated with a single trypanosome can produce multiple variants, thus establishing that antigenic variation is a property of the individual trypanosome, rather than the population. The upper limit to the number of variants is unknown, but a single trypanosome of *T. equiperdum* has been shown to give rise to over 100 distinct antigenic types, with no indication that the repertoire had been exhausted (Capbern *et al.*, 1977).

The possibility that this diversity is generated by the accumulation of point mutations (in a fashion analogous to antigenic drift in influenza) can be discounted completely, for the following reasons. All trypanosomes assume a common antigenicity on ingestion by the tsetse fly, yet often identical antigenic variants can be detected in infections initiated after cyclical transmission (Gray, 1966). The same effect can be observed in syringe-passaged populations. When trypanosomes isolated in the course of an infection are transmitted to a new, non-immune host, variants detectable at an early stage of the original infection are frequently re-expressed (Gray, 1965). Such variants were originally termed "predominant types". Closer investigation of this phenomenon revealed that when cloned trypanosomes were allowed to relapse to new antigenic types, a small proportion (10^{-4}–10^{-5}) of the trypanosome population spontaneously changed to express new variants according to a statistically definable order of priority which was different for each parent variant (Van Meirvenne *et al.*, 1975; Doyle, 1977; Miller, E. N. and Turner, 1980). In the absence of antibody, clones can relapse to antigenic types which occurred earlier in the sequence. Although there is no absolute requirement for any clone to relapse to any other in this situation, some variants do seem to have a greater probability of expression by all parental variants, and these seem to correlate with the predominant types described by Gray. Computer analysis of all the available data is fully consistent with the view that predominance is not a function of differential growth rate, but of differences in gene expression, or a selective environmental effect (Kosinski, 1980). In either case, all these data show that change to a new variant type does not eliminate the genes coding for earlier variants — that is, phenotypic rather than genotypic changes are responsible for antigenic variation. If further evidence is needed, it will be seen below that the biochemistry of the variant antigens is incompatible with their generation by any reasonable rate of mutation.

Different isolates of the same species obtained at different geographical

locations frequently express different repertoires of variants (Gray, 1966). This has given rise to the concept of the serodeme, the lowest stratum of classification of the salivarian trypanosomes. A serodeme has been defined as "a population of trypanosomes, each of which can express the same variant antigen type repertoire" (WHO, 1978). Certain variants may, in addition, be expressed by more than one serodeme, and in more than one species, and such variants (isotypes) will be discussed in further detail later. This level of classification is useful, both in allowing comparison of results of experiments carried out in different laboratories, and in epidemiological studies.

All bloodstream (haematozoic) forms of salivarian trypanosomes are distinguished by the presence of an electron dense surface coat, 12–15 nm thick, external to the limiting membrane bilayer (Vickerman, 1969). In those species capable of cyclical transmission, the surface coat is lost in the fly midgut, and reappears only with development of infective metacyclic trypomastigotes (Vickerman, 1969). (In *T. vivax*, the surface coat does not seem to reappear until after transmission to the mammalian host; Tetley *et al.*, 1981.) The evidence that antigenic variation is associated with changes in the composition of the surface coat may be summarized as follows. Firstly, when bloodstream trypomastigotes are cultured at 26°C in suitable medium, they differentiate into a form indistinguishable from that found in the insect midgut. Such procyclic trypomastigote forms, which are incapable of undergoing antigenic variation, lack a surface coat and assume a common surface antigenicity irrespective of the antigenic variant from which they were derived (Seed, 1964). Secondly, loss of the surface coat is paralleled by loss of the ability of variant specific antibodies to agglutinate the trypanosomes (Barry and Vickerman, 1979). Such variant specific antibodies, prepared by immunization with cloned trypanosomes, have been shown to bind to the surface coat of homologous but not heterologous trypanosomes (Vickerman and Luckins, 1969). Lastly, surface labelling with formylmethionine-sulphonemethylphosphate labels a single glycoprotein which may be readily purified to homogeneity (Cross, 1975). The composition of this glycoprotein is variant specific, and it can stimulate the production of variant specific immunity (Cross, 1975). Antisera from such immunized animals react with the surface coat of homologous, but not heterologous, trypanosomes (Cross, 1975). The biochemistry of these variant antigens of each species will now be discussed in detail.

B. *Trypanosoma brucei brucei*

Most of the research on variant antigens has been conducted on this subspecies, because of its ready adaptation to growth in laboratory rodents to

produce high parasitemias, and because of its non-infectivity towards humans. This latter factor should not be taken for granted, for there is at least one documented example of an apparently well-characterized strain of *T. brucei* which produced a serum-resistant, human-infective antigenic variant (Robertson and Pickens, 1975).

Early studies on variant antigens of *T. brucei* centred around the so-called "exoantigen". Weitz (1960) reported that immunization of rats with serum from trypanosome-infected rats produced an antiserum which gave a single precipitin arc in immunoelectrophoresis. Serum from rabbits chronically infected with *Trypanosoma brucei* gave an identical precipitin band to that obtained with infected rat serum, suggesting that the antigen, termed "exoantigen", was truly of trypanosome origin. Antisera raised both in rabbits and rats, using exoantigen-containing serum as immunogen, agglutinated living suspensions of trypanosomes *in vitro*, and this agglutination was inhibited when the antisera were absorbed with serum from infected, but not from uninfected, rats. Weitz therefore concluded that the exoantigen was an agglutinogen occurring on the surface of the trypanosomes. Since uncloned populations of trypanosomes were used, no conclusion could be drawn regarding the specificity of the exoantigen.

Miller, J. K. (1965) also started with an uncloned population of *T. b. brucei*, but from it obtained variant populations generated during a chronic infection of a rabbit, by inoculation of mice with infected blood obtained at 7-day intervals from the rabbit. Five variant populations were obtained and by immunization of rabbits with serum from rats infected with each population, variant specific antisera were obtained which gave precipitin lines only with homologous infected rat serum, and agglutinated only the homologous trypanosome population.

In a series of experiments, performed mainly with *T. b. rhodesiense* but to some extent with *T. b. brucei* also, Brown and Williamson (1962, 1964) and Williamson and Brown (1964) demonstrated that variant specificity seemed to be associated with two protein fractions, sedimentation coefficients 4S and 1S, which could be isolated from the cell sap of trypanosome homogenates. The 4S fraction in particular could stimulate the formation of variant specific antibodies. Allsopp *et al.* (1971) compared exoantigen in serum of infected rats with 4S antigen prepared from homogenates of the same strain, and showed that immunochemically they were indistinguishable. The important observation was also made that exoantigen was detectable only in plasma prepared from blood which had been left to stand after bleeding. It was estimated that the concentration of exoantigen increased 300-fold within 5 hours after bleeding, and that the increase in detectable exoantigen correlated with a drop in the pH value of the plasma and with declining trypanosome viability. When the trypanosomes were allowed to

stand for 6 hours in a phosphate-buffered salts solution at pH 8·0, containing 1% glucose, they remained highly motile, and no precipitin line was detectable in supernatants after removal of the trypanosomes, suggesting that no antigen was released. Vickerman (1969), in his original observations on the surface coat, had noted that this structure was absent from dead trypanosomes and moreover Wright *et al.* (1970) had reported the shedding of the surface coat in the form of filamentous plasmanemes when trypanosomes were incubated at 37°C or room temperature in physiological saline. Vickerman and Luckins (1969) prepared variant specific antisera by infection of rabbits with cloned trypanosomes and bleeding 6 days later. Such sera, when ferritin conjugated, could be seen to bind to the surface coat of homologous, but not of heterologous trypanosomes. The source of the variant specific exoantigen thus seemed clear, and Allsopp *et al.* (1971) proposed that the surface coat of trypanosomes could be shed to produce soluble 4S exoantigen under conditions in which trypanosome viability was impaired. Isoelectric focussing experiments (Allsopp *et al.*, 1971; Njogu and Humphryes, 1972) showed that 4S antigen contained five components of pI 5·5–6·0, and that three of these were present in exoantigen preparations. Differential staining showed that all the bands contained both carbohydrate and protein. Two neutral sugars, D-mannose and D-galactose, were identified in the acid hydrolysates of these antigens. Unfortunately, 4S preparations from antigenically distinct cloned populations were not compared, for, with the benefit of hindsight, it is clear that this would have given considerable insight into the nature of the variant antigen. This technique was ultimately successfully used by Cross (1975) to purify variant specific antigens. However, considerable purification of the 4S antigen was obtained by ion-exchange chromatography on diethylaminoethyl (DEAE)-Sephadex equilibrated in 1 mM phosphate buffer, pH 6·1. Under these conditions, the 4S antigen did not bind to the resin, but many other components of the mixture were absorbed.

Le Page (1968a, b) performed a remarkably detailed analysis of soluble antigens produced from cloned and uncloned variants isolated in the course of a chronic infection in a rabbit. Large numbers of antigenically homogeneous variants were prepared by rapid serial transfers from mice to rats, and the trypanosomes, when harvested, were disrupted by sonication on ice at pH 8·6 for 4 minutes. Such homogenates contained soluble variant specific antigens which differed in their charge, as shown both by immunoelectrophoresis and agar gel electrophoresis. In general, these antigens had rather low electrophoretic mobilities. Each preparation showed a rather heterogeneous pattern of variant specific bands on electrophoresis. In part, this was due to the presence of different molecular weight species of protein. Thin-layer gel chromatography suggested the presence of species

of molecular weight (M_r) comparable to lactate dehydrogenase (M_r 133 000), and to haemoglobin (64 000), and something smaller. This was almost certainly due to the presence of dimers in the population of antigen molecules (Auffret and Turner, 1981). Le Page purified the variant antigens from sonicated homogenates by ammonium sulphate fractionation, followed by DEAE-cellulose ion-exchange chromatography in 0·01 M phosphate buffer, pH 8·0. Irrespective of the variant used to prepare the homogenate and regardless of the differences in electrophoretic mobilities of the antigens, most of the variant antigen was always found in the first effluent fractions. These fractions were concentrated, and applied to Sephadex G100 or G200. The gel filtration columns were uncalibrated, but the variant antigens were excluded from Sephadex G100. Such preparations were judged to be antigenically homogeneous by immunoelectrophoresis. As a further criterion of purity, analytical ultracentrifugation was employed, and a single symmetrical peak was observed. The molecular weight of the variant antigen was measured in the ultracentrifuge both by sedimentation equilibrium and sedimentation diffusion. Each gave a value of around 90 000, with a frictional ratio of 1:6, corresponding to an axial ratio of about 10:1. Under dissociating conditions (6 M guanidine–HCl) the molecular weight fell to about 40 000, and in the presence of 6 M guanidine plus 2-mercaptoethanol, to about 20 000. Le Page therefore concluded that each antigen molecule contained two pairs of covalently-bound polypeptides, associating non-covalently. It now appears that the antigens exist in solution as non-covalently bound dimers, and occasionally as higher oligomers (Auffret and Turner, 1981).

So-called "5S antigen", contained in the void volume after Sephadex G100 chromatography, was prepared from clones obtained from sequentially isolated variant populations of *T. brucei*, and their tryptic "fingerprints" were compared. Each antigen had a unique tryptic fingerprint, demonstrating that the different variants of a single strain of trypanosomes rely for their distinction on radical changes in the primary amino acid sequence. Le Page concluded that such changes could not be accommodated by any reasonable rate of point mutation, and that therefore antigenic variation resulted from the use of different genes. This was confirmed in an experiment in which a clone was isolated, and a second clone of the same type was re-isolated after several intervening cycles of antigenic variation in a different host (i.e. this corresponds to the phenomenon of reversion to a "predominant" antigenic type in syringe-passaged populations observed by Gray, 1965). Antigen was purified from each, and the tryptic fingerprints were observed to be identical—the gene for each variant is therefore conserved during variation. Amino acid compositions of 5S proteins isolated from different variants were thought to show a basic overall similarity, but the differences between

antigenically distinct proteins were much greater than between replicate analyses of the same protein. It is a matter of great regret that this remarkable piece of work (Le Page, 1968b) did not reach a wider audience.

Lanham and Taylor (1972) showed that a semi-purified, soluble, variant specific preparation from *T. b. brucei* could produce variant specific immunity in mice, but it was not until 1975 that definitive experiments establishing the composition of the surface coat and its relationship to variant specific immunity were reported. In what must be regarded as the seminal contribution to this field, Cross (1975) described the identification, purification, and some properties of clone-specific glycoproteins, shown to constitute the surface coat of *T. b. brucei*. Four variant populations were isolated from a chronic infection of a rabbit in much the same way as described by Miller, J. K. (1965), and clones were produced from each variant population. In addition, a fifth clone was prepared from infective culture forms derived from the same strain, and a sixth from the original uncloned stabilate material. Trypanosomes, purified from red blood cells on DEAE-cellulose (Lanham and Godfrey, 1970), were labelled with [^{35}S]formylmethionine sulphonemethylphosphate (FMSMP), an *N*-acylating reagent which reacts with cell surface components only, because it cannot penetrate the cell membrane. Rupturing the trypanosomes, by high speed homogenization in the presence of ballotini beads at 0°C, released most of the label into a form soluble after centrifugation at 15 000 *g* for 15 minutes. A sodium dodecyl sulphate (SDS)–polyacrylamide gel of this soluble fraction revealed that only one component of the mixture, $M_r \sim 63\,000$, was labelled under these conditions. In contrast, if the cells were disrupted before labelling, a highly complex mixture of radioactively-labelled products was formed, which paralleled the distribution of total protein in the homogenate. The labelling reaction therefore seemed to be labelling a single surface component. The fact that this was solubilized by homogenization was consistent with the data of Vickerman (1969) and Allsopp *et al.* (1971) which suggested that the surface coat was lost when cell viability was impaired. The labelled, soluble material was further purified in two stages. Firstly, after centrifugation at 105 000 *g* for 1 hour, the supernatant was applied to a column of DEAE-cellulose in 10 mM phosphate buffer, pH 8·0, as described by Le Page (1968b). All the radioactivity was found in the first effluent peak, although 50–70% of the total protein was absorbed. The radioactive fractions were pooled and further purified by preparative isoelectric focussing. Most of the radioactivity was found in one major and one minor peak. The profile of radioactivity did not quite correlate with the protein profile, which also gave a major and a minor peak, but this was consistent with the loss of one net positive charge per protein molecule as a consequence of acylation with FMSMP. The corresponding protein and radioactive peaks co-migrated on

SDS–polyacrylamide gels to give a single band, $M_r \sim 65\,000$. The same protocol was used to purify the principal surface component from all six clones, and in each case a pure product was obtained, $M_r \sim 65\,000$. Subsequent analysis showed that these were in fact glycoproteins, and these molecules, originally termed VSSAs (variant specific surface antigens), have become known as VSGs (variant surface glycoproteins). The purification protocol is now routinely used in many laboratories.

TABLE 4

Distribution of radioactivity in soluble and insoluble fractions derived from trypanosomes labelled with [^{35}S]FMSMP (from Cross, 1975)[a]

Fraction	% initial radioactivity recovered
(1) Cell homogenate	(100)
(2) First 15 000 *g* supernatant	67
(3) First 15 000 *g* pellet	29
(4) Second 15 000 *g* supernatant	7
(5) Second 15 000 *g* pellet	20
Combined 15 000 *g* supernatants [(2) + (4)]	74
(6) 165 000 *g* supernatant	56
(7) 165 000 *g* pellet	18
Combined 15 000 *g* and 165 000 *g* pellets [(5) + (7)]	38
(8) Sephadex G-25 exclusion peak	46
(9) DEAE-cellulose exclusion peak	50
(10) DEAE-cellulose salt-eluted proteins	0
(11) Principal peak on isoelectric focussing column	45
(12) Remainder of electrofocussing fractions	5

[a] Compiled from the results of two experiments using trypanosomes of the same antigenic type.

The recovery of radioactivity at each stage is shown in Table 4. Table 5 shows the original amino acid composition of four VSGs purified by Cross. Two of the clones isolated from the rabbit appeared to be identical, suggesting that a complete relapse of this type was not achieved on its first appearance in the circulation. Furthermore, the clone isolated from the uncloned starting material and that obtained from culture were also the same. Hence four different variants were obtained, and it can be seen that each glycoprotein could be characterized by its isoelectric point (pI). The amino acid compositions differ in reproduceable fashion. Cross emphasized that the successful preparation of a single, homogeneous glycoprotein depended on careful control of the high levels of endogenous protease activity, by working at 0–4°C, and by the addition to buffers of the serine protease inhibitor phenylmethioninesulphonyl fluoride (PMSF). The high levels of proteolytic

activity present in trypanosome homogenates could explain the heterogeneous nature of antigen preparations described by earlier workers (e.g. 4S and 1S antigen of Brown and Williamson, 1962 and 1964; 4S antigen of Allsopp *et al.*, 1971; heterogeneous 5S antigen of Le Page, 1968b).

TABLE 5

Amino acid compositions and isoelectric points of four VSGs purified from clones of T. b. brucei *(from Cross, 1975)*[a]

Amino acid	Clone: 048	049	055	057
Asx	80	55	60	63
Thr	68	57	74	69
Ser	32	34	42	25
Glx	67	84	62	81
Pro	14	18	26	17
Gly	36	54	32	42
Ala	75	76	98	72
Lys	16	14	13	15
Val	18	22	18	18
Met	4	9	1	11
Ile	18	19	25	17
Leu	51	52	54	64
Tyr	20	15	17	17
Phe	11	11	9	7
His	6	12	12	8
Lys	69	74	55	62
Arg	16	7	17	14
pI	6·93	7·55	8·19	6·50

[a] Results calculated as residues per 65 000 molecular weight.

Immunochemical evidence for the identity of the purified cell surface component with the variant specific surface coat came from experiments in which mice were immunized with purified glycoprotein and challenged with a small number of trypanosomes ($\sim$ 70) of homologous or heterologous type. Essentially complete protection was provided against challenge by the homologous, but not the heterologous, variant. The purified surface component therefore seems to be the source of the variant specific immunity described by Lanham and Taylor (1972). Using antisera raised in rabbits, Cross (1975) reported that homologous combinations of antibody and glycoprotein gave a single precipitin line in agar diffusion, and heterologous combinations gave none. Such sera agglutinated homologous trypanosomes only. Cross therefore concluded, on the basis of all the data available, that the surface coat comprised a single glycoprotein, unique to

each variant. Cross calculated that, from the yield of purified VSG, each trypanosome had about 7×10^6 VSG molecules on its surface, and that given molecular dimensions of about $10 \times 4 \times 4$ nm, this was consistent with the arrangement of the VSG in a monolayer, with the long axis of the VSG perpendicular to the plasma membrane. Such a calculation is, of course, fraught with possible sources of inaccuracy, in particular in the estimation of surface area, in the calculation of yield, and in the molecular dimensions. The surface area was estimated from the diameter of hypotonically swollen, spherical trypanosomes, and such stress could produce considerable deviation from the surface area under isotonic conditions. The calculation of the yield was based on the release of material labelled with [^{35}S]FMSMP, essentially all of which appears to be VSG. Cross quotes 70–80% release of the radiolabel after homogenization of trypanosomes, centrifugation at 15 000 *g*, and one wash of the 15 000 *g* pellet (see Table 4), with about 20% remaining associated with the 15 000 *g* pellet. However, it should be noted from Table 4 that a further 18% sediments at 165 000 *g*, and overall about 50% of the total radiolabel is recovered in the VSG peak from the electrofocussing column. The yield Cross used in his calculation therefore seems to be an underestimate. Is 50% of the antigen left in "insoluble" form? Cross (1975) gave the results of an experiment in which viable trypanosomes were treated with trypsin or pronase, to demonstrate that the surface coat and the 65 000 dalton glycoprotein were both destroyed by such treatment. After enzymatic digestion, the cells were either fixed for electron microscopy, or lysed and separated into "soluble" and insoluble fractions by centrifugation at 5 000 *g*. Electron microscopy revealed that the surface coat was destroyed by such treatment, and SDS–polyacrylamide gels of both the soluble and insoluble fractions showed that the VSG was also removed. However, the SDS–polyacrylamide gel of control trypanosomes, untreated with enzyme but lysed and fractionated, showed that the major protein in the insoluble fraction was still the VSG. Two groups (Voorheis *et al.*, 1979; Rovis and Baekkeskov, 1980) have described the purification of plasma membrane from *T. b. brucei*, and both found a protein or glycoprotein having the same molecular weight as the homologous VSG to be a major constituent, judged by SDS–polyacrylamide gel electrophoresis. No formal proof that this material was truly membrane-bound VSG was presented, however. Rovis *et al.* (1978) have shown that, in *T. congolense* at least, most of the remaining surface labelled material can be solubilized by detergent. However, in a recent experiment, we have looked at the yield of VSG from *T. b. brucei* more carefully (L. Almeida and M. J. Turner, unpublished observations). From 10^{11} trypanosomes, 68 mg of VSG was purified, using exactly the protocol described by Cross. However, a further five washes of the 15 000 *g* pellet released 60 mg of protein, which SDS–polyacrylamide gel electrophoresis

showed was virtually pure VSG. The pellet still contained some VSG, which could be selectively solubilized with Triton X100, giving a further 12 mg. Hence it seems that trypanosomes contain twice as much VSG as originally supposed.

Are there any data to support Cross's assumed dimensions of the VSG? Le Page (1968b) reported a frictional ratio of 1·6, derived from ultracentrifugal analysis, suggesting an axial ratio of 10: 1, which is incompatible with dimensions of 10 × 4 × 4 nm. However, in view of the fact that Le Page's estimates of the molecular weight are not in agreement with those reported by Cross (1975), and no absolute criterion of purity was reported, these values should be treated with caution. Grundy (1980) performed a careful analysis of the solution properties of a purified VSG and noted that the molecules formed dimers in solution. Ultracentrifugal analysis of both purified VSG and VSG cross-linked into covalently-bound dimers, using bifunctional cross-linking reagents, produced an estimated molecular weight of 86 000 for both forms — in remarkably good agreement with the value of 90 000 reported by Le Page (1968b). However, Grundy did not derive a value for the frictional ratio. Clearly, more complete analysis of the solution properties of purified VSGs is needed, but even then estimation of dimensions of VSG bound to the trypanosomes is sure to be difficult. Nevertheless, it is clear that there could be a large margin of error in the calculation of surface density, and hence the arrangement on the cell membrane. It should be pointed out that Cross was merely trying to establish that sufficient VSG was present to form a cohesive surface coat, but the notion that the VSG forms a monolayer has entered the dogma, with no real experimental evidence to support it. The exact arrangement of VSGs on the surface of the trypanosome must still remain open to question, and will be discussed further. It is interesting to note, for example, that Wright and Hales (1970) reported that, when *T. b. brucei* was fixed in phosphate-buffered glutaraldehyde, two layers could be distinguished in the cell coat. Since this could not be observed with other fixatives, the possibility of a fixation artefact cannot be ruled out. Nevertheless, there is an interesting correlation with the distribution of carbohydrate in such sections (see below).

In the early 1970s, the question of whether the variant antigen of trypanosomes is of host or parasite origin was much debated. Taylor and Cross (1977) demonstrated that a protein, biochemically and immunologically indistinguishable from VSG, was synthesized and incorporated into the surface coat when *T. b. brucei* was grown *in vitro* in a fully defined culture medium, thus proving that the surface antigen of this species was of parasite origin. Eggitt *et al.* (1977) isolated messenger ribonucleic acid (mRNA) from trypanosomes, and after its translation in a cell-free system, were able to immunoprecipitate VSG polypeptide. Recombinant deoxyribonucleic acid

(DNA) techniques have now allowed the unequivocal demonstration of genomic DNA sequences, coding for VSG (see below).

This demonstration of the composition of the surface coat and the purification of the VSGs allowed many hitherto inaccessible questions to be tackled. What is the extent of the differences between VSGs? Are the differences in amino acid composition reflected in extensive sequence diversity? Are there constant and variable sequence domains, comparable to those found in immunoglobulin? What is the nature of the carbohydrate and where is it located? How are the VSGs attached to the membrane, if they are so readily removed? How are the VSGs organized on the cell surface? Each of these questions will be considered in turn.

1. *Organization of the VSGs on the cell surface*

With *T. b. brucei*, carbohydrate does not seem to be exposed on the cell surface. Steiger (1975) showed that a negligible amount of concanavalin A (Con A) bound to untreated trypanosomes. Wright and Hales (1970), using cytochemical staining techniques, demonstrated the presence of a carbohydrate component in the pellicle of bloodstream forms of *T. brucei*. A heavily stained layer about 3 nm thick was observed at the surface of the trypanosome, with some sections indicating a fainter staining outside this layer. One section clearly showed two equally dense 3 nm lines separated by a 2·5 nm space. The precise location of the carbohydrate was not immediately apparent from the Thiery staining reactions, but two lines of evidence indicated that the carbohydrate was in the surface coat. Trypanosomes were centrifuged together before fixing, staining, and sectioning. Sections stained normally showed that the surface coats of adjacent trypanosomes were in contact under such conditions. The distance between the cell membranes of adjacent trypanosomes was 20–24 nm, whereas the distance between the various carbohydrate staining layers was about 5 nm. The distance between microtubules and the cell membrane was about 8 nm, and between microtubules and the carbohydrate about 10 nm. On this basis, Wright and Hales proposed the distribution of carbohydrate in the surface coat shown in Fig. 1. Digestion of trypanosomes with trypsin removed the surface coat, and staining for carbohydrate suggested that much, but not all, of the carbohydrate was removed, depending on the duration of trypsinization. The conclusion, therefore, was that trypanosomes have a carbohydrate-containing layer adjacent to the cell membrane, and that this carbohydrate is attached to the surface coat protein, which masks the carbohydrate from the external environment.

Cross and Johnson (1976) extended these findings in the light of information on the composition of the surface coat (Cross, 1975). Using radiolabelled Con A, it was shown that this lectin could bind to the trypanosome surface

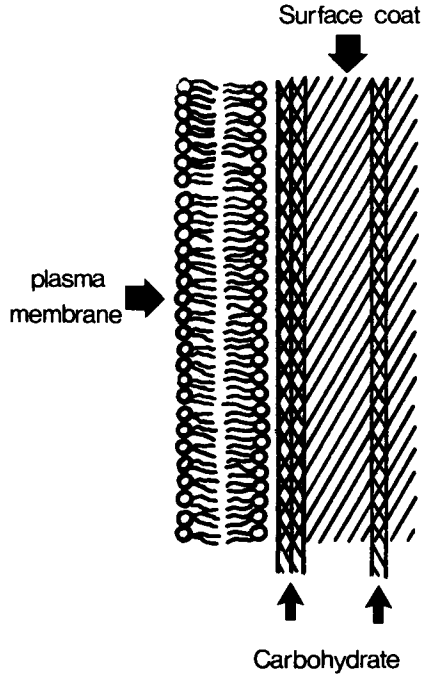

FIG. 1. Distribution of carbohydrate within the surface coat of *T. brucei* and its relationship to the plasma membrane, as deduced by electron microscopy (Wright and Hales, 1970).

after brief trypsinization. Under the conditions used, binding was optimal after about 12 minutes trypsinization, when the surface coat was no longer visible in the electron microscope. After 30 minutes, binding was again negligible, trypanosome viability was poor and lysis was occurring. Con A binding after trypsinization was also demonstrated cytochemically, and Thiery staining revealed that carbohydrate, which again was found adjacent to the plasma membrane, was still present after trypsinization. Cross and Johnson (1976) and Johnson and Cross (1979) showed that limited proteolysis of purified VSGs cleaved the molecule into two "domains". The large fragment (M_r 48 000–52 000) contained the *N*-terminus. The smaller fragment (M_r 13 000–17 000) was assumed by analogy to contain the *C*-terminus, and in the case of at least one VSG, it contained all the carbohydrate. Cross (1978) described unpublished results of Johnson, claiming that after binding Con A to trypsinized cells, the ligand-receptor complex could be solubilized by rupturing trypanosomes in conventional fashion. Dissociation of the complex with α-methylglucoside allowed the purification of a glycopeptide having a composition very similar to the purified *C*-terminal domain prepared by limited

proteolysis of purified VSG. On the basis of such findings Cross and Johnson (1976) proposed a model for the organization of VSG on the cell surface as shown in Fig. 2. The principal features of the model are that the glycosylated *C*-terminal domain of the VSG is presumed to contain the membrane attachment site, viewed in this instance as a hydrophobic sequence, capable of penetrating the lipid bilayer. Later studies of the amino acid composition of the *C*-terminal domain showed a polar composition suggesting that a different binding mechanism might be employed (Cross, 1978; Johnson and Cross, 1979). Johnson and Cross (1979) studied the cleavage of five purified VSGs into large *N*-terminal and smaller *C*-terminal fragments, as outlined above, and measured the isoelectric points of the cleavage products of two. Variant 099 produced fragments with molecular weights, measured by gel filtration, of approximately 48 000 (f48), 40 000 (f40) and 17 000 (f17).

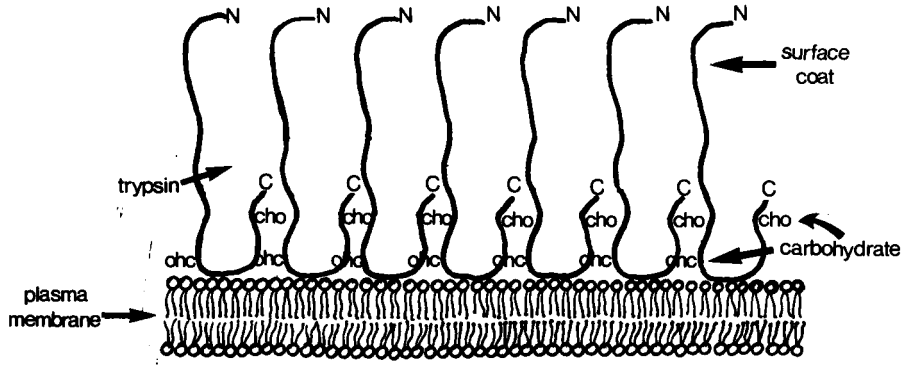

FIG. 2. Arrangement of VSG on the plasma membrane of *T. brucei*, and distribution of carbohydrate within the VSG, as deduced from biochemical studies on the VSG and the effect of trypsin on lectin binding to trypanosomes (Cross and Johnson, 1976).

Fragment f48 had an *N*-terminal amino acid sequence identical with that of the intact VSG, and the sum of the amino acid compositions of f48 and f17 agreed well with that of the native VSG. Hence f17 must contain the *C*-terminus of the molecule, although the possibility that a few amino acids were lost by proteolysis could not be excluded. Fragment f40 was presumed to be a degradation product of f48. The iso-electric points of f48, f40 and f17 were 8·66, 8·20 and 5·67, whereas that of native 099 VSG was 6·1. Similarly, variant 060 produced a *C*-terminal 13 000 dalton peptide having a pI of 4·4, whereas the native VSG had a pI of 6·46 (Cross, 1975). Such data suggested that the distribution of charge was unequal throughout the two domains, and that an electrostatic binding mechanism might be responsible for the attachment of the VSG to the plasma membrane. Cross (1978) pointed out that following the loss of surface coat, cell "ghosts" carry a strong negative charge, as do coatless culture forms (Hollingshead *et al.*, 1963), and these

observations prompted the suggestion that ionic interactions between lysine residues in the *C*-terminus of the VSG and a negatively charged receptor, possibly a glycolipid, hold the VSG to the membrane. However, although the lysine content of purified *C*-terminal glycopeptides is high, the isoelectric focussing data presented above suggest a net negative charge in this region of the molecule. Therefore, either "sub-domains", rich in lysine, are present; or a local region of high lysine content is formed by conformational changes; or a different binding mechanism operates. The problem of membrane attachment will be discussed again later.

More recent work has shown that VSG molecules contain at least two carbohydrate attachment sites. One is at the extreme *C*-terminus of the VSG, while the second is internal (Holder and Cross, 1981; see below). The exact location of the second glycosylation site is known for only one antigen (Boothroyd *et al.*, 1980), in which it is some 50 amino acid residues from the *C*-terminus. Cross and Johnson (1976) suggested that the outer layer of carbohydrate seen by Wright and Hales (1970) might be found only in variants containing relatively large amounts of carbohydrate in the VSG. A second possibility is that this layer represents carbohydrate bound at the second attachment site, and a third is that the surface coat is composed of a bilayer of VSG molecules.

An important factor in discussing VSG arrangement on the plasma membrane must be a comparison of the conformation of the glycoprotein in solution, and on the plasma membrane. Evidence that they are related comes from the work of Cross and Johnson (1976), Cross (1978) and Johnson and Cross (1979), which showed that VSGs could be cleaved by trypsin at the same or at similar sites *in vitro* and *in vivo* (see above). However, Pearson *et al.* (1980) used monoclonal antibodies to investigate the arrangement of antigen on the trypanosome surface. Mice to be used as spleen cell donors for hybridoma production were immunized with purified VSG. When the monoclonal antibodies were tested for specificity of reaction against VSG, some gave a positive, variant-specific reaction in radioimmunoassay using purified VSG, but did not react with the VSG exposed on the surface of living trypanosomes in immunofluorescence tests. Two interpretations of these results are possible. Either, such variant specific determinants are not exposed on the surface of living trypanosomes because of the close packing of antigen molecules in the surface coat, or release of the VSG induces conformational changes and the presentation of VSG-specific conformational determinants. With the former explanation, it is hard to imagine why there should be such cryptic variant sites, since such regions of the molecule should be under no selective pressure to vary. Perhaps sequence alterations are needed at non-exposed sites to allow exposed epitopes to adopt the tertiary structure needed for the formation of a compact surface coat. The "cryptic" variant

specific determinants were exposed on acetone-fixed trypanosomes. Similar findings have been reported using monoclonal antibodies directed against VSGs of *T. b. rhodesiense* (Lyon *et al.*, 1981; see below).

Auffret and Turner (1981) showed that purified *T. b. brucei* VSGs exist as dimers and occasionally as higher oligomers in solution as judged both by gel filtration and by SDS–polyacrylamide gel electrophoresis after treatment with bifunctional cross-linking reagents. Eight different VSGs were examined in cross-linking experiments, and all eight could be cross-linked to a greater or lesser extent (range 10–95%). Two of the eight produced a mixture of dimers and trimers. Three of the cross-linking reagents used were designed to react with lysine residues, but cross-linking was also achieved by treatment with sodium *meta*periodate, followed by reduction with sodium borohydride. In this case, the cross-linkage occurs by reduction of a Schiff's base, formed between the ε-amino group of lysine, and the aldehyde produced by periodate cleavage of sugar residues, suggesting that carbohydrate side chains are in the vicinity of the point of interchain contact. The suggestion that the site of dimerization was within the *C*-terminal domain was reinforced by the finding that a *C*-terminal CNBr peptide formed dimers in solution. The dimers were not held together by interchain disulphide bonds, although one such example has since been found (Miller, E. N., 1980). Hydrophobic interactions seemed to be involved, since dimers were dissociated in detergent solution. Is the formation of dimers and higher oligomers an artefact of solubilization, or do the antigens exist as dimers on the surface of the trypanosome? Auffret and Turner (1981) were unable to define suitable conditions for cross-linking VSGs on viable trypanosomes, However, Grundy (1980) confirmed the findings of Auffret and Turner (1981) and reported that VSGs could be cross-linked into dimers on the cell surface without affecting trypanosome viability. This is a difficult experiment to control properly, and until such time as the site of cross-linking can be shown to be identical in VSGs cross-linked in solution and those on the cell surface, the existence of such dimers on the trypanosome must remain an open question.

Barry (1979) studied the "capping" of VSGs on trypanosomes by antibody, as a means of studying the arrangement of molecules at the surface. Capping is usually a two-stage phenomenon, involving intermediate patch formation. However, patch formation was not observed in the capping of VSG molecules, presumably because the surface density of VSG is so high that further concentration of VSG molecules into a patch is a physical impossibility. In view of this supposed compression, it was therefore surprising that direct cap formation, with anti-VSG, could not be observed. Addition of an anti-globulin was necessary to achieve indirect capping. The usual interpretation of such a finding would be that the antigen molecules are, on average, more than 12 nm apart—that is, greater than the span of two antibody

combining sites. Whatever the merits or demerits of Cross's calculation of surface VSG density, it is very hard to accommodate such a highly dispersed distribution of antigen molecules in any reasonable model of surface coat structure. Barry suggested two explanations. Either binding of one immunoglobulin (Ig) molecule to a VSG determinant prevents the binding of a second molecule to a second determinant on the same molecule, because of steric hindrance, or indeed only one antigenic determinant per VSG molecule is exposed on the cell surface. Either would prevent lattice formation, and thus capping, in the absence of a second layer of antibody. The use of monoclonal antibodies reacting with known VSG determinants should allow the resolution of these two alternatives.

Barry (1979) noted that capping exposed underlying membrane determinants, which are common to all trypanosomes of whatever antigenic type. This disposed of the suggestion that a "replacement layer" of heterologous variable antigen might be present. The inability of anti-VSG to induce capping seems to eliminate this as a mechanism for inducing antigenic variation. Indeed, 3 hours after removing VSG from the surface by capping, the same VSG was re-expressed on the cell surface (Barry, 1979).

Freeze-fracture electron micrographs of the surface membrane of trypanosomes reveal the presence of numerous intramembrane particles in both the inner and outer membrane faces, with fewer in the outer face (Smith *et al.*, 1974; Hogan and Patton, 1976). It has been suggested that the distribution of such particles is affected by the immune status of the host (Hogan and Patton, 1976). Since the intramembranous particles are 2–3 times larger than the dimensions suggested for the VSG, and the VSG density is probably some 50 times that of the particles, it seems they do not represent individual VSG molecules. Since anti-VSG does not induce cap formation, the rearrangement of particles seen in trypanosomes obtained from the blood of normal as opposed to irradiated rats (Hogan and Patton, 1976) seems unlikely therefore to be associated with the immune response to these antigens. This has not yet been directly tested, however.

2. *Amino acid sequences*

Bridgen *et al.* (1976) reported the *N*-terminal amino acid sequences of the four variants isolated by Cross (1975). Each variant gave a single sequence, confirming the homogeneity of the preparation but no homology was detected within the first 30 or so amino acids. The sequences are shown in Table 6; other than the complete lack of homology, they are unremarkable. Again, the sequence diversity of these four variants isolated from a single infection is incompatible with any reasonable mutation rate, if further proof for the phenotypic basis of variation is sought. Does this extensive sequence heterogeneity persist throughout the length of the molecule? McConnell *et al.*

TABLE 6

N-Terminal amino acid sequences of four VSGs isolated from cloned populations of trypanosomes (from Bridgen et al., *1976)*

Variant	Amino acid sequence
I	Thr Asn Asn His Gly Leu Lys Leu Gln Lys Ala Glu Ala Ile Lys Lys Met Lys Lys Glu
II	Ala Lys Glu Ala Leu Glu Tyr Lys Thr Trp Thr Asn His Lys Gly Leu Ala Ala Thr Leu Arg Lys Val Ala Thr Gly Val Leu Thr Lys
III	Thr Asp Lys Gly Ala Ile Lys Phe Glu Thr Trp Glu Pro Leu Gln Leu Leu Thr Gln Asp Phe Gly Asn Leu Tyr Asn Lys Ala Lys
IV	Ala Glu Ala Lys Ser Asp Thr Ala Ser Gly Ser Val Asn Ser Pro Gln Thr Glu Ala Thr Tyr Ala Gln Leu Ala Lys Thr Leu Gln

Note the absence of homology. The relationship of these variants to those purified by Cross (1975) was not described.

TABLE 7

Amino acid sequences of internal (1–7) and C-terminal (8–12) tryptic glycopeptides purified from VSGs of T. brucei *(from Holder and Cross, 1981)*

Glycopeptide	Amino acid sequence
(1) 55A	Thr Lys Glx Ala Asx Val Glx Leu* Glx Ala Ala (Lys, Asx_6, Thr_4, Ser, Glx_2, Gly_2, Ala_5, Ile_3, Leu_3, Lys_2)
(2) 60B	Asx Leu Ala Tyr Thr Asx• Glx Thr Gly Asx Leu Asx Thr Glx Pro Thr Leu Lys
(3) 60B[1]	Ala Lys Asx Leu Ala Tyr Thr Asx• Glx Thr Gly Asx Leu Asx Thr (Glx, Pro, Thr, Leu, Lys)
(4) 117B	Phe Asx* Ser Thr Lys
(5) 121B	Phe Asx* Ala Thr Lys
(6) 121B[1]	Lys Lys Phe Asx• Ala Thr Lys
(7) 221B	•Asx Glx Thr Ala* Gly Ile Ala Gly Lys
(8) 55	Gly Thr Ala Glu Thr Glu Asn* Thr Thr Gly Ser*
(9) 60	Ala Asx Thr Thr Gly Ser*
(10) 117	Trp Glx Asx Asx Ala Lys Lys Asx*
(11) 121	Trp Glx Gly* Glx Thr Lys Lys Asx*
(12) 221	Thr Gly Asx Thr Asx* Thr Thr Gly Ser Ser

Note. In some variants, considerable heterogeneity was observed in both the carbohydrate composition of tryptic glycopeptides and in the site of glycosylation of the *C*-terminal tryptic glycopeptides (indicated with an asterisk). All the *C*-terminal glycopeptides were glycosylated on the *C*-terminal amino acid. The carbohydrate compositions of the various glycopeptides are given in Table 10. No heterogeneity was observed in the amino acid sequences of the various tryptic glycopeptides isolated from each VSG. Peptides 60B and 121B are presumably partial digest products, since they contain internal lysine residues. Residues in parentheses were not sequenced. Note the lack of sequence homologies in the internal tryptic glycopeptides, but the *C*-terminal sequences fall into two related families (55, 60 and 221; and 117 and 121).

(1979), on the basis of analysis of amino acid composition data and on fingerprint analysis, suggested that unrelated VSGs might contain as much as 50% sequence homology, scattered in small segments throughout the length of the molecule. Such homologies would be very hard to detect on tryptic maps or by use of any similar technique. Cross and Johnson (1976) stated that tryptic mapping experiments indicated extreme structural variation in agreement with Le Page (1968b). With the exception of the so-called isotypic variant antigen types (iso-VATs), which will be discussed later, there is little immunological cross-reaction between different VSGs, and all the cross-reactions discovered involve antibody directed against a common carbohydrate determinant (see below). Again, this argues against the existence of large, conserved, sequence domains. Holder and Cross (1981) reported the amino acid sequences of tryptic glycopeptides obtained after exhaustive digestion with trypsin. Two classes of glycopeptide were obtained. One class contained the *C*-terminal amino acid of the molecule, since no lysine or arginine residue was present. The second class were internal peptides, judged by the same criterion. The amino acid sequences of each class are shown in Table 7. It can be seen that the internal glycopeptides share no homology, whereas the *C*-terminal glycopeptides show some similarities. All the variants studied belong to the same serodeme, and at present these results have not been extended to a comparison of VSGs from different serodemes. The distribution and composition of the carbohydrate within these peptides will be discussed later.

A. M. Gurnett (personal communication) noted some sequence homology in the *N*-terminal sequences of the *C*-terminal CNBr peptides isolated from two different variants. These peptides were known to contain the *C*-terminus since they contained carbohydrate but no homoserine lactone. The homology is only six amino acids in length and contains two conservative changes, but it is the first homology outside the glycosylation sites detected by peptide sequencing. Again, the two variants studied were members of the same serodeme.

The accumulation of sequence data has been greatly accelerated by the use of recombinant DNA technology to generate cDNA clones containing antigen-specific sequences. DNA sequencing is very much faster than protein sequencing, as witnessed by the recent completion of a complete VSG sequence (J. C. Boothroyd, personal communication), and some partial structures, which will shed some light on sequence relationships between VSGs.

3. *Carbohydrate*

Early work of Allsopp *et al.* (1971) and Njogu and Humphryes (1972) had suggested that the variant antigens were glycoproteins, on the basis of differential staining of isoelectric focussing gels. Allsopp and Njogu (1974)

extended this to an analysis of 4S antigen preparations for sialic acids, methyl pentoses, uronic acids, neutral sugars, and hexosamines. Only neutral sugars and hexosamines were detectable. An average composition of 3·9% neutral sugars and 3·4% hexosamine was obtained. The neutral sugars detected were mannose and galactose, and the only amino sugar detectable was *N*-acetylglucosamine. A mannose : galactose : hexosamine proportion of 1·0:1·1:1·9 was obtained. However, the purity of the antigen preparations (3–4 bands staining for carbohydrate on the isoelectric focussing gel), the sensitivity of the methods used for analysis, the variation in the results obtained from sample to sample, and the lack of any comparative analysis of different antigens, prevented any meaningful speculation on the nature or role of the carbohydrate.

TABLE 8

Carbohydrate compositions of VSGs of T. brucei, *as residues per mole of glycoprotein of molecular weight 65 000 (from Johnson and Cross, 1977)*

Variant[a]	Mannose	Galactose	Glucose	Glucosamine
055	13	15	5	10
060	12	9	3	7
099	28	6	15	14
100	18	13	8	8
104	8	5	8	4
D12	8	8	20	6

[a] Variants 055, 060 and 099 were derived from stock 427, variant 100 from stabilate LUMP 227, variant 104 from stabilate EATRO 1125, and D12 from stock S42.

Cross (1975) stated that pure VSG contained both neutral sugars and hexosamines, and Johnson and Cross (1977) presented detailed analyses showing the presence of four sugars only—mannose, galactose, glucose and *N*-acetylglucosamine. The composition of six variants is shown in Table 8. The analyses show considerable variation in the carbohydrate content of different VSGs. The presence of glucose was a surprise, since it is not commonly a component of mature, complex eukaryotic glycoproteins. Is glucose a real component of the VSG, or a contaminant? The neutral sugar content was decreased by about 40% after one passage through a BioGel P6 column, but further passages removed no more carbohydrate. Hence, all the glycoproteins were chromatographed on P6 before analysis by gas–liquid chromatography (g.l.c.), but the glucose content remained unaltered. By mixing [^{14}C]glucose with the VSG before chromatography, it was shown that all the labelled glucose could be separated from the VSG by this technique. The absence of fructose suggested that the glucose was not derived from

hydrolysed sucrose (a sucrose gradient was used in preparative isoelectric focussing of the antigen). Johnson and Cross therefore concluded that glucose was an integral component of the glycoprotein. The sugar composition suggested *N*-glucosidic linkage of the oligosaccharide to the polypeptide and, in support of this, Johnson and Cross (1977) stated that the linkage was not labile to alkali. Eight of eleven VSGs tested formed a precipitin band with the lectin Con A (specific for glucose and mannose) in double-diffusion agar gels, but none formed a precipitin band with wheat germ agglutinin (*N*-acetyl-glucosamine) or with *Ricinus communis* lectin (β-D-galactose).

Barbet and McGuire (1978) presented evidence of substantial immunochemical cross-reaction between different VSGs. This cross-reaction was not detectable by double diffusion in agar gels, or by immunofluorescence testing of living trypanosomes (confirming the results of Cross, 1975), but was readily discernible in radioimmunoassay. VSGs, labelled with iodine-125, were shown to bind to both homologous and heterologous anti-VSG. Binding of anti-VSG to heterologous ^{125}I-labelled VSG could be inhibited by any unlabelled antigen tested, whereas homologous interactions of antigen and antibody could be inhibited effectively by the homologous antigen only. These data imply that all the VSGs shared at least one common antigenic determinant. However, the ineffective inhibition of the homologous binding reaction of VSG to anti-VSG by heterologous antigens, must mean that each molecule carried a small number of common determinants compared to the total number of variant specific determinants. Further, since similar amounts of any heterologous antigen were required to inhibit all heterologous cross-reactions, then the cross-reacting determinant must be the same or similar in all antigens. Some of the results are shown in Table 9. The cross-reactions could also be detected in a complement-fixation assay. Amongst the possible locations for the cross-reacting determinant discussed was the "the membrane binding sequence, perhaps including sugars" (Barbet and McGuire, 1978).

Barbet *et al.* (1979) reported that whereas incubation of VSGs with trypsin, which promoted extensive degradation, did not inhibit cross-reaction, treatment with periodate for 24 hours at 4°C abolished cross-reaction without affecting the homologous reaction. Periodate cleaves carbon–carbon bonds of 1,2 *cis* diols, commonly found in carbohydrates. Since the periodate-treated antigen no longer bound to Con A, this strongly suggested that the cross-reacting determinant contained carbohydrate.

Cross (1979a) was able to verify the findings of Barbet and McGuire (1978) and to locate the cross-reacting determinant in the *C*-terminal domain. Again binding of anti-VSG to ^{125}I-labelled heterologous VSG could clearly be demonstrated, but when antisera were prepared using *N*-terminal tryptic peptides (M_r 40 000–50 000), no binding to heterologous VSGs could be

detected. The cross-reacting determinant must therefore have been either in the *C*-terminal domain, or a conformational determinant requiring the proximity of *N*- and *C*-terminal domains. The latter possibility was excluded by inhibition studies using a *C*-terminal glycopeptide (M_r 17 000) containing all the carbohydrate. This glycopeptide was a highly effective inhibitor of heterologous cross-reaction, whereas the *N*-terminal peptides were not. Cross (1979a) noted that concanavalin A did not block cross-reactivity, and also reported that the VSG containing most carbohydrate was the least effective inhibitor of cross-reactivity. Each of these negative observations could be construed as implying that carbohydrate was not involved. However, treatment of the *C*-terminal glycopeptide with endoglycosidase H, which cleaves high mannose-containing oligosaccharides from the polypeptide backbone, reduced by 90% the activity of this material in inhibiting heterologous cross-reactions.

TABLE 9

Cross-reaction between heterologous combinations of VSG and rabbit anti-VSG (from Barbet and McGuire, 1978)[a]

	VSG required for 50% inhibition (ng)					
Antibody	367	9C	A4	055	052	049
Anti-367	6	(0%)[a]	(0%)[a]	(14%)[a]	(18%)[a]	(0%)[a]
Anti-052	5	12	9	3	15	5
Anti-X4	12	4	10	16	16	7

[a] Each assay tube contained 4 ng of ^{125}I-labelled 367 VSG. All the antigen could be precipitated by homologous (anti-367) and heterologous (anti-052 and anti-X4) antisera, in the presence of sheep anti-rabbit IgG. Antisera dilutions were chosen to give 50% precipitation of labelled antigen, and the amount of purified VSG required to inhibit the homologous and heterologous immunoprecipitations was measured. Only the homologous antigen (367) effectively inhibited the homologous immunoprecipitation. Figures in parentheses refer to the percentage inhibition of the homologous reaction achieved with 800 ng of each of the heterologous VSGs. Heterologous immunoprecipitation could be inhibited by all six VSGs tested, showing that the cross-reacting determinant was present on each VSG.

Definitive proof that carbohydrate alone was responsible for this cross-reaction was presented by Holder and Cross (1981). As described above, glycopeptides were prepared from VSGs by digestion with trypsin or pronase. Digestion of VSG 121 with pronase allowed the purification of two glycopeptides, one of which, 121-1, had the composition $Asx_{1.0}$ $Ala_{1.0}$ $Thr_{0.9}$ $GlcNAc_{2.6}$, and the second, 121-2, had the composition $Asx_{1.0}$ $GlcNAc_{1.3}$. Analysis of the neutral sugars showed that whereas 121-1 contained only mannose, 121-2 contained mannose and galactose. Only 121-2 was an effective inhibitor of heterologous cross-reaction, indicating that VSG

121 contained at least two types of oligosaccharide, and that the cross-reacting determinants detectable in the radioimmunoassay involved only one of them. The location and composition of this cross-reacting determinant was further analysed by the preparation of tryptic glycopeptides from five variants. As outlined above, two classes of glycopeptides were identified, one of which was internal, and the second of which included the *C*-terminal amino acid of the purified VSG. The amino acid sequences of these glycopeptides have already been presented (Table 7). The carbohydrate compositions are given in Table 10. Firstly, it can be seen that variants 055 and 060 gave heterogeneous *C*-terminal glycopeptides. The heterogeneity within each variant is in the carbohydrate, and not in the amino acid sequence. The *C*-terminal glycopeptides all contain glucosamine, mannose and galactose, whereas the internal glycopeptides contain only glucosamine and mannose (with one exception, which also contains galactose). Only the *C*-terminal glycopeptides were effective in inhibiting cross-reaction (Fig. 3).

TABLE 10

Carbohydrate composition of internal (1–7) and C-terminal (8–16) tryptic glycopeptides purified from VSGs of T. brucei

Peptide	*N*-Acetylglucosamine	Mannose	Galactose
(1) 55A[1]	3·40	2·8	—
(2) 60B	2·45	3·7	—
(3) 60B[1]	2·74	n.d.	n.d.
(4) 117B	2·82	5·4	—
(5) 121B	2·00	5·7	—
(6) 121B[1]	2·95	6·3	—
(7) 221B	4·82	2.9	1·7
(8) 55B	13·05	2·1	9·2
(9) 55C	9·44	2·2	6·9
(10) 55D	3·55	4·4	3·5
(11) 55E	1·21	1·7	8·0
(12) 60A	4·55	4.4	5·4
(13) 60C	1·21	2·2	8·2
(14) 117A	1·41	2·1	4·1
(15) 121A	1.57	2·1	5·4
(16) 221A	0·99	7·7	7·7

Note. Considerable heterogeneity in the glycopeptides generated from each variant was observed, not in the amino acid sequences (Table 7) but in the carbohydrate composition. Thus variant 055 generated five *C*-terminal tryptic glycopeptides, sharing the same amino acid sequence, but with widely differing carbohydrate composition (8–11). Glycopeptides 55B, C and D were glycosylated both on the *C*-terminal serine residue and on an asparagine residue, whereas 55E appeared to be glycosylated on the *C*-terminal amino acid only. All the *C*-terminal tryptic glycopeptides were effective inhibitors of heterologous immunoprecipitation, in experiments similar to those outlined in the legend to Table 9 (see Fig. 3).

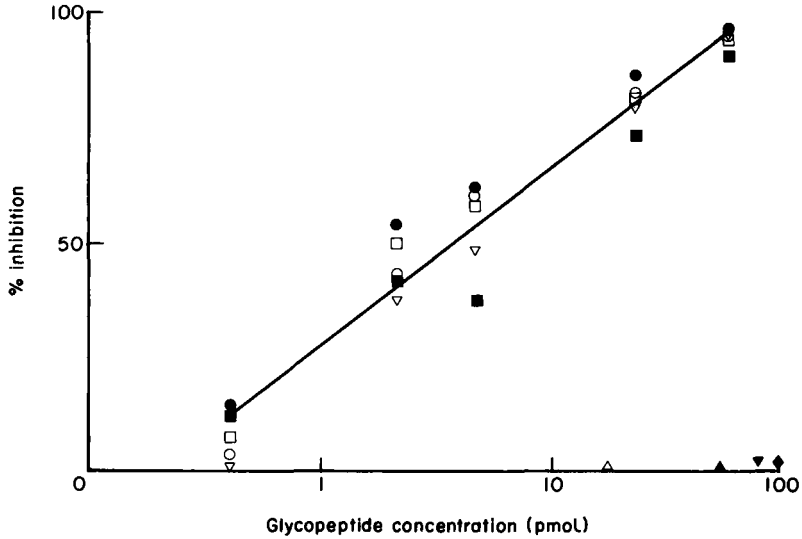

FIG. 3. Competitive inhibition of immunoprecipitation of VSG 55 by rabbit antiserum to VSG 121, using purified tryptic glycopeptides from five VSGs. Anti-VSG 121 (diluted 100-fold) was incubated with 4 ng of ^{125}I-labelled VSG 55 in the presence of different amounts of glycopeptide for 2 hours at 37°C. Immune complexes were precipitated with goat anti-rabbit IgG, and were counted. *C*-terminal glycopeptides: □, 55B; ●, 60A; ▽, 117A; ○, 121A; ■, 221A. Internal glycopeptides: △, 121B; ▲, 60B; ▼, 55A; ◆, 117B. For the internal glycopeptides, the single point on the graph shows the inhibition obtained at the highest amount tested. Where heterogeneous *C*-terminal glycopeptides were obtained (e.g. 55B, C, D, E; Table 10), each gave identical inhibition profiles. See Tables 7 and 10 for description of the glycopeptides. (Redrawn from Holder and Cross, 1981.)

Table 7, showing the sequences of the *C*-terminal glycopeptides, also indicates which residues are glycosylated. VSGs 055, 060 and 221 all contain the classic Asx-X-Ser/Thr triplet, which is universally used as the signal sequence for glycosylation of asparagine through linkage with *N*-acetylglucosamine (Marshall, 1974). VSGs 117 and 221 have a *C*-terminal asparagine or aspartate residue. Boothroyd *et al.* (1980) have shown from nucleic acid sequencing of a 117-specific cDNA clone that mRNA for this VSG encodes a 23 amino acid *C*-terminal extension, and the first two amino acids of this extension complete an Asx-Ser-Ser triplet, which can act as a glycosylation signal. However, very surprisingly, the cDNA sequence shows that the first amino acid of the triplet is aspartic acid, not asparagine, implying either a novel carbohydrate linkage, or post-translational modification of aspartate to asparagine. It is not yet known whether this peculiar finding is general for all VSGs, though Boothroyd *et al.* (in press) have reported the same phenomenon in a second VSG. Certainly, the second glycosylation site, which is some 50 amino acids from the first in VSG 117, has a con-

ventional Asn-Ser-Thr sequence. The significance of the 23 amino acid *C*-terminal extension sequence will be discussed later.

A second surprising result is that in all five VSGs studied, the *C*-terminal amino acid was always glycosylated. In VSGs 117 and 121, this was an Asx residue, but variants 055, 060 and 221 had *C*-terminal serine residues which were glycosylated in addition to glycosylated Asx residues some 5 or 6 residues from the *C*-terminus. The nature of this linkage to serine is not known. Typically, *O*-glycosidic linkages to serine should be alkali-labile, but Johnson and Cross (1977) implied that no alkali-labile carbohydrate was present in their VSG preparations. Furthermore, it is not known to which amino acid the cross-reacting carbohydrate determinant was linked. The finding that purified VSGs are always glycosylated on their *C*-terminal amino acid is most intriguing, and its significance will be discussed later.

Two further points about the carbohydrate. Firstly, the location of the cross-reacting determinant at the *C*-terminus of the VSG is consistent with the data of Wright and Hales (1970) and the model of Cross and Johnson (1976), and explains why the cross-reaction could not be detected by immunofluorescence. It is therefore most improbable that boosting antibody titres against this determinant will be of any prophylactic value whatsoever. Conceivably, however, cross-reacting antibody could be used in a serodiagnostic test if the cross-reacting determinant persists in the bloodstream but, as yet, levels of circulating antigen are unknown. Secondly, where is the glucose, reported in the first analyses? (Johnson and Cross, 1977). None of the glycopeptides isolated by Holder and Cross (1981) contained glucose, hence the original result may have been an artefact, despite the precautions taken; or there may be additional sites on the VSG to which glucose is bound; or the VSG may be a glucose-binding molecule — a suggestion made by Cross (1977).

As might be expected, the presence of carbohydrate on the VSG has been exploited in designing alternative purification procedures. Strickler *et al.* (1978) prepared a trypanosome homogenate by sonication, and removed debris by centrifugation at 12 000 *g* and 165 000 *g*. The supernatant fraction was applied to a column of *Lens culinaris* haemagglutinin coupled to Sepharose 4B, and after extensive washing, glycoproteins were eluted in buffer containing 0·5 M glucose. This fraction contained one major glycoprotein and seven to eight minor components, most of which were removed by DEAE-cellulose ion-exchange chromatography at pH 8·0. The major component did not bind under these conditions, and was then 95% pure. Antibodies prepared against the lectin eluate agglutinated homologous trypanosomes only, and provided variant specific protection in immunized mice. Furthermore, surface labelling labelled a molecule having a molecular weight (56 000) identical to that of the major glycoprotein. Strickler *et al.* (1978) concluded

that the VSG comprised 5·5% of the total cellular protein, compared to previous estimates of 5–7% (Cross, 1975).

Such purification procedures may be useful, but the variation in carbohydrate composition should be borne in mind when considering this as a general method. For example, Johnson and Cross (1977) noted that only eight out of eleven variants bound to Con A, and in our laboratory none of the VSGs from the serodemes used bind to lentil lectin. Use of lectins may of course provide a rapid method for screening for differences in carbohydrate structure between different VSGs.

4. *Membrane attachment*

The mode of attachment of VSG to the plasma membrane remains one of the most intriguing problems of trypanosome biochemistry. Under physiological conditions, the glycoproteins form a compact, stable surface coat, firmly attached to the surface of healthy, living trypanosomes. However, in the absence of glucose, or under conditions in which accumulation of pyruvate leads to a rise in acidity, the coat appears to be wholly or partially shed as "exoantigen" (see above). Disruption of cells by any mechanical or osmotic means results in the apparently spontaneous release of 50–80% of the VSG in water-soluble form. How, then, is the VSG bound to the trypanosome? At least four possibilities can be considered. Firstly, the VSGs could be bound to the membrane by interaction of a hydrophobic peptide with lipid. Release of the VSG could then be a consequence of a specific proteolytic event. Secondly, there could be a weak hydrophobic interaction, either with lipid, or with another protein, which could be disrupted under any of the conditions outlined above. Thirdly, there could be salt linkages between charged groups on the VSG and on the plasma membrane (lipid? protein?). The second and third possibilities are, of course, not mutually exclusive. The fourth possibility is that the VSG is covalently coupled to a lipid molecule, and release occurs through the activity of a lipase. What are the merits of each possibility?

The idea that VSG is released by proteolysis is appealing because of its simplicity. Most integral membrane proteins (as defined by Singer and Nicholson, 1972) characterized to date are held in the membrane by hydrophobic sequences, usually around 20 amino acids in length. However, it has not proved possible to apply the criteria used by Singer and Nicholson (1972) to determine whether VSG really is an integral membrane protein for the simple reason that no one has yet succeeded in preparing a plasma membrane preparation carrying all the VSG present on the cell surface. If proteolysis occurs, then it is extraordinarily rapid, specific, and insensitive to the common inhibitors of proteolysis. Although proteolytic enzymes are present in trypanosome homogenates (Cross, 1975), if suitable pre-

cautions are taken VSG can be prepared in which there is no apparent degradation of either the *N*- or the *C*-terminus. However, recent work of Boothroyd *et al.* (1980) has revealed that VSG specific mRNA encodes an additional 23 amino acids, not present on purified VSG, which are largely hydrophobic. Is this the membrane-binding peptide? It is tempting to think so, but several problems persist. Why have efforts to isolate a form of the VSG containing this hydrophobic peptide failed? What is the nature of the cleavage event which removes this peptide? Why is there a glycosylation site on the *C*-terminal amino acid? Some of these questions will be considered later.

If this hydrophobic *C*-terminal extension is not responsible for membrane attachment, could there be a hydrophobic sequence within the water soluble VSG? Evidence that there is comes from studies of Klein *et al.* (1980; and unpublished observations). Purified VSGs can interact with lipid monolayers in a cholesterol-dependent fashion. The interaction with sphingomyelin, phosphatidylethanolamine and phosphatidylcholine is small or non-existent, but in the presence of cholesterol, a relatively large penetration of the monolayer by VSG can be observed. The interaction with cholesterol alone is the largest of all. The specificity for cholesterol is remarkable. The interaction can be blocked with filipin, an antibiotic which complexes specifically with cholesterol, and whereas the interaction is relatively insensitive to structural changes in the hydrocarbon skeleton, any modification of the C-3 substituent which interferes with the ability to form hydrogen bonds impairs the interaction. Is it truly a hydrophobic interaction? Analysis of the pressure-area isotherms shows that the VSG actually penetrates the lipid monolayer, rather than simply adsorbing to the surface. Auffret and Turner (1981) showed that there are hydrophobic sites within VSG molecules, allowing the formation of dimers and higher oligomers, and that such sites are within the *C*-terminal domain. However, the published sequences (Cross *et al.*, 1980; Boothroyd *et al.*, 1980) do not provide evidence for a hydrophobic sequence within this region of the molecules, but the interaction of apolipoproteins with lipids shows that a linear hydrophobic sequence is not a prerequisite for lipid binding (see below). Information on the functional significance of cholesterol binding is still lacking. It may be of no relevance to membrane attachment. It may, for example, be associated with the serum sensitivity of *T. b. brucei* (Rickman and Robson, 1970). Lysis of *T. b. brucei* is known to be caused by the high density lipoprotein (HDL) component of human serum (Rifkin, 1978), which acts as the serum cholesterol carrier. Turner and Cordingley (1981) have suggested that cholesterol binding by VSGs may reflect their evolutionary origin. Certainly, much more work on the affinity of the interaction, the stoicheiometry, and the site of binding is needed.

The suggestion that VSGs are bound electrostatically rests on the observations that bloodstream trypanosomes are neutral at physiological pH values, whereas "ghosts" and culture forms are negatively charged (Cross, 1978; Hollingshead *et al.*, 1963), and *C*-terminal glycopeptides of VSGs are rich in lysine (Johnson and Cross, 1979). However, as mentioned above, the isoelectric points for two *C*-terminal glycopeptides (Johnson and Cross, 1979) suggest that these domains carry a net negative charge, and secondly the published sequences (Cross *et al.*, 1980; Boothroyd *et al.*, 1980) do not show any strikingly charged sequences.

There is no evidence for the involvement of another protein in membrane attachment. Any such protein could be expected to be present in large amounts, since the VSG comprises ~5% of the total cellular protein. Rovis and Baekkeshov (1980) reported the presence of four major proteins in purified plasma membranes, one of which was residual VSG. Of the other three, an 83 000 dalton glycoprotein was tentatively identified as a precursor of mature VSG, but observations from my laboratory show that it is not (J. McConnell, L. Almeida, J. Cordingley and M. J. Turner, unpublished observations). The two other proteins of molecular weight 56 000 and 53 000 were believed to be the α- and β-subunits of tubulin. Voorheis *et al.* (1979) prepared plasma membranes according to a different procedure and identified proteins of molecular weights 92 000, 61 000, 57 000 and 47 000. These probably corresponded to the four reported by Rovis and Baekkeshov (1980) (no proof was offered that the 61 000 dalton protein was VSG). In addition, a doublet of proteins of molecular weight ~250 000 was observed. It was suggested that this could correspond to the actin-binding protein spectrin, common to most mammalian erythrocytes (Branton *et al.*, 1981). Although spectrin is limited to the red blood cell, it does have functional and structural features in common with the ubiquitous actin-binding proteins of eukaryotic cells such as filamin (M_r 250 000) and macrophage actin-binding protein (M_r 250 000) (Tyler *et al.*, 1980), hence the 250 000 dalton doublet from trypanosomes could be the phylogenetic counterpart of these proteins. The absence of this protein from the membrane preparations of Rovis and Baekkeshov (1980) can be easily explained in terms of the different methods of preparation employed. Rovis and Baekkeshov disrupted parasites by release from a nitrogen "bomb" under 50 atmospheres pressure (~5050 kPa), and then added EDTA (ethylenediaminetetraacetic acid). These conditions, which stripped away the subpellicular microtubules, could easily disrupt the binding of an actin-binding protein, which is usually a peripheral membrane protein. However, there is no evidence that such a molecule interacts directly or indirectly (through actin) with VSG. Indeed, in *T. brucei* microfilaments have not been detected under the plasma membrane, except at the attachment region of the flagellum along the length of the trypanosome, where there is a

line of macular desmosomes, each with its attached microfilaments (Barry, 1979; Vickerman, 1969).

A really rigorous characterization of *all* the VSG present on the plasma membrane is urgently needed. Rovis *et al.* (1978) claimed that VSG of both *T. congolense* and *T. brucei* which was not released by homogenization could be solubilized by detergent—i.e. behaved as an integral membrane protein. The presence of VSG on purified plasma membranes can be taken to corroborate this. Work in my laboratory (J. McConnell, L. Almeida, J. Cordingley and M. J. Turner, unpublished observations) indicates that some of the VSG on trypanosomes, not released on rupturing the cell, lacks the cross-reacting common carbohydrate determinant, yet behaves on charge-shift electrophoresis as an integral membrane protein—that is, it contains a hydrophobic sequence, presumably identical to that detected in cDNA sequences. Hence the surface coat may be composed of a matrix of different forms of the VSG, employing different membrane-binding mechanisms.

Before leaving this topic, it is worth considering whether the trypanosome surface is unique in its apparent instability, and whether there are any other examples of "water-soluble" proteins which can also interact with membranes. In answer to the first point, trypanosomes are not unique. There is an interesting analogy with some RNA tumour viruses. Such viruses possess a lipid-containing membrane, the viral envelope, in which are embedded virally-specified protein and glycoprotein molecules, which form "knobs" or "spikes" on the surface (reviewed by Bolognesi *et al.*, 1978). Such envelope glycoproteins are common to many animal, plant and bacterial viruses, and behave as integral membrane proteins. However, the envelope glycoproteins of RNA tumour viruses are unusual in that they are released from the viral envelope under very mild conditions. In the case of murine leukaemia virus, osmotic shock, freeze-thawing, ultrasonication and chaotropic agents all release a soluble glycoprotein ($M_r \sim 70\,000$) (reviewed by Compans and Klenk, 1979). Such conditions also solubilize VSGs. The viral glycoproteins seem to interact with a protein ($M_r \sim 15\,000$) which is itself anchored in the bilayer by hydrophobic interactions with lipids. Both the glycoprotein and the protein are synthesized from a common precursor polypeptide, hence in this respect the viral and trypanosome glycoproteins differ, but the analogy provides an insight into how an apparently water-soluble molecule can be attached to a membrane.

There are several other examples of proteins which are apparently water soluble, but which can also interact with lipids. Melittin, the major toxin in bee venom, consists of four identical subunits each comprising 26 amino acids, in which 19 of 20 *N*-terminal amino acids are hydrophobic (Haberman and Jentsch, 1967) and the remaining six *C*-terminal residues are polar. It exists in solution as a tetramer, to minimize interaction of the hydrophobic residues

with water, but it penetrates natural or synthetic membranes, regardless of surface charge, by hydrophobic interactions (Sessa *et al.*, 1969). The serum complement system provides an excellent example in which all the components are water soluble, yet when the proteolytic cascade is triggered, complexes are produced which can penetrate lipid bilayers. Pyruvate oxidase of *Escherichia coli* is a peripheral membrane protein, bound *in vivo* to the inner surface of the cytoplasmic membrane. It may be extracted from the membrane fraction by salt, and purified as a tetramer without detergent (Schrock and Gennis, 1977). However, the enzyme activity is enhanced 25-fold in the presence of lipids or detergents, apparently as a result of a conformational change in the protein (Blake *et al.*, 1978). This lipid binding site can be removed by proteolysis of a 4000 dalton fragment from the *C*-terminus.

Serum lipoproteins are macromolecular lipid–protein complexes containing protein components called apoproteins, and polar lipids (phospholipids and cholesterol) in a surface film surrounding a neutral lipid core (triglycerides and cholesterol esters). The apoproteins are heterogeneous in size, ranging from 5700 to 75 000 daltons, and vary in their solubility in water. Apo A1, the principal apoprotein of HDL, is soluble to a maximum of about 10 mg ml^{-1} (Swaney and O'Brien, 1978), and can exist in solution as an equilibrium mixture of monomers and higher oligomers (Vitello and Scanu, 1976; Swaney and O'Brien, 1978). This self association is insensitive to changes in pH and ionic strength, and hence is not electrostatic. Purified Apo A1 binds to phospholipid vesicles, but the binding is enhanced in the presence of cholesterol (Yokoyama *et al.*, 1980). Apolipoproteins are believed to contain large amounts of α-helix, in which the α-helices are amphipathic. One surface of the helix is charged, and is believed to interact with phospholipid head groups. The other is hydrophobic, and binds to the lipid fatty acyl chains. It has been suggested that the role of cholesterol in the binding of proteins to phospholipid surfaces is to increase the space between the charged phospholipid head groups, and allow the amphipathic helix to penetrate the surface. Once in position, there is a weak specific interaction between the polar cholesterol head group and charged groups within the otherwise apolar face of the helix (Fukushina *et al.*, 1981). Could this be the mechanism by which the VSG binds to the trypanosome surface? The cholesterol-binding data referred to above (Klein *et al.*, 1980; and unpublished results) suggest it could. A scan of published VSG *C*-terminal sequences (Cross *et al.*, 1980) reveals that it is possible to generate amphipathic helical sequences (Fig. 4). Such sequences are not strongly hydrophobic, but the interaction with the membrane is not strong either. These sequences could also provide the hydrophobic dimerization site, noted by Auffret and Turner (1981). Clearly much more work needs to be done, including further se-

quencing, and measurement of solution conformation of VSGs by circular dichroism. Such studies on soluble VSG, and on VSG complexed to lipid, could determine whether the above mechanism operates, at least *in vitro*. Whatever the mode of attachment of the VSG to the membrane, and whatever the mechanism of release, the solution to this problem probably represents the best hope in the medium term of developing a trypanosomicidal reagent in which the VSG is the target.

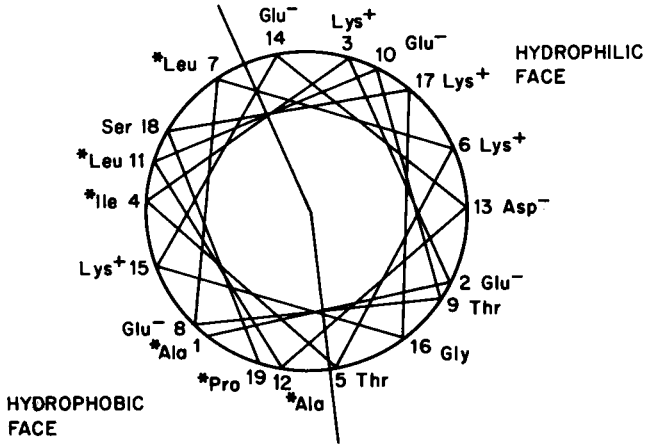

FIG. 4. Postulated amphipathic α-helix generated from published sequence of VSG 117 (Cross *et al.*, 1980). Such sequences generate hydrophobic sites which could be involved in membrane attachment, in the formation of dimers in solution, and in the binding of VSGs to cholesterol (Klein *et al.*, 1980). The hydrophobic sequence is interrupted by charged residues, as described by Fukushima *et al.* (1981). The axis of the helix is perpendicular to the plane of the paper.

5. *Biosynthesis of VSGs*

This is likely to become a growth area in trypanosome research because of the clear chemotherapeutic potential of compounds which either prevent the synthesis and thus expression of VSGs; or displace the VSG from the plasma membrane; or interfere with the mechanism of antigenic variation. The following areas of post-translational modification of the precursor VSG polypeptide (pVSG) can be considered.

Firstly, the processing of *N*-terminal signal peptides. Secretory and membrane-bound proteins are generally synthesized on ribosomes attached to the membrane of the endoplasmic reticulum through the binding of a hydrophobic *N*-terminal "signal peptide" on the nascent polypeptide chain. This interaction allows the transfer of the growing polypeptide chain through the membrane into the lumen of the endoplasmic reticulum, and during this transfer, the signal peptide is normally cleaved from the still uncompleted

chain (reviewed by Blöbel, 1978). The existence of such signal peptides in pVSG molecules has been inferred from comparisons of the molecular weights of purified VSG and antigen synthesized *in vitro* (Cordingley and Turner, 1980; Hoeijmakers *et al.*, 1980a, b). This does not constitute a formal proof, because such a comparison is complicated by the presence of carbohydrate on the VSG, and the assumed presence of a hydrophobic *C*-terminal polypeptide on the *in vitro* synthesis product, which is absent from the pure VSG. Lheureux *et al.* (1979) showed that VSGs were synthesized on membrane-bound ribosomes, which is strong circumstantial evidence for the presence of a cleavable signal peptide. There is, however, at least one example of a secreted protein (chicken ovalbumin) which is synthesized on membrane-bound ribosomes but does not have a signal peptide removed post-translationally (Lingappa *et al.*, 1978). McConnell *et al.* (1981) demonstrated unequivocally the existence of an *N*-terminal signal peptide on pVSG. The analysis was based on a size comparison of non-glycosylated *N*-terminal CNBr fragments, prepared from pure VSG, and from immunoprecipitated pVSG synthesized *in vitro*. The peptide prepared from the *in vitro* product was about 4000 daltons heavier than that from the pure VSG, demonstrating the presence of a signal peptide containing 30–40 amino acids. This is large in comparison with such peptides identified in other systems, which normally contain 15–30 amino acids (Leader, 1979). Analysis of the precursor VSGs produced when nascent polypeptide chains on *T. b. brucei* polysomes were completed in the absence of reinitiation showed, firstly, that the signal peptide was removed before translation was complete, and secondly, that the same translational start signal was used in the trypanosome, and in the rabbit reticulocyte protein synthesis system. As yet, no comparison of the signal peptidase activity of trypanosomes and that of other eukaryotes has been published. Such a comparison would indicate whether this is likely to be a useful target for chemotherapy, although by analogy with other systems, this is improbable. Indeed, it has recently been demonstrated that the signal peptide of pVSG can be accurately removed by the signal peptidase found in dog pancreatic microsomal vesicles (J. McConnell, personal communication).

Boothroyd *et al.* (1981) have presented nucleotide sequence data containing the amino terminus of two pVSGs. By comparison with the known amino acid sequence of the purified VSGs, both pVSGs could be seen to contain *N*-terminal signal peptides. One sequence, of 29 residues, was preceded by a stop codon in phase, and further upstream, indicating that the methionine codon at position −29 is almost certainly the initiation site for translation. Since there is a second methionine codon at position −15, this could theoretically also be the initiation codon, but comparison with the data of McConnell *et al.* (1981) suggests that the longer signal peptide is

TABLE 11

Proposed N-*terminal signal peptide sequences of VSGs 117 and 221 of* T. brucei

Variant	−30	−20	−10	−1 ↓
117	Met Asp Lys His Thr Lys Glu Thr Leu Gly Val Thr Gln	Trp Arg Arg Ser Thr Met Leu Thr Leu Ser	Leu Leu Tyr Ala Ile Thr Pro Ala Asp	Gly Ala
221	...(Thr Arg His Val Arg Gly) Met Pro Ser Asn Gln Glu	Ala Arg Leu Phe Leu Ala Val Leu Val Leu	Ala Gln Val Leu Pro Ile Leu Val Asp	Ser Ala

Note. Sequences were determined from nucleotide sequencing data of Boothroyd *et al.* (1981). Arrow indicates the amino terminal residue of the mature purified VSG. Underlined residues are hydrophobic. The methionine residue at position −26 in variant 221 is the postulated translation initiation site, but since no in-phase stop codon was detected further upstream, the residues in parentheses could also form part of the signal peptide, with the actual initiation site being further upstream at a site not contained in the ds.cDNA insert sequenced in this analysis.

used. The second variant contains a methionine at position −26, which could be used for initiation but as it is not preceded by an in-phase stop codon, translation may begin further upstream at a site not represented in the cDNA inserts of the recombinants sequenced. The sequences are shown in Table 11.

The second area of modification of the pVSG is the addition and processing of oligosaccharide side chains. The oligosaccharide linkage in glycoproteins can be either through covalent attachment to asparagine (*N*-linked) or to serine or threonine (*O*-linked). As noted above, some VSGs appear to contain both types of linkage (Holder and Cross, 1981). *N*-linked oligosaccharides are divided into two major classes. The "complex" chains contain *N*-acetylglucosamine, mannose, galactose, fructose and sialic acid, and the "high mannose" chains contain *N*-acetylglucosamine and variable amounts of mannose (Kornfeld and Kornfeld, 1980). The two classes are synthesized via the same pathway, involving a lipid-linked oligosaccharide intermediate (reviewed by Parodi and Leloir, 1979). The first step in the pathway is the transfer of *N*-acetylglucosamine-1-phosphate from UDP-*N*-acetylglucosamine to dolichyl phosphate, the phosphate ester of a C-55 isoprenoid alcohol. This can then be elongated by the addition of one *N*-acetylglucosaminyl, nine mannosyl, and three glucosyl residues. After elongation is complete, the oligosaccharide is transferred from the lipid to the growing polypeptide chain, within the lumen of the endoplasmic reticulum. The large oligosaccharide is then processed in a fashion dependent on whether it is to become "complex" or high mannose. This processing begins in the rough endoplasmic reticulum with the removal of glucosyl residues, and continues as the protein moves through the Golgi membranes. To what extent is this pathway utilized by trypanosomes? Holder and Cross (1981) have distinguished two classes of oligosaccharide in VSGs. Firstly, an "internal" class, which is not immunogenic, and which contains *N*-acetylglucosamine, mannose and galactose. This composition is compatible with oligosaccharides of the "high mannose" category and has been confirmed by the observation that the oligosaccharide can be cleaved with endoglycosidase H, an enzyme that cleaves the β1–4 linkage between the two core *N*-acetylglucosamine residues of "high mannose" carbohydrates (C. A. Auffret and M. J. Turner, unpublished observations). Hence this internal oligosaccharide is probably transferred from a dolichol-type intermediate in the lumen of the endoplasmic reticulum. The second class of oligosaccharide distinguished by Holder and Cross (1981) is the *C*-terminal carbohydrate which stimulates the production of cross-reacting antibody. This contains *N*-acetylglucosamine, mannose and galactose, yet is not susceptible to the action of endoglycosidases H or D, specific for "high mannose" and "complex" oligosaccharides respectively (C. A. Auffret and M. J. Turner, un-

published observations). Furthermore, cDNA sequencing has shown that in one variant at least, the glycosylated amino acid is encoded as aspartic acid, not asparagine (Boothroyd *et al.*, 1980). It is not known whether the sugar is attached to aspartate, or whether the aspartate is modified to asparagine after translation. Analysis of this carbohydrate is further complicated by the finding that in some VSGs, the *C*-terminal amino acid is not asparagine, but serine, which is glycosylated in addition to asparagine residues within five or so amino acids of the *C*-terminus (Holder and Cross, 1981). Bear in mind that the original report on carbohydrate composition of VSGs implied that the attachment was not alkali-labile. Hence far more information is needed on the structure and linkage of this unusual carbohydrate.

Experimental observations on the glycosylation of VSGs are limited. Strickler and Patton (1980) examined the effect of tunicamycin on glycosylation of VSGs, in an *in vitro* culture system in which trypanosomes could be maintained in the absence of feeder layer cells for up to 2 hours. Tunicamycin is a lipophilic analogue of UDP-*N*-acetylglucosamine, which inhibits the first step in the formation of lipid-linked oligosaccharide, namely the transfer of *N*-acetylglucosamine-1-phosphate to dolichyl phosphate. At a concentration of 100 ng ml^{-1}, tunicamycin effectively inhibited the uptake of [^{3}H]mannose after a 15–30 minute lag phase. The lag was attributed to the presence of a pool of dolichol-PP-*N*-acetylglucosamine to which [^{3}H]-mannose was transferred. Only when this pool was exhausted could the tunicamycin be effective. In the absence of tunicamycin, 90% of the label was incorporated into VSG, and in the presence of tunicamycin and [^{14}C]serine, the molecular weight of the pVSG dropped from 58 000 to 55 000 daltons, presumably because no oligosaccharide had been incorporated. Cycloheximide was also found to inhibit incorporation of [^{3}H]mannose, but the kinetics of inhibition differed from those for inhibition of protein synthesis. Incorporation of [^{3}H]mannose was inhibited by 30–40% during the first 45 minutes and by 100% thereafter, whereas [^{14}C]serine incorporation was inhibited linearly by 70% over the entire time course of the experiment. Even in the absence of any inhibitors, the extent and kinetics of [^{3}H]mannose incorporation differed from that of [^{14}C]serine, the former being linear over 60 minutes, and then levelling off, the latter being linear over 90 minutes. On the basis of this, and on the differential effects of tunicamycin and cycloheximide on mannose and serine uptake, it was proposed that glycosylation occurs post-translationally, possibly after transport from the endoplasmic reticulum to the Golgi. This would accord with the observations of Steiger (1973), who found by thiocarbohydrazide/silver albumin staining of thin sections of *T. b. brucei* that carbohydrate is associated with the cytoplasmic membrane, Golgi-derived vesicles and membranes of the Golgi

complex, but not with the cisternae of the rough endoplasmic reticulum. However, it seems that the difference in uptake of mannose and serine observed in the absence of inhibitors was not significant, given that the culture system supported trypanosomes for only 2 hours (labelling was followed over 90 minutes), and that a comparison of two labels, one of which labels essentially one product only, whereas the other labels every protein in the cell, is unfair. In the absence of specific information about incorporation of mannose and serine into pVSG alone, it still seems possible that glycosylation could be linked to translation.

Unfortunately, the carbohydrate composition and distribution of the VSG of the variant used by Strickler and Patton (1980) was not characterized, so it is not possible to say whether inhibition of incorporation into internal, *C*-terminal, or both oligosaccharide side chains was observed. Inhibition of mannose uptake was complete after 30 minutes equilibration, which would suggest that both glycosylations were inhibited. Holder and Cross (1981) showed that, in general, more mannose is associated with the internal than with the *C*-terminal oligosaccharide. It is possible that the distribution of mannose between these sites was skewed in the variant used by Strickler and Patton (1980). If so, operationally, glycosylation of one site only could have been observed. However, the observation that tunicamycin inhibits glycosylation is consistent with *N*-glycosidic linkage. Clearly, in view of the unusual nature of the carbohydrate on the VSG, a very carefully controlled examination of the steps in the glycosylation pathway is needed.

Rovis and Dube (1981) studied the sequence of glycosylation reactions in several clones of *T. brucei*. Trypanosomes were biosynthetically labelled with [^{35}S]methionine for 10 minutes, and the label was then "chased" with cold methionine for two hours. Cells were lysed with detergent, and VSG was immunoprecipitated with either homologous anti-VSG serum, or with a heterologous anti-VSG serum, recognizing the shared carbohydrate determinant. Surprisingly, not all the VSG synthesized contained the cross-reacting determinant; only 20–60% (depending on the clone examined) of the total VSG immunoprecipitated with homologous antiserum could be precipitated with the heterologous antiserum. The time of addition of the cross-reacting determinant was investigated by lysing trypanosomes after chasing for 0, 30, 60, 90 and 120 minutes and analysing the amount of radiolabel which could be precipitated by homologous and heterologous antisera. At zero time, a small proportion of the total VSG was immunoprecipitated by the heterologous antiserum; this proportion rose to a maximum at about 1 hour, and thereafter declined slightly. Interestingly, SDS–polyacrylamide gels of the immunoprecipitates at any stage of the pulse–chase experiment showed only one band ($M_r \sim 60\,000$) – i.e. the presence or absence of the cross-reacting determinant had no effect on the molecular

weight of the pVSG, implying that its addition could be concomitant with the removal of, for example, the hydrophobic *C*-terminal peptide. When similar experiments were performed after 2 hours' preincubation with tunicamycin, addition of carbohydrate was judged to be inhibited since the amount of pVSG immunoprecipitated by homologous anti-VSG was 5–10% less than that from control trypanosomes incubated in the absence of tunicamycin. However, although some inhibition of the addition of the cross-reacting determinant was observed, complete inhibition of the heterologous immunoprecipitation was never achieved.

From such experiments, Rovis and Dube (1981) drew the following conclusions. Firstly, the transfer of some oligosaccharide must occur immediately after or concomitantly with synthesis of the protein as no incompletely glycosylated form (analogous to those observed in the presence of tunicamycin) could be detected at any time during the pulse–chase experiments. Secondly, the cross-reacting determinant must be heterogeneous, since the addition of this determinant could be partially but not wholly inhibited by tunicamycin, showing that different proportions of the oligosaccharides expressing the cross-reacting determinant are *N*-glycosidically linked to the VSGs examined. In one clone, addition of the cross-reacting determinant was completely resistant to the effects of tunicamycin. Thirdly, the pulse–chase experiments showed that addition of the cross-reacting determinant is a post-translational event. The location of the glycosylation events was not firmly established. Presumably, the first transfer must take place in the endoplasmic reticulum. Rovis and Dube (1981) suggest that the VSG is then transported to the plasma membrane, and that addition of the cross-reacting determinant (and processing of the hydrophobic *C*-terminus?) occurs there. These results again suggest that a careful analysis of all forms of VSG on the cell surface is needed—in particular, to determine what proportion of the VSG carries the cross-reacting determinant.

Brett and Voorheis (1980) claimed that the formation of the oligosaccharides of glycoproteins in the bloodstream forms of *T. b. brucei* occurs through a pathway differing from that used in mammals. Firstly, the dolichol phosphate–sugar pathway seemed to be either absent, or present at a very low level. Cells were extracted with acetone and then chloroform/methanol. The extract was treated with alkali, and after neutralization, the organic phase (in the acetate form) was applied to a DEAE-cellulose column. Neutral lipids were eluted with chloroform and methanol, and acidic lipids with ammonium formate. This fraction contained dolichol phosphate in a parallel extraction of rat liver, but no dolichol phosphate was detectable in the trypanosome extract. Pre-treatment of the extract under conditions in which lipid-linked oligosaccharides are hydrolysed did not allow the subsequent detection of dolichol phosphate. Such findings are clearly at

odds with those of Strickler and Patton (1980) and Rovis and Dube (1981). Secondly, Brett and Voorheis (1980) concluded that the oligosaccharide chains of plasma membrane glycoproteins were elongated within the plasma membrane. This was shown by incubating various subcellular fractions (isolated as described by Voorheis *et al.*, 1979) with UDP-[^{14}C]GlcNAc, and measuring the radioactivity incorporated into material precipitable by trichloroacetic acid. Between 70% and 80% of the total incorporation occurred in the plasma membrane fraction; the remainder, demonstrated in other fractions, was held to be due to contamination with plasma membranes. Although glycosyltransferase activity has been found in the plasma membrane of some mammalian cells, levels are usually small compared to those found in the microsomal fraction (Schlacter, 1974). In the trypanosome, just the reverse was found. Furthermore, in contrast again to mammalian systems in which transferase activity has been detected on the exterior surface of the plasma membrane, in the trypanosome this activity was located on the cytoplasmic face of the membrane. Base-catalysed hydrolysis of protein–sugar linkages in the presence of borohydride (conditions under which both *N*- and *O*-glycosidic linkages are cleaved) followed by acid hydrolysis and chromatography, showed that all the incorporated radioactivity was released as *N*-acetylglucosamine. This proved that the UDP-GlcNAc was being used for chain elongation, and not for initiation, because under such conditions the protein-linked glycosyl monomer only is reduced to its alcohol. Therefore, the absence of any radiolabelled *N*-acetylglucosaminitol indicated that chain elongation only was occurring. After cleavage of oligosaccharides from the acceptor molecule, gel filtration suggested that the chains incorporating radioactivity were quite uniform in length, containing about seven linked monomer units. Both *O*- and *N*-linkages were detected, in a ratio of about 1 to 3. In addition to *N*-acetylglucosamine, glucose, galactose and mannose could be donated to acceptors within plasma membrane preparations when presented as their respective nucleotide sugars. The subcellular distribution of these different glycosyl transferases was not examined. Steiger *et al.* (1980) proposed that galactosyl transferase of *T. brucei* is a constituent of the Golgi apparatus, while not ruling out that some activity could also be present in the plasma membrane.

What proportion, if any, of this activity was directed towards modification of the VSG? SDS–polyacrylamide gel electrophoresis of solubilized, labelled membranes used as substrate for glycosylation, showed that the sugars were incorporated into a number of different proteins, with the pattern of incorporation differing markedly for each of the four sugars tested. However, little incorporation was seen into material which co-migrated exactly with purified VSG. This should not be considered surprising, since post-translational modifications will affect the migration rates on SDS–

polyacrylamide gels. Brett and Voorheis (1980) suggested that a higher molecular weight component ($M_r \sim 90\,000$) which acted as an acceptor molecule could be a precursor of the mature VSG. Unfortunately, no immunochemical experiment was performed to demonstrate whether any carbohydrate was incorporated into specifically immunoprecipitable material, irrespective of size. Recent experiments have shown that pVSGs produced by *in vitro* translation may differ in size from mature VSG, but only to a maximum of about 2000 daltons difference (McConnell *et al.*, 1981). Hence, if there is a large precursor in the plasma membrane, it must differ in carbohydrate content rather than protein. Attempts to immunoprecipitate such a precursor have been unsuccessful (J. McConnell, unpublished observations).

In the light of the results obtained with tunicamycin (Strickler and Patton, 1980; Rovis and Dube, 1981), there is a need to repeat the search for dolichol phosphate in *T. brucei*. Unless trypanosomes use a hitherto unknown glycosylation pathway which nevertheless tunicamycin sensitive, the explanation for the apparent absence of dolichol phosphate will probably be a purely technical one.

What role, if any, do plasma membrane-bound glycosyl transferases play in VSG biosynthesis? Firstly, it has not yet been proved whether or not membrane-bound pVSGs are modified by such enzymes. This could readily be investigated using standard immunochemical techniques if sufficient radioactivity could be incorporated. If the pVSG is modified, then the obvious suggestion in the light of the findings of Rovis and Dube (1981) is that the cross-reacting determinant is added, or modified, here, and that this is the trigger for the third post-translational modification, specific cleavage of the hydrophobic *C*-terminus. This could be viewed as occurring during maturation of the VSG, with the hydrophobic tail acting as the anchor in transporting pVSG from the endoplasmic reticulum through the Golgi apparatus to the plasma membrane. At that point a different membrane-binding mechanism could be adopted after glycosylation, and the hydrophobic *C*-terminus removed. Alternatively, glycosylation could act as a hair-trigger priming the molecule for proteolysis which occurs only when the membrane is damaged. The necessity for such a system is not immediately clear, unless the mechanism is important in antigenic variation.

6. *Mechanism of antigenic variation*

No area of parasite biochemistry has attracted as much attention in the past 2 years as the mechanism of antigenic variation in *T. b. brucei*. This is because, in many ways, the system is perfect for analysis using modern methods of recombinant DNA technology, and because the mechanism involved may be of general importance in understanding how genomic rearrangements take place in eukaryotic organisms. As often happens, the

mechanism appears more complex than initially seemed likely. The following section outlines the methods used in the production of VSG-specific complementary DNA (cDNA) clones, and how such clones may then be used to probe the structure of the genome.

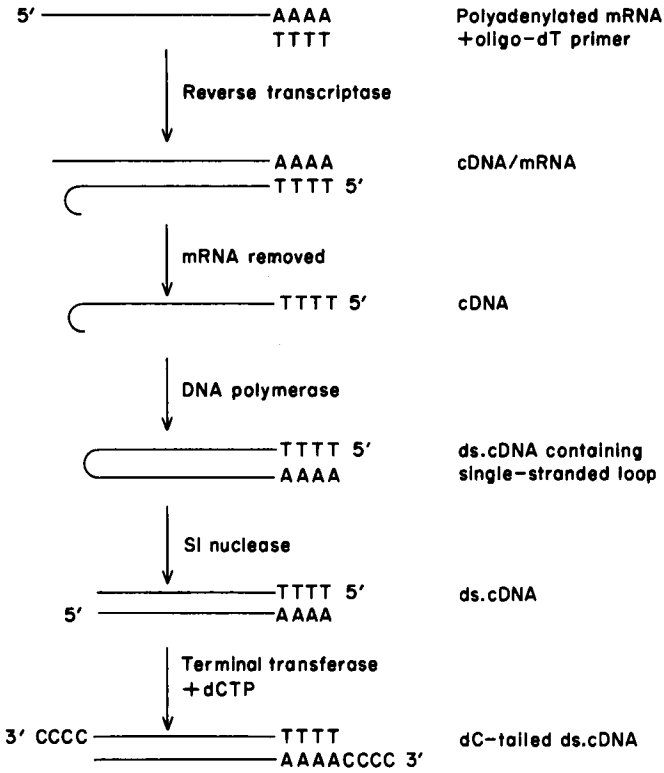

FIG. 5. Preparation of double-stranded cDNA (ds.cDNA) for use in the preparation of recombinant cDNA clones (see text).

7. *Methods*

The first essential in all the experiments to be discussed is the preparation of cDNA clones containing variant antigen-specific sequences. Polyadenylated (PA^+) mRNA is purified from trypanosomes and used as template for the synthesis of a complementary DNA strand (cDNA) using the enzyme reverse transcriptase (see Fig. 5); the cDNA is made double stranded, using DNA polymerase. The mRNA is usually destroyed, either before or after second strand synthesis. Covalent linkages between the two strands are removed by the action of the enzyme S1 nuclease, producing flush

ends in the double-stranded cDNA (ds.cDNA). This can then be inserted into a plasmid cloning vector, using any one of several techniques. Usually, a plasmid is chosen which carries genes conferring resistance to two antibiotics, for example ampicillin and tetracycline in the case of plasmid pBR 322 (Fig. 6). Insertion of a DNA sequence into one of these genes results in continued resistance to one antibiotic, but sensitivity to the second.

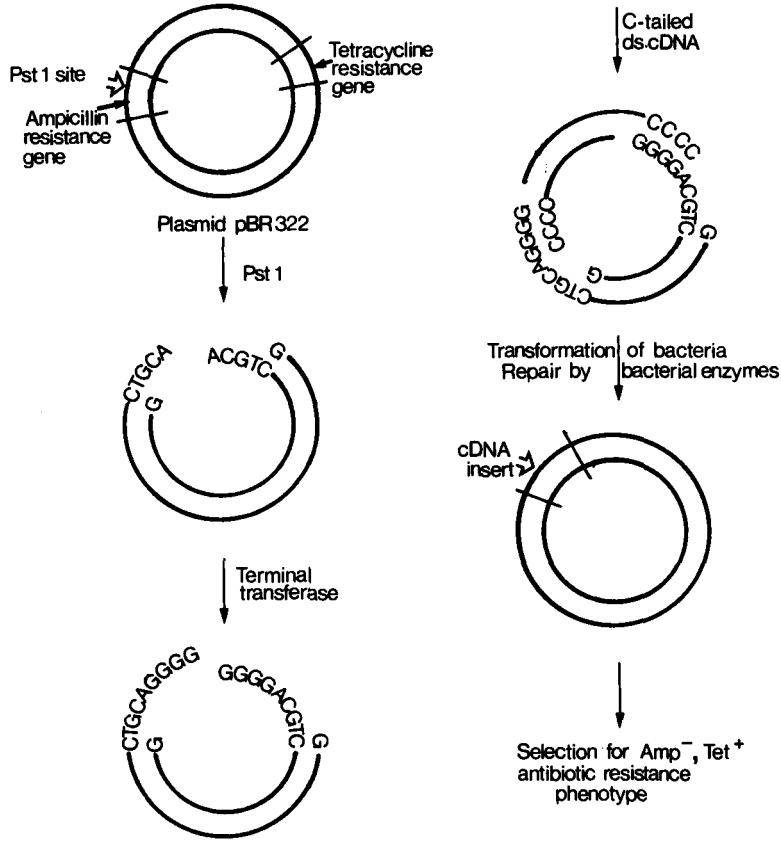

FIG. 6. Preparation of recombinant cDNA clones from ds.cDNA and a plasmid carrying antibiotic resistance markers (see text).

Resistance to the first can be used as a selective method for isolating those bacteria that contain a plasmid, and amongst this population, those bacteria that have lost resistance to the second antibiotic must carry a plasmid containing an insert. This happy state of affairs is achieved by cutting the plasmid within the antibiotic resistance gene, using a restriction enzyme.

Restriction enzymes are sequence-specific endonucleases, and many plasmids have been engineered to contain, within an antibiotic resistance gene, a single site susceptible to attack by a particular restriction endonuclease. For example, pBR322 contains one site in the ampicillin resistance gene susceptible to the endonuclease *Pst*I. A common method of inserting sequences into such linearized plasmids is by G-C tailing. The 3′-ends of the linearized plasmid are extended with 10–30 residues of deoxyguanosine, using the enzyme terminal transferase. The 3′-ends of the ds.cDNA are similarly tailed with the complementary base deoxycytosine, and the two-tailed DNA species thus formed are annealed to form open, circular molecules, held together by G-C base pairing. Bacterial enzymes eventually fill the gaps in the regenerated circular DNA molecule and seal the connection between the inserted DNA and the plasmid DNA.

Incubation of the plasmid and a bacterial host under appropriate conditions leads to the uptake of plasmid by a very small proportion of the bacteria, which can, however, be selectively grown by use of antibiotics as described above. Colonies having the appropriate antibiotic resistance phenotype (i.e. loss of one of the two antibiotic resistance genes) are then screened for the presence of variant specific sequences. Colonies are grown on nitrocellulose filters, and the bacteria are lysed *in situ* under conditions in which the DNA is released, denatured and then bound to the filters. Radioactive probes are prepared by synthesizing cDNA from MRNA isolated from two antigenically distinct variants, and the probes are hybridized to replica filters. Colony lysates containing sequences common to both variants will hybridize to both probes, whereas those containing variant specific sequences will hybridize only to the probe prepared from homologous mRNA. Final proof that such colonies contain sequences specific to the VSG polypeptide is obtained by demonstrating that plasmid preparations bind to VSG mRNA alone. This can be done in two ways. Firstly, total polyadenylated mRNA and purified plasmid can be incubated together under conditions in which the RNA hybridizes to complementary sequences in the DNA. The mixture of mRNA, DNA and mRNA–DNA hybrids is then used to prime *in vitro* message-dependent protein synthesis and the translation products are assayed, by immunoprecipitation and by SDS–polyacrylamide gel electrophoresis, for the presence of pVSG. Since mRNA molecules hybridized to DNA cannot be translated into protein, the absence of pVSG indicates that the plasmid contains pVSG sequences. This is known as hybrid-arrested translation. In the second technique, plasmid DNA is denatured and bound to a solid-phase support, usually chemically activated cellulose. A solution of total polyadenylated mRNA is applied to the cellulose under conditions in which RNA–DNA hybrids can form, and unbound mRNA is removed by washing. Bound mRNA can then be eluted and

translated *in vitro*, and the translation products analysed for pVSG. In this hybrid-selection test, the presence of pVSG, and pVSG only, in the translation assay indicates that the plasmid contains pVSG sequences. The insert can then be characterized with respect to size, the distribution of restriction enzyme sites can be mapped, and the insert can be sequenced if desired.

Such variant-specific cDNA clones can be used to probe the arrangement of the VSG genes in the genome, using a technique known as Southern blotting. Nuclear DNA is digested with a restriction enzyme, or mixture of enzymes, and the resulting DNA fragments are separated according to their size by electrophoresis in agarose. If the digest products are visualized on the gel, they usually appear as a long streak or smear of DNA. This is, however, a ladder composed of many hundreds or thousands of discrete bands, the size of each of which reflects the separation between restriction enzyme sites. This separation clearly is a function of the sequence of the DNA, and of the restriction enzyme used. All the bands can be transferred faithfully from the gel by "blotting" on to nitrocellulose filters, or diazobenzyloxymethyl-(DBM-)cellulose. Those bands containing antigen-specific sequences can be identified by hybridizing radiolabelled variant-specific plasmid DNA to the blot. This approach has now been used by at least four groups, and their results will be discussed in detail below.

8. *The arrangement of VSG genes*

This subject is highly controversial at present. Whereas all groups working on the problem agree that the variant antigen genes can be physically rearranged, there is a disagreement on the significance of such changes. One point of view is that rearrangement produces an additional copy of a variant antigen gene at an altered chromosomal site in trypanosomes expressing that antigen. Antigenic variation occurs when this expression-linked copy is displaced, and replaced by another variant antigen gene copy. The second viewpoint is that the position of the variant antigen genes is highly variable at all times, and that genomic rearrangements are not correlated with expression at all.

Williams *et al.* (1978), as a preliminary to cloning VSG sequences, characterized mRNA activities and their sequence complexities from a clone of *T. b. brucei*. Polyadenylated mRNA was prepared from polysomes, dissociated in SDS and EDTA, by elution from oligo-dT cellulose [cellulose with a mixture of polythymidylic acid chains]. This material was active in translation *in vitro*, and pVSG (M_r 58 000) could be immunoprecipitated from the translation products. The mRNA was further fractionated on 3·5% polyacrylamide gels containing 98% formamide, and mRNAs were eluted from gel fractions and tested by *in vitro* translation. The predominant cell-free translation product directed by fraction 3 from the gel (containing

1·9 to 2·5 thousand bases or kb) was pVSG, indicating that partial purification of VSG mRNA could be obtained in this way. Hybridization studies, using cDNA probes prepared from total cytoplasmic PA$^+$ mRNA and from mRNA from fraction 3, and using homologous PA$^+$ mRNA as driver, supported the cell-free translation data, as it was shown that electrophoresis allowed the purification of a fraction of low complexity RNA. When probe prepared from fraction 3 was hybridized to heterologous PA$^+$ mRNA, isolated from a different variant, the level of hybridization achieved with low complexity RNA was diminished, again suggesting that the purified fraction was enriched in variant specific sequences. Williams *et al.* (1979) therefore used size fractionation of PA$^+$ mRNA to enrich for VSG-specific mRNA used in the preparation of cDNA clones. This enrichment was accomplished by two cycles of density gradient centrifugation. Double-stranded cDNA was prepared on this template, and was inserted into the plasmid vector pBR322 by G-C tailing. Transformed bacteria containing plasmids with VSG-specific inserts were identified by *in situ* filter hybridization, and by hybrid-arrested translation, as described above. In this way, a plasmid was identified containing 902 base pairs of VSG coding sequence, equivalent to about half the length of the antigen molecule. The inserted sequence was excised, labelled by nick-translation, and used as a probe in Southern blots of fragments produced by digestion with the restriction endonuclease *Hin*dIII, of nuclear DNA from four different *T. brucei* clones, A, B, C and X. Clones A, B and C were related, in that A was generated from a relapse infection of clone C, and clone B from a relapse infection of clone A. Clone X was from an unrelated trypanosome stock. The recombinant cDNA clone containing VSG-specific sequences was derived from trypanosome clone A. It was known that the VSG-specific insert contained a single *Hin*dIII site 293 bases from one end hence, if the same site were present in genomic DNA, at least two genomic *Hin*dIII fragments should be observed, hybridizing to the two segments of the probe sequence. This was seen in digests of nuclear DNA from trypanosome clones B and C. One of the fragments was the same size in both clones (4·5 kb), whereas the second contained 7·0 kb in clone B and 8·5 kb in clone C. Clone A contained one strongly hybridizing band of 5·0 kb, and three weakly hybridizing fragments of 11, 8 and 6 kb. Clone X produced one band of 13 kb which hybridized to the probe. The authors state that in similar experiments using genomic DNAs digested with the restriction enzymes *Hin*dII, *Hin*fI and *Xho*I, differences in hybridization patterns were always observed between trypanosome clones A, B, C and X in all cases. These results show that the DNA sequences surrounding the variant antigen gene expressed in trypanosome clone A are different from those in clones B, C and X, which are not expressing the antigen. Moreover, although the gene is present in clones B, C and X, the sequences surrounding

it differ in each clone. Hence there are rearrangements around the VSG genes, which do not correlate with expression.

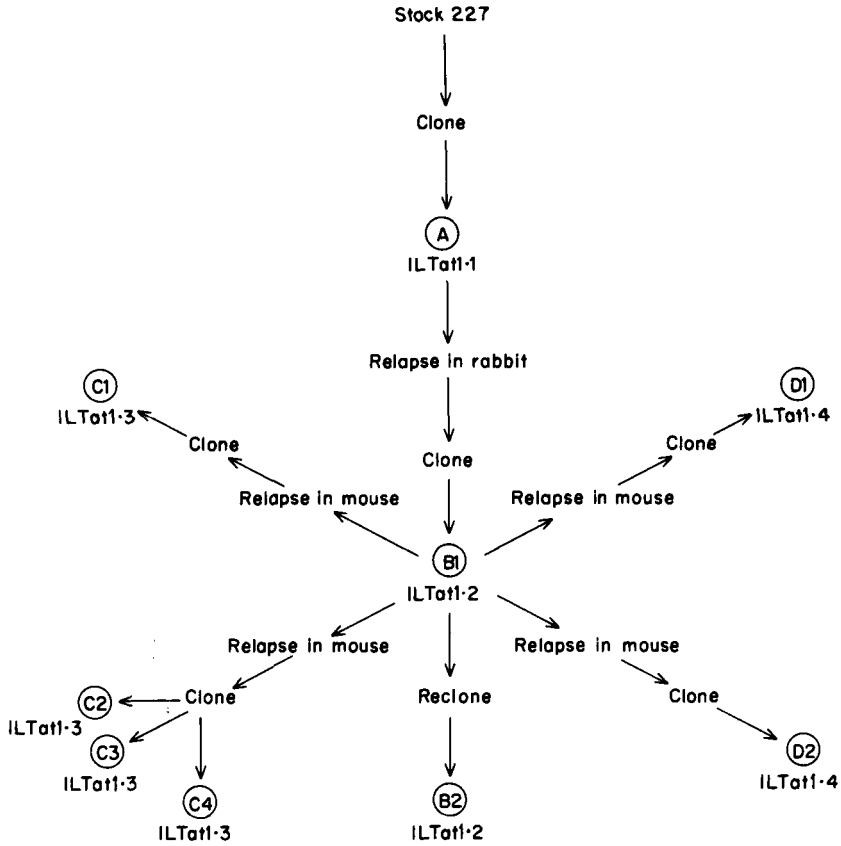

FIG. 7. Derivation of trypanosome clones, derived from stabilate LUMP 227, used by Williams *et al.* (1980a, b) in the analysis of genomic rearrangements of VSG genes. All clones were maintained in lethally irradiated mice or rats.

Williams *et al.* (1980a, b) were able to extend these observations, using a plasmid containing a larger VSG-specific insert (1·53 kb), but of the same variant type (ILTat 1·2; clone A of Williams *et al.*, 1979). Since the nomenclature of trypanosome clones used was altered in the time between publication of these two papers, the derivation and nomenclature of some of the clones used is given in Fig. 7. In addition, clones X and Y (derived from stock 427) were used; the restriction enzyme map of the cDNA insert is given in Fig. 8. From sequencing studies, it was known that the 5′ end of the

coding sequence (i.e. the *N*-terminal end of the protein) was at the left of the *Hin*dIII site shown in Fig. 8. In two trypanosome clones, B_1 and B_2, each expressing the same antigen (ILTat 1·2), the cDNA probe specific for ILTat 1·2 showed that the genomic sequences surrounding the gene were altered. No difference was detectable in the VSG expressed by these two populations, either by serology or by two-dimensional electrophoresis. Since B_2 was recloned from a population of B_1, this suggests a high rate of genomic rearrangement. Since the cDNA insert contained a single *Hin*dIII site, nuclear DNA from B_1 and B_2 digested with this enzyme should produce two bands of hybridization. This was so with clone B_1, but B_2 showed three bands. A similar result was obtained with clone C_1, which also produced three fragments hybridizing to the whole plasmid. When fragments of the cDNA insert were used as probe, those on the 5′ side of the *Hin*dIII site (fragments 2 and 4 in Fig. 8) hybridized only to a 1·1 kb fragment in digests of nuclear DNA of clones C_1 and B_2, whereas all the plasmid fragments (1, 3 and 6 in Fig. 8) prepared from the 3′ side of the site hybridized to both of the larger genomic fragments from C_1 and B_2. Since all three plasmid fragments hybridized to both genomic fragments, the presence of an intron containing a *Hin*dIII site could be eliminated, and the conclusion therefore was that in each cell clone, one expressing and one not expressing this gene, there were two copies of the ILTat 1·2 gene, with *Hin*dIII sites at different distances from the 3′ end of the coding sequence. The 1·1 kb 5′ genomic fragment is common to both copies, and therefore produces one band of greater intensity in the blot.

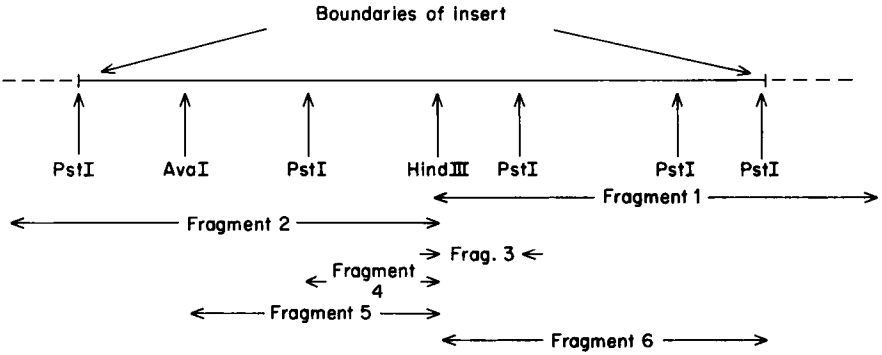

FIG. 8. Restriction map of the VSG-specific insert in plasmid PCB1352, used by Williams *et al.* (1980a, b) in the analysis of genomic rearrangements of VSG genes. The cDNA insert in this plasmid contains 1·53 kb. The derivation of the restriction fragments used in the analysis is also shown.

Heterogeneity of the sequence at the 3′ end of the gene was shown in other trypanosome clones. In each case, digestion with *Hin*dIII produced one band of 1·1 kb, which hybridized with fragments from the 5′ end of the

insert, and a number of larger bands which hybridized to fragments prepared from the 3′ end. Three different isolates of variant antigen type ILTat 1·3, trypanosome clones C_1, C_2 and C_3, each produced different patterns of hybridization to 3′ fragments, and a constant 1·1 kb band. The analysis was extended to a number of trypanosome clones, using many different restriction enzymes. The results fell into three categories. Firstly, enzymes that cleaved the cDNA sequence (*Xho*I, *Mbo*I, *Ava*I and *Ava*II) produced results similar to those obtained with *Hin*dIII. In each case a constant fragment was produced, common to each trypanosome clone which, however, varied in size with each enzyme. In addition, one or two larger hybridizing fragments were produced, the size of which varied in each trypanosome clone and with each restriction enzyme. However, the size *difference* between these large fragments was constant, irrespective of the restriction enzyme used. A second group of enzymes which did not cut the cDNA sequence (*Hgi*AI, *Sal*I, *Pvu*I) each produced two genomic fragments, and again although the size of the fragments varied, depending on the enzyme and the trypanosome clone used, the difference in size between the fragments was the same as that observed between the large variable fragments seen with the first group of enzymes. Since the data obtained using fragments of the cDNA probe to hybridize to genomic *Hin*dIII fragments showed that the variable fragments differed at the 3′ ends, it seems that all the observed differences seen with both groups of enzymes were confined to this end of the gene. A third group of enzymes (*Pvu*II, *Eco*R1, *Bam*H1 and *Bgl*II) all produced such large fragments (>20 kb) that different gene copies could not be resolved.

Such results could be explained by the presence of two closely related, but different genes showing extensive cross hybridization even under the stringent conditions employed, but Williams *et al.* (1980a, b) preferred the possibility that two alleles in a diploid genome were being observed. What could be the nature of the observed rearrangements? If the entire gene was moved to a new site, then the flanking sequences at *both* ends of the gene would be expected to change. No change was observed at the 5′ end, but because of the size range of fragments hybridizing to 5′ cDNA fragments, the possibility that there were sequence rearrangements at a distance greater than 1·5 kb "upstream" from the 5′ end of the cDNA sequence could not be ruled out. Since different enzymes produced identical size differences among genomic fragments at the 3′ end of the gene, the data are consistent with the insertion and deletion of lengths of DNA between the 3′ end of the cloned cDNA sequence, and a group of restriction enzyme sites. The cDNA probe did not contain the *C*-terminus of the coding sequence, hence it is not possible to determine from these results whether the alterations take place within the coding sequence. The possible site of insertion could be

only very roughly mapped, and the proposed model is illustrated in Fig. 9. Again there appears to be no correlation between rearrangement and expression, and hence no obvious mechanism for antigenic variation is suggested by these results.

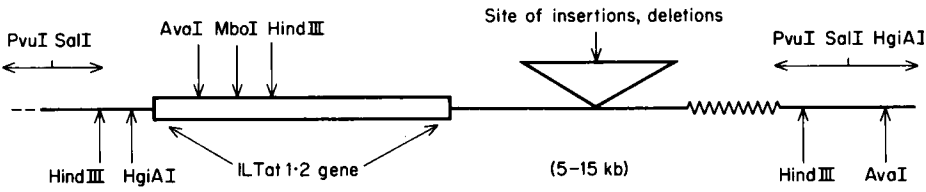

FIG. 9. Proposed genomic context of VSG ILTat 1·2, deduced by Williams *et al.* (1980, in press), showing the approximate location of a "hot spot" for insertions and deletions between the 3′ end of the coding sequence and a group of restriction enzyme sites 5–15 kb from that end of the gene.

Marcu and Williams (1981) presented preliminary results obtained with cDNA clones specific for other VSGs of the same serodeme used in earlier studies (Williams *et al.*, 1980a, b). A cDNA clone specific for ILTat 1·3 (expressed by trypanosome clones C1–C4 in Fig. 7) gave results comparable to those obtained above, in that 4–6 bands were observed in different cell clones, with four of these being variable in all clones. In contrast, cDNA clones specific for ILTat 1·1 (trypanosome clone A) and ILTat 1·4 (clones D1 and D2) hybridized to 4 and 3 bands, respectively, in all trypanosome clones, but the size of the bands was constant, irrespective of which member of the serodeme was examined.

Hoeijmakers *et al.* (1980a) described the preparation of cDNA clones specific for VSGs of variants first isolated by Cross (1975) and Johnson and Cross (1979). Polyadenylated mRNA was isolated from cloned trypanosomes by phenol extraction and oligo-dT cellulose chromatography. *In vitro* translation and immunoprecipitation of translation products with anti-VSG serum showed that such preparations contained variant-specific mRNA, and that the only major difference in the translation products produced by mRNA from each trypanosome clone was in the pVSGs. The mRNA preparations were used to prime cDNA synthesis, and cDNA clones were constructed in plasmid vector pBR322 by G-C tailing, as described above. Bacterial colonies containing VSG-specific recombinant plasmids were identified by *in situ* colony hybridization, using cDNA probes prepared from homologous and heterologous mRNA, and were further characterized by hybrid selection of VSG-specific mRNA. To determine the extent of sequence homology in the cloned VSG cDNAs of the different variants, alkali-fragmented PA^+ mRNA of each variant, labelled *in vitro* with polynucleotide kinase, was hybridized to the recombinant plasmids. Hybridization was observed only in homologous com-

binations of plasmid and mRNA, implying little sequence homology between the cloned sequences of the different variants. The size of the largest cloned cDNA inserts is compared with the size of the homologous VSG mRNA and protein for each variant in Table 12. It can be seen that the cDNA inserts are between one-half and two-thirds the size of VSG mRNA. Since the inserts hybridized well to homologous cDNA of limited size, synthesized on PA$^+$ mRNA primed with oligo-dT, and therefore predominantly containing 3′ sequences, the inserts presumably covered the 3′ ends of the VSG mRNA, equivalent to the *C*-terminal of the protein. Hence, the lack of hybridization between heterologous combinations of plasmid and PA$^+$ mRNA-primed cDNA indicates that there is no conserved sequence domain comparable to that found in immunoglobulin, in this region of the molecule. The evidence for a conformational domain in the *C*-terminus was discussed earlier in this review (Section III B1, p. 84), as was the extent of sequence homology (Section III B2, p. 89). The fact that the screening method used was so successful clearly indicates that VSG synthesis is not under translational control—only one species of VSG-specific mRNA seems to be present in cloned trypanosome populations.

TABLE 12

Comparison of the size of cloned VSG-specific cDNAs with the size of the homologous mRNA, pVSG and VSG (from Hoeijmakers et al., *1980a)*[a]

Variant	Molecular weight of: pVSG	VSG	Size of VSG-specific: mRNA (no. of nucleotides)	cDNA insert (no. of base pairs)
117	62 000	61 000	2250	820 (1600)
118	57 000	58 000	2050	1500 (1600)
121	59 500	58 500	2150	800 (1150)
221	52 000	53 000	1950	1200

[a] The size of the VSG was determined by SDS–polyacrylamide gel electrophoresis, and that of the pVSG by SDS–polyacrylamide gel electrophoresis of the translation product of variant-specific mRNA isolated by hybrid-selection using variant-specific cDNA clones. Size of the mRNA was determined by size fractionation of glyoxal-treated PA$^+$ mRNA by gel electrophoresis, transfer to DBM paper, and detection of variant-specific mRNA by hybridization to variant-specific cDNA plasmids. The variant-specific cDNA clones were first isolated by differential hybridization to homologous and heterologous [^{32}P]cDNA, and the size of the inserts was measured by gel electrophoresis. Sizes refer to the largest cDNA inserts proved to be variant-specific by hybrid-selection of variant-specific mRNA. Figures in parentheses refer to the size of the largest variant-specific cDNA clones contained in the collection detected by hybridization to variant-specific plasmids identified by hybrid selection.

Having thus carefully characterized cDNA clones specific for four different antigenic types, Hoeijmakers *et al.* (1980b) were able to use them in an

analysis of genomic rearrangements occurring during antigenic variation, using the technique of Southern blotting. The results obtained differ significantly from those of Williams *et al.* (1979, 1980a, b). When variant-specific cDNA probes were hybridized to restriction enzyme digests of nuclear DNA from *T. b. brucei* variant 118, each cDNA was shown to hybridize to a different set of bands. Two of the probes (specific for 118 and 121) hybridized mainly to one or two bands in each digest, indicating that they were primarily recognizing one gene, whereas the other two probes (specific for variants 117 and 221) hybridized to many different bands, indicating cross hybridization to related VSG genes. Under more stringent washing conditions, many of these bands could be removed. This aspect of the work will be discussed at more length below. Having shown that each probe recognized different genes (i.e. hybridized to different bands), the hybridization of cDNA probes to fragments from homologous and heterologous nuclear DNA was studied. In each case, cDNA probes recognized an additional band in digests of homologous DNA, compared to heterologous DNA. For example, the 118 cDNA probe recognized one band in digests of 117 nuclear DNA with *Pst*I, *Eco*R1, or *Hin*dIII, whereas hybridization to comparable digests of 118 nuclear DNA revealed two bands. In *Bam*H1 digests, the 118 probe recognized three bands in the homologous and two in the heterologous digest. In all, the 118 cDNA probe recognized an extra band in digests performed with seven different restriction enzymes. Similar results were obtained with 117 and 121 cDNA probes, although digests prepared with two enzymes did not produce extra bands in the homologous nuclear DNA. The presence of extra bands implied the presence of an extra gene copy in which the sequences flanking the gene were altered, and hence produced different restriction fragments. Enzymes which did not recognize an additional copy must therefore have been cutting at a site between the sequence recognized by the probe, and the altered sequence. Since the extra gene copy was found only in homologous combinations of cDNA probe and nuclear DNA, it must presumably be related to the expression of the homologous VSG, and it was therefore christened the expression-linked copy. It will be recalled that no such change associated with expression was observed by Williams *et al.* (1979, 1980a, b).

A second difference is that, whereas Williams *et al.* (1979, 1980a, b) observed genomic rearrangements in comparisons not only of homologous with heterologous variants, but also in different heterologous variants and even in different clones of the homologous variant, Hoeijmakers *et al.* (1980b) reported no such non-expression linked alteration in comparisons of different heterologous variants. Thus the same fragments were seen to hybridize with 117 cDNA probes in digests of 118, 121 and 221 nuclear DNA. Different isolates of the homologous variant were not examined. Williams *et al.*

(1980a, b) could interpret their data only on the basis of the presence of two slightly differing copies of each VSG gene, both in expressing and non-expressing clones of trypanosomes; however, Hoeijmakers *et al.* (1980b) showed that restriction mapping of the basic copy of the 118 gene was consistent with the presence of only one basic 118 VSG gene, containing no intron. The expression-linked and basic copies seem to be present in equal numbers, based on hybridization intensity, suggesting one expression-linked and one basic copy per nucleus if trypanosomes are haploid and two if diploid. Recent evidence (Tait, 1980) suggests that trypanosomes are diploid.

How can an expression-linked copy be generated? Either a copy of the gene must be transposed into an expression site, or an insertion sequence could be transposed to flank an extra copy. The first model has a precedent. Control of yeast mating type expression proceeds through such a cassette mechanism (Hicks *et al.*, 1979), although yeast mating types are confined to two recognizable phenotypes as opposed to 100–1000 in trypanosomes. Clearly a structural analysis of the sequences surrounding the basic and the expression-linked copies would be extremely informative. The results of Hoeijmakers *et al.* (1980b) are summarized schematically in Fig. 10.

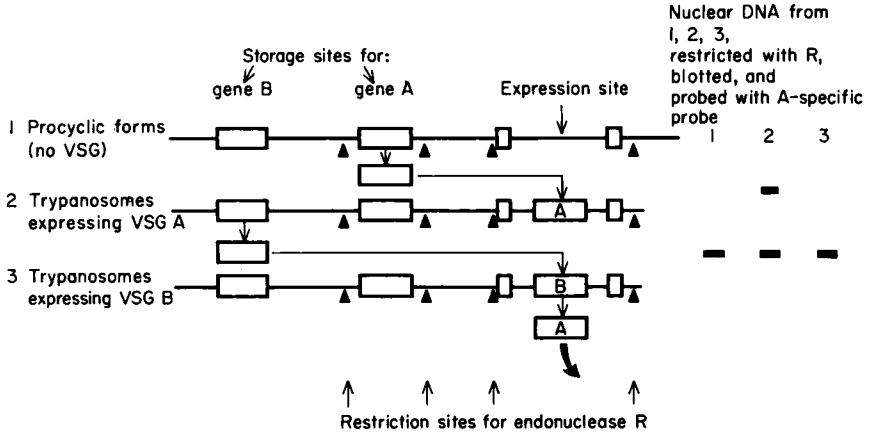

FIG. 10. Model showing how transposition of VSG gene can generate the "expression-linked copy" of the VSG gene, observed by Hoeijmakers *et al.* (1980b) and Pays *et al.* (1981a).

Some restriction mapping studies on the sequences surrounding basic and expression-linked copies have been reported (Borst *et al.*, 1980a, b; Frasch *et al.*, 1980). The 118 gene was chosen for study as it produced the simplest hybridization patterns. The maps are shown in Fig. 11. It can be seen that the cDNA sequence is uninterrupted in both copies, and that both genes

probably contain the complete mRNA sequence (but see Boothroyd *et al.*, 1981). Hoeijmakers *et al.* (1980b) had suggested that generation of the expression-linked copy could provide the 5′-leader sequence and signal peptide needed for each VSG, but this does not seem to be so in this case. The 3′ and 5′ flanking sequences do differ, in the basic and expression-linked copies, and the differences support the interpretation that the expression-linked copy is generated by transposition of a duplicate of the basic copy into a new location. Borst *et al.* (1980b) reported that preliminary studies on the expression-linked copies of the 121 and 221 genes showed that the sequences surrounding the expression-linked copies are not conserved. Hence, a simple cassette model, with one expression site, can be ruled out.

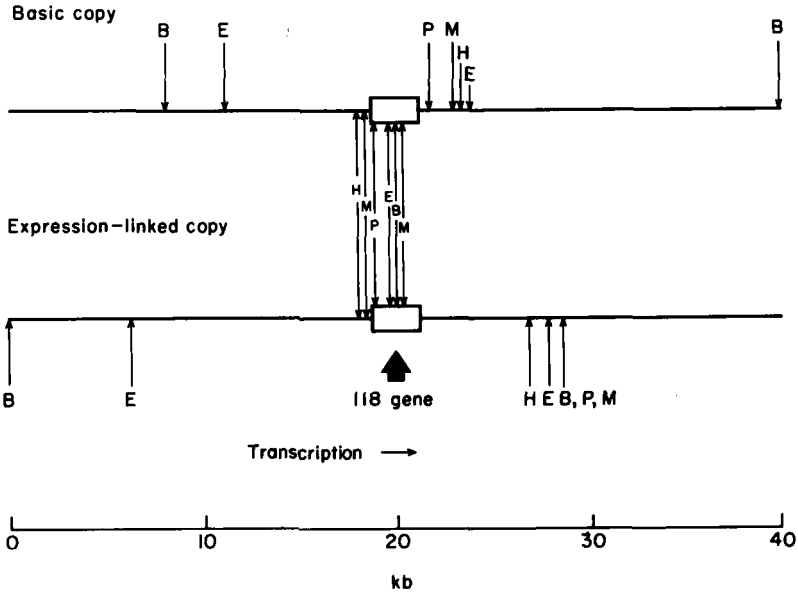

FIG. 11. Restriction endonuclease map of the basic and expression-linked copies of the VSG 118 gene. Sequences on both sides of the gene are altered in the expression-linked copy relative to the basic copy. This is most consistent with a gene transposition model. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; M, *Msp*I; P, *Pst*I. (From Borst *et al.*, 1980.)

Investigation of the conditions under which multiple genes can be recognized by a single cDNA probe (e.g. 117 and 221 cDNA probes; Hoeijmakers *et al.*, 1980b) has yielded much information on familial relationships in VSG genes, and on the evolution of the VSG genes (Borst *et al.*, 1980a, b; Frasch *et al.*, 1980). Again, the 118 genes were examined, since under less stringent hybridization conditions, 118 cDNA probes hybridized to many more

bands than were seen under standard conditions. Probes were prepared which recognized the 5′ or 3′ end of the gene, taking advantage of the presence of restriction enzyme sites within the cDNA insert. The multiple bands observed after low stringency hybridization were recognized only by 3′ probes (corresponding to the *C*-terminus of the VSG). A comparison of the intensity of hybridization with 5′ and 3′ probes revealed that the family of genes recognized contained, not identical 5′ ends and diverse 3′ ends, but rather a related set of 3′ ends associated with 5′ ends so diverse that no homology could be detected by hybridization. Similar results were obtained with the 221 family of genes. It should be noted that the conserved sequences were "family specific"—that is they were recognized by homologous probes only. The exact location of such sequences has not been mapped, although Borst *et al.* (1980b) point out that a 117 cDNA probe lacking the coding sequence for the last 29 *C*-terminal amino acids and the 3′ non-translated bases still recognized the 117 gene "family". Assuming that the VSG gene segments detected using 3′ probes recognizing the 3′ half are part of complete and functional VSG genes (and this has not been rigorously proven), Borst *et al.* (1980a, b) proposed that VSG genes evolved by gene duplication followed by sequence divergence caused by mutation. The 5′ half of the genes, corresponding to the *N*-terminus of the VSG, evolves faster, presumably because this part of the molecule is exposed to the immune system, and the 3′ half, containing the membrane binding site and carbohydrate attachment sites, is constrained to evolve more slowly. Borst *et al.* (1980a, b) then asked whether new VSG genes were still being generated by duplication, and if so, whether old genes were being discarded. Different stocks of *T. brucei* and *T. evansi* were examined for the presence in their genome of genes belonging to the 118, 121 or 221 "families" of VSGs. Probes recognizing the 5′ half of the 221 gene failed to recognize any sequence in stocks other than that from which this variant was isolated, whereas the large number of sequences hybridizing to 3′ probes was more or less conserved. In contrast, the 118 gene appeared to be present intact in some stocks and absent from others, as judged by hybridization with 5′ probes. All the strains contained segments of the 3′ half family, some of which were not detectably altered. An interesting result was obtained with 121 probes. In contrast to 117 or 118, 121 probes hybridized to multiple genes (3 or 4) in all stocks, even under stringent conditions. Since it was shown that in this case, 5′ fragments hybridized to more fragments in blots than 3′ probes, the 121 genes must represent a stable family of closely related genes, generated by gene duplication. Thus gene duplication still appears to be continuing, and is probably balanced by deletions. Since those genes which are present in different stocks appeared to be little altered (the restriction sites in and around the gene were largely conserved), Borst *et al.* (1980a, b)

proposed that the timescale of the evolution of the VSGs is very much greater than the timescale of the evolution of the different trypanosome stocks tested.

Boothroyd *et al.* (1981) have recently published the sequences of cDNA clones encoding the *C*-terminus of three VSGs, the hydrophobic *C*-terminal extension, and the 3′ non-translated bases. The presence of substantial homology was noted, extending from the last three residues of the hydrophobic tail through the non-translated bases. Preliminary (unpublished) results suggested that by juxtaposing such homologous sequences with otherwise complete VSG-genes, active VSG genes could be generated. Such a result would be consistent with the R-loop mapping results of Borst *et al.* (1930b), in which a genomic clone containing a 117 basic gene was hybridized to 117 PA^+ mRNA. The hybrid formed was continuous (confirming the absence of introns) but with a long single-stranded RNA tail of about 310 nucleotides, mapped to the 3′ side of the gene. The existence of such a long tail could indicate the absence of 3′ terminal sequences in the basic copy which are present in the mRNA. A similar result has been reported by Marcu and Williams (1981), who state that as yet they have been unable to identify genomic clones containing complete genes. Mapping studies, to determine whether the 5′ half or 3′ half is missing, have not been reported.

Lheureux *et al.* (1979) purified VSG-specific PA^+mRNA from *T. b. brucei* by immunoprecipitation of polysomes. This approach to the purification of VSG-specific mRNA has been successfully used by a number of groups (Cordingley and Turner, 1980; Shapiro and Young, 1981; Merritt, 1980). The VSG mRNA was found to be associated with membrane-bound polysomes, and a 15-fold immunological purification of this mRNA was obtained, using partially purified anti-VSG IgG and inactivated *Staphylococcus aureus* Cowans strain A. VSG-specific mRNA purified in such a way from two variants was used to prime cDNA synthesis, and ds.cDNA was inserted into pBR 322 both by G-C tailing, and by blunt-end ligation. Two clones were selected, one containing a 1700 bp insert derived from variant AnTat 1·1, the other a 1770 bp insert from AnTat 1·8 (Pays *et al.*, 1980). Hybrid arrest and hybrid selection were used to confirm that the inserts were variant-specific. Neither cross-hybridization nor restriction mapping of these sequences revealed the existence of common segments. The inserts were comparable in size to their respective mRNAs, indicating that the cloned VSG sequences were complete, or nearly so. These clones were then used in an analysis of genomic rearrangements.

Pays *et al.* (1981a) examined the arrangement of the AnTat 1·1 (abbreviated to A.1) and AnTat 1·8 (A.8) genes within the genome of both homologous and heterologous trypanosomes, and observed both expression-linked copies and genomic rearrangements which were not expression-linked. Each of the two

variant-specific probes showed several bands when hybridized to Southern blots of nuclear DNA from four trypanosome clones expressing the antigenic types A.1, A.8, A.3 and A.13, even under the most stringent conditions of hybridization. The number of bands observed was small (five for A.1 and six for A.8 in *Pst*1 digestions), and in this respect the A.1 and A.8 genes seem to be intermediate between the 118 gene (1 copy) and the 117 and 221 genes (multigene families) of Hoeijmakers *et al.* (1980b). Both A.1 and A.8 probes recognized an extra band in the DNA of trypanosomes of the homologous antigenic type, and since in other respects the hybridization pattern was conserved, the extra band was presumed to contain the expression-linked copy of the basic gene. From the known restriction enzyme map of the A.1 and A.8 probes, the production of internal fragments of about 500 bp could be predicted by digestion with *Hin*dIII in the case of A.1, and by a double digest with *Pst*I plus *Sal*I in the case of A.8. Such fragments were observed in DNA digests of all four variants tested, and were present at approximately twice the intensity in digests of the homologous variant. This showed, firstly, that the same internal restriction enzyme sites were present in the expression-linked copy, and that there is probably one additional expression-linked copy per basic gene in both cases. Analysis of two different isolates of trypanosomes of type A.1 revealed that probably the same basic copy of the gene was duplicated and transposed at the same expression site, although slight differences in the sequences surrounding the expression-linked copy in each isolate were noted. Additional gene copies not correlated with expression were also observed. However, in contrast to the basic and expression-linked copies, these could be removed under more stringent hybridization conditions.

Use of 3′ specific probes revealed that the 3′ end of the coding sequences of both variants contained a repetitive element, as observed by Borst *et al.* (1980a, b). Again, the repetitive elements appeared to be variant-specific, because although the 3′ probes hybridized with many sequences present in nuclear DNA, they specifically hybridized with homologous mRNA only. The exact location of the repetitive element within the cDNA clone, with respect to the mRNA transcript, was not reported. The sequences recognized by 3′ probes were rearranged in different variants, thus resembling the non-expression linked rearrangements which could be seen using some probes under less stringent hybridization conditions. The effect of altering the hybridization conditions on the sequences recognized by 3′ probes was not discussed.

Pays *et al.* (1981a) demonstrated that procyclic forms generated by *in vitro* culture of A.1 bloodstream trypanosomes did not contain the expression-linked copy of the A.1 gene. Such procyclic forms lack a surface coat, and are analogous to the forms which develop in the midgut of the tsetse fly

after ingestion. Since no surface coat is detectable on any of the developmental stages of the trypanosome found in the insect until the appearance of infective metacyclic forms in the salivary glands, some 15–40 days after ingestion, clearly the deletion of an expression-linked copy can be dissociated from the expression of the next antigenic type. By using probes specific for different parts of the A.1 coding sequence, it was possible to assess which of the sequences hybridizing to probes specific for A1 contained the complete A.1 gene. Of the six major *Pst*I fragments detected with A.1 probes in digests of A.1 nuclear DNA, only two seemed to contain a complete sequence—a 6·4 kb fragment and the 2·0 kb expression-linked copy. The 6·4 kb fragment was therefore judged to contain the true basic copy of the A.1 gene. Pays *et al.* (1981b) then isolated nuclei from trypanosomes expressing antigen A.1, and submitted them to mild digestion by DNAse I to determine which of the genomic sequences specific for A.1 were susceptible to treatment by this enzyme. After digestion, the nuclear DNA was extracted with phenol, digested with *Pst*I, and Southern blots were hybridized with probes specific for A.1. It was found that the 2·0 kb genomic fragment was preferentially digested with DNAse I. The only firmly established difference between active and inactive chromatin in eukaryotes is that the transcribed sequences present in active chromatin are preferentially digested by DNAse I, hence it can reasonably be assumed that the expression-linked copy of the VSG gene is in an active configuration and is therefore the one transcribed.

Agabian *et al.* (1980a) prepared cDNA clones specific for two variants, termed A and D, of the ISTAR-1 serodeme. When digests of nuclear DNA from variants A and D were analysed by hybridizing D-specific probes to Southern blots, an additional fragment, interpreted as the expression-linked copy, was detected in the homologous DNA. This is compatible with the findings of Borst and his colleagues (1980a, b), and Pays *et al.* (1980, 1981a, b). However, the same cDNA probe, when used to analyse D-antigen gene arrangements in five other sequential variants produced in a chronically infected mouse, revealed that the gene was present in different genomic contexts in several instances—i.e. gene D showed both expression-linked and non-expression-linked rearrangements (Agabian *et al.*, 1980b). In contrast, the authors stated that the gene for antigen A showed no rearrangement under any conditions (it is not clear whether this included the absence of an expression-linked copy of A in homologous nuclear DNA). In an important control, a tubulin-specific cDNA clone was isolated, and it was shown that the environment of the tubulin genes remained constant during both antigenic variation and the trypanosome life cycle. The genomic rearrangements were probably limited therefore to variant antigen genes. A similar result has been obtained using probes specific for rRNA genes, which also do not rearrange (G. Hasan, M. J. Turner and J. Cordingley, unpublished observations).

It can be seen that the picture is confusing. What conclusions can be drawn regarding the mechanism of antigenic variation? Firstly, consider the paradoxes—in particular, why is the expression-linked copy not found in all cases? Is it a function of the ploidy of the trypanosomes? Isoenzyme analysis (Tait, 1980) of the variants used in different laboratories would be useful in this regard. Perhaps there are mechanisms other than the generation of an expression-linked copy which can activate VSG genes. In variants which do not show an expression-linked copy, are any of the gene copies susceptible to the action of DNAse I? If a particular copy could be shown to be transcribed in variants expressing that gene but not in non-expressing variants, this would at least provide an assay for the active gene, allowing a search for the factors involved in its activation. As Borst *et al.* (1980b) have pointed out, the generation of an expression-linked copy may not be the only way in which a VSG gene can be activated. It is, of course, much easier to construct a model for the mechanism of antigenic variation that involves the generation of an expression-linked copy, but even so there are anomalies in the data. For example, Hoeijmakers *et al.* (1980b) reported that probes specific for variant 121 recognized an additional expression-linked copy in homologous nuclear DNA, but also recognized a different extra gene in nuclear DNA from variant 221. Furthermore, probes specific for variant 221 recognized more than one gene in heterologous nuclear DNA, and these genes were differently arranged in different variants; in homologous 221 DNA, however, only a single gene was recognized (Borst *et al.*, 1980b). All groups reported non-expression-linked rearrangements, and, where analysed, there was agreement that these rearrangements occurred at the 3′ end of the gene. From the data of Boothroyd *et al.* (1981) it seems that activation may involve the fusion of a sequence containing the *C*-terminus and 3′ untranslated bases of the VSG mRNA to an inactive, incomplete copy of the gene. Abortive rearrangements could be envisaged, in which the incorrect sequence is inserted, to account for non-expression-linked rearrangements. The nature of the events which then lead to the expression of a correctly completed gene must still remain open to conjecture. Recent results of Matthyssens *et al.* (1981) also imply that active VSG genes are generated by juxtaposition of incomplete basic copy genes with sequences encoding the hydrophobic *C*-terminus and 3′ untranslated bases in an expression site.

c. *Trypanosoma brucei gambiense*

Little work has yet been reported on the variant antigens of *T. b. gambiense* and *T. b. rhodesiense*, because of the natural preference of investigators to work with non-human-infective material. *T. b. gambiense* is certainly

worthy of investigation, since it displays a much more limited range of antigenic types in natural infections than does *T. b. brucei.* Gray (1972) obtained isolates of *T. b. gambiense* from different areas of Nigeria, and noted that all the isolates produced many antigens in common after adaptation to growth in rodents. Gray concluded that all the isolates could be grouped as a single strain. Extension of this work to isolates from Zaire, Senegal and Uganda revealed that trypanosomes from all four locations seemed to express common antigenic repertoires. At the very least, the same "predominant" antigenic types were expressed (Gray, 1974). The occurrence of such common predominant types has allowed the development of a very elegant diagnostic test for *T. b. gambiense*, the Card Agglutination Test, which detects circulating antibody specific for a small range of predominant variants in patients' serum (Magnus *et al.*, 1978). If the repertoire of variants is more stable in *T. b. gambiense* it may be easier to determine the mechanism of antigenic variation, and we may expect to see an analysis of genomic rearrangements during antigenic variation in *T. b. gambiense* in the near future. Indeed, Merritt (1980) has described the purification of VSG-specific mRNA from *T. b. gambiense* by immunoprecipitation of polysomes.

Dodin and Fromentin (1962) reported the presence of soluble "exoantigen" in the serum of animals infected with *T. b. gambiense*, analogous to that found in *T. b. brucei* by Weitz (1960). Seed (1972) described the preparation of variant specific protective antigens from relapse populations of *T. b. gambiense*, by ion-exchange chromatography of an ammonium sulphate fraction prepared from sonicated trypanosomes. No biochemical analysis was performed. Osaki *et al.* (1979) produced protective immune responses in mice immunized with antigens from mouse blood infected with *T. b. gambiense*, and described the production of plasmanemes. Vervoort *et al.* (1981) prepared VSG from *T. b. gambiense* clones by the method of Cross (1975). Biochemical analysis showed a close resemblance to the VSGs of *T. b. brucei* (see p. 140). Two VSGs were isolated, both of which had a molecular weight of 60 000 under reducing conditions, but one of which appeared as a disulphide-linked dimer in the absence of 2-mercaptoethanol.

D. *Trypanosoma brucei rhodesiense*

Seed and Weinman (1963) demonstrated the presence of strain-specific antigens in the serum of rats infected with *T. b. rhodesiense*, and achieved partial purification by ammonium sulphate fractionation and elution from calcium phosphate gels. It was concluded that the fraction producing strain-specific immunity was a protein. Brown and Williamson (1962, 1964) fraction-

ated homogenates of *T. b. rhodesiense* into particulate and soluble fractions, and concluded that the cytoplasmic fraction contained highly immunogenic material, capable of giving strain-specific protection. This activity was associated with material of sedimentation coefficient 4S. The relationship between 4S antigen and exoantigen in *T. b. brucei* was reported by Allsopp *et al.* (1971) and Njogu and Humphryes (1972), and was described earlier in this review. Campbell *et al.* (1979) described the isolation and characterization of a new serodeme of *T. b. rhodesiense*. Thirteen distinct VATs were isolated. Variant-specific antisera were raised by immunization either with irradiated trypanosomes, or with a saline extract prepared by incubating isolated trypanosomes in 0·9% NaCl containing 50 μM 2-mercaptoethanol, buffered to pH 5·6. After 21 hours at 0°C, the suspension was centrifuged at 800 *g* for 20 minutes, and the supernatant was used as an immunogen after Millipore filtration. The authors stated that at the end of the low pH incubation the trypanosomes were intact but non-motile. Saline extracts produced much better variant-specific antibody than did irradiated trypanosomes. Similar low pH extractions have been used in the preparation of VSG from *T. equiperdum*, and will be discussed later. No chemical study of the acid-soluble material was reported. Olenick *et al.* (1981) described the isolation of VSG from *T. b. rhodesiense* variants, using affinity chromatography of similar acid-soluble antigens on Con A-Sepharose. A set of clones was prepared from stabilates drawn from a rabbit with a chronic infection initiated with a clone of *Trypanosoma rhodesiense*. VSGs were isolated either by solubilization overnight at pH 5·6, followed by lectin affinity chromatography, or by taking the supernatant from frozen and thawed trypanosomes, passing it down a DEAE-Sephacel column, and applying the unretarded material to a column of Con A-Sepharose. Preparations from four clones gave only one band on SDS–polyacrylamide gels, both under reducing and non-reducing conditions, with molecular weights ranging from 58 000 to 67 000. Each glycoprotein showed charge heterogeneity on isoelectric focussing, irrespective of the precautions taken to avoid proteolysis and of the original extraction procedure. Amino acid compositions were comparable to those obtained for *T. brucei* VSGs (Cross, 1975). Immunization with each glycoprotein gave clone-specific protection.

Lyon *et al.* (1981) demonstrated that all the major components of such charge heterogeneous preparations of *T. rhodesiense* VSG could be immunoprecipitated by a single monoclonal antibody. Similar findings have been reported with variants of *T. brucei* (Pearson and Anderson, 1980). All the monoclonal antibodies reacted by immunofluorescence with acetone-fixed smears of the variant studied; hence it was concluded that the charge heterogeneity was derived not from heterogeneous populations of parasites, but from post-translational modification or limited proteolysis. Further,

at least two different epitopes were recognized by the monoclonal antibodies tested, since only one antibody could react with living trypanosomes. Analogous results obtained with *T. b. brucei* were discussed earlier in this review (Pearson *et al.*, 1980). Vervoort *et al.* (1981) described the isolation of ^{125}I-labelled *T. b. rhodesiense* VSG after surface labelling intact trypanosomes. The procedure of Cross (1975) was followed, yielding VSG of molecular weight 60 000 (see below).

E. *Trypanosoma evansi*

Gill (1965) reported the presence of soluble antigen in the plasma of rats infected with *T. evansi*, and noted that immunization of mice with infected rat plasma produced some protection against reinfection with trypanosomes of the same stock. Cross (1977) purified VSG from two clones of different strains of *T. evansi* (both akinetoplastic, and so perhaps more properly termed *T. equinum*). In one case, the VSG was isolated as disulphide-linked dimers. Each subunit was about the size of a *T. brucei* VSG, although subsequently (Cross, 1979b) it was shown that SDS–polyacrylamide gel electrophoresis under reducing conditions yielded a major band at either 64 000 or 45 000 daltons, depending on the fraction separated by isoelectric focussing. The second clone of *T. evansi* examined expressed a VSG immunochemically indistinguishable from that of two clones of *T. b. brucei*. Such serologically indistinguishable variants are known as isotypes or isoVATs, and will be discussed below. VSG from the two clones of *T. b. brucei* was readily isolated, and although the amino acid compositions were similar, the isoelectric points were not. In contrast, the yield of soluble VSG from the *T. evansi* clone was very low. The amino acid composition was very similar to that of the two *T. b. brucei* VSGs, but the isoelectric point could not accurately be determined. When the preparation was repeated using surface-labelled cells, it was apparent that most of the VSG was not released following disruption of the trypanosomes. Electron microscopy confirmed that the surface coat had been disorganized, but not released. The *T. evansi* VSG, although immunochemically indistinguishable from the *T. b. brucei* VSGs, therefore must have unique structural features. Vervoort *et al.* (1981) also isolated VSG from two clones of *T. evansi*, and noted that whereas both had molecular weights of 60 000 on SDS–polyacrylamide gel electrophoresis under reducing conditions, one was a disulphide-linked dimer in the absence of 2-mercaptoethanol (see below).

F. *Trypanosoma equiperdum*

T. equiperdum is interesting insofar as more variants have been detected in a single stock of this species (101) than in any other (Capbern *et al.*, 1977).

Baltz *et al.* (1976) isolated VSG from three clones of *T. equiperdum*. Trypanosomes were purified from infected rat blood by the method of Lanham and Godfrey (1970), and after centrifugation, the pellet of trypanosomes was suspended in 6 volumes of 0·12 M phosphate buffer, pH 5·5, containing 1% glucose. After gentle agitation for 16 hours at 4°C, the trypanosomes were centrifuged, the supernatant fraction containing soluble VSG was applied to a column of Con A-Sepharose, and glycoproteins were eluted with 2% methylmannoside. Interestingly, some of the glycoprotein could be eluted only in the presence of 0·2% Triton X100. Does this represent material containing a hydrophobic *C*-terminal peptide? Such findings underline the need emphasized earlier for a careful characterization of all the forms of the VSG present on the cell membrane. It might also be interesting to know what proportion of the VSG is solubilized by this unusual low pH treatment. All three glycoproteins had monomer subunit molecular weights of about 65 000, but one existed as a disulphide-linked dimer. Again, charge heterogeneity was detected in all preparations, attributable either to post-translational modification or to minor proteolytic degradation under the conditions of isolation. These studies were extended to an examination of five VSGs isolated from variants which characteristically appeared at different stages of infection (Baltz *et al.*, 1977). BoTat-1, considered to be the "basic" antigenic type of the strain, and BoTat-2, appeared during the first 10 days of infection in rabbits. BoTat 78 and BoTat 100 always developed in 3–4 weeks, and BoTat 28 appeared later during the course of infection. Immunization with the purified VSG produced clone-specific immunity. Amino acid analyses showed a degree of variability similar to that found in *T. brucei*, with a suggestion that the different glycoproteins were particularly rich in aspartate, glutamate, threonine, alanine and lysine and differed most markedly in their leucine content. The authors stated that preliminary results indicated absence of homology in the *N*-terminal sequences of the VSGs. Carbohydrate analysis showed that *N*-acetylglucosamine, galactose and mannose were present in roughly constant amounts (7–8%, w/w) and proportions (see Table 13). In addition, small amounts of glucose were present. Labastic *et al.* (1981) investigated the distribution of carbohydrate within one VSG (BoTat 28), which could be cleaved by trypsin into two major fragments, F_1 (M_r = 38 000) and F_2 (28 000). Fragment F_1 had the same *N*-terminal sequence as intact BoTat 28, and contained 30% of the carbohydrate. Fragment F_2 had a different *N*-terminal sequence and contained the remaining 70% of the carbohydrate, including virtually all of the galactose. Antisera to BoTat 28 were shown to precipitate ^{125}I-labelled BoTat-1 VSG. This heterologous cross-reaction could be completely inhibited by fragment F_2 but not by F_1. Hence, as with VSGs of *T. brucei*, carbohydrate seems to be located primarily in the *C*-terminal half of the molecule, which also carries a cross-reacting

determinant. Whether that determinant is identical in both *T. brucei* and *T. equiperdum* has not yet been established.

TABLE 13

Carbohydrate content of purified glycoproteins from three variants of T. equiperdum (*Baltz* et al., *1977*)[a]

Sugar	Variant BoTat-1	BoTat-78	BoTat-28
Mannose	14·4	14·9	14·4
Galactose	8·3	7·7	8·6
Glucose	0·7	2·1	2·3
N-Acetylglucosamine	2·9	3·4	2·9

[a] Values have been converted to moles per mole of glycoprotein (M_r 65 000) to allow comparison with Table 8.

Jackson (1977) studied agglutination of *T. equiperdum* by lectins, and obtained no reaction of bloodstream trypomastigotes with Con A, phytohemagglutinin, wheat germ agglutinin, *Dolichus biflorus* lectin or *Ulex europeus* lectin. Treatment of trypanosomes with trypsin, pronase, papain or chymopapain exposed binding sites for Con A, phytohemagglutinin and wheat germ agglutinin. Baltz *et al.* (1976) noted that only 5 of 10 antigenic variants of *T. equiperdum* were agglutinated by Con A, although surface antigens isolated from all the variants bound to Con A. Baltz *et al.* (1977) also reported that at least two variants could be agglutinated by both Con A and *Ricinus communis* lectin. Hence, there appears to be considerable variation between clones in the extent of exposure of carbohydrate at the trypanosome surface.

G. *Trypanosoma* (*Nannomonas*) *congolense*

The antigens of *T. congolense* show some interesting differences from those of *T. brucei.* Rovis *et al.* (1978) surface-labelled trypanosomes of a clone of *T. congolense*, both by lactoperoxidase iodination with ^{125}I and by galactose oxidase followed by NaB^3H_4 reduction. The labelled coat material was extracted by repeated freezing and thawing, followed by high-speed centrifugation. Between 60% and 80% of the total radioactivity was present in the soluble supernatant fluid. Most of the remaining radioactivity was solubilized by treating the insoluble pellet with the nonionic detergent NP40. Electrophoresis on SDS–polyacrylamide gels showed that both the water-soluble and detergent-soluble fractions contained a single labelled glyco-

protein, molecular weight 56 000. Both the water-soluble and detergent-soluble material could be immunoprecipitated with variant-specific antisera. Isoelectric focussing showed some charge heterogeneity of the labelled glycoproteins, in comparison with labelled glycoproteins of *T. brucei*, which was ascribed to proteolysis, since rapid breakdown of the 56 000 dalton glycoprotein was observed in the absence of inhibitors of proteolysis. This study, then, revealed several overall similarities between the surface coats of *T. congolense* and *T. brucei*.

Similar results were obtained by Reinwald *et al.* (1978), who surface labelled trypanosomes of a clone of *T. congolense* using the diazonium salt of ^{35}S-labelled sulphanilic acid. After labelling, trypanosomes were washed and lysed with distilled water, and the 15 000 *g* supernatant fraction was analysed by SDS–polyacrylamide gel electrophoresis and isoelectric focussing. Up to 80% of the radioactivity was found in one band, corresponding to a molecular weight of about 57 000; another band with a molecular weight of about 50 000 accounted for most of the remaining radioactivity. Trypanosomes labelled after treatment with trypsin or pronase produced no soluble radioactivity after water lysis. The 57 000 dalton product of the labelling reaction had an isoelectric point of 6·25, with little or no evidence of the charge heterogeneity observed by Rovis *et al.* (1978).

Reinwald *et al.* (1979) produced a VSG preparation of homogeneous size using an interesting technique for disrupting the surface coat. Again, surface labelling with [^{35}S]diazoniobenzenesulphonate was employed to monitor coat release, which was achieved by shaking a suspension of the trypanosomes under controlled conditions. After pre-incubation for 3–4 hours at 0°C, trypanosomes at a concentration of 10^9 ml^{-1} were distributed in 400 µl volumes into 50 ml siliconized glass tubes and shaken at 200 rev./min. in a gyratory shaker for 90 minutes at room temperature. Under these conditions, the surface label was completely released in a molecule of molecular weight 57 000. Electron microscopic inspection of unshaken and shaken trypanosomes revealed that the shaken trypanosomes had lost the internal granular structure, indicating that much of the cytoplasmic material had leaked out. Indeed, many proteins in addition to coat protein were observed on SDS–polyacrylamide gels of the supernatant fraction from shaken trypanosomes. However, many of the internal membranes seemed to be intact, suggesting that this method avoided the release of lysosomal proteases. The shaken trypanosomes showed no evidence for the presence of a surface coat.

Reinwald *et al.* (1981) developed a third solubilization technique, because the procedure outlined above was found to be time consuming and laborious when working with large numbers of cells, and because the tendency of the protein to aggregation and adhesion was found to reduce the protein recovery enormously. Trypanosomes were suspended in a buffer containing

5% dioxane, plus inhibitors of proteolysis, for between 30 and 90 minutes, after which time the appearance of the cells was comparable to that obtained by shaking. The solubilized proteins were applied to a column of con A-Sepharose, but the VSG could not be efficiently eluted by methyl mannoside, borate or ethylene glycol. Efficient elution was accomplished only by electrophoretic desorption in the presence of the nonionic detergent Triton X-100, with concomitant separation of bound glycoproteins in a granulated electrofocussing gel. Even under these conditions some 15–20% of the VSG remained bound to Con A, and could not be eluted. This was ascribed to the hydrophobic nature of the *T. congolense* VSG. Con A is known to bind hydrophobic molecules, in addition to glycoproteins, and the tendency of the VSG to aggregate and precipitate in aqueous buffers strongly suggests a more hydrophobic character for this VSG. Hence the requirement for Triton X-100 in the electrophoretic desorption. Dioxane itself is quite a hydrophobic solvent, which could explain its efficacy in releasing VSG. The authors pointed out, however, that at the concentration used, dioxane had no effect on the permeability of the red blood cell membrane. It would be most interesting to analyse the VSG remaining bound to Con A-Sepharose. The electroeluted material apparently showed a single band of molecular weight 57 000 on SDS-polyacrylamide gels, but more careful analysis showed three very closely spaced polypeptides, all three of which were glycosylated. Differences in affinity for other lectins suggested that the size heterogeneity was due to differences in the carbohydrate structures. Charge heterogeneity was also observed. From the particular variant studied, three glycoprotein bands of isoelectric points 5·9, 6·2 and 6·5 were separated, each of which showed similar slight heterogeneity of size.

Richards *et al.* (1981), describing their results on the biochemistry of *T. congolense* VSG, stated that freeze–thawing and shearing of the trypanosomes released an apparently homogeneous protein of molecular weight 53 000 from each of two variants studied. Both preparations showed charge heterogeneity in isoelectric focussing gels, each producing three bands. Further, the three components were said to show small but reproducible differences in competitive radioimmunoassay, implying slight differences in their antigenic determinants. Such differences could reflect heterogeneity in the carbohydrate side chains, as proposed by Reinwald *et al.* (1981), or heterogeneity in the polypeptide due to proteolysis. However, most surprisingly, the authors stated that this charge heterogeneity was also observed in the cell-free translation products of the VSG-specific mRNA. Such a finding, if corroborated, would imply the co-expression of closely linked genes, or the rapid development within cloned populations of closely related variants. Clearly, more work is needed to clarify these results.

Richards *et al.* (1981) also reported that a higher molecular weight form of

the VSG (M_r 63 000) was observed, which could form the "anchor" by which the remainder of the VSG is held to the membrane. However, no biochemical comparison of the 63 000 and 53 000 molecular weight forms of the VSG were made, nor was the size of the *in vitro* translation product of VSG-specific mRNA reported. It should be relatively easy to determine whether differences in glycosylation or in polypeptide sequence were responsible for the size discrepancy. The two VSGs isolated by Richards *et al.* (1981) were said to exhibit 27% sequence homology when residue 1 of VSG 1 was aligned with residue 4 of VSG 2. The sequences were not given but the sequence data were cited as indirect evidence for the proposal that sequentially duplicated genes are sequentially expressed by trypanosomes during relapsing infections.

Carbohydrate seems to be exposed at the surface of *T. congolense*. Jackson *et al.* (1978) noted that *T. congolense* bloodstream trypomastigotes could be agglutinated efficiently by Con A, fucose binding protein, phytohemagglutinin, wheat germ agglutinin and soybean agglutinin. The lectin binding sites were localized on the surface of the trypanosomes, as judged from electron microscopy. The carbohydrate specificity of the lectins implies the presence of mannose (or glucose), *N*-acetylgalactosamine and /or galactose, *N*-acetylglucosamine, and, surprisingly, fucose. This is the first report of fucose-like saccharides on the surface of a bloodstream form of any salivarian species. Carbohydrate analyses of purified VSG have not yet been reported. An uncloned population of *T. congolense* seems to have been used throughout. Rautenberg *et al.* (1980) also reported that both cloned and uncloned populations of *T. congolense* could be agglutinated by Con A, and that this reaction was localized to the cell surface. Trypsinization of intact trypanosomes led to the loss of the surface coat, but not to a complete loss of Con A binding; hence the possibility remained that all or some of the binding was to surface receptors other than the VSG. As purified VSG binds to Con A, this must be considered unlikely. Such exposed carbohydrate may have a role in specifying the interaction of *T. congolense* with its environment, for example in mediating its known ability to bind to the walls of the microvasculature and to circulating erythrocytes (Banks, 1978; Bungener and Muller, 1976).

H. *Trypanosoma* (*Duttonella*) *vivax*

The surface coat of *T. vivax* is less compact than that found in the bloodstream stages of the subgenera *Trypanozoon* and *Nannomonas* (Vickerman, 1969). This led to the suggestion that the surface coat of *T. vivax* may function rather differently from that of other salivarian trypanosomes (Vickerman

and Preston, 1976). Desowitz and Watson (1953) reported that, to obtain growth of *T. vivax* in rodents, the host must be given supplementary injections of sheep serum until adaptation occurs. Such rodent-adapted strains appear to bind host serum proteins avidly. Desowitz and Watson (1953) reported that lysis of such *T. vivax* by homologous antiserum occurred only if the parasites were separated from the blood and washed, whereas Ketteridge (1971) found that they could be both agglutinated and lysed by antisera prepared against host serum. There has therefore been considerable speculation that such host proteins form an integral component of the parasite's surface coat. Antigenic variation does occur in both natural and rodent-adapted *T. vivax* (reviewed by Gray and Luckins, 1976), hence there must be a variant-specific component of the surface coat. Leeflang *et al.* (1976) discovered strains of *T. vivax* which adapted immediately to growth in rodents without any requirement for sheep serum supplement. By surface-labelling such strains, de Gee and Rovis (1981) demonstrated the presence of a molecule of molecular weight 50 000 which could be immunoprecipitated by antisera specific to the variant population examined. The trypanosomes were not agglutinated or lysed by antisera prepared against host serum, and the anti-*T. vivax* antiserum showed no cross-reaction with mouse serum components. None of the surface-labelled material could be immunoprecipitated by anti-mouse serum. The analysis was complicated by the fact that variant populations rather than clones were used, since clones of *T. vivax* seem to be antigenically unstable (de Gee and Rovis, 1981), but there was no evidence for the presence of host serum components in association with the *T. vivax* strains used. This phenomenon therefore seems to be an artefact. Indeed, Tabel and Losos (1980) reported that *T. vivax* isolated from cattle have no detectable host protein on their surface.

I. ISOTYPIC SURFACE GLYCOPROTEINS

Van Meirvenne *et al.* (1977) described the isolation of cloned populations of trypanosomes, derived from widely different field isolates and species, which cross-reacted in immune lysis and immunofluorescence tests. Such "isotypic" trypanosomes, or "isoVATs", therefore seem to be expressing similar VSGs, even though they might be isolated from different species or subspecies. Clearly, it is of considerable interest to determine what degree of structural homology exists between such isotypic VSGs. Indications that they are not identical were reported by Cross (1979b), who compared two clones of *T. b. brucei* and one of *T. evansi* which appeared to be expressing identical VSGs. The VSG purified from the clone of *T. evansi*, however, showed considerable differences in solution properties (see above). A more

detailed study has been reported by Vervoort *et al.* (1981). Two groups of isoVATs were studied. Group I comprised one clone of *T. b. brucei*, one of *T. b. rhodesiense*, one of *T. b. gambiense*, and one of *T. evansi* (isolated from a capybara in South America). The second group comprised one clone of *T. b. gambiense* and one of *T. evansi*, each isolated from the same serodeme as the representatives of these two species in group I.

Each representative of each group could be recognized in both immunofluorescence and immune lysis by sera raised by infection against any other member of the same group, but there was no cross-reactivity between the two groups. There was some difference in the titres obtained with heterologous and homologous combinations of trypanosomes and antisera, suggesting that the sera were not recognizing identical groups of determinants; this was confirmed by radioimmunoassay. The VSGs were prepared from each clone, and used to raise variant-specific antisera. All antisera stained all trypanosomes within the same isotype group, but not other, heterologous, trypanosomes. The VSGs themselves all had the same molecular weight on SDS–polyacrylamide gels under reducing conditions ($M_r \sim 60\,000$), but the two representatives of group II existed as disulphide-linked dimers in the absence of 2-mercaptoethanol. However, the VSGs each had different pI values, showing nonidentity. Radioimmunoassay showed that homologous reactions between an isotype VSG and its antiserum could be effectively inhibited by heterologous VSGs from the same isotype group, although the maximum inhibition obtained varied from 19% to 93%. Such inhibition of homologous combinations of VSG and antiserum could not be achieved using non-isotypic VSGs, suggesting a marked degree of structural homology within each isotype group. In addition, the isotype VSGs were shown to possess the common antigenic determinant shared by most VSGs (Barbet and McGuire, 1978; Cross, 1979b). (See p. 39, above.)

Peptide maps of VSGs labelled with either ^{125}I or ^{14}C revealed a large number of overlapping peptides when any two members of group I were compared. Iodinated VSGs produced 12–15 labelled peptides, the majority of which overlapped, whereas ^{14}C-labelled VSGs (labelled by reductive methylation of ε-amino groups of lysine) produced 40–60 labelled peptides, the majority of which overlapped. Hence the isotypic VSGs of group I were clearly very similar, although not identical. The two isotypic VSGs of group II generated more non-overlapping peptides in similar comparisons, implying a lower degree of homology between these two VSGs. This was also reflected in the results of inhibition tests in competitive radioimmunoassay.

The existence of such isotypic VSGs must be a reflection of the intra- and interspecies hybridization observed with VSG-specific DNA probes (Borst *et al.*, 1980b; Frasch *et al.*, 1980). As yet it is not known whether trypanosomes expressing VSGs partially homologous in amino acid sequence can be

generated in the course of infection initiated by a single organism. Presumably, they could only be detected by passage of relapse populations into non-immune hosts, because such a degree of immunological cross-reaction would lead to the elimination of isoVATs appearing late in the sequence. It would be fascinating to know whether the serum-sensitive and serum-insensitive forms of the same antigenic type described by Van Meirvenne *et al.* (1976) expressed non-identical isotypic VSGs, and hence whether serum sensitivity can be related to VSG structure. More detailed examination of the distribution of isoVATs should also indicate whether detection of anti-isoVAT antibody is likely to prove a reliable method of serodiagnosis.

J. METACYCLIC ANTIGENS

Little work has been done on the biochemistry of antigens expressed by metacyclic trypanosomes of any species, simply because the amount of material available is so limited. There is intense interest in the antigenic composition of metacyclic trypanosomes since if the repertoire is restricted, vaccination could be a possibility. The serological evidence suggests that (a) the repertoire of metacyclic VATs is restricted, and (b) there is limited overlap between VATs present in metacyclic and in bloodstream populations (Jenni, 1977a, b; Le Ray *et al.*, 1978; Barry *et al.*, 1979; Barry and Hajduk, 1979). The potential value of metacyclic trypanosomes as immunogens providing strain-specific immunity has also been demonstrated with *T. congolense* (Nantulya *et al.*, 1980). Several groups are currently developing monoclonal antibodies which recognize metacyclic antigens, hence the extent of the antigenic heterogeneity within metacyclic populations should soon be established. Recent developments in the culture of metacyclic trypanosomes should allow surface labelling experiments to be carried out (Jenni and Brun, 1981).

IV. CONCLUSIONS

Enormous strides in our understanding of antigenic variation have been made in the past 5 or 6 years but, as usual, many more questions have been raised than answered. We now know much more about the mechanism of antigenic variation, but the true relationship between the observed genomic rearrangements of VSG genes and the expression of a particular VSG, and the mechanism by which such rearrangements are generated, remain subjects of intense speculation. We now know that VSGs are synthesized with a hydro-

phobic *C*-terminus absent from purified VSG, but the precise temporal relationships of the various post-translational modifications of the VSG molecules still have to be characterized. In what way are such modifications of the VSG related to the mechanism of membrane attachment, and to the ease of VSG release? How are the VSG molecules arranged in the surface coat? What can be the role of a buried carbohydrate structure, conserved in many VSGs, even in different species? Is this cross-reacting determinant sufficiently widely distributed for its detection in patients' serum to be used for serodiagnosis? Is there any relationship between VSG structure and serum sensitivity? And what, if anything, is the significance of the charge heterogeneity which has been observed in VSGs of all species, when looked for? Will an answer to any of these questions provide the basis for the development of VSG-directed chemotherapy, or even for the production of improved methods of serodiagnosis, or for vaccine production?

These are exciting times for those working on antigenic variation. Developments are occurring so rapidly that areas of this review will be outdated by the time of publication, and the answer to several of the questions I have posed will most certainly be known by then. We must hope that the next generation of questions to be answered will concern the exploitation of our knowledge of antigenic variation and VSG structure in the development of new methods of controlling this most versatile of parasites.

Acknowledgements

My opinions about the biochemistry of antigenic variation have been greatly influenced by many stimulating discussions and arguments with colleagues and collaborators in the Molteno Institute. I wish to acknowledge my gratitude to Lucia Almeida, Cathy Auffret, Chung-Lit Choi, Esther Choolun, John Cordingley, Anne Gurnett, Pauline Harris, Gaiti Hasan, Benito Infante, Roger Klein, Josie McConnell, Nancy Miller, Karen Ovington and Kim Tuffnell. They are, of course, absolved of any responsibility for the opinions expressed in this review, or for the errors of omission or interpretation, which are mine! Also, many thanks to Carol Throup, who typed the manuscript under considerable pressure.

References

Agabian, N., Thomashow, L., Milhausen, M. and Stuart, K. (1980a). Structural analysis of variant and invariant genes in trypanosomes. *American Journal of Tropical Medicine and Hygiene* **29**, 1043–1049.

Agabian, N., Thomashow, L., Stuart, K. and Milhausen, M. (1980b). Molecular cloning of parasite genes. *In* "Cancer Immunology and Parasite Immunology" (L. Israël, P. Lagrange and J. C. Salomon, eds), Les Colloques de l'INSERM, Vol. 97, pp. 425–439. INSERM, Paris.

Allsopp, B. A. and Njogu, A. R. (1974). Monosaccharide composition of the surface glycoprotein antigens of *Trypanosoma brucei*. *Parasitology* **69**, 271–281.

Allsopp, B. A., Njogu, A. R. and Humphryes, K. C. (1971). Nature and location of *Trypanosoma brucei* subgroup exoantigen and its relationship to 4S antigen. *Experimental Parasitology* **29**, 271–284.

Auffret, C. A. and Turner, M. J. (1981). Variant specific antigens of *Trypanosoma brucei* exist in solution as glycoprotein dimers. *Biochemical Journal* **193**, 647–650.

Baltz, T., Baltz, D. and Pautrizel, R. (1976). Affinity of concanavalin A for *Trypanosoma equiperdum:* purification of the antigenic type specific glycoprotein fraction. *Annales d'Immunologie* **127**, 761–774.

Baltz, T., Baltz, D., Pautrizel, R., Richel, C., Lamblin, G. and Degand, P. (1977). Chemical and immunological characterisation of specific glycoproteins from *Trypanosoma equiperdum* variants. *FEBS Letters* **82**, 93–96.

Banks, K. L. (1978). Binding of *Trypanosoma congolense* to the walls of small blood vessels. *Journal of Protozoology* **25**, 241–245.

Barbet, A. F. and McGuire, T. C. (1978). Cross-reacting determinants in variant-specific antigens of African trypanosomes. *Proceedings of the National Academy of Sciences of the U.S.A.* **75**, 1989–1993.

Barbet, A. F., McGuire, T. C., Musoke, A. J. and Hirumi, H. (1979). Cross-reacting determinants in trypanosome surface antigens. *In* "Pathogenicity of Trypanosomes" (G. Losos, and A. Chouinard, eds), pp. 38–43. International Development Research Centre, Ottawa.

Barry, J. D. (1979). Capping of variable antigen of *Trypanosoma brucei* and its immunological and biological significance. *Journal of Cell Science* **37**, 287–302.

Barry, J. D. and Hajduk, S. L. (1979). Antigenic heterogeneity of bloodstream and metacyclic forms of *Trypanosoma brucei*. *In* "Pathogenicity of Trypanosomes" (G. Losos, and A. Chouinard, eds), pp. 51–56. International Development Research Centre, Ottawa.

Barry, J. D. and Vickerman, K. (1979). *Trypanosoma brucei*: loss of variable antigens during transformation from bloodstream to procyclic forms *in vitro*. *Experimental Parasitology* **49**, 281–300.

Barry, J. D., Hajduk, S. L., Vickerman, K. and Le Ray, D. (1979). Detection of multiple variable antigen types in metacyclic populations of *Trypanosoma brucei*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **73**, 205–208.

Blake, R., Hager, L. P. and Gennis, R. B. (1978). Activation of pyruvate oxidase by monomeric and micellar amphiphiles. *Journal of Biological Chemistry* **253**, 1963–1971.

Blöbel, G. (1978). Mechanisms for the intracellular compartmentation of newly synthesised proteins. *FEBS Symposia* **43**, 99–108.

Bolognesi, D. P., Montelaro, R. C., Frank, H. and Schäfer, W. (1978). Assembly of Type C oncornaviruses: a model. *Science* **199**, 183–186.

Boothroyd, J. C., Cross, G. A. M., Hoeijmakers, J. H. J. and Borst, P. (1980). A variant surface glycoprotein of *Trypanosoma brucei* synthesised with a *C*-terminal hydrophobic "tail" absent from purified glycoprotein. *Nature* (*London*) **288**, 624–626.

Boothroyd, J. C., Paynter, C. A., Cross, G. A. M., Bernards, A. and Borst, P. (1981). Variant surface glycoproteins of *Trypanosoma brucei* are synthesised with cleavable hydrophobic sequences at the carboxy and amino termini. *Nucleic Acids Research* **9**, 4743–4745.

Borst, P., Frasch, A. C. C., Bernards, A., Hoeijmakers, J. J., Van der Ploeg, L. H. T. and Cross, G. A. M. (1980a). The genes for variant antigens in trypanosomes. *American Journal of Tropical Medicine and Hygiene* **29**, 1033–1036.

Borst, P., Frasch, A. C. C., Bernards, A., Van der Ploeg, L. H. T., Hoeijmakers, J. H. T., Arnberg, A. C. and Cross, G. A. M. (1980b). DNA rearrangements involving the genes for variant antigens in *Trypanosoma brucei*. *Cold Spring Harbor Symposia on Quantitative Biology* **45**, 935–943.

Branton, D., Cohen, C. M. and Tyler, J. (1981). Interaction of cytoskeletal proteins of the human erythrocyte membrane. *Cell* **24**, 24–32.

Brett, C. T. and Voorheis, H. P. (1980). Glycoprotein biosynthesis in *Trypanosoma brucei*. The glycosylation of glycoproteins located in and attached to the plasma membrane. *European Journal of Biochemistry* **109**, 139–150.

Bridgen, P. J., Cross, G. A. M. and Bridgen, J. (1976). *N*-Terminal amino acid sequences of variant specific surface antigens from *Trypanosoma brucei*. *Nature (London)* **273**, 613–614.

Brown, K. N. and Williamson, J. (1962). Antigens of *brucei* trypanosomes. *Nature (London)* **194**, 1253–1255.

Brown, K. N. and Williamson, J. (1964). The chemical composition of trypanosomes IV. Location of antigens in subcellular fractions of *Trypanosoma rhodesiense*. *Experimental Parasitology* **15**, 69–86.

Bungener, W. and Muller, G. (1976). Adharenzphänomene bei *Trypanosoma congolense*. *Tropenmedizin und Parasitologie* **27**, 370–371.

Campbell, G. H., Esser, K. M., Wellde, B. T. and Diggs, C. L. (1979). Isolation and characterisation of a new serodeme of *Trypanosoma rhodesiense*. *American Journal of Tropical Medicine and Hygiene* **28**, 974–983.

Capbern, A., Giroud, C., Baltz, T. and Mattern, P. (1977). *Trypanosoma equiperdum*: étude des variations antigènique au cours de la trypanosomose expérimentale du lapin. *Experimental Parasitology* **42**, 6–13.

Compans, R. W. and Klenk, H. D. (1979). Viral membranes. *In* "Comprehensive Virology" (H. Fraenkel-Conrat and R. R. Wagner, eds), Vol. 13, pp. 293–407. Plenum Press, New York.

Cordingley, J. S. and Turner, M. J. (1980). Isolation of *Trypanosoma brucei* variant specific antigen mRNA by immunoprecipitation of polysomes. *Molecular and Biochemical Parasitology* **1**, 129–137.

Cross, G. A. M. (1975). Identification, purification and properties of clone-specific glycoprotein antigens constituting the surface coat of *Trypanosoma brucei*. *Parasitology* **71**, 393–417.

Cross, G. A. M. (1977). Isolation, structure and function of variant-specific surface antigens. *Annales de la Société Belge de Médecine Tropicale* **57**, 389–399.

Cross, G. A. M. (1978). Antigenic variation in trypanosomes. *Proceedings of the Royal Society of London, B* **202**, 55–72.

Cross, G. A. M. (1979a). Cross-reacting determinants in the *C*-terminal region of trypanosome variant surface antigens. *Nature (London)* **277**, 310–312.

Cross, G. A. M. (1979b). Biochemistry of variant antigens. *In* "Pathogenicity of Trypanosomes" (G. Losos and A. Chouinard, eds), pp. 32–37. International Development Research Centre, Ottawa.

Cross, G. A. M. and Johnson, J. G. (1976). Structure and organisation of the variant specific surface antigens of *Trypanosoma brucei*. *In* "Biochemistry of Parasites and Host-Parasite Relationships" (H. Van den Bossche, ed.), pp. 413–420. North Holland Publishing Company, Amsterdam.

Cross, G. A. M., Holder, A. A., Allen, G. and Boothroyd, J. C. (1980). An introduction to antigenic variation in trypanosomes. *American Journal of Tropical Medicine and Hygiene* **29**, 1027–1032.

De Gee, A. L. W. and Rovis, L. (1981). *Trypanosoma vivax:* absence of host proteins on the surface coat. *Experimental Parasitology* **51**, 124–132.

De Raadt, P. and Seed, J. R. (1977). Trypanosomes causing disease in Africa. *In* "Parasitic Protozoa" (J. P. Kreier, ed.), Vol. 1, pp. 175–239. Academic Press, New York.

Desowitz, R. S. and Watson, H. J. C. (1953). Studies on *Trypanosoma vivax* IV. The maintenance of a strain in white rats without sheep serum supplement. *Annals of Tropical Medicine and Parasitology* **47**, 62–67.

Dodin, A. and Fromentin, H. (1962). Mise en évidence d'un antigène vaccinant dans le plasma de souris expérimentalement infectées par *Trypanosoma gambiense* et par *Trypanosoma congolense*. *Bulletin de la Société de Pathologie Exotique* **55**, 128–138.

Doyle, J. J. (1977). Antigenic variation in the salivarian trypanosomes. *In* "Immunity to Blood Parasites of Animals and Man" (L. H. Miller, J. A. Pino and J. J. McKelvey, eds), pp. 31–63. Plenum Press, London.

Eggitt, M. J., Tappenden, L. and Brown, K. N. (1977). Translation in a reticulocyte cell-free system of RNA isolated from bloodstream and culture forms of *Trypanosoma brucei*. *Parasitology* **75**, 133–142.

Frasch, A. C. C., Bernards, A., Van der Ploeg, L. H. T., Borst, P., Hoeijmakers, J. H. J., Van den Burg, J. and Cross, G. A. M. (1980). The genes for the variable surface glycoproteins of *Trypanosoma brucei*. *In* "The Host-Invader Interplay" (H. Van den Bossche, ed.), pp. 235–239. Elsevier/North Holland Biomedical Press, Amsterdam.

Fukushima, D., Yokoyama, S., Kézdy, F. J. and Kaiser, E. T. (1981). Binding of amphiphilic peptides to phospholipid/cholesterol unilamellar vesicles: a model for protein–cholesterol interaction. *Proceedings of the National Academy of Sciences of the U.S.A.* **78**, 2732–2736.

Gashumba, J. K. (1981). Sleeping sickness in Uganda. *New Scientist* **89**, 164.

Gill, B. S. (1965). Properties of soluble antigen of *Trypanosoma evansi*. *Journal of General Microbiology* **38**, 357–361.

Gray, A. R. (1965). Antigenic variation in clones of *Trypanosoma brucei*. I. Immunological relationships of the clones. *Annals of Tropical Medicine and Parasitology* **59**, 27–36.

Gray, A. R. (1966). The antigenic relationships of strains of *Trypanosoma brucei* isolated in Nigeria. *Journal of General Microbiology* **44**, 263–271.

Gray, A. R. (1972). Variable agglutinogenic antigens of *Trypanosoma gambiense* and their distribution among isolates of the trypanosomes collected in different places in Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **66**, 283–284.

Gray, A. R. (1974). Antigenic similarities among isolates of *Trypanosoma gambiense* from different countries in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **68**, 150–151.

Gray, A. R. and Luckins, A. G. (1976). Antigenic variation in salivarian trypanosomes. *In* "Biology of the Kinetoplastida" (W. H. R. Lumsden and D. A. Evans, eds), pp. 493–542. Academic Press, London.

Grundy, S. N. (1980). "Studies on purified *Trypanosoma brucei* variant specific surface antigens: immunological specificity and properties in solution." Ph.D Thesis, University of London.

Haberman, E. and Jentsch, J. (1967). Sequenzenanalyse des Melittins aus den tryptischen und peptischen spaltstuchen. *Physiologische Chemie* **348**, 37–50.

Hicks, J., Strathern, J. N. and Klar, A. J. S. (1979). Transposable mating type genes in *Saccharomyces cerevisiae*. *Nature* (*London*) **282**, 478–484.

Hoare, C. A. (1964). Morphological and taxonomic studies on mammalian trypanosomes. X. Revision of the systematics. *Journal of Protozoology* **11**, 200–207.

Hoeijmakers, J. H. J., Borst, P., Van den Burg, J., Weissmann, C. and Cross, G. A. M. (1980a). The isolation of plasmids containing DNA complementary to messenger RNA for variant surface glycoproteins of *Trypanosoma brucei*. *Gene* **8**, 391–417.

Hoeijmakers, J. H. J., Frasch, A. C. C., Bernards, A., Borst, P. and Cross, G. A. M. (1980b). Novel expression-linked copies of the genes for variant surface antigens in trypanosomes. *Nature* (*London*) **284**, 78–80.

Hogan, J. C. and Patton, C. L. (1976). Variation in intramembrane components of *Trypanosoma brucei* from intact and X-irradiated rats: a freeze-cleave study. *Journal of Protozoology* **23**, 205–215.

Holder, A. A. and Cross, G. A. M. (1981). Glycopeptides from variant surface glycoproteins of *Trypanosoma brucei*. *C*-terminal location of antigenically cross-reacting carbohydrate moieties. *Molecular and Biochemical Parasitology* **2**, 135–150.

Hollingshead, S., Pethica, B. A. and Ryley, J. F. (1963). The electrophoretic behaviour of some trypanosomes. *Biochemical Journal* **89**, 123–127.

Jackson, P. R. (1977). Lectin binding by *Trypanosoma equiperdum*. *Journal of Parasitology* **63**, 8–14.

Jackson, P. R., Honigberg, B. M. and Holt, S. C. (1978). Lectin analysis of *Trypanosoma congolense* bloodstream trypomastigote and culture procyclic surface saccharides by agglutination and electron microscopic techniques. *Journal of Protozoology* **25**, 471–481.

Jenni, L. (1977a). Comparisons of antigenic types of *Trypanosoma* (*T.*) *brucei* strains transmitted by *Glossina m. morsitans*. *Acta Tropica* **34**, 35–41.

Jenni, L. (1977b). Antigenic variants in cyclically transmitted strains of the *T. brucei* complex. *Annales de la Société Belge de Médecine Tropicale* **57**, 383–386.

Jenni, L. and Brun, R. (1981). *In vitro* cultivation of pleomorphic *Trypanosoma brucei* stocks: a possible source of variable antigens for immunization studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 150–151.

Johnson, J. G. and Cross, G. A. M. (1977). Carbohydrate composition of variant-specific surface antigen glycoproteins from *Trypanosoma brucei*. *Journal of Protozoology* **24**, 587–591.

Johnson, J. G. and Cross, G. A. M. (1979). Selective cleavage of variant surface glycoproteins from *Trypanosoma brucei*. *Biochemical Journal* **178**, 689–697.

Ketteridge, D. (1971). Host antigens adsorbed by rodent adapted *Trypanosoma vivax*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **65**, 260.

Klein, R. A., Turner, M. J. and Infante, R. B. (1980). The interaction of variant-specific antigens from *Trypanosoma brucei* with lipid monolayers. *Parasitology* **81**, xxvii–xxviii.

Kornfeld, R. and Kornfeld, S. (1980). The structure of glycoproteins and their oligosaccharide units. *In* "The Biochemistry of Glycoproteins and Proteoglycans" (W. J. Lennarz, ed.), pp. 1–34. Plenum Press, New York.

Kosinski, R. J. (1980). Antigenic variation in trypanosomes: a computer analysis of variant order. *Parasitology* **80**, 343–357.

Labastic, M. C., Baltz, T., Richet, C., Giroud, C., Duvillier, G., Pautrizel, R. and Degand, P. (1981). Variant surface glycoproteins of *Trypanosoma equiperdum*: cross-reacting determinants and chemical studies. *Biochemical and Biophysical Research Communications* **99**, 729–736.

Lanham, S. M. and Godfrey, D. G. (1970). Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Experimental Parasitology* **28**, 521–534.

Lanham, S. M. and Taylor, A. E. R. (1972). Some properties of the immunogens (protective antigens) of a single variant of *Trypanosoma brucei brucei*. *Journal of General Microbiology* **72**, 101–116.

Leader, D. P. (1979). Protein biosynthesis on membrane-bound ribosomes. *Trends in Biochemical Sciences* **4**, 205–208

Leeflang, P., Buys, J. and Blotkamp, C. (1976). Studies on *Trypanosoma vivax*: infection and serial maintenance of natural bovine isolates in mice. *International Journal of Parasitology* **6**, 413–417.

Le Page, R. W. F. (1968a). Further studies on the variable antigens of *T. brucei*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **62**, 131.

Le Page, R. W. F. (1968b). "Antigenic variation in *Trypanosoma brucei*." Ph.D Thesis, University of Cambridge.

Le Ray, D., Barry, J. D. and Vickerman, K. (1978). Antigenic heterogeneity of metacyclic forms of *Trypanosoma brucei*. *Nature* (*London*) **273**, 300–302.

Lheureux, M., Lheureux, M., Vervoort, T., Van Meirvenne, N. and Steinert, M. (1979). Immunological purification and partial characterisation of variant-specific surface antigen messenger RNA of *Trypanosoma brucei*. *Nucleic Acids Research* **7**, 595–609.

Lingappa, V. R., Shields, D., Woo, S. L. C. and Blobel, G. (1978). Nascent chicken ovalbumin contains the functional equivalent of a signal sequence. *Journal of Cell Biology* **79**, 567–572.

Lyon, J. A., Pratt, J. M., Travis, R. W., Doctor, B. P. and Olenick, J. G. (1981). Use of monoclonal antibody to immunochemically characterise variant-specific surface coat glycoprotein from *Trypanosoma b. rhodesiense*. *Journal of Immunology* **126**, 134–137.

Magnus, E., Vervoort, T. and Van Meirvenne, N. (1978). A card-agglutination test with stained trypanosomes for the serological diagnosis of *T. b. gambiense* trypanosomiasis. *Annales de la Société Belge de Médecine Tropicale* **58**, 169–176.

Marcu, K. B. and Williams, R. O. (1981). Microbial surface elements: the case of variant surface glycoproteins (VSG) genes of African trypanosomes. *In* "Genetic Engineering" (J. K. Setlow and A. Wollaender, eds), Vol. 3, pp. 129–155. Plenum Press, New York.

Marshall, R. D. (1974). The nature and metabolism of the carbohydrate peptide linkages of glycoproteins. *Biochemical Society Symposia* **40**, 17–26.

Matthyssens, G., Michiels, F., Hamers, R., Pays, E. and Steinert, M. (1981). Two variant surface glycoproteins of *Trypanosoma brucei* have a conserved C-terminus. *Nature* (*London*) **293**, 230–233.

McConnell, J., Cordingley, J. S. and Turner, M. J. (1979). The extent of variability of variant-specific antigens of *T. b. brucei*. *Parasitology* **79**, vi.

McConnell, J., Gurnett, A. M., Cordingley, J. S., Walker, J. E. and Turner, M. J. (1981). Biosynthesis of *Trypanosoma brucei* variant surface glycoprotein. Part I. Synthesis, size and processing of an *N*-terminal signal peptide. *Molecular and Biochemical Parasitology* **4**, 225–242.

Merrit, S. C. (1980). Purification and cell-free translation of mRNA coding for a variant-specific antigen from *Trypanosoma brucei gambiense*. *Molecular and Biochemical Parasitology* **1**, 151–166.

Miller, E. N. (1980). "Biological aspects of antigenic variation in African trypanosomes." Ph.D Thesis, University of Cambridge.

Miller, E. N. and Turner, M. J. (1980). Analysis of antigenic types appearing in first relapse populations of clones of *Trypanosoma brucei*. *Parasitology* **82**, 63–80.

Miller, J. K. (1965). Variation of the soluble antigens of *Trypanosoma brucei*. *Immunology* **9**, 521–528.

Nantulya, V. M., Doyle, J. J. and Jenni, L. (1980). Studies on *Trypanosoma* (*Nannomonas*) *congolense*. II. Antigenic variation in three cyclically transmitted stocks. *Parasitology* **80**, 123–131.

Njogu, A. R. and Humphryes, K. C. (1972). The nature of the 4S antigens of the *brucei* subgroup trypanosomes. *Experimental Parasitology* **31**, 178–187.

Olenick, J. G., Travis, R. W. and Garson, S. (1981). *Trypanosoma rhodesiense*: chemical and immunological characterisation of variant specific surface coat glycoproteins. *Molecular and Biochemical Parasitology* **3**, 227–238.

Osaki, H., Furuya, M. and Oka, M. (1979). Protective immune response in mice immunised with antigens from *Trypanosoma gambiense*-infected mouse blood. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* **245A**, 254–261.

Parodi, A. J. and Leloir, L. F. (1979). The role of lipid intermediates in the glycosylation of proteins in the eucaryotic cell. *Biochemica et Biophysica Acta* **559**, 1–37.

Pays, E., Delronche, M., Lheureux, M., Block, J., Gannon, F. and Steinert, M. (1980). Cloning and characterisation of cDNA sequences coding for two variant-specific antigens of *Trypanosoma brucei brucei*. *In* "The Host-Invader Interplay" (H. Van den Bossche, ed.), pp. 241–244. Elsevier/North Holland Biomedical Press, Amsterdam.

Pays, E., Van Meirvenne, N., Le Ray, D. and Steinert, M. (1981a). Gene duplication and transposition linked to antigenic variation in *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences of the U.S.A.* **78**, 2673–2677.

Pays, E., Lheureux, M. and Steinert, M. (1981b). The expression-linked copy of surface antigen gene in *Trypanosoma* is probably the one transcribed. *Nature* (*London*) **292**, 265–267.

Pearson, T. and Anderson, L. (1980). Analytical techniques for cell fractions. XXVIII. Dissection of complex antigenic mixtures using monoclonal antibodies and two-dimensional gel electrophoresis. *Analytical Biochemistry* **101**, 377–386.

Pearson, T. W., Pinder, M., Roelants, G. E., Kar, S. K., Lundin, L. B., Mayor-Withey, K. S. and Hewett, R. S. (1980). Methods for the derivation and isolation of anti-parasite monoclonal antibodies. *Journal of Immunological Methods* **34**, 141–154.

Rautenberg, P., Reinwald, E. and Risse, H.-J. (1980). Evidence for concanavalin A binding sites on the surface coat of *Trypanosoma congolense*. *Parasitology* **80**, 113–122.

Reinwald, E., Risse, H. J. and Sälker, R. (1978). Diazoniobenzene-sulphonate as a marker for cell surface proteins: study of the surface coat of *Trypanosoma congolense*. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* **359**, 939–944.

Reinwald, E., Rautenberg, P. and Risse, H. J. (1979). *Trypanosoma congolense*: mechanical removal of the surface coat *in vitro*. *Experimental Parasitology* **48**, 384–397.

Reinwald, E., Rautenberg, P. and Risse, H. J. (1981). Purification of the variant antigens of *Trypanosoma congolense*. A new approach to the isolation of glycoproteins. *Biochemica et Biophysica Acta* **668**, 119–131.

Richards, F. F., Rosen, N. L., Onodera, M., Bogucki, M. S., Neve, R. L., Hotez, P., Armstrong, M. Y. K. and Konigsberg, W. H. (1981). Antigenic variation and the surface glycoproteins of *Trypanosoma congolense*. *Federation Proceedings of the American Societies for Experimental Biology and Medicine* **40**, 1434–1439.

Rickman, L. R. and Robson, J. (1970). The testing of proven *Trypanosoma rhodesiense* strains by the blood incubation infectivity test. *Bulletin of the World Health Organization* **42**, 911–916.

Rifkin, M. R. (1978). Identification of the trypanocidal factor in normal human serum: high density lipoprotein. *Proceedings of the National Academy of Sciences of the U.S.A.* **75**, 3450–3454.

Ritz, H. (1916). Über Rezidive bei experimenteller Trypanosomiasis. II. Mitteilung. *Archiv für Schiffs-und Tropenhygiene* **20**, 397–420.

Robertson, D. H. H. and Pickens, S. (1975). Accidental laboratory infection with *Trypanosoma brucei rhodesiense*. A case report. *Communicable Diseases in Scotland* **32**, iii–vi.

Rovis, L. and Baekkeskov, S. (1980). Sub-cellular fractionation of *Trypanosoma brucei*. Isolation and characterization of plasma membranes. *Parasitology* **80**, 507–524.

Rovis, L. and Dube, D. K. (1981). Studies on the biosynthesis of the variant surface glycoprotein of *Trypanosoma brucei*: sequence of glycosylation. *Molecular and Biochemical Parasitology* **4**, 77–93.

Rovis, L., Barbet, A. F. and Williams, R. O. (1978). Characterisation of the surface coat of *Trypanosoma congolense*. *Nature* (*London*) **271**, 654–656.

Schlacter, H. (1974). The sub-cellular sites of glycosylation. *Biochemical Society Symposia* **40**, 57–71.

Schrock, H. L. and Gennis, R. B. (1977). High affinity lipid binding sites on the peripheral membrane enzyme pyruvate oxidase. Specific ligand effects on detergent binding. *Journal of Biological Chemistry* **252**, 5990–5995.

Seed, J. R. (1964). Antigenic similarity among culture forms of the *brucei* group of trypanosomes. *Parasitology* **54**, 593–596.

Seed, J. R. (1972). *Trypanosoma gambiense* and *Trypanosoma equiperdum*. Characterisation of variant-specific antigens. *Experimental Parasitology* **31**, 98–108.

Seed, J. R. and Weinman, D. (1963). Characterisation of antigens isolated from *Trypanosoma rhodesiense*. *Nature* (*London*) **198**, 197–198.

Sessa, G., Freer, J. H., Colacicco, G. and Weissmann, G. (1969). Interaction of a lytic polypeptide, melittin, with lipid membrane systems. *Journal of Biological Chemistry* **244**, 3575–3582.

Shapiro, S. Z. and Young, J. R. (1981). An immunochemical method for mRNA purification: application to messenger RNA encoding trypanosome variable surface antigen. *Journal of Biological Chemistry* **256**, 1495–1498.

Singer, S. J. and Nicholson, G. L. (1972). The fluid mosaic model of the structure of cell membranes. *Science* **175**, 720–731.

Smith, D. S., Njogu, A. R., Cayer, M. and Jarlfors, U. (1974). Observations of freeze-fractured membranes of a trypanosome. *Tissue and Cell* **6**, 223–241.

Steiger, R. (1973). On the ultrastructure of *Trypanosoma* (*Trypanozoon*) *brucei* in the course of its life cycle, and some related aspects. *Acta Tropica* **30**, 64–168.

Steiger, R. F. (1975). Ultracytochemistry of the surface coat/pellicle complex of *Trypanosoma brucei*. *Acta Tropica* **32**, 152–158.

Steiger, R., Opperdoes, F. R. and Bontemps, J. (1980). Sub-cellular fractionation of *Trypanosoma brucei* bloodstream forms with special reference to hydrolases. *European Journal of Biochemistry* **105**, 163–175.

Strickler, J. E. and Patton, C. L. (1980). *Trypanosoma brucei brucei*: inhibition of glycosylation of the major variable surface glycoprotein by tunicamycin. *Proceedings of the National Academy of Sciences of the U.S.A.* **77**, 1529–1533.

Strickler, J. E., Mancini, P. E. and Patton, C. L. (1978). *Trypanosoma brucei brucei*: isolation of the major surface coat glycoprotein by lentil lectin affinity chromatography. *Experimental Parasitology* **46**, 262–276.

Swaney, J. B. and O'Brien, K. (1978). Cross-linking studies of the self-association properties of Apo AI and Apo AII from human high-density lipoprotein. *Journal of Biological Chemistry* **253**, 7069–7077.

Tabel, H. and Losos, G. J. (1980). Absence of host proteins from the surface of *T. vivax* of cattle. *Veterinary Parasitology* **7**, 297–303.

Tait, A. (1980). Evidence for diploidy and mating in trypanosomes. *Nature* (*London*) **287**, 536–538.

Taylor, D. W. and Cross, G. A. M. (1977). The synthesis of variant-specific antigen of *Trypanosoma brucei in vitro*. *Parasitology* **74**, 47–60.

Tetley, L., Vickerman, K. and Moloo, S. K. (1981). Absence of a surface coat from metacyclic *Trypanosoma vivax*: possible implications for vaccination against *vivax* trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 409–414.

Turner, M. J. and Cordingley, J. S. (1981). Evolution of antigenic variation in the salivarian trypanosomes. *In* "Molecular and Cellular Aspects of Microbial Evolution" (M. J. Carlile, J. F. Collins and B. E. B. Moseley, eds). *Society for General Microbiology Symposia* **32**, 313–347. Cambridge University Press, Cambridge.

Tyler, J., Anderson, J. M. and Branton, D. (1980). Structural comparison of several actin-binding macromolecules. *Journal of Cell Biology* **85**, 489–495.

Van Meirvenne, N., Magnus, E. and Janssens, P. G. (1975). Antigenic variation in syringe-passaged populations of *Trypanosoma* (*Trypanozoon*) *brucei*. I. Rationalization of the experimental approach. *Annales de la Société Belge de Médecine Tropicale* **55**, 1–23.

Van Meirvenne, N., Magnus, E. and Janssens, P. G. (1976). The effect of normal human serum on trypanosomes of distinct antigenic type (ETat 1 to 12) isolated from a strain of *Trypanosoma brucei rhodesiense*. *Annales de la Société Belge de Médecine Tropicale* **56**, 55–63.

Van Meirvenne, N., Magnus, E. and Vervoort, T. (1977). Comparison of variable antigenic types produced by trypanosome strains of the subgenus *Trypanozoon*. *Annales de la Société Belge de Médecine tropicale* **57**, 409–423.

Vervoort, T., Barbet, A. F., Musoke, A. J., Magnus, E., Mpimbaza, G. and Van Meirvenne, N. (1981). Isotypic surface glycoproteins of trypanosomes. *Immunology* **44**, 223–232.

Vickerman, K. (1969). On the surface coat and flagellar adhesion in trypanosomes. *Journal of Cell Science* **5**, 163–193.

Vickerman, K. (1974). Antigenic variation in African trypanosomes. *In* "Parasites in the Immunized Host: Mechanisms of Survival" (R. Porter and J. Knight, eds). *Ciba Foundation Symposia* **25** (new series), 53–80. Associated Scientific Publishers, Amsterdam.

Vickerman, K. and Luckins, A. G. (1969). Localisation of variable antigens in the surface of *Trypanosoma brucei* using ferritin conjugated antibody. *Nature (London)* **224**, 1125–1126.

Vickerman, K. and Preston, T. M. (1976). Comparative cell biology of the kinetoplastid flagellates. *In* "Biology of the Kinetoplastida" (W. H. R. Lumsden and D. A. Evans, eds), Vol. 1, pp. 35–130. Academic Press, London.

Vitello, L. B. and Scanu, A. M. (1976). Studies on human serum high density lipoproteins: self association of apolipoprotein A-1 in aqueous solutions. *Journal of Biological Chemistry* **251**, 1131–1136.

Voorheis, P. H., Gale, J. S., Owen, M. J. and Edwards, W. (1979). The isolation and partial characterisation of the plasma membrane from *Trypanosoma brucei*. *Biochemical Journal* **180**, 11–24.

Weitz, B. (1960). The properties of some antigens of *Trypanosoma brucei*. *Journal of General Microbiology* **23**, 589–600.

WHO (1978). Proposals for the nomenclature of salivarian trypanosomes and for the maintenance of reference collections. *Bulletin of the World Health Organization* **56**, 467–480.

WHO (1979). The African trypanosomiases. *Technical Report No. 635*. World Health Organization, Geneva.

Williams, R. O., Marcu, K. B., Young, J. R., Rovis, L. and Williams, S. C. (1978). A characterisation of mRNA activities and their sequence complexities in *Trypanosoma brucei*: partial purification and properties of the VSSA mRNA. *Nucleic Acids Research* **5**, 3171–3182.

Williams, R. O., Young, J. R. and Majiwa, P. A. O. (1979). Genomic rearrangements correlated with antigenic variation in *Trypanosoma brucei*. *Nature (London)* **282**, 847–849.

Williams, R. O., Young, J. R., Majiwa, P. A. O., Doyle, J. J. and Shapiro, S. Z. (1980a). Analyses of variable antigen gene rearrangements in *Trypanosoma brucei*. *American Journal of Tropical Medicine and Hygiene* **29**, 1037–1042.

Williams, R. O., Young, J. R., Majiwa, P. A. O., Doyle, J. J. and Shapiro, S. Z. (1980b). Contextural genomic rearrangements of variable antigen genes in *Trypanosoma brucei*. *Cold Spring Harbor Symposia on Quantitative Biology* **45**, 945–949.

Williamson, J. and Brown, K. N. (1964). The chemical composition of trypanosomes. III. Antigenic constituents of *brucei* trypanosomes. *Experimental Parasitology* **15**, 44–68.

Wright, K. A. and Hales, H. (1970). Cytochemistry of the pellicle of bloodstream forms of *Trypanosoma* (*Trypanozoon*) *brucei*. *Journal of Parasitology* **56**, 671–683.

Wright, K. A., Lumsden, W. H. R. and Hales, H. (1970). The formation of filopodium-like processes by *Trypanosoma* (*Trypanozoon*) *brucei*. *Journal of Cell Science* **6**, 285–297.

Yokoyama, S., Fukushima, D., Kupferberg, J. P., Kezdy, F. J. and Kaiser, E. T. (1980). The mechanism of activation of lecithin: cholesterol acyltransferase by apolipoprotein A-1 and an amphiphilic peptide. *Journal of Biological Chemistry* **255**, 7333–7337.

ADDENDUM (April, 1982)

Considerable progress has, as forecast, been made in the past eight months. The problem of surface packing of VSGs has been made more acute by the finding in our own laboratory that the amount of VSG per trypanosome is almost exactly double the original estimate. Quantitation was achieved by radioimmunoassay of detergent lysates of trypanosomes (L. Almeida and M. J. Turner, unpublished results). In the variant tested, all the VSG carried the cross-reacting determinant. Whether any of the VSG contains the *C*-terminal hydrophobic extension remains problematical. The existence of the coding potential for such extensions seems to be a general feature of VSG mRNA (Boothroyd *et al.*, 1981; Matthysens *et al.*, 1981; Rice-Ficht *et al.*, 1981).

Strickler and Patton (1982a, b) examined oligomer formation of VSGs both in solution and on intact cells, and concluded that whereas VSGs existed predominantly as dimers in solution, oligomers of eight or more VSG molecules could be formed on the cell surface. Whether this was the result of cross-linking dimeric forms on the cell surface could not be resolved. By examining the effect of tunicamycin on surface and solution properties of VSGs, the *N*-linked oligosaccharide side chains were deemed not to be essential for the maintenance of the integrity of the surface coat or for the formation of oligomers at the cell surface, but were necessary for dimer formation in solution. This finding accords with the report (Auffret and Turner, 1981) that VSGs could be cross-linked in solution using reagents that couple carbohydrate to protein. It has also recently been shown in this laboratory that different VSGs can interact to form heterodimers (L. Almeida and M. J. Turner, unpublished data).

A great deal of sequence information has been published (Matthysens *et al.*, 1981; Rice-Ficht *et al.*, 1981) which allows some generalization about VSG structure. The division of VSGs into two groups according to their *C*-terminal sequence (Holder and Cross, 1981) seems to be general. Most of the sequence homologies within each group are in the *C*-terminal 70 or so amino acids; in the hydrophobic tail; and in 3′ non-coding sequences of the mRNA. Rice-Ficht *et al.* (1981) noted that the *C*-terminal domain contained both hydrophobic and hydrophilic regions, whose position was conserved between different VSGs, although the actual sequences may vary. The clustering of four cysteines near the *C*-terminus in both VSG groups was particularly striking, the more so since each cysteine was almost invariably followed by a lysine residue. The significance of this remains to be determined. The published sequences do not support the existence of amphipathic α-helical domains, but the conserved hydrophobic domains could serve as the membrane attachment site, and/or the site of dimerization, and/or the site of cholesterol attachment.

The mode of attachment of VSG to the membrane has not become any more apparent, but Bowles and Voorheis (1982) suggested that VSG release can be triggered by calcium ion uptake and inhibited by zinc ions. The sequence of events linking cause to effect has yet to be established.

McConnell *et al.* (in press) have shown that heterologous dog pancreatic microsomal vesicles are capable of removing *N*-terminal signal peptides and of adding asparagine-linked oligosaccharides to precursor VSGs, but are incapable of adding the cross-reacting determinant or removing the *C*-terminal hydrophobic extension. The nature of the linkage between the cross-reacting determinant and the polypeptide backbone remains unknown.

The mechanism of antigenic variation has become clearer, at least in the case of variants which contain an expression-linked copy gene. Bernards *et al.* (1981) showed that the expression-linked copy is used as the template for mRNA synthesis. Cloned basic copy genes were shown to contain the nucleotide sequence of the corresponding mRNA, with the exception of the last 100–150 nucleotides preceding the poly A tail. In this region the sequences differed by multiple point mutations, insertions and deletions. Since the expression-linked copy and the mRNA were shown both to contain a restriction enzyme site in this area that the basic copy lacked, the authors concluded that generation of the expression-linked copy involved gene duplication followed by a transposition event which led to the replacement of the 3′ end of the gene.

Majiwa *et al.* (in press) have now observed both expression-linked and non-expression-linked VSG gene rearrangements. Concerning the latter, there is still no information as to which gene copy is in an environment allowing expression.

Additional References

(References cited in the Addendum and not listed here will be found in the main list on pp. 142–151.)

Bernards, A., Van der Ploeg, L. H. T., Frasch, A. C. C., Borst, P., Boothroyd, J. C., Coleman, S. and Cross, G. A. M. (1981). Activation of trypanosome surface glycoprotein genes involves a duplication-transposition leading to an altered 3′ end. *Cell* **27**, 497–505.

Bowles, D. J. and Voorheis, H. P. (1982). Release of the surface coat from the plasma membrane of intact bloodstream forms of *Trypanosoma brucei* requires Ca^{2+}. *FEBS Letters* **139**, 17–21.

Majiwa, P. A. O., Young, J. R., Donelson, J. E., Englound, P. T., Rovis, L. and Williams, R. O. (in press). Two distinct forms of surface antigen gene rearrangements in *T. brucei*. *Nature* (*London*).

Matthysens, G., Michiels, F., Hamers, R., Pays, E. and Steinert, M. (1981). Two variant surface glycoproteins of *Trypanosoma brucei* have a conserved *C*-terminus. *Nature* (*London*) **293**, 230–233.

McConnell, J., Cordingley, J. S. and Turner, M. J. (in press). The biosynthesis of *Trypanosoma brucei* variant surface glycoprotein: *in vitro* processing of signal peptide and glycosilation using heterologous rough endoplasmic reticulum vesicles. *Molecular and Biochemical Parasitology*.

Rice-Ficht, A. C., Chen, K. K. and Donelson, J. E. (1981). Sequence homologies near the *C*-termini of the variable surface glycoproteins of *Trypanosoma brucei*. *Nature* (*London*) **294**, 53–57.

Strickler, J. E. and Patton, C. L. (1982a). *Trypanosoma brucei*: nearest neighbour analysis on the major variable surface coat glycoprotein – cross-linking patterns with intact cells. *Experimental Parasitology* **53**, 117–132.

Strickler, J. E. and Patton, C. L. (1982b). *Trypanosoma brucei*: effect of inhibition of *N*-linked glycosilation on the nearest neighbour analysis of the major variable surface coat glycoprotein. *Molecular and Biochemical Parasitology* **5**, 117–132.

This Page Intentionally Left Blank

Transmission of Parasites Across the Placenta

Y. W. LOKE

Division of Experimental and Cellular Pathology, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, U.K.

I. Introduction

To my knowledge, there have not been many general reviews written about the transplacental transmission of protozoan and metazoan parasites collectively as a group, although articles on individual organisms, particularly *Toxoplasma*, are readily available. Furthermore, the emphasis so far has chiefly concerned the frequency with which various maternal infections affect the foetus and the pathological consequence to the foetus of such infections. Although this approach is of undoubted value to medical and veterinary scientists in that it focusses attention on the magnitude of the problem and how to recognize it when it occurs, it nevertheless fails to examine some of the more fundamental biological questions involved.

The placenta, being the organ which is interposed between maternal and foetal circulations would be expected to play a central role in all discussions concerning intra-uterine infections. Reproductive biologists have long been interested in the question as to whether the placenta fulfills its remarkably efficient barrier function passively by virtue of its structural characteristics or whether it plays a more active role by possessing cell populations which are actually capable of defence against pathogenic organisms. Should the latter be the case, then it could explain why those organisms like *Toxoplasma* which have evolved mechanisms for survival in the intracellular environment of host phagocytic cells, are also the ones that are most able to traverse the placenta barrier. Recent data have shown that the human placenta does, indeed, contain a large number of mononuclear phagocytes, so much so that it may justifiably be included as an organ of the mononuclear-phagocyte system among the more established members like the liver, spleen and lymph nodes.

Many concepts derived from basic reproductive immunology are relevant to intra-uterine infections. The immune response during pregnancy is depressed by a variety of factors and this would be expected to have a bearing on the severity of certain parasitic infections and hence provides a greater opportunity for transplacental spread to occur. Much evidence has now accumulated to support the conclusion that ontogeny of immunocompetence in man and perhaps also in other species develops very early in embryogenesis so the foetus *in utero* is not entirely defenceless against any pathogenic organisms which may have breached the placental barrier.

The passive transfer of maternal antibodies will further help to protect the foetus. On the other hand, many pathological lesions are the results of hypersensitivity reactions, so that the ability of the foetus to mount an active response to parasitic antigens or the passive transfer of maternal antibodies specific for these antigens may not necessarily be beneficial, but may lead

instead to immunopathological consequences. Thus, maternal infections can be harmful to the foetus without the actual transmission of the pathogenic organisms.

Overt, clinical disease in the foetus resulting from intra-uterine infection is easily recongnizable, but there may be less obvious manifestations. Early contact with parasitic antigens could modulate the foetus' future immune response, leading either to sensitization or to the development of a state of tolerance at the next encounter with similar parasites. This could markedly alter the pathological spectrum of future infections, thus changing the whole pattern of parasitic diseases among indigenous populations of endemic areas.

The present review is written from the veiwpoint of a reproductive immunobiologist. It is an attempt to examine the problem of intra-uterine infections from the wider context of the foetal-maternal interaction as a whole, in the hope that an approach from this angle may result in a fresh look at the subject and perhaps give rise to new ideas to be explored in the future.

II. Routes of Infective Agents from Mother to Foetus

The possible routes by which infective organisms may be transmitted from mother to foetus *in utero* have been discussed in detail by Benirschke (1960) and reiterated by Blanc (1961).

These are as follows.

1. Haematogenous route across the placenta. The transplacental route is probably the most important, especially for those diseases where the organisms circulate in the maternal blood stream. This route, therefore, is applicable to many bacteria, viruses and those parasites that have a parasitaemic phase.

2. Across the placenta through the substance of the umbilical cord. A variation of the transplacental route is that the organisms need not be transferred directly from the maternal to the foetal circulation but, instead, they can make their way across the foetal membranes along the substance of the cord. Although relatively less important than the haematogenous route, it is conceivable that the tissue migratory larvae of some helminths may employ this mode of transmission. The observation of larvae of *Toxocara canis* in the cords of puppies by Scothorn *et al.* (1965) may be such an example.

3. Ascending infection from the cervix. This mode of transmission occurs mainly with bacterial infections, presumably because many varieties of bacteria colonize the female genital tract. Foetal infection is usually associated with premature rupture of membranes. There are no records of any parasitic infections being transmitted by this route although this must remain a possibility for organisms like *Trichomonas* which have a genital habitat.

4. From the peritoneal cavity. Organisms from the maternal peritoneal cavity may reach the foetus either via the fallopian tubes or directly through the uterine wall. This presupposes a peritonitis complicating pregnancy. A protozoal infection that may have such a clinical outcome would be amoebiasis which has penetrated the intestine. This may be the cause of foetal death associated with *Entamoeba histolytica* occasionally reported in the literature (Czeizel *et al.*, 1966).

III. The Placental Barrier

Since it is clear from the preceeding discussion that the transplacental route is likely to be the most important for intra-uterine transmission of parasitic infections, it becomes necessary to examine in some detail those features of this organ that may be relevant to this transmission.

It was in the late 19th Century that investigators on the human placenta made the cardinal observation that placental villi were covered by a continuous layer of epithelial cells of foetal origin which was given the name 'trophoblast' (Boyd and Hamilton, 1970). With this came the realization that foetal and maternal blood were not in direct communication with each other but totally separated. This was the structural basis for the concept of the placental barrier.

A. COMPARATIVE MORPHOLOGY

Unlike conventional organs, the placenta shows a wide variation in structure among different mammalian species. Although this has provided an area of fruitful research for comparative anatomists, it is a serious disadvantage to reproductive biologists because it means that experimental animal data cannot be extrapolated to man with any degree of confidence. A structural difference which may be important to transmission of infective organisms is the number of cellular layers that are interposed between blood in the foetal circulation and maternal blood in the uterine sinusoids. This was the basis of Grosser's original classification of the chorio-allantoic placenta. Any reader interested in the details may consult the definitive work on 'Comparative Placentation' edited by Steven (1975). Theoretically, the layers of tissues which are available to separate foetal from maternal blood are shown in Fig. 1.

From this diagram, it can be seen that there are effectively six layers. In fact, the number is even greater if one includes the various basement membranes but these are usually not included in the total count. This is

because very little is known about these structures but this may soon change as there is now much interest in the collagen composition of placental basement membrane which appears to be different in type from those found in other organs.

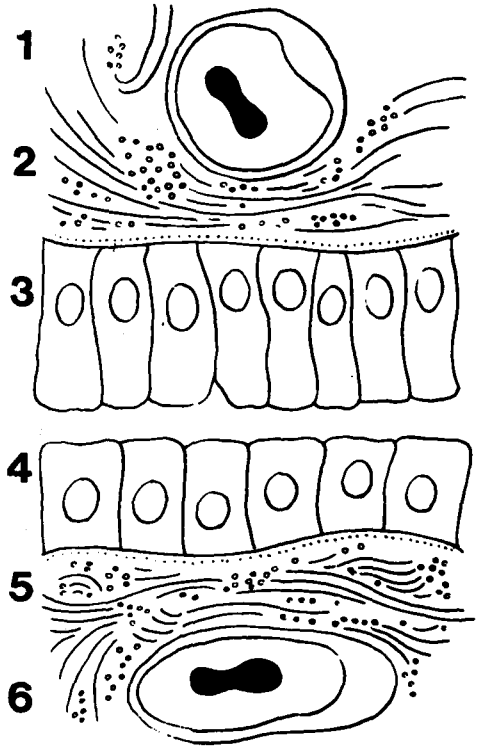

FIG. 1. Layers of tissues that are theoretically available to separate foetal blood from maternal blood. 1, Foetal capillary endothelium; 2, chorionic villus mesenchyme; 3, trophoblast; 4, uterine epithelium; 5, uterine mesenchyme; 6, uterine blood vessel endothelium. (From Steven, 1975.)

On the basis of the presence or absence of foetal and maternal layers, chorio-allantoic placentae can be classified into different types. These features are summarized in Table 1 where the different categories are set out in descending order according to the number of intervening layers present. From this table, it can be seen that in the haemochorial type, of which man is an example, no maternal tissue remains and the foetal chorion is bathed directly by maternal blood flowing through uterine sinusoids. Thus, the chorionic trophoblast assumes great importance as it becomes the tissue of the foetal–maternal interface.

TABLE 1

Classification of different types of chorio-allantoic placentae according to the number of maternal and foetal layers present

Type of placenta	Maternal tissues			Foetal tissues			Examples
	Endothelium	Connective tissue	Uterine epithelium	Chorion	Connective tissue	Endothelium	
Epitheliochorial	+	+	+	+	+	+	Horse, pig
Syndesmochorial	+	+	−	+	+	+	Most ruminants, e.g. sheep, cow
Endotheliochorial	+	−	−	+	+	+	Most carnivores, e.g. cat, dog
Haemochorial	−	−	−	+	+	+	Man, primates, rodents

(Adapted from Amoroso, 1968)

The number of trophoblast layers in haemochorial placentae varies with different species. This permits the further subdivision of this type of placentation into three categories: (1) haemotrichorial, e.g. rat, mouse, hamsters; (2) haemodichorial, e.g. rabbit; (3) haemomonochorial, e.g. man, guinea pig, armadillo, chipmunk. The classification of the human placenta as haemomonochorial rather than haemodichorial deserves a brief explanation. Human placental villi are indeed covered by two layers of trophoblast—an inner cellular layer of cytotrophoblast and an outer layer made up of syncytium called syncytiotrophoblast. However, the cytotrophoblast is discontinuous, and over large areas only the syncytiotrophoblast is interposed between maternal blood and foetal tissues. This may not be apparent under light microscopy but is revealed quite clearly when examined by the electron microscope (Fig. 2).

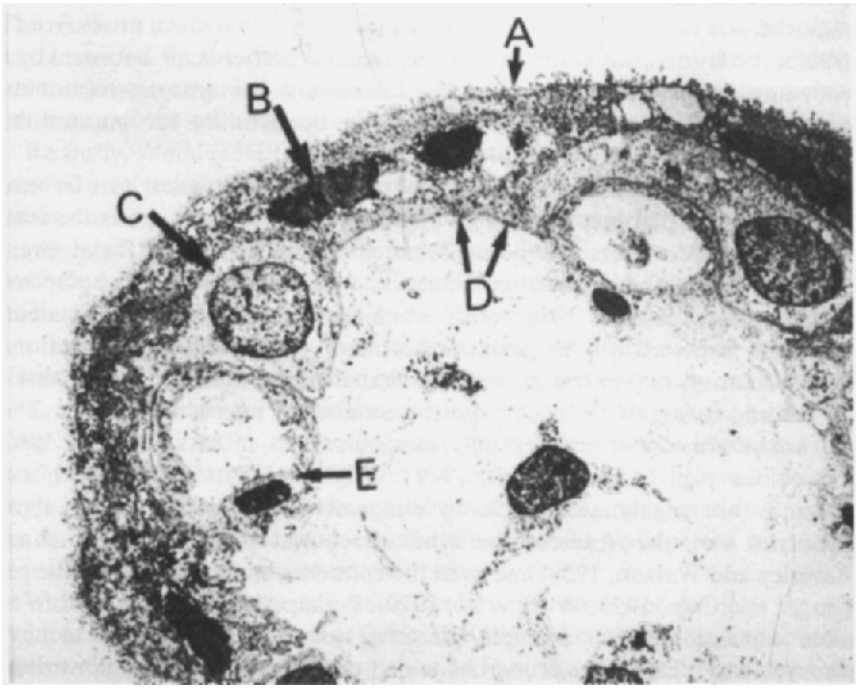

FIG. 2. Transmission electron micrograph of human chorionic villus. A, Microvilli of syncytiotrophoblast; B, nuclei of syncytiotrophoblast; C, cytotrophoblast; D, basement membrane; E, foetal capillary endothelium. (From Ockleford and Whyte, 1977.)

Furthermore, the cytotrophoblast cells are believed to gradually decrease in number as pregnancy advances (Boyd and Hamilton, 1970). This thinning of the trophoblast may have a bearing on the relative frequency of congenital

toxoplasmosis when the mother contracts the disease during the later stages of pregnancy (Thalhammer, 1962; Desmonts and Couvreur, 1974; Plotkin, 1975). It must be pointed out that the trophoblast basement membrane, on the other hand, appears to thicken progressively as the placenta ages (Boyd and Hamilton, 1970) with an average thickness of 220 μm in a placenta associated with a foetus of 13 cm CR length to 310 μm at full term. If the basement membrane does have a functional barrier effect, then this corresponding increase in thickness during a period when there is a thinning of the trophoblast may well compensate for a loss of integrity of the placenta. There is now much interest in the structure and biochemistry of placenta basement membrane and it appears that the type of collagen found here differs markedly from that found in basement membranes of other tissues. It remains to be seen whether this structural finding can be correlated with the functional requirements of a membrane which needs to be highly selective in what it allows to pass through. A further point of interest which arises from a structural consideration is that, since the placental barrier layer is formed by a syncytium, maternal organisms must pass *through* the syncytiotrophoblast to reach the foetal circulation. There is no opportunity for intercellular penetration.

From the above discussion on comparative placentation, it can be seen that the human placenta belongs to the category in which there is the least number of tissue layers interposed between the maternal and foetal circulations. If indeed foetal infection is due to active penetration of the placenta by maternal organisms then, on anatomical grounds, man and rodents would be expected to be highly susceptible to intra-uterine infections. This conclusion makes the assumption that the placental barrier is entirely passive and is largely dependent on the number of intervening layers. This may not be entirely correct. Certainly, man, mice, rats, rabbits (Beverley, 1960) and guinea pigs (Adams *et al.*, 1949; Wright, 1972) with haemochorial placentae are highly susceptible to congenital toxoplasmosis, but these organisms can also traverse the syndesmochorial placenta of the sheep (Beverley and Watson, 1959) and even the epitheliochorial placenta of the pig (Sanger and Cole, 1955; Work *et al.*, 1970). Perhaps other factors need to be taken into account. For example, placental cells have been shown to have pinocytic and phagocytic properties which could be expected to contribute towards the organ's defensive capabilities. Thus, the possibility must be considered that the placental barrier against infections is an active one.

B. PHAGOCYTOSIS BY PLACENTAL CELLS

In his study of malarial placentae, Garnham (1938) was very interested in the phagocytic system within this organ but he came to the conclusion that,

unlike that of the spleen, bone-marrow and liver, this activity in the placenta was confined to the intervillous space. The phagocytic cells that were mixed with the parasites in these spaces were probably derived from the blood, while the placental cells themselves appeared to play no part. Wislocki (1921) also did not believe that placental cells were capable of phagocytosis because when he injected indian ink intravenously into pregnant dogs, cats, rabbits or guinea pigs, no carbon particles were observed in the placenta. In contrast, most recent evidence appears not to be in agreement with these conclusions. Moskalewski *et al.* (1974) harvested a mixed suspension of cells by enzymatic dispersion of the mouse placenta during early and late gestation. These cells were all capable of phagocytosing *Candida albicans* to varying degrees. The greatest activity was shown by placental macrophages but trophoblast cells as well as endothelial cells also did so to a limited extent. Human placental cells are also capable of phagocytosing *Candida albicans* (Foldes *et al.*, 1975). The authors made this observation after their cultures were accidentally contaminated by the fungus. They noted with some surprise that, whereas other cell types rounded up and died, placental cell cultures not only survived but many of the cells had actually phagocytosed the fungi.

Recently, Wood (1980) carried out a quantitative analysis and reported that over 60% of cells obtained by trypsinization of human placentae had characteristics of mononuclear phagocytes as demonstrated by morphology, the presence of Fc and C3 receptors and the ability to phagocytose opsonized red blood cells which were used to form EAC rosettes. Additional studies on frozen whole placental sections using EA rosettes and fluorescein-labelled immune complexes demonstrated that the majority of the cells in the villous stroma were Fc-receptor positive, indicating they were probably mononuclear phagocytes. Certainly macrophages designated as Hofbauer cells have long been identified by morphological criteria in the human placenta (Fox, 1967; Boyd and Hamilton, 1970) but the data of Wood (1980) would seem to suggest that these cells are more numerous than was appreciated in the past. As an organ, the human placenta, therefore, would be expected to possess considerable phagocytic properties.

We ourselves have observed phagocytosis of a variety of particles by cells isolated from human placentae (Table 2; Y. W. Loke and S. Day unpublished observations). It can be seen that over 50% of the total cell population are capable of phagocytosing the smaller particles, while fewer cells have taken up the larger ones. When latex particles of increasing size were used, placental cells were found to be capable of phagocytosing particles of up to 8·3 μm in diameter. Unlike *Staphylococcus aureus*, heat-killed *Streptococcus pyogenes* were not phagocytosed by placental cells unless they were first opsonized by specific antibody (Table 3). This indicates that surface characteristics of the organisms as well as size are important factors determining their susceptibility to phagocytosis by placental cells.

TABLE 2

Percentages of cells phagocytosing different particles after various periods of incubation.
(From Y. W. Loke and S. Day, unpublished observations)

Particles	% of phagocytic cells after incubation for: 30 min	1 hr	2 hr	24 hr
Staphylococcus aureus	61	56	58	67
Latex (1 μm)	50	54	60	68
Latex (5·7 μm)	12	21	24	25

TABLE 3

Phagocytosis of heat-killed Streptococcus pyogenes (*untreated and treated with specific antibody*) *by placental cells after various periods of incubation.*
(From Y. W. Loke and S. Day, unpublished observations)

Streptococcus pyogenes	% cells containing organisms after incubation for: 30 min	1 hr	2 hr	24 hr
Untreated	0	0	0	0
Sensitized with 1: 100 antibody	0	2	6	10
Sensitized with 1: 50 antibody	0	5	5	25

These phagocytic cells express Fc and C3 receptors and many of them also have HLA-DR antigens on their surface so we are in agreement with Wood (1980) that they probably belong to the macrophage series.

Besides possessing the requisite surface receptors for immune phagocytosis, human placental cells also appear to contain the necessary enzymes for the intracellular digestion of phagocytosed material (Contractor and Krakauer, 1976a, b). In our own experiments, ingested bacteria were found to be rapidly lysed.

Therefore, the available evidence would seem to indicate that the human placenta, as an organ, can act as an active barrier against the materno-foetal transmission of infective organisms by virtue of its phagocytic properties. Perhaps, initially, the integrity of the syncytiotrophoblast does have a passive barrier effect but if this layer should be breached by leaks and tears, then the second-line defensive role is assumed by the villous mesenchymal macrophages.

It could be argued that parasites, unlike bacteria and viruses, are really too large to be dealt with in this manner. We have observed that human placental cells are capable of phagocytosing latex particles of up to 8·3 μm in diameter, which would be sufficient to deal with many protozoal organisms. Indeed, amastigotes of *Trypanosoma cruzi* have been observed inside human trophoblast cells as well as within the cytoplasm of Hofbauer cells (Bittencourt, 1976). It was demonstrated experimentally that transplacental transmission of *Trypanosoma cruzi* could only be achieved in mice if the R-E system of the animals was first depressed by treatment with thorium dioxide, indicating that phagocytic efficiency of the placenta is an important factor in its barrier effect (Delgado and Santos-Buch, 1978). Similarly, *Toxoplasma gondii* have been detected lying within human syncytiotrophoblast cells (Elliott, 1970) and in mouse syncytiotrophoblast where the parasites were actually found to be undergoing active multiplication (Cowen and Wolf, 1950b). *Toxoplasma* appear to have evolved a mechanism for evading intracellular digestion by host cells by blocking the delivery of lysosomal constituents to phagocytic vacuoles (Hirsch *et al.*, 1974). Thus, not only are they not killed, but they are now able to create for themselves a microenvironment which permits their replication and in which they are sheltered from the effects of host antibody. Can this be the reason why congenital toxoplasmosis occurs so frequently compared to other parasitic infections?

C. TRANSMISSION OF CELLS ACROSS THE PLACENTA

Many protozoa are intracellular parasites and it is possible they may be carried across the placenta within the cells they inhabit. For example, *Plasmodium* is said to be transmitted inside erythrocytes (Thomas and Chan, 1980) and *Toxoplasma* via infected leucocytes or macrophages (Remington *et al.*, 1961a). Available data on cellular exchange across the placenta are based mainly on clinical observations. In the present context, it is, of course, the transmission of cells from the direction of the mother to the foetus that is relevant.

1. *Erythrocytes*

Early studies on the transfer of erythrocytes from mother to foetus employed the infusion of red blood cells labelled with radioactive ^{32}P (Naeslund and Nylin, 1946), radioactive ^{59}Fe (Naeslund, 1951) or radioactive ^{51}Cr Smith *et al.*, 1961) into the maternal circulation and then testing foetal blood after delivery for level of radioactivity. Smith *et al.* (1961) detected significant radioactivity in the foetal blood in 13 out of 18 patients after infusion of labelled erythrocytes while Zarou *et al.* (1964) found 7 out of 19

cases were positive. It was calculated that volumes of from 1 ml to 13 ml of blood could be transmitted. Other investigators preferred the alternative approach of using erythrocytes with some inherent definitive characteristics which could be recognized in the foetal circulation after infusion into the mother. Hedenstedt and Naeslund (1946) infused elliptocytes into two pregnant women and recovered similar cells in the blood of one infant. Mengert *et al.* (1955) infused cells with the sickling trait into two pregnant women at term and found sickle cells in the blood of both infants. Further evidence was presented by Macris *et al.* (1958) who found sickle cells in the cord blood of newborn infants in three out of 25 cases where the mother was transfused with whole blood from donors with the sickle-cell trait. Fujikura and Klionsky (1975) found there was a 100% concurrent incidence of sickling in maternal and foetal blood in women with the sickling trait. The transplacental passage of maternal erythrocytes was inferred because foetal erythrocytes with the sickling trait would not usually sickle because of their high content of haemoglobin F.

More recently, investigators have looked for the presence of Rh positive cells in the blood of Rh negative infants born to Rh positive mothers as an indicator for the transmission of maternal erythrocytes. Using a fluorescent antibody technique, Jennings and Clauss (1978) detected Rh positive cells in only two cases out of 105 (1·9%) and therefore concluded that the materno-foetal transfer of erythrocytes is rare. In contrast, Carapella-de Luca *et al.* (1978) using a mixed agglutination method, detected Rh positive maternal cells in 47 out of 86 newborns, which is a frequency of over 50%. The latter authors claimed that this technique was highly sensitive and could detect one foreign cell among 10 000.

Most investigators are, therefore, agreed that maternal erythrocytes can be transmitted to the foetus but there is some difference in opinion as to how often this occurs. The conflicting data may be a reflection of the sensitivity of the techniques used. It must be remembered that the finding of maternal cells in the blood of newborn infants does not necessarily imply that these cells have crossed the placenta during pregnancy. It may well be that the actual moment of transfer occurs during the separation of the placenta at delivery. Current opinion views the transplacental transmission of cells as a passive event with blood passing through breaks in the placental barrier. This cellular exchange can take place throughout gestation but appears to increase as pregnancy advances (Cohen *et al.*, 1964) probably as a result of progressive deterioration of the trophoblast layers, with the most significant transmission occurring during parturition.

Another factor to be considered is the direction of blood flow through the placental leaks, which is dependent on the prevailing pressure differential between the foetal and maternal circulations. This appears to favour the

transmission of cells from foetus to mother rather than in the reverse direction because Cohen and Zuelzer (1964) found that, in 82 pregnant women studied immediately after birth, maternal red blood cells were demonstrable in foetal blood in only three (3·6%) cases while, at the same time, foetal red blood cells were found in maternal blood in 34 (41·5%) cases.

Even if maternal erythrocytes have managed to break through the trophoblast barrier, they may be stopped by the mesenchymal phagocytic cells mentioned in a preceeding section. Red blood cells have been observed to be phagocytosed by sheep (Myagkaya and Vreeling-Sindelarova, 1976), mouse, rat and guinea pig placentae (Schwartz *et al.*, 1974) under physiological conditions. Wood (1980) noted phagocytosis of opsonized sheep erythrocytes by human placental cells and we ourselves have seen that 20–30% of human placental cells are capable of phagocytosing opsonized ox erythrocytes (Y. W. Loke and S. Day, unpublished observations).

Thus it may be concluded that both the structural characteristics and haemodynamics of the placenta, together with the phagocytic activity of its cells, can all combine to discourage the transmission of maternal cells to the foetus during gestation. If the transplacental transmission of *Plasmodium* is similarly dependent on the passive transfer of infected erythrocytes, then the inefficiency of this mode of transport provides a valid explanation why congenital malaria is relatively uncommon.

2. *Leucocytes*

Data relating to the materno-foetal transmission of leucocytes are based mainly on the detection of 46XX cells in cord blood of male infants. The results obtained by various groups of investigators are summarized in Table 4. It can be seen from this table that maternal leucocytes are not easily detectable in foetal blood, with only four positive cases out of 279 newborn infants tested and only 22 female cells seen from a total of 22 626 cells analysed. Assuming that the mechanism of leucocyte transmission is like that for erythrocytes and is dependent on the passive transfer of maternal blood across leaks in the placental barrier, then it is not surprising that the number of maternal leucocytes which have succeeded in doing so is small. Therefore, the suggestion that *Toxoplasma gondii* may be transported across the placenta via infected leucocytes during a chronic phase of the infection (Huldt, 1960; Remington *et al.*, 1961a) cannot be considered as a significant mode of transmission, if it occurs at all.

3. *Neoplastic cells*

Further supporting evidence that the human placenta does not usually permit the transfer of maternal cells to the foetus can be seen in studies of pregnant women with cancer. In a survey of the world literature spanning a

period of 100 years from 1866–1966, Potter and Schoeneman (1970) could only find 24 reported cases where maternal cancer had metastasized to the placenta. Of these 24 cases, only eight had succeeded in breaching the placental barrier to affect the foetus. Even maternal leukaemia did not appear to be transmitted to the foetus (Vitums and Sites, 1968).

TABLE 4.

Summary of the results from different groups of investigators concerning the detection of maternal leucocytes in the foetal circulation.
(From Loke, 1978)

Reference	No. of newborn young tested	No. of cases where 46 XX cells detected	No. of 46 XX cells present	No. of cells analysed
Turner *et al.* (1966)	183	2	14	5490
Angell and Adinolfi (1969) (Cited by Adinolfi (1975))	18	0	0	6482
Anderson and Ferguson-Smith (1971)	5	0	0	534
Kay and Margoles (1971)	33	0	0	895
Olding (1972)	14	0	0	1772
Adinolfi and Gorvette (1973) (Cited by Adinolfi (1975))	16	1	4	1600
Schröder (1974)	10	1	4	5853
Total	279	4	22	22 626

When detailed histological studies were made, it could be seen that even when tumour masses came to occupy the inter-villous spaces of the maternal sinusoids, no tumour cells ever succeeded in invading the chorionic villi (Bender, 1950; Horner, 1960; Freedman and McMahon, 1960; Diamondopoulos and Hertig, 1963). This is really a remarkable finding, for cancer cells are highly invasive for most tissues. If trophoblast can resist penetration by malignant tissue, then it should come as no surprise that it can also deal effectively with parasite migration.

IV. EFFECTS OF PREGNANCY ON THE SEVERITY OF PARASITIC INFECTIONS

During pregnancy, there is a depression of maternal immune response. In the main, this lowered reactivity is directed specifically towards foetal

allo-antigenic determinants and this process is probably concerned with maternal non-rejection of her immunologically alien conceptus. In addition, there appears to be also a large element of non-specific immune depression mediated by immuno-suppressive substances like pregnancy-specific proteins and hormones. This would compromise maternal defence against pathogenic organisms and, thus, have an adverse effect on many parasitic infections. An increased severity of the disease will lead to a greater chance of dissemination of the organisms to the placenta and hence to the foetus.

A. AMOEBIC DYSENTERY

Lewis and Anita (1969) from Ibadan in Nigeria reported a statistically significant association between amoebic colitis and pregnancy. This was later confirmed in detailed post-mortem studies (Abioye and Edington, 1972; Abioye, 1973) where it was shown that, while amoebiasis, typhoid and other enterocolites were responsible for 42%, 42·1% and 52·2% respectively of deaths in women of all age groups, the picture was very different when causes of death in pregnant women were analysed. The figures now were 68% for amoebiasis, 17·1% for typhoid and 12·5% for other enterocolites. In fact, amoebiasis appears to be the main cause of maternal death in Tanzania (Armon, 1978). A case of a young woman with fatal, post-partum invasive amoebic colitis who died in spite of intensive therapy was reported by Rivera (1972). On post-mortem, evidence of organization and fibrosis was seen in her liver abscess, indicating that the patient had had the disease for some time. Somehow, the relatively dormant parasite must have suddenly become invasive again during the latter part of pregnancy.

The increased production of various substances during pregnancy may enhance the growth of *Entamoeba histolytica*. For example, the rise in serum cholesterol could provide an important growth factor for this protozoan (Armon, 1978). Corticosteroids have long been known to exacerbate latent amoebiasis (Armon, 1978), although whether its mode of action is by stimulating the growth of the parasite or by decreasing host inflammatory reaction is not clear. The plasma level of 17-hydrocorticosteroids rises progressively during pregnancy, sometimes reaching levels which even exceed those found in patients with Cushing's syndrome (Bayliss *et al.*, 1955).

B. MALARIA

It is an established fact that latent malaria has the tendency to develop into acute overt attacks during pregnancy (Clark, 1915), which could be due to

a lowering of immunity. In an endemic area, malarial pyrexia is rare among the indigenous population after the fourth year of life, although a low grade parasitaemia is maintained. This host–parasite equilibrium can break down during pregnancy, resulting in pyrexia and heavy parasitaemia developing, especially in primigravidae (McGregor and Smith, 1952), and most noticeable in the first trimester (Bray and Anderson, 1979). In a prospective study of 60 primigravidae in Nigeria, Gilles *et al.* (1969) found that the frequency of malarial parasitaemia and density of infection was higher in pregnant women, compared to either the same individuals before pregnancy or to a control group of similar age. Bruce-Chwatt (1952) also found that the parasite rate in parturient women was 12% higher than in the rest of the adult indigenous population of Lagos. The cases of congenital Quartan malaria described in the United States (McQuay *et al.*, 1967; Harvey *et al.*, 1969) may be examples of the reactivation of a latent infection during pregnancy. These patients were symptomless and, since they were now living in a non-endemic area, reinfection was unlikely. The source of the foetal infections must have been from latent maternal infections: *Plasmodium malariae* is known to remain in the host for long periods.

Humoral immunity plays an important role in the host defence against malaria (Butcher *et al.*, 1973), so the exacerbation of infection during pregnancy could be due to a depression of antibody production, particularly that of IgG class. It has been reported from a Gambian community that the mean values for IgG and IgA in pregnant women were significantly lower ($P = 0{\cdot}001$) than for non-pregnant controls, with the levels of IgG declining progressively to reach the lowest level at the last 10 weeks of pregnancy. IgM and IgD showed the same tendency to be lower in pregnant women but the figures did not reach statistical significance (McGregor *et al.*, 1970). Longitudinal follow-up studies of individual women during different stages of gestation confirmed a significant decrease in IgG and IgA levels in the second and third trimesters (Amino *et al.*, 1978). Pacsa and Pejtsik (1977) tested the sera from 297 pregnant women randomly for rubella, herpes simplex and toxoplasma antibodies. They found antibody titres were relatively high at 12–20 weeks, fell to a low level at delivery, and then returned to early pregnancy levels 30–45 days post partum.

The evidence would seem to confirm the hypothesis that there is a depression of antibody production during pregnancy. However, it should be pointed out that the reduction of IgG values observed in pregnant women may be apparent rather than real. The progressive increase in plasma volume and extracellular fluid could serve to dilute the amount of antibody present, although the method of calculation by Amino *et al.* (1978) based on total serum proteins goes some way to exclude a potential haemodilution bias. Significant amounts of IgG could also be transmitted to the foetus or are

absorbed by the placental mass, thereby being sequestered from the maternal circulation.

As regards specific anti-malarial antibody, it has recently been observed that there is a significant reduction of the Indirect Fluorescent Antibody (IFA) titre in the third trimester of pregnancy when compared to non-pregnant controls (Campbell *et al.*, 1980) although this was not apparent to earlier investigators (see Taufa, 1978). This raises the question as to whether this antibody is indeed a true measure of acquired protective immunity against malaria as is generally supposed. Nevertheless, depression of humoral immunity during pregnancy would seem to be the most logical explanation for the increased susceptibility to malarial attacks, although other non-specific factors like increased protein requirements or hormonal output must also be considered.

C. TOXOPLASMOSIS

Pregnant mice are found to be about three times more susceptible to experimentally induced toxoplasma infections than non-pregnant controls, indicating that the pregnant animal is a more receptive host for the parasite (Cowen and Wolf, 1950a). If this should be true also for pregnant women, then it offers an explanation why there is such a high frequency of human congenital toxoplasmosis. Foetal involvement even during the chronic latent phase of maternal disease may be due to the fact that, during pregnancy, immunity is reduced to such a low level that parasitic cysts in the uterus which are otherwise quiescent, are now permitted to liberate organisms to invade the placenta and foetus (Werner *et al.*, 1963; Jacobs, 1967).

Certainly, *Toxoplasma gondii* (together with certain fungi, Gram-negative bacteria and DNA viruses) appears to be emerging as an important opportunist pathogen in patients with impaired immunological mechanisms like cancer patients and those undergoing immunosuppressive therapy (Remington, 1970). The immunodepression associated with pregnancy would be expected to lead to a similar enhancement of susceptibility. In the preceeding section, we have already reviewed the evidence that there may be a deficiency in antibody production in pregnant women. Free from the constraints of high titres of specific antibody, toxoplasma cysts may become active again to give to periodic parasitaemia, thus enabling the organisms to reach the placenta and the foetus.

Since the protozoan is an obligate intracellular parasite, cell-mediated immunity would also play an important role in host defence, although the exact mode of action in relation to toxoplasmosis has not been clearly established.

Much evidence has now accumulated that maternal cell-mediated immunity is depressed during human pregnancy (see Loke, 1978). Although this lowered reactivity can be shown to be directed specifically at foetal antigens, a factor which may contribute to the non-rejection of the foetal allograft, there is also an element of non-specific depression. In a study of the mixed lymphocyte reaction (MLR) which is an *in vitro* measure of the inherent ability of lymphocytes from two genetically non-identical individuals to be stimulated by each other, maternal lymphocytes were found to respond poorly to allogeneic cells (Jones and Curzen, 1973) and also to rubella virus (Thong *et al.*, 1973). Maternal T cell immunocompetence, as measured by phytohaemagglutinin (PHA) transformation, also appears to be low.

It appears that the lowered response observed in these experiments is mainly due to factors present in maternal serum rather than to any inherent defect of maternal cellular reactivity (Bissenden *et al.*, 1980). These inhibitory factors are detectable in the serum of pregnant women and in experimental animals and do not appear to be antigen specific nor even species specific (Noonan *et al.*, 1979). A large variety of substances have been suggested as being responsible, either acting alone or in concert with others. They include products generated by the allergic response towards foetal antigens like antibody, immune complexes and suppressor cells which may act as negative feedback homoeostatic mechanisms. Placental hormones can have a dampening effect on maternal cellular reactivity. Both steroid hormones like 17-hydroxycorticosteroid, oestrogen or progesterone and protein hormones like human chorionic gonadotrophin (HCG) have been observed to have a wide variety of non-specific immunosuppressive functions *in vitro* and *in vivo*. The foetal protein, alpha-foetoprotein (AFP), similarly has a marked depressive action on lymphocyte reactivity in the murine as well as human systems. Nearly all these substances inhibit mainly T cell functions so this will eventually affect both cell-mediated immunity as well as antibody production. The precise mechanisms of action have yet to be elucidated. They may simply coat lymphocyte surfaces and thus prevent antigen recognition, but certain observations with AFP would seem to suggest that its mode of action is likely to be more complex than the mere competitive blocking of receptor sites.

Maternal neutrophil activity also appears to be diminished during pregnancy. The migration (Senelar and Bureau, 1979) and chemotactic response of these cells are significantly depressed (Björksten *et al.*, 1978). Phagocytosis of live and heat-killed bacteria is inefficient compared to non-pregnant controls and this seems to be mediated by some inhibitory serum factors present in pregnant women (Björksten *et al.*, 1978; Persellin and Leibfarth, 1978) which appear from 16 weeks gestation, remain throughout and are no longer detectable by 2 days post partum (Persellin and Thoi, 1979). The intra-

cellular digestive capacity of neutrophils are also impaired in pregnant women as indicated by the observations of El-Maallem and Fletcher (1980) where the killing of ingested candida was reduced to about 65% of that of non-pregnant controls. This was associated with a fall in the myeloperoxidase enzyme content of the cells to 55% of non-pregnant neutrophils. The authors proposed that this reduced activity was due to the fact that the neutrophils were already partially exhausted by their constant phagocytosis of antigen–antibody complexes generated by the foetal–maternal immunological interaction. Since neutrophils play a very important role in the inflammatory reaction, a depression of their activity would be expected to compromise further the defence capabilities of a pregnant host against invasion by pathogenic organisms.

D. HELMINTHIC INFECTIONS

Direct evidence for any effects of pregnancy on helminthic infection is scanty. Perhaps *Toxocara canis* may be cited as an example. Intra-uterine infection with these parasites can occur even when the bitches are not harbouring intestinal worms (Yutuc, 1949; Webster, 1958). This may be due to larvae which have been encapsulated in somatic tissues of the bitch becoming reactivated during pregnancy and then migrating to the placenta and across to the foetus (Scothorn *et al.*, 1965). Only a few larvae need to be involved at any one time, with those remaining repeating the process during subsequent gestations. This provides a potential reservoir for prenatal infections of puppies over several pregnancies without the need for the bitch to be reinfected (Koutz *et al.*, 1966). At present, it is not clear whether this reactivation of somatic larvae is due to a lowering of maternal immunity or to the hormonal changes associated with pregnancy.

V. Parasitic Infections of the Placenta

A. MALARIA

1. *Parasites in placental sinusoids*

Any parasite that is widely disseminated in the pregnant host has an opportunity to reach the placenta, but in malaria, this organ appears to offer a particularly suitable environment for the organisms. This was already well appreciated by investigators in the beginning of this century who drew attention to the massive infection that may be present in placental blood at a

time when only a few parasites can be seen in the peripheral blood of the mother (see Covell, 1950). This led Clark (1915) to conclude that examination of placental blood may be of greater diagnostic value for malarial infection during pregnancy than examination of peripheral blood. In his 19 cases where placental blood was positive for parasites, less than half of these (eight cases) had demonstrable parasites in the peripheral blood. A random selection of 26 placentae was examined by Blacklock and Gordon (1925a) in an endemic malarious area: they found that 46% were infected but only 17% of these patients showed parasites in their peripheral blood smears. In an investigation from Stanleyville, Schwetz and Peel (1934) reported that heavy infections were found in 16% of placental blood in spite of the absence of parasites in maternal peripheral blood. The more recent findings of Jellife (1968) confirm these older reports. In an analysis of 570 Ugandan women within 22 hours of delivery, Jellife found the placentae were infected in 16·1% of cases compared to 5·6% of peripheral blood samples. It is interesting to note that, even when a mother is treated with quinine so that her peripheral blood is cleared of parasites, her placental blood film still shows abundant organisms (Wislocki, 1930). This observation, of course, has an important bearing on the treatment of malaria during pregnancy and raises the question whether the parasites are more resistant to drugs in the placenta or perhaps the penetration of drugs to the sinusoids is relatively inefficient.

The placenta as an organ of sequestration appears to apply particularly to *Plasmodium falciparum* infection. The few quartan infections examined did not show any parasite concentration in the intervillous spaces (Garnham, 1938). The most abundant forms are schizonts while gametocytes are rarely seen (Clark, 1915; Blacklock and Gordon, 1925a; Garnham, 1938; Thomas and Chan, 1980) although Jellife (1968) reported seeing gametocytes more frequently. It appears, therefore, that the placental sinusoids can provide just the right environment for the asexual development of *P. falciparum*. It has been suggested that this is because these areas have a sluggish blood flow, a low oxygen tension and correct concentration of glucose, conditions which simulate the tissue culture requirements of *P. falciparum in vitro* (Blacklock and Gordon, 1925a). These beneficial conditions are fulfilled only in the relatively mature placenta, for no parasite schizogony can be seen before the fourth month of gestation and are also confined to the intervillous spaces only. Even maternal blood vessels traversing the decidua basalis do not share in this parasite development (Garnham, 1938).

Since this great multiplication of parasites in the placental sinusoids is not accompanied by a concommitant increase of parasites in the peripheral blood, it may be concluded that the parasites are somehow trapped in the placenta. Experimentally it has been shown that schizonts of *P. falciparum*-infected *Aotus* monkeys are sequestered in the vessels of the heart, adipose tissue,

skeletal muscle and intestinal submucosa (Miller, 1969) and it is thought that the mechanism for this deep vasculature sequestration is due to some ultra structural alteration in the infected erythrocytes inducing them to bind to vascular endothelium (Luse and Miller, 1971). Can the same thing occur in the placenta? Bray and Sinden (1979) have recently looked into this question. Neither light nor electron microscopy revealed any definite attachment of parasitized erythrocytes to the trophoblast layer of placental villi so they concluded that retention of parasites in the placenta cannot be explained on the basis of binding to the lining cells of the sinusoids as postulated for the deep vasculature of other organs. They were also unimpressed with the theory that sluggish blood flow might delay the passage of infected erythrocytes through the placental circulation because they felt that the estimated pressure of 80 mm of mercury with frequent bursts of flow would not support such a mechanism. This problem must await further elucidation.

2. *Mononuclear cell infiltration*

Besides infected erythrocytes, the intervillous spaces are infiltrated by large numbers of mononuclear cells. Although Galbraith *et al.* (1980b) detected only macrophages, other investigators found also lymphocytes (Wislocki, 1930; Garnham, 1938; Thomas and Chan, 1980). The functional significance of these cells is not clear. They are not present to any extent in recent infections of susceptible subjects but are found only in chronic infections (Garnham, 1938). The complete absence of placental cellular infiltration in *P. berghei* infections of gravid mice noted by Bruce-Chwatt and Gibson (1956) may, therefore, be a similar manifestation of recent infection rather than any difference between human and murine response to placental malaria. Reticuloendothelial hyperplasia is a conspicuous feature of chronic malarial infection but it has not yet been clearly established whether this response contributes significantly to the host's acquired defences or is merely an adaptation for clearing up the increased number of damaged red blood cells which must be removed. The macrophages in placental sinusoids do contain much pigment (Wislocki, 1930; Garnham, 1938) but this finding does not distinguish an immune from a scavenging function. However, later investigators did detect intact parasites (Thomas and Chan, 1980) as well as erythrocyte debris (Galbraith *et al.*, 1980b) within the macrophages, indicating an active defensive role. One of the effector mechanisms of cell-mediated immunity is the accumulation of activated macrophages resulting from the release of lymphokines and these cells would be expected to be ideally suited for ingesting and disposing of parasite-infected host cells. This function would be further augmented in the presence of maternal antibody acting as opsonins on the surface of the parasites and perhaps even on the surface of her own infected red blood cells if they expose any new parasite-induced antigenic

determinants. It is, therefore, tempting to propose that the accumulation of mononuclear phagocytes, with their contents of pigment and parasites, in the placental sinusoids is a manifestation of the gravid host's response to malaria. Even the syncytiotrophoblast of malarial placentae have been observed to contain pigment (Galbraith *et al.*, 1980a, b). Whether this indicates that trophoblast can also fulfil an active defensive function against malarial parasites similar to that of the mesenchymal macrophages remains to be determined.

3. *Changes in the placenta*

In spite of all the parasite and cellular accumulations in the sinusoids, it is remarkable that distinctive abnormalities in the villi are not readily apparent. Wislocki (1930) observed that in human malaria, the villi, including their stroma and covering chorionic trophoblast, were not visibly affected by the organisms or their products in the intervillous spaces. In some areas, there were depositions of fibrin under which the syncytium appeared denuded to a certain extent but Wislocki regarded this as a finding that is normally present in a mature placenta and should be regarded as a degenerative or senescent phenomenon. This conclusion agrees with current opinion that fibrin deposits tend to increase as pregnancy advances, being particularly frequent in post-mature placentae (Boyd and Hamilton, 1970). In immune placentae infected with *P. berghei*, again neither the syncytiotrophoblast nor the cellular elements of the placental labyrinth showed any reaction to the parasites in the maternal blood sinusoids (Bruce-Chwatt and Gibson, 1956).

With the benefit of electron microscopy, Galbraith *et al.* (1980b) described changes in the malarial placenta which might not have been apparent to earlier investigators. They saw focal areas of syncytiotrophoblast necrosis and loss of syncytial microvilli, particularly in relation to monocyte accumulation or in syncytial cells containing pigment. This suggests that the phagocytic monocytes or their products, and malarial pigment itself, may have a toxic effect on the adjacent trophoblast layer. There is an accompanying cytotrophoblast proliferation which may reflect a non-specific response to trophoblast damage. The trophoblast basement membrane was disorganized, amorphous and grossly thickened, with deposits of osmiophilic granules and was partially traversed by tongue-like projections of syncytiotrophoblast. Immunohistological staining revealed a markedly increased amount of C3d and C9 completely encircling the trophoblast basement membrane which may be taken to indicate that the changes observed have an immunopathological origin. Immune complexes between antibody and plasmodial antigen may be involved. Within the villi, the only consistent abnormality noted was an increased number of collagen fibres.

The above observations, therefore, indicate that pathological features are

associated with a malarial placenta but it is somewhat difficult to decide how definitive these changes really are in relation to malarial infection. These authors examined ten placentae from Gambian patients of which only three had evidence of parasites. The remainder contained only pigment, but was presumed to have had malaria sometime during the pregnancy. Furthermore, some of the changes described may not be restricted to malarial placentae but can be found even in normal uninfected organs. For example, Boyd and Hamilton (1970) have described the presence of osmiophilic granules in normal trophoblast basement membrane with tongues protruding into this structure from the overlying trophoblast. The attenuation or absence of syncytial microvilli, similarly, may not be significant for even in the normal placenta, "electron micrographs frequently show that, on one and the same villus, regions with developed microvilli can be present close to areas with a smooth surface" (Boyd and Hamilton 1970). Thus, it could be highly profitable for readers who are not familiar with the detailed ultrastructure of the human placenta to consult that excellent and superbly illustrated monograph by these authors before making up their own minds regarding the significance of changes described in association with diseases of this organ.

B. TOXOPLASMOSIS

Like the central nervous system, the placenta is a particularly receptive site for *Toxoplasma gondii*. A sensitive method for determining whether a placenta is infected is to inoculate a sample of placental homogenate after tryptic digestion into susceptible animals like mice. In this way, organisms have been isolated from the placentae of guinea pigs (Wright, 1972), dogs (Chamberlain *et al.*, 1953) and man where 25% of the placentae were found to be infected in primary maternal toxoplasmosis acquired during pregnancy (Desmonts and Couvreur, 1974).

The parasites can be observed in histological material from the placenta but a relatively large number of sections need to be examined. Both free trophozoits and encysted forms of toxoplasma can be seen in placental villi (Werner *et al.*, 1963; Glasser and Delta, 1965; Garcia, 1968; Elliot, 1970; Altshuler, 1973; Altshuler and Russell, 1975). In some areas, the parasites form clusters of several dozen or more, indicating local multiplication (Cowen and Wolf, 1950b). Parasites are found mainly in the stroma of the villi but it is interesting to note photomicrographs depicting organisms lying within the syncytiotrophoblast (Elliot, 1970) and in endothelial cells of the chorionic vessels (Garcia, 1968). In experimentally induced toxoplasmosis in gravid mice, groups of *Toxoplasma* are also seen in the cytoplasm of the syncytiotrophoblast, located mainly adjacent to the nuclei (Cowen and Wolf,

1950b). These observations suggest that transmission of *Toxoplasma* from mother to foetus may be by initial invasion of, and multiplication within, the trophoblast layer. This is followed by the organisms undergoing the same process in cells of the villous stroma and then in the endothelial lining of the chorionic vessels, finally gaining access to the foetal circulation.

The organisms are capable of growth for a time inside the syncytiotrophoblast without causing any significant changes in them, but eventually these cells will begin to die (Cowen and Wolf, 1950b). There is a gradual loss of cytoplasmic basophilia with nuclei showing fragmentation and lysis, leading to small focal areas of syncytial necrosis which is a characteristic histological feature of placental toxoplasmosis. Leucocyte infiltration and other signs of acute inflammation are absent. Instead, the histology is that of a chronic low-grade villitis with stromal fibrosis, infiltration by lymphocytes, histiocytes and even giant cells to give rise to almost a granulomatous reaction (Garcia, 1968; Elliot, 1970; Fox, 1978). In many cases, a conspicuous feature is hydropic degeneration of the chorionic villi. This hydrops is detectable macroscropically where the placenta is seen to be large, bulky and pale. This appearance should raise the suspicion of placental toxoplasmosis (Altshuler, 1973).

C. CHAGAS' DISEASE

The most detailed study on placental pathology in Chagas' disease is by Bittencourt (1976). This author noticed large numbers of parasites in 14 infected human placentae, the organisms occurring as amastigotes rounded up within the cytoplasm of Hofbauer cells in the placental villi. In one case, probably that of a recent infection, a photomicrograph was published showing amastigotes distending a localized area of the syncytiotrophoblast. Severe placental infection was coincident with a severe foetal infection. Thus, it seems that *Trypanosoma cruzi* follows a similar route from mother to foetus as that seen for *Toxoplasma*, with the organisms initially invading the trophoblast layer where a period of multiplication occurs. They are then liberated into the stroma and phagocytozed by Hofbauer cells. Those that are not killed will undergo further multiplication cycles at the end of which trypomastigotes will be liberated to invade the foetal circulation through the chorionic blood vessels. The frequency of foetal infection is therefore dependent on the efficiency of intracellular survival of the parasite during its sojourn across the placenta.

Inflammatory reaction can be found both in the villous stroma as well as in the intervillous spaces and takes the form of a chronic granulomatous infiltration mainly of mononuclear cells and some giant cells. There is

villous oedema and an increase in the number of Hofbauer cells, findings that are somewhat reminiscent of placental toxoplasmosis. Indeed, the placenta in erythroblastosis foetalis due to Rh incompatibility also presents a very similar appearance, which leads one to conclude that perhaps these are non-specific changes to be found in any pathological condition that leads to malfunction of the foetal–placental unit.

D. HELMINTHIC INFECTIONS

There is little of significance in the literature about helminthic infections of the placenta. That larvae can occasionally make their way to this organ is evident from the observations of Kimmig (1979) of microfilariae of *Brugia pahangi* found in the placenta and those of Bittencourt *et al.* (1972b) of schistosomula infiltrating the labyrinth of the rat and rabbit placentae. Schistosome ova can be found in the placenta but, in marked contrast to other organs like the liver, inflammation around the eggs appears to be minimal (see Altshuler and Russell, 1975). It is not clear why there is this absence of cellular infiltration. The granulomatous reaction around schistosome eggs is generally considered to be due to a delayed hypersensitivity (Type IV) response and it is conceivable there may be insufficient time for this to develop fully within the life-span of the placenta, especially if the ova have reached this organ only during the latter part of gestation. Recently, Bittencourt *et al.* (1980) tabulated the cases of schistosome eggs affecting the human placenta which have been reported in the literature and added four more cases of their own. Eggs were demonstrable in the intervillous spaces as well as within the chorionic villi. In some areas, there is no inflammatory infiltrate around the eggs, a finding which agrees with that of Altshuler and Russell (1975), but in other areas, a chronic granulomatous lesion is induced. There is therefore a need for differential diagnosis from other chronic granulomatous placentitis like Chagas' disease and toxoplasmosis. The authors felt that the incidence of placental infiltration by schistosome ova is probably much greater than reported. At times in their own study, very large numbers of placental sections needed to be cut in order to find an egg. Perhaps the employment of some kind of digestive procedures of placental tissue may prove to be more efficient in identifying positive cases.

Of the four pregnancies described by these authors, one resulted in the birth of a normal infant but the other three were stillborn. There was no evidence of foetal infection so foetal death probably was secondary to placental pathology.

Larvae of *Toxocara canis* (Scothorn *et al.*, 1965) and microfilariae of *Onchocerca volvulus* (Brinkmann *et al.*, 1976) have been seen in the tissues

of the umbilical cord. This suggests that the route of transmission of helminthic larvae from mother to foetus does not necessarily have to involve an haematogenous route but could occur via the connective tissue of the umbilical cord. This mode of transmission would, no doubt, favour those helminthic species with a tissue migratory larval phase in their life cycles.

VI. Parasites that are Transmitted from Mother to Foetus *in utero*

A. *Toxoplasma*

1. *Animal studies*

Naturally acquired or experimentally induced congenital toxoplasmosis has been recorded in a wide variety of animal species. All types of placentation are represented. Foetal infections have been found in pigs (epitheliochorial), sheep (syndesmochorial) and dogs (endotheliochorial) and in mice, rats, rabbits and guinea pigs (haemochorial). Mice (Eichenwald, 1948; Cowen and Wolf, 1950a, b; Beverley, 1959; Remington *et al.*, 1961a) and rats (Hellbrugge *et al.*, 1953) are experimental animals most frequently used. The guinea pig is also highly susceptible and infection of the pregnant animal at any stage of gestation (Huldt, 1960) will be transmitted to the foetuses in all cases (Adams *et al.*, 1949; Wright, 1972). This occurs even with the Rabbit Beverley strain of organism which is considered to be of relatively low virulence (Wright, 1972). It is tempting to suggest that this could be a reflection of placental structure because the guinea pig, like man, has a haemochorial placenta and therefore has the least number of layers interposed between foetal and maternal circulations. Unfortunately, it is not possible to come to any definite conclusions regarding the relative frequency of transplacental transmission of toxoplasma between animal species of different placental types because the data are not directly comparable. For example, the evidence in sheep was based on an examination of 39 aborted foetuses where six (15%) were found to harbour the organisms (Beverley and Watson, 1959). These figures, therefore, reveal that abortion in sheep can be caused by *toxoplasma* but do not give any indication as to the frequency of transplacental transmission. A further uncontrolled factor is the experimental procedures used to infect the pregnant mother. These range from feeding the animals with *Toxoplasma*-infected tissue (Eichenwald, 1948; Work *et al.*, 1970) to injecting organisms intravenously (Hellbrugge *et al.*, 1953; Work *et al.*, 1970), intraperitoneally (Wright, 1972) or via the vagina (Cowen and Wolf, 1950a). It is not clear if the degree of dissemination of the organisms to the

placenta is the same in these different routes of infection. Finally, it cannot be entirely certain that the foetal infection has been acquired *in utero*. Delivery of the foetuses by Caesarian section (Adams *et al.*, 1949) would go some way to ensure that infection had not been caused by birth trauma. Even collecting the foetuses aseptically would not necessarily avoid contamination (Chamberlain *et al.*, 1953; Sanger and Cole, 1955). Organisms have been observed in the milk of lactating bitches (Chamberlain *et al.*, 1953) so the possibility of post-natal transmission by suckling must also be borne in mind (Eichenwald, 1948; Beverley 1959) in those experiments where this had not been controlled. The dose of organisms administered to experimental animals would also have a bearing on congenital transmission because it has been observed that there is an earlier onset and increased degree of parasitaemia associated with the size of inoculum (Jacobs and Jones, 1950) and that residual infectivity in rat tissues is also proportional to inoculum size (Remington *et al.*, 1961a).

The overall conclusion from available evidence is that transplacental transmission of toxoplasma probably occurs in a wide variety of animal species but whether this transmission occurs with equal regularity and efficiency in all of them is difficult to determine. Many factors must be considered, like differential susceptibility to various strains of *Toxoplasma* by different animals (Jacobs, 1963) but, above all, the structural characteristics of the placenta would be expected to play a dominant role. It is best therefore, that animal data are not too readily extrapolated to man.

2. *Human studies*

In human infections, "The main problem of congenital toxoplasmosis is to know how much of a problem it really is" (Fleck, 1973) and many studies have been concerned with estimating the true magnitude of this problem (Stagno, 1980). The numbers of pregnant women at risk of being infected with toxoplasmosis for the first time can be estimated by observing the proportion showing serological conversion from a negative into a positive Sabin-Feldman dye test. In Britain, the figure is about 2·3 per 1000 pregnancies (Ruoss and Bourne, 1972). Data from the United States are comparable to those in Britain. In New York, six out of 2765 seronegative women were observed to convert during pregnancy (Kimball *et al.*, 1971) and in Oregon, 22 out of 10 298 did so (Beach, 1979), both surveys therefore giving a figure of just over 2 per 1000 pregnancies. The women in France seem to have a greater risk of contracting toxoplasmosis with about 10 per 1000 seroconverting during pregnancy (Desmonts and Couvreur, 1974). The reason for this has frequently been suggested as being due to the French love of eating undercooked meat (Fleck, 1973; Lancet, 1973) which may harbour viable cystic forms of the organisms. It is generally believed that the eating of infected meat is the main method of horizontal transmission of toxoplasmosis

but the demonstration of the oocyst form of the parasite in the faeces of cats has revealed a possible alternative route of human infection. Since the relative degrees of risk of these two modes of spread is not known, it might be wise for pregnant women to avoid close contact with cats and to cook meat well.

Maternal toxoplasmosis acquired during pregnancy does not necessarily give rise to foetal infection. In their study, Desmonts and Couvreur (1974) estimated that about 33% will do so. Since in France, about 10 per 1000 pregnant women are at risk of acquiring toxoplasmosis, the incidence of congenital toxoplasmosis will be in the region of 3 per 1000 births. Based on a similar calculation, the rate of congenital toxoplasmosis in Britain should be just below 1 per 1000. However, the observed figure appears to be in the region of 1 in 5000 (Lancet, 1980) so that additional factors, like severity of maternal infection, may determine the frequency of foetal involvement. The data from different areas of the world certainly differ widely and range from a rate of 0·2 per 1000 live births in Sweden to 18·3 per 1000 in Mexico (Stevenson, 1977). These statistics probably comprised only those cases with overt clinical signs which are apparent at birth. Many with mild or subclinical infections will be missed. It has been estimated that between two and four undetected cases exist for each case recognized (Stevenson, 1977).

Although the figures for the incidence of congenital toxoplasmosis are likely to be underestimated, it is generally agreed that, in a country like Britain, the scale of the problem is not a significant one. This makes it even more difficult to arrive at a policy to identify the few mothers and foetuses who are most at risk. Screening mothers for seroconversion would involve testing their sera twice or perhaps three times during pregnancy. It has been estimated that the detection of a single case would need the testing of 40 000 sera samples at a cost of £200 000 (Lancet, 1980). Even then, only just over 30% of the foetuses born to mothers who have acquired toxoplasmosis during pregnancy are likely to be affected. Many of these newborn infants will not exhibit any overt symptoms at birth and must be followed up if they are found to have raised serum levels of IgM. Thus, the routine surveillance of pregnant women and their children for congenital toxoplasmosis in a country where this disease is not a major public health problem might be regarded as somewhat impracticable and financially prohibitive.

3. *Acute and chronic maternal infections*

A major point of controversy in congenital toxoplasmosis is whether foetal infection follows only an acute maternal infection, i.e. where the mother acquires a primary infection during the relevant pregnancy, or whether a chronic persistent infection acquired in the past can also affect the foetus. The answer to this question is, of course, of great practical importance

because if only acute infections are relevant, then congenital toxoplasmosis can be effectively eradicated if mothers are prevented from being infected while pregnant. Desmonts and Couvreur (1974) believe that only acute maternal infection can involve the foetus because in their study of 59 cases of congenital toxoplasmosis, 55 (93%) were born to mothers with acute infections, as evidenced by seroconversion, a rise in antibody titre and the presence of lymphadenopathy. Further support for this view is provided by the observations of Sabin *et al.* (1952), Feldman and Miller (1956) and Beverley (1960) that the subsequent children born to women who already had a congenitally infected child were all uninfected. It was suggested that only acute infections could give rise to a sufficiently significant parasitaemia in order to infect the placenta and then the foetus (Eichenwald, 1948). Thalhammer (1962) and Kimball *et al.* (1971) also commented that only primary infections during pregnancy can affect the foetus.

Contrary evidence, however, is also available and this has resulted in the formation of a rival group of investigators who believe that chronic maternal toxoplasmosis can give rise to congenital infections. There are many instances in experimental animals where congenital infections have occurred even when the mothers are infected for various periods before the onset of pregnancy. Rats injected with *Toxoplasma* as long as 5 months before pregnancy and who no longer had visible parasitaemia were observed to give rise to foetal infection (Hellbrugge *et al.*, 1953). This is confirmed by the experiments of Remington *et al.* (1961a) where 12 pregnant rats with chronic infections had three offspring infected although the frequency of congenital transmission was not as high as that observed when mothers had acute infections. Experiments with guinea pigs have led to similar results. Wright (1972) infected 44 female animals with *Toxoplasma* cysts during various periods before mating and subsequently found 5 out of 17 foetuses (30%) to be infected. Inoculations of the mother as long as 17 weeks before conception has been observed to lead to congenital infection (Huldt, 1960).

The original reports by Sabin *et al.* (1952) and by Feldman and Miller (1956) that mothers who have had a congenitally infected child will not give birth to another have been contradicted by other investigators. Beverley (1959) was one of the first authors to report that he had observed toxoplasmosis to be transmitted up to five generations in mice. In the guinea pig experiments of Wright (1972), two congenitally infected offspring who survived were subsequently mated. One animal from each litter was found to be infected, indicating the infection was passed through two generations. In man, repeated congenital infections of offspring from the same mother was reported by Langer (1963), and Garcia (1968) documented the case of a Portuguese mother with two consecutive children with toxoplasmosis.

From the evidence presented, it would seem that primary maternal toxo-

plasmosis is not an absolute prerequisite for congenital transmission but that chronic infections can also affect the foetus, albeit with a lower frequency. If this conclusion is valid, then a question that follows is how are the organisms transmitted to the foetus during chronic maternal disease. It is generally accepted that in acute infections of the mother, organisms find their way to the placenta during the parasitaemic phase and then cross to affect the foetus. With the onset of humoral immunity, the parasites are usually cleared from the blood so that, in chronic infections, they exist mainly in the form of tissue cysts. These cysts can occur in a variety of host tissues, but of particular relevance in the present context is their demonstration in uterine endometrial tissue of women who showed positive serological evidence of past infection, either by direct histological examination of currettings (Werner *et al.*, 1963) or by inoculation of digested material into susceptible animals (Remington *et al.*, 1960). In this latter case, the organisms were presumed to be encysted because proliferative trophozoites would not be able to withstand enzymatic digestion.

The presence of viable, encysted, organisms in uterine tissue has led to the postulate that congenital infections occurring during chronic maternal disease are due to local spread of the organisms (Remington *et al.*, 1961a; Werner, 1962; Elliott, 1970). The invading trophoblast causes rupture of the parasitic cysts, liberating organisms which will invade the placenta and subsequently gain access to the foetal circulation. Thus, foetal infection can result even in the absence of systemic maternal parasitaemia. However, it must be pointed out that, although an uncommon occurrence, spontaneous parasitaemia can occasionally occur in chronic infections and this has been observed in animals (Jacobs and Jones, 1950; Remington *et al.*, 1961b) as well as in man (Prior *et al.*, 1953; Miller *et al.*, 1969). One of Miller's patients had demonstrable parasitaemia over a 14-month period despite several courses of therapy. Some cases of foetal infection during chronic maternal infection may, therefore, be due to blood-borne infection of the placenta.

B. *Plasmodium*

1. *Frequency of congenital malaria*

Prenatal transmission of malaria is well documented, and the older literature has been systematically reviewed by Covell (1950). From 1873 to 1950, a total of 135 individual case reports have been accepted as being due to congenital malaria. The criteria used are either that parasites were observed in the infant within 7 days of birth, or by reason of locality where mosquito transmission could be excluded. Much data has also been collected from malarial endemic areas relating to the frequency of congenital infections.

However, care is needed in the interpretation of these results because some are based on the number of infected infants born to mothers who are definitely infected while others relate to the total number of births with the assumption that, in an endemic area, the majority of the mothers will be infected. From Covell's (1950) review of the literature from endemic areas of Africa, South America and Asia, a total of 16 cases of congenital malaria have been recorded out of a total of 5324 births, a frequency of 0·03% which is extremely low. Subsequent reports have confirmed this low incidence. Bruce-Chwatt (1952) did not see a single case of congenital malaria in 332 consecutive babies born in Southern Nigeria, although 33% of the mothers had parasitaemia and 23·8% of the placentae were infected. In the series from Uganda reported by Jelliffe (1968), there was only one case of congenital malaria in 570 women while parasites were demonstrable in peripheral blood and in placental blood in 5·6% and 16·1% of the cases respectively. The situation in Gambia appears to be very similar (Logie and McGregor, 1970) with no cases of congenital infections detected in the infants born to 227 mothers in spite of a parasitaemia rate of 28% among the mothers and placental blood being positive in 32%.

There are a few results, however, which point to a higher frequency of congenital transmission. In the French Cameroons, Perves (1947) observed 63 cases of congenital malaria among 661 newborn infants, a rate of 9·6% while Mackay (1934) recorded a rate of 32% in Tanganyika and Jean and van Nitsen (1929) a rate of 41% in the Congo. A more recent article from Eastern Nigeria (Okeke, 1970) reported that parasites were found in cord blood in over one-third of the infants born to 50 consecutive pregnant women suffering from malaria.

At present, it is not clear whether the disparity in results is due to methodology or whether it truly reflects a difference in susceptibility to congenital infection among population groups in various endemic areas. A factor which may have a bearing on the matter is the immune status of the mothers. There is a suspicion that congenital transmission of malaria is relatively more common among non-indigenous European women living in Africa (Jones, 1950; Pabst, 1950) with rates of from 2–8%. Among the indigenous populations, variation in immune status in different areas could be affected by the widespread but inadequate use of antimalarials.

The rarity of congenital malaria in man is confirmed by animal studies. Inoculations of parasites into pregnant rats, mice and hamsters at different intervals before delivery did not result in foetal infection (Werner, 1956), in spite of a heavy concentration of parasites in the decidual sinuses and the meshes of the placental labyrinth (Bruce-Chwatt, 1954). Foetal blood and tissues were all free of parasites as monitored by the examination of blood smears and sub-inoculation into recipient animals. Only in the two litters

which were delivered by Caesarian section because the mothers were too ill, were the foetuses found to be infected (Bruce-Chwatt and Gibson, 1956), but it is not clear whether the transmission in these cases is due to mechanical or functional deficiency of the placental barrier. Das Gupta (1940) infected a pregnant monkey with *Plasmodium knowlesi* and observed that placental blood was massively infected but foetal blood and organs were all free of parasites.

Thus, the available evidence from human observations as well as from animal studies indicates that congenital malaria is an unusual occurrence. The efficiency of the placenta in preventing malarial parasites from being transmitted must be an important factor and only under exceptional circumstances which lead to functional and mechanical derangement of this barrier will foetal infection result. In a preceeding section, we have already noted how both the structural characteristics and haemodynamics of the placenta combine to discourage the passage of maternal cells to the foetus. However, it must be pointed out that failure of the parasites to cross the placenta may not be the sole explanation. Failure of development of the parasites in the foetal environment may also occur. It is conceivable that the presence of a large number of immature red blood cells and of foetal haemoglobin is incompatible for parasite growth. In addition, the presence of passively transferred maternal antibodies would be able to act against the parasites. It seems, therefore, that a variety of factors are probably involved to protect the foetus *in utero* against maternal malarial infection.

2. *Species of* Plasmodium *causing congenital malaria*

Any of the human *Plasmodium* species can lead to congenital infections (Covell, 1950). It is of interest to note that, of the reported cases of congenital malaria in non-endemic countries like the United States, *P. malariae* features very frequently (Keital *et al.*, 1956; McQuay *et al.*, 1967; Harvey *et al.*, 1969; Thompson *et al.*, 1977). This presumably is because this species can survive for extremely long periods in the host so that the mother could have acquired the infection in an endemic country many years previously before returning to America. At the time of birth of the congenitally infected child, some of the mothers were asymptomatic and had not had a malarial attack for many years (McQuay *et al.*, 1967; Harvey *et al.*, 1969) indicating that febrile malaria is not essential for prenatal transmission as was sometimes postulated in the past (see Covell, 1950).

C. *Trypanosoma*

1. *Human South American trypanosomiasis*

In a survey of the literature up to 1973, Bittencourt (1976) uncovered 89

reported cases of congenital Chagas' disease, all in the Spanish language. The frequency of congenital transmission in an endemic area was estimated to be 2% from a study of 500 consecutive deliveries (Bittencourt *et al.*, 1972a) and this was confirmed by Santos-Buch (1979) who quoted a rate of 2·4% of all live births. However, if the mother has definite evidence of the disease, then the rate of congenital transmission rises to 10·5% (Bittencourt *et al.*, 1972a). It has been suggested that organisms probably reach the foetus via the placenta by the haematogenous route following maternal parasitaemia. Since the trypomastigotes can actively penetrate epithelial cells, they may similarly transverse the trophoblast layer. Once in the villous mesenchyme, the organisms round up into amastigotes, and after a period of development, will then invade the foetal circulation again as trypomastigotes (Bittencourt, 1976). The histological findings in Chagasic placentae have already been described in a preceeding section. However, many mothers with definite parasitaemia do not transmit the disease to their foetuses nor are all cases of congenital infections associated with mothers with demonstrable parasites in their blood. In a detailed study of 30 cases of congenital *T. cruzi* infections seen in Santiago, Chile, only one mother had clinical evidence of infection and she was the only one with detectable parasitaemia in a wet blood film. Parasites were found in wet blood films of all the children (Howard and Rubio, 1970). A further point of interest arising from this study was the observation that none of the 26 subsequent children born to the same mothers after the affected siblings showed any evidence of congenital infection. This may indicate a similar pathogenic mechanism as that postulated for toxoplasmosis in that only acute maternal infection, and not chronic disease, can lead to foetal involvement. Yet, maternal parasitaemia does not appear to be a necessary prerequisite for transplacental transmission. Perhaps the rarity of congenital infection following chronic maternal disease may be explained by a transfer of passive immunity from mother to foetus rather than due to the relative absence of parasitaemia.

2. *Human African trypanosomiasis*

The only report of a possible case of congenital infection in Africa appears to be that of Olowe (1975) who presented a case of a 17-day-old baby girl with signs of meningo-encephalitis. Organisms identified as *T. gambiense* were isolated from her cerebrospinal fluid. Similar parasites were detected in her mother's blood. Since the infant was thought not to have been exposed to insect bites and since organisms do not usually appear in the body until about 21 days after initial infection, it was concluded that the infection was transmitted *in utero*.

It is not clear whether the lack of reports of cases of congenital African trypanosomiasis in the literature is due to failure of diagnosis or that it is

indeed a rare event. If the latter interpretation is correct it may be postulated that perhaps the absence of the ability to survive as intracellular amastigotes (like *T. cruzi*) has allowed placental phagocytic defence to take full effect.

3. *Trypanosomiasis in animals*

Experiments with white Swiss mice infected with strains of *T. cruzi* have revealed that these animals are very refractory to congenital infections (Delgado and Santos-Buch, 1978). Injections of both pathogenic and non-pathogenic strains did not result in foetal infection. Transplacental transmission was only achieved with pathogenic organisms after the recipient pregnant animals' R-E system was blocked by thorium dioxide. In this group, seven out of 79 (8·9%) foetuses were found to be infected. These results confirm that the placenta plays an active rather than merely a passive role in defending the foetus against invading maternal infections. Our own observations on *T. brucei* infections of pregnant Balb/c mice confirm the rarity of transplacental transmission (Y. W. Loke and S. Day, unpublished observations). Thirty-six pregnant mice were injected intraperitoneally with parasites at various periods ranging from 1 to 3 weeks before mating. Subsequently, a total of 22 dead and 30 live foetuses were recovered. In none of these foetuses could trypanosomes be detected either by direct blood smear or by subinoculation into other recipient animals. All the mothers had a heavy parasitaemia at the time of birth. When two near-term pregnant mice were killed and the foetuses and placentae carefully dissected away from the uterine wall, no trypanosomes were detected from cord blood but blood from the maternal side of the placentae showed large numbers of parasites. This indicates that the organisms can get to the placenta via maternal blood but are somehow prevented from gaining access to the foetal circulation.

Transplacental transmission of *T. vivax* in a sheep has been reported (Ikede and Losos, 1972). It seems that congenital infection in other animal species, including guinea pigs, rats, dogs, camels, cows and goats, and caused by a variety of trypanosomes like *T. equinum*, *T. rhodesiense*, *T. gambiense*, *T. brucei*, *T. evansi*, *T. vivax* and *T. therilei*, has been documented (see Dikmans *et al.*, 1957).

D. *Leishmania*

The biology of this organism, with its ability to survive in the intracellular environment of host cells like *Toxoplasma*, would seem to be particularly favourable for evading placental defence. Surprisingly, congenital leishmaniasis has only occasionally been reported (Manson-Bahr, 1960). Gastebled *et al.* (1973) concluded this must be an exceptional occurrence.

These investigators described a woman with severe Kala-azar who delivered a child who was entirely free from infection and a search of the literature over several years has revealed no reports of naturally occurring cases of congenital leishmaniasis. Experimentally, it has been shown that *Leishmania* can traverse the placenta of the Syrian hamster (Bossie and Lupasco, 1969). Five female hamsters became pregnant at intervals of 3, 4, 10, 30 and 48 days respectively from the time of being infected with *L. donovani*. From a total of 27 foetuses, hepatic but not splenic smears were always positive for the organisms, which again points to a transplacental haematogenous route as the most likely source of foetal infection. Also, in three cases, foetal infection was correlated with placental infection. Thus under experimental conditions, congenital transmission of leishmaniasis is by no means unusual. Perhaps the apparent rarity of this occurrence in man is due to failure of recognition of foetal infection and it seems prudent to await further data before coming to any definite conclusions.

E. HELMINTHS

Any helminth which has a wandering larval stage in its life cycle has the chance to reach the placenta and to be disseminated to the foetus. The greatest opportunity for this to occur would be in nematodes while a few trematodes may also do so. The wandering phase of a cestode life cycle takes place in the intermediate host so that, in man, examples of congenital infection should be looked for in infections like hydatid disease or cysticercosis caused by larvae of the pork tapeworm.

1. *Nematodes*

The ascaroids of dogs have been most studied in relation to congenital infection. The original studies of Fülleborn (1921), Shillinger and Cram (1923) and Augustine (1927) all confirmed that pregnant bitches at various stages of gestation infected with *Belascaris marginata*, either by being fed parasite eggs or injected subcutaneously with infective larvae, will transmit the helminths to the puppies in 100% of the cases. When the pups were sacrificed at various intervals after birth and their tissues examined, large numbers of *Belascaris* larvae were first noted mainly in the liver immediately after birth. Larvae then appeared in the lungs a few days later and finally could be found in the stomach and small intestine. The initial sequestration of larvae in the foetal liver lends support to the idea that congenital infection is most likely to be via the placenta. It is of interest to note that when the two infected pregnant bitches in Augustine's (1927) experiment who had given birth to infected pups were killed after birth, no worms were found in one bitch while the

other had only two young specimens in her duodenum. This raises the possibility that pups *in utero* may have a greater predilection for infection than adult bitches.

Belascaris marginata is now known as *Toxocara canis* and reports using this new nomenclature have confirmed that the primary mode of transmission of these helminths in dogs is prenatal. Griesmer *et al.* (1963) demonstrated larvae in 100% of 95 pups from 34 litters. Second-stage larvae are found only in foetal liver (Koutz *et al.*, 1966) which again suggests a transplacental route of migration. The demonstration of larvae in the substance of the umbilical cord by Scothorn *et al.* (1965) indicates that the helminth may reach the foetus by active migration along the cord as well as via cord blood. In the liver, larvae transform into the third-stage but remain here until the pulmonary circulation becomes operational at birth when migration continues to the lungs (Scothorn *et al.*, 1965). By the first week of life, fourth-stage larvae and young adults can be found in the intestines and eggs appear in the faeces by 31–50 days so that the pups are now infective from this age onwards (Griesmer *et al.*, 1963).

Congenital infection can occur even when the bitches are treated repeatedly with anthelmintic until they no longer show any evidence of intestinal *Toxocara* infection (Yutuc, 1949; Webster, 1958). This finding has led to the conclusion that an active intestinal infection in the bitch is not necessary for congenital infection to occur. Larvae which are dormant in somatic tissues may become reactivated during pregnancy. These larvae may have been acquired by the bitch during a past infection, thereby providing a potential reservoir for pre-natal infection over several pregnancies without the bitch having to be re-infected.

While congenital infection with *T. canis* is well documented, the situation with the dog hookworm *Ancylostoma caninum* is not at all clear. In the older literature, Alder and Clark (1922), in an investigation in Freetown, Sierra Leone where the infection is very common, examined 13 pups from eight different litters ranging from 2–15 days old for evidence of *A. caninum* infestation. They found four pups were positive for worms and three were positive for ova. Similarly, Foster (1932) found all 13 pups from the litters of two bitches infected with *A. caninum* to have acquired the infection. In one premature, stillborn pup, five filariform larvae were recovered from its organs in a Baermann apparatus, thus strongly suggesting that the infection had been acquired before birth. It was estimated that from 10 to 20% of the larvae given to the mother by mouth found their way to the pups which indicates a very widespread migration of these larvae to the foetus.

In contrast, Griesmer *et al.* (1963) found that when bitches were infected with *T. canis* and *A. caninum*, only *T. canis* was transmitted to the foetus. No hookworm larvae were demonstrable in the pups. It is not clear why

there is this inconsistency in results. There is a suspicion that the earlier studies had not strictly controlled against the possibility of post-natal transmission and the pups might have been infected by suckling because there are reports that larvae of *A. caninum* can be recovered from colostrum and milk in parturient bitches (Miller, 1971). This form of materno-foetal transmission has also been documented in man for infection with *Strongyloides fuelleborni* (Brown and Girardeau, 1977). Because of this possibility, some investigators think that only 2% or less of the so-called cases of prenatal *A. caninum* infections are really acquired *in utero* (Miller, 1971; Krakowka, 1977). It seems that additional data from well-controlled experiments are needed before any definite conclusions can be reached regarding the frequency of congenital infection by this helminth. Since the larvae of this species are very active migrators, there is no logical reason why they should not traverse the placenta as efficiently as *T. canis*.

Among members of the Filarioidea, transplacental transmission of *Dirofilaria immitis* in the dog has been reported (Mantovani and Jackson, 1966). A pregnant bitch with a parasitaemia of 40 000 microfilariae per ml of blood gave birth to five pups. The blood of all five pups was positive for microfilariae with counts of 5–30 larvae per ml of blood. A few microfilariae were also found in centrifuged amniotic fluid so that the possibility that the route could be by swallowing infective amniotic fluid cannot be entirely discounted. In the cat, Kimmig (1979) studied eight kittens born to two mothers infected with *Brugia pahangi*. No microfilariae were seen in the peripheral blood of any of the kittens but in the lung of one kitten killed 2 days after birth, 30 microfilariae were found. From the histology, the author concluded that larvae reached the placenta either via the blood or alternatively from secretions of the uterine glands. In human infections, microfilariae of *Onchocerca volvulus* were found in skin snips of two out of 11 babies born to mothers infected with the helminth (Brinkmann *et al.*, 1976). Microfilariae were also found in the umbilical cords of the two infected babies but were not demonstrable in cord blood. This suggests that microfilariae of *O. volvulus*, like the larvae of *T. canis*, also get to the foetus via the connective tissue of the cord rather than by transmission from maternal to foetal circulation across the placenta. This could explain why those species of human filariae which circulate in the blood stream like *Tetrapetalonema perstans* are not readily transmitted to the foetus (Jean and van Nitsen, 1929) although they are found in placental blood (Garnham, 1938). In contrast, the microfilariae of *Wuchereria bancrofti* appear to cross the placenta although they appear to be unable to establish an active infection in the foetus (Bloomfield *et al.*, 1978). *Trichinella spiralis* also appear to be unable to traverse the placenta. Augustine (1934) has made a detailed study in rabbits, rats and swine. Although all the mothers had demonstrable larvae in their muscles, the

foetuses were free from infection when monitored by five different methods: (1) skin and precipitation test; (2) comparison of eosinophil count between mother and newborn; (3) histological examination of newborn's tissues for larvae; (4) search for larvae in peptic digested carcases of newborns; (5) histological search for larvae in the placenta. The same article also presented a case of a human patient who contracted trichinosis 3 weeks before giving birth. No larvae were found in several sections of the placenta. Five days after birth, the mother's eosinophil count was 24% while the child's remained at 3·5% and at 6 months, skin test with *Trichinella* antigen was positive for the mother but negative for the infant.

From the preceeding survey it is fair to conclude that, although many species of nematodes can affect the foetus *in utero*, only the dog ascarids appear to do so with any regularity. It is not clear why this is so. Perhaps an ability to penetrate placental and cord tissue and a preference for foetal tissue during larval development may contribute to the success of these helminths in colonizing the foetus.

2. *Cestodes*

A survey of the older literature by Shillinger and Cram (1923) revealed several reports which may represent human congenital infections by *Echinococcus granulosus*. There was a case of a hydatid cyst found in the liver of a 12-day-old child, another case of a new born child with a cyst in the abdomen and a third where hydatid cysts were found in the placenta and cord of a 7-month-old foetus. The comment from the authors was that there was some doubt whether these were really hydatid cysts or were, in reality, foetal malformations.

3. *Trematodes*

The earlier reports by Japanese workers (Fujinami and Nakamura, 1911; Narabayashi, 1914) would seem to indicate that *Schistosoma japonicum* can give rise to pre-natal infection in experimental animals as well as in natural human infections. When pregnant bitches were infected with the cercariae of *S. japonicum*, parasites could subsequently be recovered in most of the embryos and in the examination of 32 specimens of faeces from newborn infants, three were found to contain schistosome ova. However, more recent investigations have not confirmed these results. Bittencourt *et al.* (1972b) infected 24 pregnant rabbits with *S. mansoni* during different stages of gestation but could find no evidence of transmission to the foetuses. Schistosomula may occasionally reach the placenta to lie in a maternal labarynthine blood vessel but they do not appear to be able to reach the foetal circulation.

VII Effects of Maternal Parasitic Infections on the Foetus

Maternal parasitic infections can affect the foetus in two ways: (1) by direct invasion of the foetus by the parasites; and (2) indirectly, by lowering maternal health to such a level as to interfere with foetal well-being.

The extent of foetal damage directly attributable to foetal infections is difficult to assess but according to Dudgeon (1968), it is comparatively small relative to other forms of reproductive failure even when viruses and bacteria are taken into account beside parasites. However, if damage does occur, then the effects can be severe. These range from early foetal death resulting in abortion and stillbirth to growth retardation. The foetus may be born alive but dies soon in infancy or may survive but suffers from some malformations. This last manifestation is usually caused by viruses because these organisms have subtle, low grade effects on infected cells (Mims, 1976), while large organisms like parasites tend to cause widespread damage and kill the foetus. Finally, foetal infection may not lead to any overt clinical effects but could modulate its future immune response to the same parasite depending on whether sensitization or tolerance has occurred. This aspect will be discussed in greater detail in the next section.

A. MALARIA

Foetal malaria infection *in utero* can present with similar symptoms as in adults, with hepatosplenomegaly, jaundice and haemolytic anaemia (Dimson, 1954; Thompson *et al.*, 1977). When this occurs in a country not endemic for malaria, the problem of differential diagnosis will inevitably arise. Other congenital infections like syphilis, rubella, toxoplasmosis, cytomegalovirus and herpes infections can all be present with hepatosplenomegaly and these must be excluded. The case in England described by Dimson (1954) of an infant with severe jaundice and hepatosplenomegaly 8 days after birth was, in fact, diagnosed as infective hepatitis, and malaria was not suspected until it was known that the mother had just returned from West Africa. Haemolytic anaemia in an infant also has a large number of causes, mostly of non-infective origin (Thompson *et al.*, 1977). Foetal–maternal incompatibility for Rhesus and ABO blood groups immediately springs to mind. Maternal auto-allergic conditions can result in the passive transfer of antierythrocyte antibodies to haemolyse foetal red blood cells. Finally, foetal anaemia may be due to some inherited defect which interferes with red blood cell survival like spherocytosis or enzyme deficiency.

The nephrotic syndrome is a condition frequently associated with adult quartan malaria so it is interesting to note that it may similarly occur in

congenital quartan malaria (Keital *et al.*, 1956). Since the pathogenesis of this disease is postulated to be due to a Type III immune-complex mechanism, this case will be discussed further in the next section.

The observation of Blacklock and Gordon (1925b) raises an interesting point. They noted that those children born to malaria-infected mothers and who died within 7 days of birth, no parasites were demonstrable in the children's blood or tissues. However, in several cases, pigment could be seen in their internal organs. Could this pigment be derived from haemolysed foetal red blood cells? Since no parasites were detected in foetal tissues, the authors postulated that perhaps some malarial toxin might have traversed the placenta from mother to foetus and was responsible for the haemolysis. If this is applicable to other infections then the transfer of some noxious agent across the placenta, rather than of the parasites themselves, must be considered as an important mechanism for direct foetal involvement.

There can be no doubt that a large proportion of the reproductive failure found in malaria must be due to indirect effects on the foetus resulting from maternal illness. Malaria causes severe constitutional disturbances and it may be assumed that these are likely to be detrimental to foetal wellbeing. Fever of any origin is said to be an important cause of abortion so that the high fever of malarial attacks may lead to a similar outcome. The conclusion by Covell (1950) from a survey of the world literature was that malaria was responsible for abortions, miscarriages and stillbirths but mainly in European women in the tropics or during epidemics. The association was not so strong with regard to the influence of this disease on pregnancy failure among the indigenous population of highly endemic areas. This may be because these inhabitants seldom succumb to overt malarial attacks.

Maternal anaemia resulting from malaria is another factor which could lead to poor foetal nutrition. Harrison (1976) in a study of maternal anaemia due to various causes like malaria, folate deficiency and haemoglobinopathies concluded that a maternal haematocrit of less than 50% was associated with a retardation of foetal growth. If at the end of pregnancy this anaemia was not corrected, a 2% drop in maternal haematocrit reduced foetal birth weight by about 100 g. A further factor in malaria which may be involved in determining foetal birth weight is a disturbance of placental function. As we have noted in a preceeding section, there is a massive accumulation of parasites and mononuclear cells in the placental sinusoids, particularly in *P. falciparum* infections. This could lead to interference with placental blood flow and hence the transfer of oxygen and nutrients to the foetus. Much evidence has certainly accumulated to indicate that mothers with infected placentae tend to give birth to smaller babies (Table 5).

From Table 5, it can be seen that all investigators are agreed that babies

born to mothers with infected placentae have a lower mean birth weight. It is not clear as to what extent the lower mean birth weight is contributed by a high incidence of premature births. Many African mothers tend to be hazy about their menstrual dates so it is difficult to be exact about the gestation age of the baby at birth. However, if the figure of 2500 g which is designated by the World Health Organisation as the minimum weight of a full term baby is used as a yardstick, then the incidence of prematurity would appear to be higher in mothers with infected placentae than in controls (Table 6).

TABLE 5

Summary of the results from different groups of investigators on the mean birth weight of foetuses born to mothers with infected and uninfected placentae

References	Mean birth weight (g)	
	Non-infected placentae	Infected placentae
Bruce-Chwatt (1952)	3048	2903
Archibald (1956)	2892	2722
Archibald (1958)	3076	2778
Cannon (1958)	2920	2610
Spitz (1959)	2940	2851
Jellife (1968)	3063	2085

TABLE 6

Incidence of prematurity (baby less than 2500 g at birth) in mothers with infected and non-infected placentae

Reference	Percentage of premature births	
	Non-infected placentae	Infected placentae
Archibald (1956)	16·5	29·4
Cannon (1958)	13	33
Spitz (1959)	27	41·2
Jellife (1968)	10	19·6

Further support for a direct association between malaria infection and foetal birth weight is seen in the results of treatment. In the study by Morley *et al.* (1964) in North East Ibadan where malaria was holoendemic, 429 pregnant women were divided into two groups. The first group received 50 mg of pyrimethamine monthly while the second acted as controls, being given placebos. It was found that the infants born to treated mothers had a mean

birth weight 157 g greater than in the control group. Similar results were obtained by MacGregor and Avery (1974) who studied the effects on birth weight following malaria eradication programs in the British Solomon Islands. They found that the mean birth weight rose substantially within months of starting anti-malarial operations with an increase averaging 252 g in babies of primips and an increase of 165 g for all babies. Also, the proportion of premature babies (i.e. less than 2500 g) fell by 8% overall and by 20% among babies of primips. These observations, therefore, indicate that the adverse effects of malaria on infant wellbeing can be stopped if the transmission of the disease in the community is interrupted or if infected mothers in endemic areas are treated monthly by anti-malarials. Some investigators have urged caution regarding the latter of these procedures. Malarial immunity is maintained in an endemic population by the constant antigenic challenge provided by a low grade parasitaemia. It has not been established what effects treatment has on maternal antibody production so that the passive transfer of protective immunity to the foetus may be jeopardized in an effort to raise foetal birth weight.

An interesting observation was made by Jellife (1968) that the sex ratio at birth was reversed in mothers with malaria-infected placentae. The human sex ratio (M: F) at birth is approximately 109: 100 with a slight variation in different population groups. In Jellife's (1968) data, the sex ratio in mothers with infected placentae was 41: 51. This could be due to an intra-uterine selection against the male foetus because of its greater susceptibility to malaria. This differential susceptibility may not be restricted to malaria but could be applicable to all acute infections. In a survey of the world's literature and the records of the Johns Hopkins Hospital, Washburn *et al.* (1965) concluded that male children were indeed more susceptible to acute infections than female children and postulated a genetic hypothesis that this could be due to a gene locus on the X-chromosome which is involved with the synthesis of immunoglobulins. This hypothesis is supported by the observation of Butterworth *et al.* (1967) that the IgM levels of females are significantly higher than in corresponding groups of males although the actual difference is small, in the region of 20 mg $100ml^{-1}$. Production of IgM may be linked to the X-chromosome. Rhodes *et al.* (1969) observed that mean IgM values were highest in XXX women, intermediate in normal XX women and lowest in normal XY men. In seven cases of XXY individuals, the mean IgM values were identical to normal XX women and in three cases of XXXY individuals, the mean IgM values were identical to XXX women. These findings therefore suggest that IgM secretion is influenced by the X-chromosome and must be exerted before X-inactivation. No such sex linkage was observed for IgA or IgG secretion. It has been further suggested that the X-linked enzyme glucose 6-phosphate dehydrogenase (G-6-P-D) may

also be related to the increased male susceptibility to infections (Schlegel and Bellanti, 1969). This enzyme plays a role in phagocytosis. The female might be expected to enjoy the advantages of increased genetic variability by virtue of her mosaicism for maternally and paternally derived G-6-P-D genes. Her brothers, on the other hand, must suffer the consequences of bearing only the G-6-P-D genes derived from their mothers. In the context of transplantation biology between the mother and foetus, the male foetus is already handicapped because of its H-Y antigen which is not possessed by the mother and would be expected to be rejected more frequently (see Loke, 1978). If it should turn out that malaria, or any other infections, should place a further burden on male foetal survival, then congenital infections would become a very important influence on the determination of the sex ratio in human populations, especially in those areas of the world where parasitic diseases are endemic.

B. TOXOPLASMOSIS

Toxoplasmosis produces a disease in the mother which is usually mild or subclinical. Any foetal pathology, therefore, is likely to be attributable to direct foetal involvement by the transplacental spread of the organisms. The effects on the foetus will depend on several factors: the gestational age of the foetus when infected; the size of the infecting dose of organisms which has succeeded in reaching the foetal circulation; and the virulence of the strain of toxoplasma involved (Beverley, 1960).

1. *Intra-uterine death*

Severe infection of a foetus early on in gestation would be expected to result in intra-uterine foetal death with abortion, miscarriage or stillbirth as the clinical outcome. There is no doubt that this can happen but it is the frequency of occurrence which is open to question. Positive histological identification of toxoplasma in the foetal tissues was reported by Garcia (1968) in two consecutive children born to a Portuguese mother. The first child was premature and died 24 hours later, while the second child, who was conceived 5 months after delivery of the first, ended in abortion at 6 months gestation age. Indirect evidence of association is provided by reports such as those of Jones *et al.* (1966, 1969) that there is a greater frequency of prior abortion among women with high antibody titres than in controls. This is confirmed by the later findings of Sharf *et al.* (1973) who studied 228 women with obstetric histories which included spontaneous abortions, premature deliveries and stillbirths. *Toxoplasma* antibodies, as detected by the Sabin–Feldman dye test, was found in a titre of 1 : 64 to 1 : 2024 much more frequently

in these women (16·6%) than in a group of 184 women with normal obstetric histories (1·1%). In addition, treatment of these women with abnormal obstetric histories was found to increase the chances of a subsequent successful pregnancy outcome in 71% of the cases, further confirming that toxoplasmosis is a significant cause of pregnancy interruption. From India, Oumachigui *et al.* (1980) also reported a positive association between maternal infection and foetal wastage. Of 240 women with high antibody titre (i.e. greater than 1 in 256 as measured by indirect haemagglutination), 13% were habitual aborters, 6·7% had sporadic abortions, 8·3% had foetuses with congenital anomalies, 13% had stillbirths and 7·2% had premature births.

In contrast, Kimball *et al.* (1971) found no evidence at all for any association between acute toxoplasmosis and abortions in a series of 260 cases. There was no correlation between any rise in maternal antibody titre, maternal seroconversion during pregnancy and positive isolation of *Toxoplasma* from aborted tissues.

From a review of the literature, Weinman (1960) came to the conclusion that toxoplasmosis has been held to be responsible for abortion and stillbirth with a frequency ranging from 0·3% to 3%. These figures were based on cases where it was possible either to isolate the organisms from aborted tissues by animal inoculation or to observe the parasites in histological sections. The higher of these figures was also reached by the study of Desmonts and Couvreur (1974) who observed that there were 11 (3%) abortions in 378 pregnant women with high initial toxoplasma antibody titres or seroconversion during pregnancy, but no parasite isolation was done from the aborted tissues. It must be stressed that while the demonstration of parasites in aborted foetal tissues is the best proof of aetiological relationship, this is only so if direct foetal infection is the sole pathogenic mechanism for abortion. There remains an indirect mode of causation whereby latent cysts in chronic toxoplasmosis may establish a state of chronic endometritis which interferes with foetal implantation and development (Werner *et al.*, 1963). It may be concluded from the available evidence that, although congenital toxoplasmosis can result in abortion or stillbirth "this infection remains an infrequent cause of such events" (Desmonts and Couvreur, 1974).

2. *Infection in the neonate*

A broad clinical spectrum may be recognized (Beverley, 1960; Desmonts and Couvreur, 1974; Stevenson, 1977). The onset of parasitaemia will lead to a generalized systemic disease in the foetus. The organisms are phagocytosed by reticulo-endothelial cells in organs like lymph nodes, spleen, liver and bone-marrow but the ability of toxoplasma to survive intracellular digestion by these cells (Hirsch *et al.*, 1974) permit them to continue to multiply in these tissues resulting in focal areas of necrosis and cellular

proliferation. Thus, the infant at this stage of infection will present with lymphadenopathy and hepatosplenomegaly together with symptoms associated with derangement of the haemopoetic system like jaundice, anaemia and even thrombocytopenic purpura. Differential diagnosis from other congenital infections and haemolytic disease will be needed.

It is with the subsidence of the visceral stage that the well-known symptoms of congenital toxoplasmosis appear, consisting of the classical triad of chorioretinitis, hydrocephalus and cerebral calcifications (Eichenwald, 1957). In fact, *Toxoplasma gondii* and *Treponema pallidum* are considered to be two of the most important causes of congenital eye diseases (Zimmerman, 1970). In toxoplasmosis, chorioretinitis was a well-documented manifestation of congenital infection long before the protozoan was incriminated as a cause of the disease. Also, congenital toxoplasmosis appears to be the only known form of the disease which present with eye lesions.

Wolf and colleagues (1939a, b) were the first to demonstrate that congenital central nervous system (CNS) lesions can be caused by *Toxoplasma*. They described a 3-day-old infant who died with widespread encephalomyelitis. Parasites were present in great numbers in the patient's meningeal exudate. Emulsion from the brain and spinal cord of the infant taken at post-mortem and injected intracerebrally into mice, guinea pigs, rabbits and chickens resulted in CNS lesions in all these animals. Since this original observation, it is now well documented that the brain and spinal cord are frequent sites of involvement but the cerebellum by contrast, is seldom affected (Beverley, 1960). There may be such extensive loss of cortical tissue that microcephaly results. From the nervous tissue, the infection can spread to the ventricles to start an ependymitis which then progresses to meningitis. Should large portions of infected tissue be detached, blocking of the aqueduct and obstructive internal hydrocephalus may occur. Thus, the microcephalus and hydrocephalus are both the results of destructive lesions and should not be considered as teratogenic effects of the parasite.

It is not clear why lesions in congenital toxoplasmosis are so frequently restricted to the CNS while this tissue is seldom involved in adult infection. It has been suggested that both organisms and maternal antibody are transmitted across to the foetus at the same time. The antibody can largely control the infections in most foetal organs except the CNS because of the difficulty in penetrating the blood/brain barrier (Weinman, 1952). This hypothesis is supported by the experiments of Eichenwald (1949) who treated congenitally infected mice by a variety of regimes. Treatment with antibody alone resulted in lesions mostly in the brain but visceral lesions predominated when the animals were treated with sulphadiazine only. If treated with both antibody and sulphadiazine, half the foetuses were cured while the rest harboured parasites mainly in the brain as chronic carriers.

Even if the infants recover from these cerebro-ocular lesions, the residual consequences may be significant. In a detailed follow-up of 150 infected neonates for 5 years or more, Eichenwald (1957) recorded the following outcome: (1) overall mortality was 12%; (2) of the survivors, 90% showed some degree of mental retardation; (3) 75% developed convulsions, spasticity and palsies; (4) 50% had impaired eye-sight. A corollary approach arrives at the same conclusion. Thalhammer (1962) looked at the sera from 1332 children aged 0–14 years with signs of cerebral palsy, epilepsy and mental retardation for evidence of *Toxoplasma* antibody. A dye-test titre of 1 in 4 or more was considered positive. It was found that a positive serum was detected in 17% more children with cerebral symptoms than in controls. Even when there is no evidence of overt mental retardation, congenital toxoplasmosis may be associated with some degree of intellectual impairment (Saxon *et al.*, 1973). The question of what proportion of all cases of mental deficiencies are due to congenital toxoplasmosis is more difficult to answer, the main reason being that it is almost impossible to estimate exactly the number of subclinical infections (Hume, 1972). According to Stern *et al.* (1969) congenital infection by Cytomegalovirus is probably the most important cause of mental deficiency while rubella and *Toxoplasma* together are responsible for no more than 2–3% of all cases of mental retardation.

Many infants give evidence of active infection continuing during the early months of life which raises the hope that efficient treatment after birth may arrest further progression of the lesions (Plotkin, 1975). The observation by Saxon *et al.* (1973) that there was no detectable intellectual impairment in those children with congenital toxoplasmosis who were treated immediately after birth may be interpreted as indicating that a significant amount of brain damage from this infection occurs after delivery and can be slowed or ameliorated with adequate therapy. This would be a very important concept for neonatal management if applicable to other congenital infections. Further studies of this kind to gauge the effectiveness of treatment would be valuable data in deciding whether the cost for mounting programmes for the management of high risk groups of neonates can be justified (Wilson and Remington, 1980).

C. TRYPANOSOMIASIS

In an earlier section, we have seen that congenital transmission of trypanosomes is relatively uncommon but when it does occur the effect on the foetus can be very severe in both South American and African trypanosomiasis. At worst, abortion, stillbirth or macerated foetus may occur (Bittencourt, 1976). Out of the 10 cases of congenital Chagas' disease, eight

resulted in macerated foetuses. Leishmanoid forms of the parasite and inflammatory infiltration were found in sections from heart, skeletal muscles and oesophagus (Bittencourt *et al.*, 1972a). If the foetus is born alive, then the subsequent mortality rate is high. In a follow up by Howard and Rubio (1970) of 30 cases of congenital Chagas' disease in Chile since 1957, 15 of the children had since died with the oldest attaining 8 years at the time of the report. The outcome of a further 66 cases was documented by Bittencourt (1976) as follows: five died within 1 day of birth, 23 died before the fourth month and nine died between 4 and 9 months post-partum. Szarfman *et al.* (1975) collected together six children with congenital *T. cruzi* infection of which four subsequently died at 30 days, 63 days, and 5 months after birth. Two died of encephalitis, one of heart failure and one of pulmonary infection. There is now much evidence to indicate that production of lesions in trypanosomiasis is by some immunopathological mechanism. This will be dealt with in the next section of foetal immune response.

A case of congenital African trypanosomiasis was reported from Lagos by Olowe (1975). A 17-day-old girl presented with signs of meningo-encephalitis. Parasites identified as *T. gambiense* were recovered from her cerebrospinal fluid (CSF) but curiously none were detected in her blood. In contrast, her mother had a positive parasitaemia but no parasites in the CSF. The neonate's course of infection, therefore, appears to be very different from that of adult African trypanosomiasis where CNS manifestations generally come late in the disease. From our previous discussion, we see a parallel in toxoplasmosis where congenital infections and not adult infections present with cerebral symptoms. Perhaps a similar explanation may be offered that, in trypanosomiasis as in toxoplasmosis, protective maternal antibody cannot penetrate the blood/brain barrier.

Abortion and early neonatal death also occurs in domestic animals infected with congenital trypanosomiases (Dikmans *et al.*, 1957; Ikede and Losos, 1972). Whether this represents a major economic problem in endemic areas has not been established.

Like malaria, trypanosomiasis can give rise to severe constitutional disturbances in the mother, so that foetal wastage need not be solely due to foetal infection but may be an indirect consequence of maternal illness. The data from our own experiments on *T. brucei* infection of pregnant mice (Y. W. Loke and S. Day, unpublished observations) support this conclusion (Table 7).

From Table 7, it can be seen that interruption of pregnancy is most marked when infection of the mother commences early in gestation and is manifested as infertility, abortion and stillbirth. In some of the cases of infertility with no litters, the presence of residual decidua on histological examination of the uterus indicates that the pathogenic mechanism is

likely to be early foetal loss with resorption rather than the absence of fertilization. No organisms are detected in the aborted and stillborn foetuses. Therefore, abortion and foetal death appear not to be a result of transplacental infection but is probably secondary to such severe maternal illness that pregnancy can no longer be sustained.

TABLE 7

Effects of T. brucei *infection on the outcome of pregnancy*
(From Y. W. Loke and S. Day, unpublished observations)

Group	Number of animals	Number with no litters	Number with dead litters	Number with live litters
1. Injected with parasites 1 week after mating	12	8	4	0
2. Injected with parasites 2 weeks after mating	12	9	2	1
3. Injected with parasites 3 weeks after mating	12	3	3	6
Control 1. Injected with phosphate-buffered saline 1 week after mating	12	0	0	12
Control 2. Injected with phosphate-buffered saline 2 weeks after mating	12	0	1	11
Control 3. Injected with phosphate-buffered saline 3 weeks after mating	12	0	1	11

D. HELMINTHIC INFECTIONS

Helminthic infections are generally well tolerated by the host and foetal consequences are not common. This seems to apply also to congenital helminthic infections which do not usually lead to reproductive failure. The only report to the contrary is by Foster (1932) where all 13 pups infected prenatally by *A. caninum* died within 25 days of birth. It is possible that the tissue migration of a heavy burden of larvae can compromise foetal well-being to some extent. Present interest is directed not so much at any overt pathological lesions which may be caused, but on the subtle modulation of the foetus' immunological reponse as a result of this early contact with helminthic antigens. This will be discussed in more detail in the next section.

VIII. Foetal Immune Response to Intra-uterine Infections

There is now increasing evidence that, in the human foetus at any rate, ontogeny of immunocompetence begins very early during embryonic development (see Loke, 1978) and it seems that this may occur at the same physiologically equivalent age in all mammalian species (Solomon, 1971). The foetus *in utero*, therefore, does not remain passive when confronted with parasitic organisms, or their antigens but will be expected to mount an active immune response.

A. Evidence for foetal sensitization to parasites

Human congenital parasitic infections are frequently accompanied by a raised cord serum level of immunoglobulins, especially of IgM and IgA classes. Since IgM and IgA (unlike IgG) are poorly transmitted across the human placenta, it has been inferred that these immunoglobulins are produced by the foetus in response to intra-uterine infections and not passively transferred from the mother. This conclusion is supported by the observation of Cederqvist *et al.* (1977) who reported that infants with congenital toxoplasmosis all had elevated serum IgM and IgA levels but children born to mothers with toxoplasmosis who themselves were not infected did not have raised serum immunoglobulins of these classes. The specificity of these foetal antibodies was demonstrated by Eichenwald and Shinefield (1963) who obtained sera from cord blood of infants with congenital toxoplasmosis and compared the anti-*Toxoplasma* activity of 7S and 19S fractions with those from mothers. It was found that the 7S fractions from both cord and maternal sera had high anti-*Toxoplasma* activity but only the 19S fraction from cord sera had this activity. This indicates that IgM anti-*Toxoplasma* antibody was probably produced by the congenitally infected foetus while the IgG fraction was entirely or partially transmitted from the mother. This is further substantiated by the finding that infants who were not infected with toxoplasmosis but were born to mothers with high titres of anti-*Toxoplasma* antibody showed anti-*Toxoplasma* activity only in the 7S globulin fraction of their sera.

Congenital infection by malaria may also result in the production of IgM and some IgA antibodies by the foetus. It was postulated that the high cord blood levels of IgA and IgM found in Nigerian infants relative to infants in Britain is probably almost entirely due to congenital malaria infections since the incidence of the other more common intra-uterine infections like rubella, cytomegalovirus disease and syphilis was no greater in Nigeria (Okafor *et al.*, 1978). The case of congenital malaria due to

P. malariae described by Harvey *et al.* (1969) and that due to *P. falciparum* by Thomas and Chan (1980) both had demonstrable specific anti-malarial IgM antibody in the infant's blood.

Dissanayake *et al.* (1980) from Sri Lanka, a country where *Wuchereria bancrofti* is endemic, reported that 12 out of 340 cord blood samples had specific IgM antibodies directed against microfilariae antigens. This may be an example of foetal response to the transplacental transmission of helminthic larvae or their antigens. Microfilariae of *W. bancrofti* can cross the placenta but cannot establish an active infection in the foetus (Bloomfield *et al.*, 1978), but their transient presence would appear to be sufficient to lead to foetal sensitization.

The ability of the foetus to produce a specific IgM response has become a useful diagnostic measure of intra-uterine infections, especially in diseases like toxoplasmosis where there are inapparent or subclinical manifestations. Thus, Alford *et al.* (1969) screened a series of cord sera for elevated levels of IgM (i.e. IgM levels above the first S.D. of the mean) and found a 30-fold increase in infections in those infants with elevated levels compared to controls. Many of these infants were symptomless at birth but developed CNS symptoms later. Since, as we have discussed in an earlier section, much of the CNS damage in congenital toxoplasmosis continues after birth and may be arrested by prompt treatment, the screening for IgM levels in newborn infants therefore offers a means of delineating high-risk groups.

Unfortunately, not all infants with congenital toxoplasmosis (Alford *et al.*, 1969) or congenital malaria (Woods *et al.*, 1974) mount an active IgM response so that a negative finding must not be interpreted as excluding congenital infection. In fact, only about 28% of children with congenital toxoplasmosis exhibit a positive IgM-IFA test (Wilson *et al.*, 1980). Presumably, the degree of antigenic stimulation by the parasites and the immune responsiveness of the foetus are important considerations. The reverse situation of false positives must also be guarded against and can arise if the antibody used to detect IgM is not sufficiently specific but has residual cross-reactivity against maternally derived IgG in foetal serum (Remington and Desmonts, 1973).

Recently, a more sensitive method for detecting foetal sensitization to congenital toxoplasmosis was reported by Wilson *et al.* (1980). This is based on foetal lymphocyte transformation in response to *Toxoplasma* antigen, measured either as increased counts per minute or as stimulation index, when compared to controls. This transformation test was found to be as specific (100% correlation) as isolating the organisms from the placenta and almost as sensitive (84% correlation) as *Toxoplasma* isolation from the placenta (94% correlation). In comparison, only 28% of congenitally infected infants showed a positive IgM-IFA test. In addition, of four infants

studied before they produced IgM antibodies, three were positive in the transformation test, indicating that cellular sensitization can be detected prior to specific antibody synthesis. Stray-Pedersen (1980) has also found that, in three children with definite evidence of having been infected with *Toxoplasma in utero*, all had a strong *in vitro* lymphocyte stimulation index while two had raised IgM and IgA levels. None had demonstrable specific IgM antibody as tested by the indirect fluorescent antibody test. This confirms that lymphocyte sensitivity is perhaps the most reliable measure of foetal *Toxoplasma* infection. It must be remembered, however, that there may be a long period of several months before this test becomes positive so that a negative finding during the early neonatal period does not necessarily signify absence of infection.

Although it is possible that the vast majority of infants with specific transforming lymphocytes in response to parasitic antigens has been sensitized by congenital infections, it must be pointed out that other theoretical possibilities exist. Soluble parasitic antigens from maternal circulation may traverse the placenta to sensitize foetal lymphocytes. Alternatively, the observed foetal sensitivity could be mediated by the passive transfer of sensitized maternal lymphocytes or the transmission of some transfer factor. In these situations, therefore, an observed foetal lymphocyte sensitivity towards a particular parasitic antigen is not necessarily indicative of foetal infection.

B. PROTECTIVE IMMUNITY

It is generally considered that protection of the foetus against intra-uterine infections is mediated largely by passively transferred maternal antibodies in those species where this occurs *in utero* but it is not known to what extent the foetus' own immune response contributes to this protection. In theory, there is no reason why the specific immune response discussed in the preceding sections should not play a part. The specific IgM antibody mounted by the foetus against malaria, for example, would be expected to be effective, as one of the effector immune mechanisms against this parasite is the non-complement dependent agglutination of merozoites and blocking of their red blood cell receptors, thus preventing further re-invasion of erythrocytes (Butcher *et al.*, 1973). There is also evidence that specific antibody against toxoplasma may increase the rate of intracellular digestion of these parasites by macrophages (Hirsch *et al.*, 1974) so, again, the IgM response of the foetus against congenital toxoplasmosis may be protective to a certain extent.

It is possible that the foetus may not be too well equipped to deal with those diseases, like trypanosomiasis, where immune protection is by complement-dependent antibody-mediated parasiticidal effects. Although the ontogeny of the complement system in the human foetus begins early in gestation, it seems that there is a gradual maturation which is not complete

until a few months after birth so that the foetus is deficient in complement components for both the classical and alternate pathways during much of its developmental period *in utero* (see Loke, 1978). Since various complement components are involved not only in classical immune lysis, but also in chemotactic response and phagocytosis, the human foetus *in utero* would be expected to be somewhat inefficient in these immunological reactions.

In the preceding section, it was also noted that a specific lymphocyte sensitization against toxoplasmosis could be demonstrated in congenitally infected infants so it seems that the foetus *in utero* may also mount a form of cell-mediated response. This would be useful against intracellular tissue parasites like *Leishmania*, *Toxoplasma* and *T. cruzi*. It appears that many other cellular activities are well developed in the human foetus. Foetal lymphocytes, as early as the first trimester of gestation, have been found to be able to respond to allogeneic stimulation in the mixed lymphocyte reaction and antibody mediated cytotoxic activity (K-cell killing) is readily demonstrable (see Loke, 1978). It is interesting to note that cells from the foetal liver respond particularly strongly to allogeneic stimulation. Since the foetal liver is the first organ traversed by blood returning from the placenta, it is tempting to postulate that this effector function by liver lymphoid tissue could be a mechanism by which the foetus is protected from those maternal allogeneic predator cells which have eluded the placental barrier. Whether this applies also to foreign pathogens remains to be determined.

C. IMMUNOPATHOLOGY

It is now well established that in many adult parasitic infections, the host's hypersensitivity reactions against the parasite plays a big part in the production of pathological lesions. The question therefore arises as to whether this can occur *in utero*.

Keitel *et al.* (1956) presented a case from Washington, where postnatal mosquito infection was unlikely, of a child with congenital quartan malaria who developed nephrotic syndrome. In adult quartan malaria, immunofluorescent studies of kidney biopsies usually show the characteristic 'lumpy-bumpy' deposition of immune complexes, so it seems possible that a similar Type III immunopathological mechanism may be responsible for the kidney lesions in this case. The child was already 21 months old when diagnosed, an age when all residual passively transferred maternal antibodies are likely to have been catabolized. It was therefore assumed that the antibody involved in the formation of the complexes was generated by the foetus itself in response to the intra-uterine infection. Congenital toxoplasmosis has also been known to give rise to nephrotic syndrome in the foetus (Wickbom and

Winberg, 1972). Like quartan malaria, the pathogenic mechanism may again be due to Type III hypersensitivity for experimentally it has been shown that mice infected with a low-virulent strain of toxoplasma frequently have immune complexes deposited in the glomeruli (Huldt, 1971). It is not clear why nephrotic syndrome is, in fact, not more frequently reported as a manifestation of congenital toxoplasmosis. Perhaps a proper balance of antigenic load and foetal immune response is needed. As pointed out by Peters and Lachmann (1974), for chronic immune-complex nephritis to develop, there must be a persistent source of antigen and an antibody response to it. The immune response must provide sufficient antibody to generate the immune complexes but not so much as to result in the over-rapid elimination of antigen.

In congenital Chagas' disease, organisms can be very difficult to find in foetal tissues in spite of the presence of extensive inflammatory lesions and this has led to the suspicion that the pathology may be due to some immunopathological mechanism (Bittencourt *et al.*, 1972a). In the detailed study of six children with congenital Chagas' disease by Szarfman *et al.* (1975) the following findings were recorded. In three cases, circulating IgM antibody was detected which cross-reacted with *T. cruzi* and endocardium, vascular structures and striated muscles. In two cases, these antibodies reacted with the foetus' own tissues and therefore appear to be auto-antibodies. Muscle biopses from three children showed deposits of IgM and/or IgG bound to the plasma membrane. From these observations, several interesting conclusions may be reached. It appears that a cross-reactive Type II as well as an immune-complex Type III hypersensitivity reaction may be responsible for pathological lesions in congenital parasitic infections. The antibody produced is auto-reactive and is directed against certain host's own tissues, a situation rather reminiscent of that of post-streptococcal rheumatic fever. The pathogenesis of congenital Chagas' disease, therefore, may be said to be due to an auto-allergic mechanism. The offending cross-reactive antibody is probably produced by the foetus in response to the infection because it is largely of IgM class. However, the finding of some IgG in the muscle biopses raises the suspicion that passively transmitted maternal auto-antibodies may also play a part in the production of foetal lesions. This, of course, has very important implications for it means that pregnant women with Chagas' disease can affect the foetus by the transfer of maternal auto-antibodies without the need for transmission of the parasites themselves. This possibility should be considered also for other congenital parasitic infections like malaria where the haemolytic episodes (Thompson *et al.*, 1977) could equally be due to the passive transfer of maternal anti-erythrocyte auto-antibody. In support of this hypothesis it should be mentioned that the transplacental transfer of maternal auto-antibodies in many auto-allergic diseases is certainly well documented

(see Loke, 1978). For example, the auto-antibodies produced in maternal connective tissue diseases and thyroid disorders are known to cross the placenta to give rise to transient manifestations of these conditions in the foetus.

Finally, it should be recognized that the transmission of parasitic antigens from mother to foetus may also give rise to immunopathological lesions in combination with an intact foetal immune response. However, the main interest in this aspect is not the overt pathology which can result *in utero*, but rather, in the extent to which the foetus' future immune response can be modulated. This will now be discussed in the next section.

D. MODULATION OF FOETAL IMMUNE RESPONSE BY PARASITIC ANTIGENS

In the preceeding section, we have seen that in the human foetus with its early maturation of immunocompetence, confrontation with parasitic antigens from the mother will usually result in foetal sensitization. This active sensitization *in utero* can confer on the foetus the ability to mount a secondary immune response when it next encounters the relevant parasite after birth. Thus, the experiments of Palmer (1978) showed that a group of weanling rats from mothers infected with *Plasmodium berghei* would mount a rapid secondary antibody response when challenged with *P. berghei* compared to control groups from non-infected mothers. The neonates were themselves not infected, so sensitization could have resulted from the transplacental transfer of soluble malarial antigens. These antigens have been demonstrated in the circulation of rats with acute *P. berghei* infections (Cox *et al.*, 1968).

In contrast to foetal sensitization, it must be remembered that acquired immunological tolerance to foreign antigens can similarly be evoked if these antigens are introduced during a period when the animals have not passed beyond a critical period in their immunological maturation. In this situation, the neonate's subsequent immune response to the same antigen will be very different from that of the prenatally sensitized one. The occurrence of prenatal tolerance and the establishment of a subsequent carrier state seems well established in congenital viral diseases but it is not clear how important this is in parasitic infections. The parasitic disease which has been investigated in greatest detail is schistosomiasis.

Camus *et al.* (1976) carried out a study in Brazil on the reaction to schistosomal delayed skin test between: (1) a group of 27 children from mothers infected with *S. mansoni* but who themselves were not infected; (2) a control group of 40 children from non-infected mothers. The ages ranged from 7–34 months. It was found that 13 out of 27 (48·1%) children from group (1)

were positive for the delayed skin test compared to 3 out of 40 (7·5%) in control group (2). In addition, the mean diameter of the skin lesion in group (1) was 26·27 ± 34·84 mm² S.D. compared to 3·20 ± 4.62 mm² S.D. in group (2). It was therefore concluded that maternal schistosomiasis can sensitize the foetus *in utero* leading to a positive delayed skin reaction to schistosomal antigen after birth. These results were confirmed by Tachon and Borojevic (1978) who found the values for delayed skin reactions to be significantly higher in children born to mothers infected with *S. mansoni* than in control children born to uninfected mothers. In addition, the macrophage migration inhibition test by cord blood cells in the presence of schistosomal antigens was positive in 40% of the children from infected mothers. Controls were all negative. However, the data presented by Moriearty and Lewert (1974) do not seem to fit readily into this pattern. These investigators found there was a higher incidence of delayed skin test to schistosomal antigens among European immigrant populations to Uganda compared to the Ugandans themselves and postulated that this could be due to the absence of prenatally induced tolerance among the non-indigenous population. An alternative interpretation of their data is that there could be genetically determined reactivity to schistosomiasis between different groups with an inherent resistance among the negroid populations (Bina *et al.*, 1978).

A further method of measuring whether sensitization or tolerance has been induced *in utero* is by monitoring the severity of pathological lesions in subsequent infections by schistosomes. Warren (1972) has established that the granulomatous reactions formed around schistosome eggs is essentially a cell-mediated response and is a manifestation of delayed hypersensitivity. From this, it will be expected that sensitized animals would show a more severe reaction on challenge with schistosomal eggs compared to tolerant animals. This was subjected to direct experimentation by Lewert and Mandolowitz (1969) who injected 1000 *S. mansoni* eggs into the caudal veins of offspring from mice infected with *S. mansoni* just before pregnancy. Offspring from non-infected mice were similarly injected and served as controls. The offspring were then killed and the size of the egg granulomas in the lungs were estimated in the two groups. It was found that the mean diameter was 101 ± 2 μm in the group from infected mothers compared to 139 ± 4 μm in offspring from non-infected mothers, the difference being significant to the level of $P < 0{\cdot}001$. These data, therefore, point to an induction of tolerance in the offspring by maternal infection resulting in a less severe pathology. This may explain why the disease is milder in Africans compared to Europeans in an endemic area in spite of a heavier worm burden in the former.

Later experiments of similar design with mice by Hang *et al.* (1974) have indicated that the antigenic dose given to the mothers has an important bearing on the effects on the offspring. Massive infection of the

mothers with 110 cercariae resulted in offspring who were hyporesponsive to subsequent egg challenge with a mean granuloma diameter of 112 $\pm$ 5 μm compared to 168 $\pm$ 5 μm in control offspring. Less severe maternal infections with 60 or 20 cercariae did not alter the offspring's immune response to egg challenge. This is further confirmed by giving mothers soluble egg extracts where a dose of 600–900 μg administered resulted in offspring who were hyporesponsive to egg challenge. Interestingly, mothers given small doses (100 μg) of egg extracts gave birth to offspring who were sensitized. Therefore, it would appear that the dose of antigen experienced by the foetus *in utero* is an important factor in determining whether tolerance or sensitization results. This is not really surprising, and similar results have been observed in other immunological situations. The data of Hang *et al.* (1974) further showed that only when measured at 8 days is the mean diameter of the egg granulomas in hyporesponsive offspring smaller than in controls. By 32 days the mean diameter becomes as great, if not greater, than in control animals. This indicates that the tolerance induced prenatally is, at most, only transient.

Instead of measuring the diameter of egg granulomas, Borojevic *et al.* (1977) have used hepatic and spleen weights as a measure of sensitization or tolerance in offspring born to infected and non-infected mothers. They infected C_3H female mice with 50 cercariae of *S. mansoni* and they were then mated 40 days later. Sucklings from the subsequent litters were exposed to 50 cercariae and then sacrificed 8–10 weeks later and their liver and spleen weights measured. A group of sucklings from uninfected mice were treated in the same way. They found that hepato-splenomegaly was more pronounced in neonates born to infected mothers than in controls. It is difficult to judge the relevance of these results. Hepato-splenomegaly is presumably due to an overall proliferation of the R.E. system in response to infection. Whether the degree of this response can be correlated with sensitization or tolerance in the same way as measuring the size of the egg granulomas is not clear at present. Hang *et al.* (1974) could not find any difference in liver and spleen weights between tolerant offspring and controls and concluded that prenatal modulation of immune response was measurable only by variation in egg granuloma diameter and did not appear to affect hepatosplenic manifestations. In defence of the experimental protocol of Borojevic *et al.* (1977), it may be said that their challenge of neonatal mice by cercarial penetration is more akin to the natural route of infection than the intravenous injection of eggs as applied by Hang *et al.* (1974).

Since congenital schistosomiasis is uncommon, the modulation of foetal immune response must be mediated by mechanisms other than the direct transmission of the parasites themselves. Even schistosome eggs are rarely found in the foetus or placenta. The passive transfer of sensitized maternal lymphyocytes or some transfer factor may be involved (Camus *et al.*, 1976)

and it appears that immunological tolerance can also be passively transmitted from mother to foetus in a similar manner (Auerbach and Clark, 1975).

However, the most likely candidate is probably the materno-foetal transmission of soluble schistosomal antigen. According to Smithers and Terry (1969), in schistosomiasis, circulating antigens, defined as soluble substances released by the parasite into the blood stream of the host, are the main antigenic stimuli of the host's immune response to these helminths. The transplacental transmission of these antigens, therefore, would be expected to have significant effects on foetal immune response. These antigens have been demonstrated in the sera of a wide variety of animals infected with *S. mansoni* or *S. haematobium* (Bergyren and Weller, 1967; Gold *et al.*, 1969; Bawden and Weller, 1974; Nash *et al.*, 1974; Deelder *et al.*, 1976) and also in human patients (Madwar and Voller, 1975). These circulating antigens can be detected as early as the 19th day after infection of hamsters and mice with 800 cercariae (Bawden and Weller, 1974) and there is a linear relationship between serum concentration of antigen and worm burden (Gold *et al.*, 1969; Bawden and Weller, 1974). Some of these antigens are also found in urine (Gold *et al.*, 1969; Carlier *et al.*, 1975; Deelder *et al.*, 1976; Santoro *et al.*, 1977a) and in the milk of lactating mothers (Santoro *et al.*, 1977b). This latter finding points to an additional route for the transfer of schistosomal antigens from mother to foetus.

There is probably more than one antigenic entity (Deelder *et al.*, 1976). Immunofluorescent studies have localized the epithelium of the worm gut as a potential source of antigens (Lichtenberg *et al.*, 1974; Nash 1974). Many investigators have concluded that many of the antigens are certainly derived from adult worms and not from eggs because antigens appear in the circulation before egg deposition has occurred (Gold *et al.*, 1969; Bawden and Weller, 1974). In contrast, the results of Nash *et al.* (1974) indicated that it is the egg antigen from maternal infection which is the tolerogenic stimulus to the foetus. They showed that massive unisexual infections of the mothers or massive bisexual infections 1 week before mating the mothers (i.e. before the worms have reached the stage of egg deposition) did not result in any detectable modulation of foetal immune response but injections of the mothers with eggs or egg extracts will clearly do so. For the present, therefore, it seems best to reserve judgement as to what is the source of schistosomal antigen which is most relevant to transplacental transmission.

In chronic schistosomiasis, much of the antigenic material may be in the form of immune complexes in the circulation (Phillips and Draper, 1975; Bout *et al.*, 1977) and this may lead to some difficulty in detection. These complexes can be dissociated in the breast or kidneys, presumably under the low pH condition that dissociates low affinity complexes, with the antigen passing through the mammary or urinary epithelium to be secreted into

milk or urine (Carlier *et al.*, 1975; Santoro *et al.*, 1977b). One wonders whether a similar mechanism can operate in the placenta.

The general conclusion from the available evidence, therefore, would seem to indicate that maternal parasitic infections can modulate foetal immune response. Either tolerance or sensitization may result and this seems to be related to the antigenic load presented to the foetus. Perhaps of greatest potential significance is the observation that overt parasitic infection of the foetus in not necessary, but the transmission of antigens from the mother is sufficient to lead to the modulation of foetal immune response. Thus, parasitic infections of the mother during pregnancy may have a much wider ranging influence on foetal development than hitherto realized.

IX. Conclusion

The placental form of gestation in mammals may be considered to be highly successful because the foetus can now be nourished safely by the mother for long periods in the relatively germ-free internal environment of the uterus and thereby avoids a high rate of reproductive wastage. In order for the placenta to fulfil this protective role, an important requirement is that it should function as a barrier against the transmission of infective organisms from the mother. Indeed, the pregnant mother is particularly vulnerable to infection as there appears to be a generalized depression of her immune response during gestation which can lead to an increase in severity or to a reactivation of a dormant disease, as occurs for example in amoebic dysentery, malaria and toxoplasmosis.

From a survey of the available evidence, it would appear that, for the vast majority of parasitic infections, transmission from mother to foetus is the exception rather than the rule. The main protozoan culprit is *Toxoplasma* but even this organism does not always lead to foetal involvement. It is estimated that only about a third of the cases of maternal toxoplasmosis acquired during pregnancy will affect the human foetus. The frequency of congenital transmission with other protozoa like *Plasmodium* and *T. cruzi* is much lower while only sporadic reports are available for *Leishmania* and African trypanosomes. Of the helminths, the dog ascaris *Toxocara* is the one that affects the foetus most frequently. In fact, almost 100% of puppies will be infected by the mother *in utero* and this appears to be the main route of transmission of this disease. There are no data available to indicate whether or not a similar situation pertains with human ascariasis. Other species of nematodes, together with cestodes and trematodes, are rarely concerned with congenital infection. It may be said therefore that, on balance, the

placenta certainly seems to fulfil its barrier function with commendable efficiency. The multiple layers of foetal and maternal tissues which make up the placenta probably play a key role. However, small breaks in the integrity of these layers can occur during gestation, especially in the haemachorial type of placentation as in man which has the fewest intervening layers between foetal and maternal circulations. When this occurs, maternal blood from the placental sinusoids can seep into the foetus. Malarial parasites may be passively transmitted in this way inside maternal erythrocytes. A further defensive aid to the foetus is that the haemodynamics of the placental circulation is such that the pressure is higher on the foetal side. Placental tears will tend to result in foetal blood leaking into maternal sinusoids rather than in the reverse direction. These features, therefore, would restrict the transmission of all those parasites which are entirely dependent on such a passive mode of transplacental transfer.

An additional mechanism of defence in the placenta is the presence of a large number of cells capable of non-specific as well as immune phagocytosis. These are likely to destroy a variety of organisms except those that have evolved ways of evading intracellular digestion by host phagocytes. Such an example is *Toxoplasma*, and this may be a reason why these organisms can traverse the placenta with relative ease. The difference in the reported frequency of congenital infections between African trypanosomiasis and Chagas' disease may be due to the occurrence of an intracellular tissue phase in the latter's life cycle which could utilize a similar evasive mechanism. Unfortunately the data relating to a further example of an intracellular protozoan, *Leishmania*, are still too meagre to permit a definite conclusion.

From the observation with *Toxocara*, it is tempting to generalize that an active migratory tissue phase in the helminthic life cycle is a necessary prerequisite for foetal infection. It is also interesting to note that *Toxocara* appears to reach the foetus by migrating along the substance of the umbilical cord rather than via the haematogenous route. Yet, many other helminths with similar behavioural patterns do not give rise to congenital infection with such regularity. Perhaps, in the case of *Toxocara*, foetal tissue is particularly susceptible to the parasites. This is certainly an additional factor which should be considered also for other diseases. For example, malarial parasites may not succeed in colonizing foetal erythrocytes so that the infrequent occurrence of congenital malaria may be due to the parasites' inability to establish themselves in the foetal environment even if they should succed in breaching the placental barrier. Furthermore, immunocompetence in the mammalian foetus develops very early *in utero* so it is capable of mounting some forms of activity against invasion by maternal pathogenic organisms. Added to this active immunity, the passive transfer of maternal antibodies will further augment foetal defence. Possibly the reason why the CNS is so

frequently affected in congenital toxoplasmosis is because of the difficulty antibodies have in penetrating the blood/brain barrier to deal effectively with the parasites in this situation.

The effects of maternal parasitic infection on the foetus may be indirect and not necessarily due to transplacental transmission. This is particularly likely to happen in those diseases like malaria and trypanosomiasis which cause severe constitutional disturbance in the mother. High fever, whatever the cause, can kill the foetus. Maternal anaemia would be expected to lead to poor foetal nutrition and may be a contributory factor to the low birthweight and growth retardation so frequently observed in malarious endemic countries. In this disease, the characteristic massive accumulation of parasites and mononuclear cells in the placental sinusoids would further interfere with placental transfer functions. Thus, even in a disease where congenital infection is rare, adverse effects on the foetus as a consequence of maternal illness is still significant.

When foetal infection does occur, the effects on the foetus can be very severe and, depending on when the infection started, can result in early abortion, miscarriage and stillbirth. In milder cases, the foetus may be born apparently normal only to develop symptoms later as in toxoplasmosis. There is evidence that a continuing inflammatory process after birth may be responsible for much of this later pathology which raises the hope that efficient treatment postnatally may arrest further deterioration of the lesions. This, of course, necessitates the early recognition of high risk groups among pregnant mothers and their neonates which may prove to be difficult and too expensive to be of practical value. For example, in toxoplasmosis, it has still not been established whether only acute maternal infections can affect the foetus or if chronic infections can also do so. This makes it difficult to delineate which group of neonates to follow-up. Furthermore, the present method of using a raised serum IgM value in the neonate as an indicator of congenital infection is still not sufficiently sensitive and will result in many cases being missed.

The presenting clinical symptoms of many congenital infections are usually so indistinct as to create problems in differential diagnosis, especially in a non endemic country. Thus, in malaria, hepatosplenomegaly, jaundice and haemolytic anaemia in a newborn can be caused by a variety of viral, bacterial as well as many inherited disorders. Meningoencephalitis in a newborn could similarly be caused by other pathogens besides trypanosomes. Even in toxoplasmosis, the early visceral stage of RE involvement is not sufficiently specific for firm diagnosis to be made. It is the late symptoms consisting of the 'classical triad' of chorioretinitis, hydrocephalus and cerebral calcification which may be described as characteristic of the disease. Destruction of central nervous tissue and obstruction of the central ventricle

can lead to the development of microcephalus and hydrocephalus so these lesions must not be attributed to any teratogenic potential of the parasite. Only congenital viral infections appear to lead to true malformations. There may be less overt consequences of congenital infection of the CNS like mental retardation or just impairment of intellectual ability which should also be recognized.

Research in basic parasite immunology has now clearly established that the pathogenesis of many lesions in adult infections is the result of a hypersensitivity reaction by the host. With increasing maturation of foetal immune response, it is possible that much of the pathology in congenital parasitic infections is also caused by similar immunopathological mechanisms. This raises intriguing questions. Can the diffusion of parasite antigens alone from mother to foetus across the placenta, without intact parasites, stimulate foetal immune response to give rise to hypersensitivity reactions? Alternatively, can passively transferred maternal antibodies combine with soluble parasite antigens to form immune complex lesions in the foetus, like the nephrotic syndrome found in congenital malaria and myocarditis in congenital Chagas' disease? It seems that even a third possible mechanism may operate, and this is the appearance of cross-reactive antibodies directed against the foetus' own tissues leading to auto-allergy. The demonstration that these auto-antibodies are of IgG as well as IgM class suggests that some might have originated from the mother, a situation analogous to that of neonatal hyperthyroidism caused by the transplacental transmission of LATS from a thyrotoxic mother. These observations therefore indicate that maternal parasitic infections can lead to the development of foetal pathology without the need for actual foetal infection.

Early contact with parasitic antigens will affect the foetus' future immune response when it next encounters similar organisms. Either sensitization or tolerance can develop, depending on the gestation age when contact is first made and on the dose of antigen. If indeed the foetus can be sensitized in this way, then a potential clinical application would be a programme of active immunization of the foetus by administering the immunogen to the mother, so that the neonate at the time of birth will already be able to mount a secondary response to appropriate parasites. This would be particularly valuable in endemic areas of the world. On the other hand, creation of a state of tolerance may be more desirable. For instance, it is thought that the severity of schistosomal granulomas is due to a delayed hypersensitivity reaction in the host. In this case, a degree of hyporeactivity would lead to milder symptoms, a situation which may exist in natural infections, thereby explaining why schistosomiasis tends to be milder among indigenous Africans in endemic areas than in Europeans, in spite of a heavier worm burden in the former. Theoretically, therefore, maternal parasitic infections can

have far-ranging effects on foetal immune reactivity and it remains to be seen if practical methods can be devised to manipulate this to the advantage of the foetus.

Acknowledgements

I am grateful to the Ford Foundation and the American Friends of Cambridge University for financial support. Miss Susan Day provided excellent technical assistance. I would like to thank Mrs. Pauline Davies for her gallant typing efforts in getting the manuscript ready within a very tight schedule. The kind permission of D. Steven to reproduce Fig. 1 and of C. D. Ockleford and A. Whyte and the *Journal of Cell Science* to reproduce Fig. 2 is also acknowledged.

References

Abioye, A. A. (1973). Fatal amoebic colitis in pregnancy and puerperium. A new clinico-pathological entity. *Journal of Tropical Medicine and Hygiene* **76**, 97–100.

Abioye, A. A. and Edington, J. M. (1972). Prevalence of amoebiasis at autopsy in Ibadan. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **66**, 754–763.

Adams, F. H., Cooney, M., Adams., J. M. and Kabler, D. (1949). Experimental toxoplasmosis. *Proceedings of the Society of Experimental Biology and Medicine* **70**, 258–260

Adinolfi, M. (1975). The human placenta as a filter for cells and plasma proteins. *In* 'Immunobiology of Trophoblast' (A. Edwards, A. Howe and A. Johnson, eds), pp. 193–210. Cambridge University Press, Cambridge.

Adler, S. and Clark, E. J. (1922). Intra-uterine infection with Ancylostoma caninum in dogs. *Annals of Tropical Medicine and Parasitology* **16**, 353–354.

Alford, C. A., Faft, J. W., Blankenship, W. J., Cassady, G. and Benton, J. W. (1969) Subclinical central nervous system disease in neonates: a prospective study of infants born with increased levels of IgM. *Journal of Pediatrics* **75**, 1167–1178.

Altshuler, G. (1973). Toxoplasmosis as a cause of hydranencephaly. *American Journal of Disease of Children* **125**, 251–252.

Altshuler G. and Russell, P. (1975). The human placental villitides: a review of chronic intrauterine infection. *Current Topics in Pathology* **60**, 63–112.

Amino, N., Tanizawa, O., Miyai, K., Tanaka, F., Hayashi, C., Kawashima, M. and Ichihara, K. (1978). Changes of serum immunoglobulins IgG, IgA, IgM and IgE during pregnancy. *Obstetrics and Gynaecology (New York)* **52**, 415–420.

Amoroso, E. C. (1968). The evolution of viviparity. *Proceedings of the Royal Society of Medicine* **61**, 1188–1200

Anderson, J. M. and Ferguson-Smith, M. A. (1971). Nature's Transplant. *British Medical Journal* **2**, 166–167.

Archibald, H. M. (1956). The influence of malarial infection of the placenta on the incidence of prematurity. *Bulletin of the World Health Organisation* **15**, 842–845.

Archibald, H. M. (1958). Influence of maternal malaria on newborn infants. *British Medical Journal* **4**, 1512–1514.

Armon, P. J. (1978). Amoebiasis in pregnancy and the puerperium. *British Journal of Obstetrics and Gynaecology* **85**, 264–269

Auerbach, R. and Clark, S. (1975). Immunological tolerance: transmission from mother to offspring. *Science* **189**, 811–812.

Augustine, D. L. (1927). Development in pre-natal infestation of *Belascaris*. *Journal of Parasitology* **13**, 256–259.

Augustine, D. L. (1934). Studies on pre-natal trichinosis. *American Journal of Hygiene* **19**, 115–122.

Bawden, M. P. and Weller, T. H. (1974). *Schistosoma mansoni* circulating antigen: detection by complement fixation in sera from infected hamsters and mice. *American Journal of Tropical Medicine and Hygiene* **23**, 1077–1084.

Bayliss, R. I. S., McC. Browne, J. C., Round, B. P. and Steinbeck, A. W. (1955). Plasma-17-Hydroxycorticosteroids in pregnancy. *Lancet* **i**, 62–64.

Beach, P. G. (1979). Prevalence of antibodies to *Toxoplasma gondii* in pregnant women in Oregon. *Journal of Infectious Diseases* **140**, 780–783.

Bender, S. (1950). Placental metastases in malignant disease complicated by pregnancy. *British Medical Journal* **1**, 980–981.

Benirschke, K. (1960). Routes and types of infection in the foetus and the newborn. *American Journal of Diseases of Children*. **99**, 714–721.

Bergyren, W. L. and Weller, T. H. (1967). Immunoelectrophoretic demonstration of specific circulating antigen in animals infected with *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **16**, 606–612.

Beverley, J. K. A. (1959). Congenital transmission of toxoplasmosis through successive generation of mice. *Nature* (*London*) **183**, 1348–1349.

Beverley, J. K. A. (1960). Discussion on toxoplasmosis: Congenital toxoplasma infection. *Proceedings of the Royal Society of Medicine* **53**, 111–113.

Beverley, J. K. A. and Watson, W. A. (1959). Ovine abortion due to toxoplasmosis. *Nature* (*London*) **184**, 2041.

Bina, J. C., Tavaresneto, J., Prata, A. and Azevedo, E. S. (1978). Greater resistance to development of severe schistosomiasis in Brazilian negroes. *Human Biology* **50**, 41–49.

Bissenden, J. G., Ling, N. R. and Mackintosh, P. (1980). Suppression of mixed lymphocyte reactions by pregnancy serum. *Clinical and Experimental Immunology* **39**, 195–202.

Bittencourt, A. L. (1976). Congenital Chagas' Disease, *American Journal of Diseases of Children* **130**, 97–103.

Bittencourt, A. L., Barbosa, H. S., Rocha, T., Sodré, I. and Sodré, A. (1972a). Incidência da transmissão congênita da doenca de Chagas em partos prematuros na Maternidade Tsylla Balbino (Salvador, Bahia). *Revista do Instituto de medicina tropical de Sao Paulo* **14**, 131–134.

Bittencourt, A. L., Oliveira, V. S. and Andrade, C. C. Q. (1972b). Observações sobre a tentativa de transmição congénita da esquistossomose mansônica. *Revista do Instituto de medicina tropical de Sao Paulo* **14**, 106–109.

Bittencourt, A. L., Almeida, M. A. C. de., Innes, M. A. F. and Motta, L. D. C. da. (1980). Placental involvement in schistosomiasis mansoni. Report of four cases. *American Journal of Tropical Medicine and Hygiene* **29**, 571–575.

Björksten, B., Söderström, T., Damber, M-G., von Schoultz, B. and Stigbrand, T. (1978). Polymorphonuclear leucocyte function during pregnancy. *Scandinavian Journal of Immunology* **8**, 257–262.

Blacklock, B. and Gordon, R. M. (1925a). Malaria parasites in the placental blood. *Annals of Tropical Medicine and Parasitology* **19**, 37–45.

Blacklock, B. and Gordon, R. M. (1925b). Malaria infection as it occurs in late pregnancy; its relationship to labour and early infancy. *Annals of Tropical Medicine and Parasitology* **19**, 327–365.

Blanc, W. A. (1961). Pathways of foetal and early neonatal infection. *Journal of Pediatrics* **59**, 473–496.

Bloomfield, D. R., Suarez, J. R. and Malangit, A. C. (1978). Transplacental transfer of Bancroftian filariasis. *Journal of the National Medical Association* **70**, 597–598.

Borojevic, R., Santoro, F., and Grimaud, J. A. (1977). Congenital modification of murine schistosomiasis mansoni. *Revista do Instituto de medicina tropical de Sao Paulo* **19**, 109–112.

Bossie, A. and Lupasco, G. L. (1969). Transmission du parasite de la mère au foetus dans l'infection experimentale avec L. donovani du hamster doré (mesocricetus auratus). *Archives roumaines de pathologie experimental et de microbiologie, Paris* **428**, 49–55.

Bout, D., Santoro, F., Carlier, Y., Bina, J. C. and Capron, A. (1977). Circulatory immune complexes in schistosomiasis. *Immunology* **33**, 17–22.

Boyd, J. D. and Hamilton, W. J. (1970). "The Human Placenta". Heffer and Sons, Cambridge.

Bray, R. S. and Anderson, M. J. (1979). Falciparum malaria and pregnancy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **73**, 427–431.

Bray, R. S. and Sinden, R. E. (1979). The sequestration of *Plasmodium falciparum*-infected erythrocytes in the placenta. *Transactions of Royal Society of Tropical Medicine and Hygiene* **73**, 716–719.

Brinkmann, U. K., Kramer, P., Presthus, G. T. and Sawadogo, B. (1976). Transmission *in utero* of microfilariae of *Onchocerca volvulus*. *Bulletin of the World Health Organisation* **54**, 708–709.

Brown, R. C. and Girardeau, M. H. F. (1977). Transmammary passage of *Strongyloides* sp. larvae in the human host. *American Journal of Tropical Medicine and Hygiene* **26**, 215–219.

Bruce-Chwatt, L. J. (1952). Malaria in African infants and children in Southern Nigeria. *Annals of Tropical Medicine and Parasitology* **46**, 173–200.

Bruce-Chwatt, L. J. (1954). Plasmodium berghei in the placenta of mice and rats: transmission of specific immunity from mother rats to litters. *Nature* (*London*) **173**, 353–354.

Bruce-Chwatt, L. J. and Gibson, F. D. (1956). Transplacental passage of Plasmodium berghei and passive transfer of immunity in rats and mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **50**, 47–53.

Butcher, G. A., Mitchell, G. H. and Cohen, S. (1973). Mechanism of host specificity in malarial infection. *Nature* (*London*) **244**, 40–42.

Butterworth, M., McClellan, B. and Allansmith, M. (1967). Influence of sex on immunoglobulin levels. *Nature* (*London*) **214**, 1224–1225.

Campbell, C. C., Martinez, J. M. and Collins, W. E. (1980). Serioepidemiological studies of malaria in pregnant women and newborns from coastal El. Salvador. *American Journal of Tropical Medicine and Hygiene* **29**, 151–157.

Camus, D., Carlier, Y , Bina, J. C., Borojevic, R., Prata, A. and Capron, A. (1976). Sensitisation to Schistosoma mansoni antigen in uninfected children born to infected mothers. *Journal of Infectious Diseases* **134**, 405–408.

Cannon, D. S. H. (1958). Malaria and prematurity in the Western Region of Nigeria. *British Medical Journal* **2**, 877–878.

Carapella-de Luca E., Casadei, A. M., Pascone, R., Tardi, C. and Pacioni, C. (1978). Materno-foetal transfusion during delivery and sensitisation of the newborn against the Rhesus D-antigen. *Vox Sanguinis* **34**, 241–243.

Carlier, Y., Bout, D., Bina, J. C., Camus, D., Figueiredo, J. F. M. and Capron A. (1975). Immunological studies in human schistosomiasis. I. Parasitic antigen in urine. *American Journal of Tropical Medicine and Hygiene* **24**, 949–954.

Cederqvist, L. L., Kimball, A. C., Ewool, L. C. and Litwin, S. D. (1977). Foetal immune response following congenital toxoplasmosis. *Obstetrics and Gynaecology (New York)* **50**, 200–204.

Chamberlain, D. M., Docton, F. L. and Cole, C. R. (1953). Toxoplasmosis. II. Intra-uterine infection in dogs, premature birth and presence of organisms in milk. *Proceedings of The Society for Experimental Biology and Medicine* **82**, 198–200.

Clark, H. C. (1915). The diagnostic value of the placental blood film in aestivo-autumnal malaria. *Journal of Experimental Medicine* **22**, 427–443.

Cohen, F. and Zuelzer, W. W. (1964). Identification of blood group antigens by immunofluorescence and its application to the detection of the transplacental passage of erythrocytes in mother and child. *Vox Sanguinis* **9**, 75–78.

Cohen, F., Zuelzer, W. W., Gustafson, D. C. and Evans, M. M. (1964). Mechanisms of iso-immunization. I. The transplacental passage of foetal erythrocytes in homo-specific pregnancies. *Blood* **23**, 621–646.

Contractor, S. F. and Krakauer, K. (1976a). Immunofluorescent localisation of cathepsin D in trophoblastic cells in tissue culture. *Beitrage zur Pathologie* **158**, 445–449.

Contractor, S. F. and Krakauer, K. (1976b). Pinocytosis and intracellular digestion of ^{125}I-labelled haemoglobin by trophoblastic cells in tissue culture in the presence and absence of serum. *Journal of Cell Science* **21**, 595–607.

Covell, G. (1950). Congenital Malaria. *Tropical Diseases Bulletin* **47**, 1147–1167.

Cowen, D. and Wolf, A. (1950a). Experimental congenital toxoplasmosis. I. The vagina as a portal of entry in the mouse. *Journal of Experimental Medicine* **92**, 394–402.

Cowen, D. and Wolf, A. (1950b). Experimental congenital toxoplasmosis. II. Transmission of toxoplasmosis to the placenta and foetus following vaginal infection in the pregnant mouse. *Journal of Experimental Medicine* **92**, 403–416.

Cox, H. W., Milar, R. and Patterson, S. (1968). Serologic cross-reactions of serum antigens associated with acute *Plasmodium* and *Babesia* infections. *American Journal of Tropical Medicine and Hygiene* **17**, 13–18.

Czeizel, E., Hancock, M., Palkovich, I., Janko, M. and Zoltai, N. (1966). Possible relation between foetal death and *E. histolytica* infection of the mother. *American Journal of Obstetrics and Gynaecology* **96** 264–266.

Das Gupta, B. M. (1940). Malarial infection in placenta and transmission to foetus. *Tropical Diseases Bulletin* **37**, 184.

Deelder, A. M., Klappe, H. T. M., Van Den Aardweg, A. J. M. and Van Meerbeke, E. H. E. M. (1976). *Schistosoma mansoni:* demonstration of two circulating antigens in infected hamsters. *Experimental Parasitology* **40**, 189–197.

Delgado, M. A. and Santos-Buch, C. A. (1978). Transplacental transmission and foetal parasitosis of *Trypanosoma cruzi* in outbred white Swiss mice. *American Journal of Tropical Medicine and Hygiene* **27**, 1108–1115.

Desmonts, G. and Couvreur, J. (1974). Congenital toxoplasmosis: a prospective study of 378 pregnancies. *New England Journal of Medicine* **290**, 1110–1116.

Diamondopoulos, G. T. and Hertig, A. T. (1963). Transmission of leukaemia and allied diseases from mother to foetus. *Obstetrics and Gynaecology (New York)* **21,** 150–154.

Dikmans, G., Manthei, C. A. and Frank, A. H. (1957). Demonstration of *Trypanosoma theileri* in the stomach of an aborted bovine fetus. *Cornell Veterinarian* **47,** 344–353.

Dimson, S. B. (1954). Congenital malaria in England. *British Medical Journal* **2,** 1083–1085.

Dissanayake, S., Silva, L. V. K. de and Ismael, M. M. (1980). IgM antibody to filarial antigens in human cord blood: possibility of transplacental infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74,** 542–544.

Dudgeon, J. A. (1968). Breakdown in maternal protection: infections. *Proceedings of the Royal Society of Medicine* **61,** 1236–1243.

Eichenwald, H. (1948). Experimental toxoplasmosis. I. Transmission of the infection in-utero and through the milk of lactating female mice. *American Journal of Diseases in Children* **76,** 307–315.

Eichenwald, H. (1949). Experimental toxoplasmosis. II. Effect of sulfadiazine and antiserum on congenital toxoplasmosis in mice. *Proceedings of Society of Experimental Biology and Medicine.* **71,** 45–49.

Eichenwald, H. (1957). Congenital toxoplasmosis: a study of one hundred and fifty cases. *American Journal of Diseases in Children* **94,** 411–412.

Eichenwald, H. F. and Shinefield, H. R. (1963) Antibody production by the human fetus. *Journal of Pediatrics* **63,** 870.

Elliott, W. G. (1970). Placental toxoplasmosis. *American Journal of Clinical Pathology* **53,** 413–417.

El-Maallem, H. and Fletcher, J. (1980). Impaired neutrophil function and myeloperoxidase deficiency in pregnancy. *British Journal of Haematology* **44,** 375-381.

Feldman, H. A. and Miller, L. T. (1956). Congenital human toxoplasmosis. *Annals of the New York Academy of Science* **64,** 180–184.

Fleck, D. G. (1973). The problem of congenital toxoplasmosis. *In* "Intra-uterine Infection" Ciba Foundation Symposium 10, 45–52. Elsevier/North Holland.

Foldes, J. J., Schwartz, J. and Kehaty, T. (1975). Trophoblast and phagocytosis. I. In vitro phagocytosis by human cultured trophoblasts. *American Journal of Fertility* **20,** 228–230.

Foster, A. O. (1932). Prenatal infection with the dog hookworm, *Ancylostoma caninum*. *Journal of Parasitology* **19,** 112–118.

Fox, H. (1967). The incidence and significance of Hofbauer cells in the mature human placenta. *Journal of Pathology and Bacteriology* **93,** 710–717.

Fox. H. (1978). "Pathology of the Placenta". W.B. Saunders and Co. Ltd., London.

Freedman, W. L. and McMahon, F. J. (1960). Placental metastasis. Review of literature and report of case of malignant melanoma. *Obstetrics and Gynaecology (New York)* **16,** 550–560.

Fujikura, T. and Klionsky, B. (1975). Transplacental passage of maternal erythrocytes with suckling. *Journal of Pediatrics* **87,** 781–783.

Fujinami, A. and Nakamura, H. (1911). On the prophylaxis of schistosomiasis and and some investigations on infection with this disease. Cited by Augustine (1927).

Fülleborn, F. (1921) Ascarisinfektion durch Verzehren eingekapselter Larven und über gelungene intrauterine Ascarisinfektion. Cited by Augustine (1927).

Galbraith, R. M., Faulk, W. P., Galbraith, G. M. P., Holbrook, T. W. and Bray, R. S. (1980a). The human materno-foetal relationship in malaria: I. Identification of pigment and parasites in the placenta. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74,** 52–60.

Galbraith, R. M., Fox, H., Hsi, B., Galbraith, G. M. P., Bray, R. S. and Faulk, W. P. (1980b). The human placenta in maternal malaria. II. Histological, ultrastructural and immunopathological studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74,** 61–72.

Garcia, A. G. P. (1968). Congenital toxoplasmosis in two successive sibs. *Archives of Disease in Childhood* **43,** 705–710.

Garnham, P. C. C. (1938). The placenta in malaria with special reference to reticuloendothelial immunity. *Transactions of the Royal Society of Medicine and Hygiene* **32,** 13–48.

Gastebled, G., Nurit, Y., Beucler, A. and Lafaix Ch. (1973). Kala-azar et grossesse. *Journal of Gynecological Obstetrics and Biology of Reproduction* **2,** 1067–1071.

Gilles, H. M., Lawson, J. B., Sibelas, M., Voller, A. and Allan, N. (1969). Malaria anaemia and pregnancy. *Annals of Tropical Medicine and Parasitology* **63,** 245–263.

Glasser, L. and Delta, B. G. (1965). Congenital toxoplasmosis with placental infection in monozygotic twins. *Pediatrics* **35,** 276–283.

Gold, R., Rosen, F. S. and Weller, T. H. (1969). A specific antigen in hamsters infected with *Schistosoma mansoni*. Detection of antigen in serum and urine, and correlation between antigenic concentration and worm burden. *American Journal of Tropical Medicine and Hygiene* **18,** 545–552.

Griesmer, R. A., Gibson, J. P. and Elsasser, D. S. (1963). Congenital Ascariasis in gnotobiotic dogs. *Journal of the American Veterinary Medical Association* **143,** 962–964.

Hang, L. M., Boros, D. L. and Warren, K. S. (1974). Induction of immunological hyporesponsiveness to granulomatous hypersensitivity in *S. mansoni* infection. *Journal of Infectious Diseases* **130,** 515–522.

Harrison, K. A. (1976). Sickle cell disease in pregnancy. *Tropical Doctor* **6,** 74–79.

Harvey, B., Remington, J. S. and Sulzer, A. J. (1969). IgM malaria antibodies in a case of congenital malaria in the United States. *Lancet* **i,** 333–335.

Hedenstedt, S. and Naeslund, J. (1946). Investigation of the permeability of the placenta with the help of elliptocytes. *Acta Medica Scandinavica* **170,** 126–134.

Hellbrugge, T. F., Dahme, E. and Hellbrugge, J. K. (1953). Tierexperimentelle Beobach tungen zur diaplazentaren Infektion der Toxoplamen. *Zeitschrift für Tropenmedizin und Parasitologie* **4,** 312–322.

Hirsch, J. G., Jones, T. C. and Len, L. (1974). Interactions *in vitro* between Toxoplasma gondii and mouse cells. *In* "Parasites in the Immunised Host: Mechanisms of Survival," Ciba Foundation Symposium 25. Elsevier/North Holland.

Horner, E. M. (1960). Placental metastases. *Obstetrics and Gynaecology (New York)* **15,** 566–572.

Howard, J. and Rubio, M. (1970). Congenital Chagas' disease. I. Clinical and epidemiological study of thirty cases. II. Pathological findings in nine cases. *Tropical Diseases Bulletin* **67,** 142–143.

Huldt, G. (1960). Experimental toxoplasmosis. Transplacental transmission in guinea pigs. *Acta Pathologica et Microbiologica Scandinavica* **49,** 176–188.

Huldt, G. (1971). Studies on experimental toxoplasmosis. *Annals of the New York Academy of Science* **177,** 146–155.

Hume, O. S. (1972). Toxoplasmosis and pregnancy. *American Journal of Obstetrics and Gynaecology* **114,** 703–715.

Ikede, B. O. and Losos, G. J. (1972). Hereditary transmission of *Trypanosoma vivax* in sheep. *British Veterinary Journal* **128,** i–ii.

Jacobs, L. (1963). Toxoplasma and Toxoplasmosis. *Annual Review of Microbiology* **17**, 429–450.

Jacobs, L. (1967). Toxoplasma and Toxoplasmosis. *Advances in Parasitology* **5**, 1–45.

Jacobs, L. and Jones, F. E. (1950). The parasitaemia in experimental toxoplasmosis. *Journal of Infectious Diseases* **87**, 78–89.

Jean, L. and van Nitsen, R. (1929). Congenital malaria. *Tropical Diseases Bulletin* **26**, 11.

Jellife, E. F. P. (1968). Low birth-weight and malarial infection of the placenta. *Bulletin of the World Health Organisation* **38**, 69–78.

Jennings, E. R. and Clauss, B. (1978). Maternal-foetal haemorhage—its incidence and sensitising effects. *American Journal of Obstetrics and Gynaecology* **131**, 725–727.

Jones, B. S. (1950). Congenital malaria: a report of three cases. *British Medical Journal* **2**, 439–441.

Jones, E. and Curzen, P. (1973). The immunological reactivity of maternal lymphocytes in pregnancy. *Journal of Obstetrics and Gynaecology of the British Commonwealth* **80**, 608–610.

Jones, M. H., Sever, J. L., Baker, T. H., Hallatt, J. G., Goldenberg, E. D., Justus, K. M., Bonnet, C., Gilkeson, M. R. and Roberts, J. M. (1966). Toxoplasmosis, antibody level, and pregnancy outcome. *American Journal of Obstetrics and Gynaecology* **95**, 809–816.

Jones, M. H., Sever, J. L., Baker, T. H., Hallatt, J. H., Goldenberg, E. D., Justus, K. M. and Gilkeson, M. R. (1969) Toxoplasmosis and abortion. *American Journal of Obstetrics and Gynaecology* **104**, 919–920.

Kay, H. E. M. and Margoles, C. (1971). Chromosomes of human foetal lymphocytes: frequency of abnormalities and absence of maternal cells. *Lancet* **ii**, 733–735.

Keitel, H. G., Goodman, H. C., Havel, R. J. and Baxter, J. H. (1956). Nephrotic syndrome in congenital quartan malaria. *Journal of the American Medical Association* **161**, 520–523.

Kimball, A. C., Kean, B. H. and Fuchs, F. (1971). The rate of toxoplasmosis in abortion. *American Journal of Obstetrics and Gynaecology* **111**, 219–226.

Kimmig, P. (1979). Transplacental transmission of microfilariae of *Brugia pahangi* in the cat. *Zeitschrift für Parasitenkunde* **58**, 181–186.

Koutz, F. R., Groves, H. F. and Scothorn, M. W. (1966). The prenatal migration of *Toxocara canis* larvae and their relationship to infection in pregnant bitches and in pups. *American Journal of Veterinary Research* **27**, 789–795.

Krakowka, S. (1977). Transplacentally acquired microbial and parasitic diseases of dogs. *Journal of the American Veterinary Medical Association* **171**, 750–753.

Lancet (1973). Intrauterine infections: problems and prevention. *Lancet* **i**, 868–869.

Lancet (1980). Congenital toxoplasmosis. *Lancet* **i**, 578–579.

Langer, H. (1963). Repeated congenital infection with *Toxoplasma gondii*. *Obstetrics and Gynaecology* (*New York*) **21**, 318–329.

Lewert, R. M. and Mandolowitz, S. (1969). Schistosomiasis: Prenatal induction of tolerance to antigens. *Nature* (*London*) **224**, 1029–1030.

Lewis, E. A. and Anita, A. V. (1969). Amoebic colitis: review of 295 cases. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63**, 633–638.

Lichtenberg, F. V., Bawden, M. P. and Shealey, S. H. (1974). Origin of circulating antigen from the schistosome gut. An immunofluorescent study. *American Journal of Tropical Medicine and Hygiene* **23**, 1088–1091.

Logie, D. E. and McGregor, I. A. (1970). Acute malaria in newborn infants. *British Medical Journal* **3**, 404–405.

Loke, Y. W. (1978). "Immunology and Immunopathology of the Human Foetal-Maternal Interaction". Elsevier/North Holland, Amsterdam, 328 pp.

Luse, S. A. and Miller, L. H. (1971). Plasmodium falciparum malaria. Ultrastructure of parasitised erythrocytes in cardiac vessels. *American Journal of Tropical Medicine and Hygiene* **20**, 655–660.

Mackay, R. (1934). A note on congenital malaria. *Tropical Diseases Bulletin* **31**, 427.

Macris, N. T., Hellman, L. M. and Watson, R. J. (1958). Transmission of transfused sickle-trait cells from mother to foetus. *American Journal of Obstetrics and Gynaecology* **76** 1214–1218.

Madwar, M. A. and Voller, A. (1975). Circulating soluble antigens and antibody in schistosomiasis. *British Medical Journal* **1**, 435–436.

Manson-Bahr, P. (1960). Leishmaniasis. *In* "Manson's Tropical Diseases" 15th ed. p. 137. Cassell, London.

Mantovani, A. and Jackson, R. F. (1966). Transplacental transmission of microfilariae of *Dirofilaria immitis* in the dog. *Journal of Parasitology* **52**, 116.

MacGregor, J. D. and Avery, J. G. (1974). Malaria transmission and foetal growth. *British Medical Journal* **3**, 433–436.

McGregor, I. A. and Smith, D. A. (1952). A health, nutrition and parasitological survey in a rural village (Keneba) in West Kiang, Gambia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **46**, 403–427.

McGregor, I. A., Rowe, D. S., Wilson, M. E. and Billewicz, W. Z. (1970). Plasma immunoglobulin concentrations in an African (Gambian) community in relation to season, malaria and other infections and pregnancy. *Clinical and Experimental Immunology* 7 51–74.

McQuay, R. M., Silberman, S., Mudrik, P. and Keath, L. E. (1967). Congenital malaria in Chicago. A case report and a review of published reports (U.S.A.). *American Journal of Tropical Medicine and Hygiene* **16**, 258–266.

Mengert, V. F., Rights, C. S., Bates, C. R., Reid, A. F., Wolfe, C. R. and Nabors, G. C. (1955). Placental transmission of erythrocytes. *American Journal of Obstetrics and Gynaecology* **69**, 678–685.

Miller, L. H. (1969). Distribution of mature trophozoites and schizonts of Plasmodium falciparum in the organs of Aortus trivirgatus, the night monkey. *American Journal of Tropical Medicine and Hygiene* **18**, 860–865.

Miller, T. A. (1971). Vaccination against the canine hookworm disease. *Advances in Parasitology* **9**, 153–183.

Miller, M. J., Aronson, W. J. and Remington, J. S. (1969). Late parasitemia in asymptomatic acquired toxoplasmosis. *Annals of Internal Medicine* **71**, 139–145.

Mims, C. (1976). Comparative aspects of infective malformations. *British Medical Bulletin* **32**, 84–88.

Moriearty, P. L. and Lewert, R. M. (1974). Delayed hypersensitivity in Ugandan schistosomiasis. II. Epidemiologic patterns of intradermal responses. *American Journal of Tropical Medicine and Hygiene* **23**, 179–189.

Morley, D., Woodland, M. and Cuthbertson, W. F. J. (1964). Controlled trial of pyrimethamine in pregnant women in African village. *British Medical Journal* **1**, 667–668.

Moskalewski, S., Ptak, W. and Strzyzewska, J. (1974). Macrophages in mouse placenta: morphologic and functional identification. *Journal of the Reticuloendothelial Society* **16**, 9–14.

Myagkaya, G. and Vreeling-Sindelarova, H. (1976). Erythrophagocytosis of cells of the trophoblastic epithelium in the sheep placenta in different stages of gestation. *Acta Anatomica* **95**, 234–238.

Naeslund, J. (1951). Studies on placental permeability with radio-active isotopes of phosphorus and iron. *Acta obstetrica et Gynecologica Scadinavica* **30**, 231–246.

Naeslund, J. and Nyelin, G. (1946). Investigations of permeability of placenta with aid of red blood corpuscle tagged with radio-active phosphorous. *Acta Medica Scadinavica* **170**, 390–398.

Narabayashi, H. (1914). Beiträge zur Frage der kongenitalen Invasion von *Schistosoma japonicum*. Cited by Augustine (1927).

Nash, T. E. (1974). Localisation of the circulating antigen within the gut of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **23**, 1085–1087.

Nash, T. E., Prescott, B. and Neva, F. A. (1974). The characteristics of a circulating antigen in schistosomiasis. *Journal of Immunology* **112**, 1500–1507.

Noonan, F. P., Halliday, W. J., Morton, H. and Clunie, G. J. A. (1979). Early pregnancy factor is immunosuppressive. *Nature (London)* **278**, 649–651.

Ockleford, C. D. and Whyte, A. (1977). Differentiated regions of human placental cell surface associated with exchange of materials between maternal and foetal blood: coated vesicles. *Journal of Cell Science* **25**, 293–312.

Okafor, G. O., Uche, G. O., Emejuaiwe, S. O. and Marshall, W. C. (1978). Immunoglobulin levels in Nigerian cord blood sera. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **73**, 27–30.

Okeke, N. E. (1970). Acute malaria in newborn infants. *British Medical Journal* **3**, 108.

Olding, L. (1972). The possibility of materno-foetal transfer of lymphocytes in man. *Acta paediatrica Scandinavica* **61**, 73–75.

Olowe, S. A. (1975). A case of congenital trypanosomiasis in Lagos. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **69**, 57–59.

Oumachigui, A., Bhatia, V. N. and Nayak, P. N. (1980). *Toxoplasma* antibodies and foetal wastage. *Indian Journal of Medical Research* **71**, 522–525.

Pabst, M. H. van Lawick van (1950). Congenital malaria. *Tropical Diseases Bulletin* **47**, 199–200.

Pacsa, A. S. and Pejtsik, B. (1977). Impairment of immunity during pregnancy and antiviral effect of amniotic fluid. *Lancet* **i**, 330–331.

Palmer, T. T. (1978). Plasmodium berghei infection in pregnant rats: effects on antibody response and course of infection in offspring. *Journal of parasitology* **64**, 493–496.

Persellin, R. H. and Leibfarth, J. K. (1978). Studies of the effects of pregnancy serum on polymorphonuclear leucocyte functions. *Arthritis and Rheumatism* **21**, 316–325.

Persellin, R. H. and Thoi, L. L. (1979). Human polymorphonuclear leucocyte phagocytosis in pregnancy—development of inhibition during gestation and recovery in the postpartum period. *American Journal of Obstetrics and Gynaecology* **134**, 250–254.

Perves, M. (1947). Hereditary and congenital transmission of P. falciparum malaria: influence of the disease on the early months of life. *Tropical Diseases Bulletin* **44**, 277–278.

Peters, D. K. and Lachmann, P. J. (1974). Immunity deficiency in pathogenesis of glomerulonephritis. *Lancet* **i**, 58–60.

Phillips, T. and Draper, C. C. (1975). Circulating immune complexes in schistosomiasis due to *Schistosoma mansoni*. *British Medical Journal* **2**, 476–478.

Plotkin, S. A. (1975). Routes of fetal infection and mechanisms of fetal damage. *American Journal of Diseases of Children* **129**, 444–449.

Potter, J. F. and Schoeneman, M. (1970). Metastasis of maternal cancer to the placenta and fetus. *Cancer* **25**, 380–388.

Prior, J. A., Cole, C. R., Docton, F. L., Saslaw, S. and Chamberlain, D. M. (1953). Toxoplasmosis. IV. Report of three cases with particular reference to asymptomatic toxoplasma parasitaemia in a young woman. *Archives of medicine* **92**, 314–320.

Remington, J. S. (1970). Toxoplasmosis: recent developments. *Annual Review of Medicine* **21**, 201–218.

Remington, J. S. and Desmonts, G. (1973). Congenital toxoplasmosis: variability in the IgM fluorescent antibody response and some pitfalls in diagnosis. *Journal of Pediatrics* **83**, 27–30.

Remington, J. S., Melton, M. L. and Jacobs, L. (1960). Chronic toxoplasma infection in the uterus. *Journal of Laboratory and Clinical Medicine* **56**, 879–883.

Remington, J. S., Jacobs, L. and Melton, M. L. (1961a). Congenital transmission of toxoplasmosis from mother animals with acute and chronic infections. *Journal of Infectious Diseases* **108**, 163–173.

Remington, J. S., Melton, M. L. and Jacobs, L. (1961b). Induced and spontaneous recurrent parasitaemia in chronic infections with avirulent strains of *Toxoplasma gondii*. *Journal of Immunology* **87**, 578–581.

Rhodes, K., Markham, R. L., Maxwell, P. M. and Monk-Jones, M. E. (1969). Immunoglobulins and the X-chromosome. *British Medical Journal* **3**, 439–441.

Rivera, R. A. (1972). Fatal postpartum amoebic colitis with trophozoites present in peritoneal fluid. *Gastroenterology* **62**, 314–317.

Ruoss, C. F. and Bourne, G. L. (1972). Toxoplasmosis in pregnancy. *Journal of Obstetrics and Gynaecology of the British Commonwealth* **79**, 1115–1118.

Sabin, A. B., Eichenwald, H., Feldman, H. and Jacobs, L. (1952). Present status of clinical manifestations of toxoplasmosis in man. Indications and provisions for routine serologic diagnosis. *Journal of American Medical Association* **150**, 1063–1069.

Sanger, V. L. and Cole, C. R. (1955). Toxoplasmosis. VI. Isolation of toxoplasma from milk, placentas, and newborn pigs of asymptomatic carrier sows. *American Journal of Veterinary Research* **16**, 536–539.

Santoro, F., Carlier, Y., Borojevic, R., Bout, D., Tachon, P. and Capron, A. (1977a) Parasite "M" antigen in milk from mothers infected with *Schistosoma mansoni*. *Annals of Tropical Medicine and Parasitology* **71**, 121–123.

Santoro, F., Borojevic, R., Bout, D., Tachon, P., Bina, J. C. and Capron, A. (1977b). Mother–child relationship in human schistosomiasis mansoni: I. Parasitic antigens and antibodies in milk. *American Journal of Tropical Medicine and Hygiene* **26**, 1164–1168.

Santos-Buch, C. A. (1979). American trypanosomiasis: Chagas' Disease. *In* "International Review of Experimental Pathology" (G. W. Richter and M. A. Epstein, eds). Academic Press, New York, London.

Saxon, S. A., Knight, W., Reynolds, D. W., Stagno, S. and Alford, C. A. (1973). Intellectual deficits in children born with subclinical congenital toxoplasmosis: a preliminary report. *Journal of Pediatrics* **82**, 792–797.

Schlegel, R. J. and Bellanti, J. A. (1969). Increased susceptibility of males to infection. *Lancet* **ii**, 826–827.

Schröder, J. (1974). Passage of leucocytes from mother to fetus. *Scandinavian Journal of Immunology* **3**, 369–373.

Schwartz, J. Kehaty, T. and Foldes, J. J. (1974). Phagocytosis of foetal erythrocytes by trophoblast cells. *IRCS Library Compendium* **2**, 1002.

Schwetz, J. and Peel, M. (1934). Congenital malaria and placental infections among the negroes of Central Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **28,** 167–174.

Scothorn, M. W., Koutz, F. R. and Groves, H. F. (1965). Prenatal *Toxocara canis* infection in pups. *Journal of the American Veterinary Association* **146,** 45–48.

Senelar, R. and Bureau, J. P. (1979). Inhibitory effect of pregnancy on the migration of the inflammatory cells: A quantitative histological study. *British Journal of Experimental Pathology* **60,** 286–293.

Sharf, M., Eibschitz, I. and Eylan, E. (1973). Latent toxoplasmosis and pregnancy. *Obstetrics and Gynaecology (New York)* **42,** 349–354.

Shillinger, J. E. and Cram, E. B. (1923). Parasitic infestation of dogs before birth. *Journal of the American Veterinary Medical Association* **16,** 200–203.

Smith, K., Duhring, J. L., Greene, J. W., Rochlin, D. B. and Blakemore, W. S. (1961). Transfer of maternal erythrocytes across the human placenta. *Obstetrics and Gynaecology (New York)* **18,** 673–676.

Smithers, S. R. and Terry, R. J. (1969). The immunology of schistosomiasis. *Advances in Parasitology* **7,** 41–93.

Solomon, J. B. (1971). "Foetal and Neonatal Immunology." Elsevier/North Holland, Amsterdam.

Spitz, A. J. (1959). Malaria infection of the placenta and its influence on the incidence of prematurity in Eastern Nigeria. *Bulletin of the World Health Organisation* **21,** 242–244.

Stagno, S. (1980). Congenital toxoplasmosis. *American Journal of Diseases of Children* **134,** 635–637.

Stern, H., Elek, S. D., Booth, J. C. and Fleck, D. G. (1969). Microbial causes of mental retardation. The role of prenatal infections with cytomegalovirus, rubella virus, and toxoplasma. *Lancet* **ii,** 443–448.

Steven, D. (1975). Anatomy of the placental barrier. *In* "Comparative Placentation. Essays in Structure and Function" (D. H. Steven, ed.), pp. 25–57. Academic Press, London.

Stevenson, R. E. (1977). "The fetus and newly born infant. Influences of the prenatal environment," 2nd edn. pp. 319–333. C. V. Mosby, St. Louis.

Stray-Pedersen, B. (1980). Infants potentially at risk for congenital toxoplasmosis—a prospective study. *American Journal of Diseases of Children* **134,** 638–642.

Szarfman, A., Cossio, P. M., Arana, R. M., Urman, J., Kreutzer, E., Laguens, R. P., Segal, A. Coarasa, L. (1975). Immunologic and immunopathologic studies in congenital Chagas' disease. *Clinical Immunology and Immunopathology* **4,** 489–499.

Tachon, P. and Borojevic, R. (1978). Mother-child relation in human schistosomiasis mansoni: skin test and cord blood sensitivity to schistosomal antigens. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **72,** 605–609.

Taufa, T. (1978). Malaria and pregnancy. *Papua New Guinea Medical Journal* **21,** 197–206.

Thalhammer, O. (1962). Congenital toxoplasmosis. *Lancet* **i,** 23–24.

Thomas, V. and Chan, W. C. (1980). A case of congenital malaria in Malaysia with IgM malaria antibodies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74,** 73–76.

Thompson, D., Pegelow, C. Underman, A. and Powars, D. (1977). Congenital malaria: a rare cause of splenomegaly and anaemia in an American infant. *Pediatrics* **60,** 200–212.

Thong, Y. H., Steele, R. W., Monroe, M. V., Hensen, S. A. and Bellanti, J. A. (1973). Impaired *in-vitro* cell-mediated immunity to rubella virus during pregnancy. *New England Journal of Medicine* **289**, 604–606.

Turner, J. H., Wald, H. and Quinlivan, W. (1966). Cytogenetic evidence concerning possible transplacental transfer of leucocytes in pregnant women. *American Journal of Obstetrics and Gynaecology* **95**, 831–833.

Vitums, V. C. and Sites, J. G. (1968). Leukaemia in pregnancy. Data on 9 cases with important new findings. *Medical Annals of the District of Columbia* **37**, 588–593.

Warren, K. S. (1972). The immunopathogenesis of schistosomiasis: a multidisciplinary approach. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **66**, 417–434.

Washburn, T. C., Medearis, D. N. Jr. and Childs, B. (1965). Sex differences in susceptibility to infections. *Pediatrics* **35**, 57–64.

Webster, G. A. (1958). On prenatal infection and the migration of *Toxocara canis*, Werner, 1972 in dogs. *Canadian Journal of Zoology* **36**, 435–440.

Weinman, D. (1952). *Toxoplasma* and toxoplasmosis. *Annual Review of Microbiology* **6**, 281–298.

Weinman, D. (1960). *Toxoplasma* and abortion: a field for further investigation. *Fertility and Sterility* **11**, 525–530.

Werner, H. (1956). Question of placental transmission of Plasmodium berghei (congenital malaria). *Zeitscrift für Tropenmedizin und Parasitologie* **7**, 64–79.

Werner, H. (1962). Experimentelles Beitrag zur konnatalen Toxoplasmose. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* **186**, 391–402.

Werner, J., Schmidtke, L. and Thomascheck, G. (1963). Toxoplasma infection and pregnancy: histological demonstration of the intrauterine route of infection. *Tropical Diseases Bulletin* **60**, 787–788.

Wickbom, B. and Winberg, J. (1972). Coincidence of congenital toxoplasmosis and acute nephritis with nephrotic syndrome. *Acta Paediatrica Scandinavica* **61**, 470–472.

Wilson, C. B. and Remington, J. S. (1980). What can be done to prevent congenital toxoplasmosis. *American Journal of Obstetrics and Gynaecology* **138**, 357–363.

Wilson, C. B., Desmonts, G., Couvreur, J. and Remington, J. S. (1980). Lymphocyte transformation in the diagnosis of congenital toxoplasma infection. *New England Journal of Medicine* **302**, 785–788.

Wislocki, G. B. (1921). Observations upon the behaviour of carbon granules injected into pregnant animals. *Anatomical Record* **21**, 29–33.

Wislocki, G. B. (1930). Observations on the placenta from a case of malaria. *Bulletin of the Johns Hopkins Hospital* **47**, 157–161.

Wolf, A., Cowen, D. and Paige, B. H. (1939a). Toxoplasmic encephalomyelitis. III. A new case of granulomatous encephalomyelitis due to a protozoan. *American Journal of Pathology* **15**, 657–694.

Wolf, A., Cowen, D. and Paige, B. H. (1939b). Human toxoplasmosis; occurrence in infants as encephalomyelitis: verification by transmission to animals. *Science* **89**, 226–227.

Wood, G. W. (1980). Mononuclear phagocytes in the human placenta. *Placenta* **1**, 113–123.

Woods, W. C., Mills, E. and Ferrieri, P. (1974). Neonatal malaria due to *Plasmodium vivax*. *Journal of Pediatrics* **85**, 669–671.

Work, K., Eriksen, L., Fennestad, K. L., Moller, T. and Siim, J. C. (1970). Experimental toxoplasmosis in pregnant sows. I. Clinical, parasitological and serological observations. *Acta pathologica et microbiologica Scandinavica* **78**, 129–139.

Wright, I. (1972). Transmission of *Toxoplasma gondii* across the guinea-pig placenta. *Laboratory Animals* **6**, 169–180.

Yutuc, L. M. (1949). Prenatal infection of dogs with Ascarids, *Toxocara canis* and hookworms, *Anacylostoma caninum*. *Journal of Parasitology* **35**, 358–360.

Zarou, D. M., Lichtman, H. C. and Hillman, L. M. (1964). The transmission of chromium-51 tagged maternal erythrocytes from mother to foetus. *American Journal of Obstetrics and Gynaecology* **88**, 565–571.

Zimmerman, L. E. (1970). Changing concepts concerning the pathogenesis of infectious diseases. *American Journal of Ophthalmology* **69**, 947–964.

Hydatidosis/Cysticercosis: Immune Mechanisms and Immunization Against Infection

M. D. RICKARD

University of Melbourne, Veterinary Clinical Centre, Princes Highway, Werribee, Victoria, 3030, Australia

AND J. F. WILLIAMS

Department of Microbiology and Public Health, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824, U.S.A.

I. Introduction

The hydatidosis/cysticercosis disease complex is caused by infection with the larval stages of tapeworms belonging to the family Taeniidae. It is a continuing threat to the well-being of people in many countries through its effect on human health and the food animal industries. *Echinococcus* spp. which use canids as definitive hosts and a wide range of animals as intermediate hosts, are the aetiological agents of hydatidosis in humans. Man is the obligatory definitive host of *Taenia saginata* and *T. solium* with, respectively, cattle and pigs acting as intermediate hosts. Man can also act as intermediate host of *T. solium*, sometimes with severe consequences to health from neurocysticercosis. There is an urgent need for improvements in preventive medical approaches to these infections, and in the clinical management of the immunological complications of the disease processes they cause, because, despite the measures currently available for control of larval taeniids, there has been little evidence of a decline in their prevalence (Pawlowski and Schultz, 1972; Gemmell and Johnstone, 1977; Matossian *et al.*, 1977). The fundamental obstacle in applying existing control methods is that they rely upon altering complex patterns of social behaviour which are based on attitudes toward feeding pets, dietary preferences regarding meat, personal standards of hygiene, etc. There are enormous difficulties in changing these behavioural characteristics even in highly literate societies, and as a result conventional programmes have been protracted and correspondingly expensive.

There are several common taeniids that have their larval phase in sheep (*T. hydatigena* and *T. ovis*) and laboratory rodents (*T. pisiformis*, *T. taeniaeformis* and *T. crassiceps*) and these have been extensively used for research in laboratory and field situations. The recognition that immunity in the intermediate host plays a central role in determining the natural patterns of transmission of taeniid parasites (Gemmell, 1976, 1978) has stimulated a surge of interest directed towards defining the immune mechanisms and developing procedures for immunization. In this review we have focussed upon the implications of new knowledge concerning innate and acquired resistance to taeniid cestodes for the development of immunoprophylactic methods and for enhanced understanding of the pathogenesis of larval tapeworm diseases. Characterization of innate resistance is essential for the evaluation of research on acquired immunity, and may, in the long term, lead to methods for the genetic selection of resistant animals. Knowledge of the immune mechanisms involved should help to develop optimal strategies for applying vaccination procedures, and perhaps also to deal with the clinicopathological effects of infection with these parasites in man. Established metacestodes have highly effective means of evading the aggressive immune

response of the host, and by identifying these we may eventually be able to manipulate the immune system to effect destruction of the parasite. This could be of benefit in both disease control and in the alleviation or prevention of clinical signs of infection.

Emphasis throughout this review is placed on larval cestodes belonging to the family Taeniidae, but reference is made to observations on other cestode parasites where information is lacking on taeniids, or where these findings are seen as being particularly relevant or supportive. Although there have been significant recent advances in the immunological diagnosis of the cysticercoses, and especially in hydatid disease of man, they form a separate topic, and are not dealt with here.

II. Innate Resistance to Infection

Factors determining the innate resistance to infection with larval taeniid cestodes have not been studied to an extent commensurate with their significance. Not only do they play an important part in determining the epidemiology of natural infection with larval cestodes, but they have a central role in the critical evaluation and interpretation of research data concerning immunity to these parasites. Many variables which influence innate resistance have been identified including host factors such as strain, age, sex, health, as well as differences in infectivity between "strains" of parasites. This section reviews available evidence concerning variations in innate resistance to infection with special emphasis on immunologically based mechanisms.

A. Detection and Measurement

A fundamental problem in interpreting data relating to innate resistance is the variation in the types of measurement used to assess how infection differs between groups of animals. In a recent publication, Williams *et al.* (1981) have highlighted some of these issues. The most commonly used measure of suitability of a particular host has been the number of parasites established from a primary infection in animals examined at a given time after exposure. However, this kind of measurement takes no account of more subtle influences that can affect the net receptiveness of hosts of a specific strain, age or sex. Detailed consideration has to be given not only to the establishment of cestode larvae but also to their growth rate, long-term survival and ultimate infectivity for the definitive host. The physiological state of the host can markedly influence the outcome of an experiment. For instance, Williams *et al.* (1981) found that inbred lines of Wistar rats obtained from separate commercial sources showed apparent differences in susceptibility to the

establishment of infection with *T. taeniaeformis*. However, the more susceptible rats had been severely stressed during transport to the laboratory, and when a further group of rats from the same supplier was tested there was no difference between the two inbred lines.

Variation in parasite material used could also play a significant part in yielding misleading or conflicting results. Batches of eggs differ in their infectivity and widely different doses of eggs have been used by various workers. Mitchell *et al.* (1980), in their experiments on mouse strain variation in resistance to *T. taeniaeformis*, showed a clear interaction between egg dose and success of infection. They suggested that a more vigorous host response induced by a large dose of antigen militated against survival of larvae in the innately more reactive host. Similar reasoning could be used to predict that when egg batches of poor viability are administered at high doses because of their low infectivity, the immune response to less infective oncospheres may prejudice the survival of the remaining larvae.

Differences in the source of the parasites can also be of great importance. There are numerous reports concerning intra-specific variation in host infection range amongst larval cestodes. Heath and Elsdon-Dew (1972) and Ambu and Kwa (1980) commented that their respective "strains" of *T. taeniaeformis*, which had originally been isolated from rats, were infective for rats but not mice, and Williams *et al.* (1981) were unable to infect a variety of laboratory animal species, including rats and mice, with *T. taeniaeformis* obtained from cats in their locality. Olivier (1962a) infected groups of mice with eggs collected from various proglottides of worms from each of two separate cats and found wide variations in the number of cysticerci in the livers of groups given either eggs from different proglottides or from different cats.

Clearly, experiments concerning innate resistance require extreme care in standardization of host and parasite, and demand the use of large numbers of healthy, uniform animals (Williams *et al.*, 1981).

B. AGE EFFECTS

Most reports of age effects on resistance to a primary infection with eggs of taeniid cestodes have concerned *T. taeniaeformis* and *T. pisiformis* in laboratory rodents. Several authors have noted a marked increase in resistance to *T. taeniaeformis* infection in older rats (Bullock and Curtis, 1920; Curtis *et al.*, 1933; Greenfield, 1942; Rohde, 1960). The observations of Curtis *et al.* (1933) were based only on the proportion of animals infected, but Greenfield (1942), using Sherman rats, and eggs from a single proglottid, examined both quantitative and qualitative aspects of age resistance. She demonstrated convincingly that rats less than 25 and older than 60 days of age were much

less susceptible to infection. Musoke *et al.* (1975) found that young Sprague–Dawley rats did not become susceptible to infection until their third week of life. Heath and Elsdon-Dew (1972), on the other hand, could not demonstrate any increased resistance in Wistar Albino rats up to 12 months of age. These workers assessed infection only at 30 days after exposure, but nevertheless they were unable to detect any differences in either the number or survival rate of cysticerci. Ambu and Kwa (1980) also failed to demonstrate age resistance to *T. taeniaeformis* in three strains of rats, but their observation was based exclusively on numbers of parasites present at 4 weeks post-infection, and the degree of larval development in the various strains was not taken into account.

There have been reports of increased resistance of older mice to infection with *T. taeniaeformis* (Dow and Jarrett, 1960; Turner and McKeever, 1976; Mitchell *et al.*, 1977a). Dow and Jarrett (1960) examined age resistance in three strains of mice and found that in the innately more susceptible Porton strain there was no increase in resistance between 14 and 42 days of age, whereas in "A" strain or White Swiss mice, resistance became evident earlier.

Detailed information is lacking concerning age resistance to *T. pisiformis* infection in rabbits but an effect of age has been reported (Silverman, 1956; Potseleuva, 1958; Heath, 1971). In domesticated ruminants, the evidence for age resistance is conflicting. In *T. saginata* infection of cattle, some workers have shown increasing resistance with age, whereas others consider age resistance to be unimportant (Penfold, 1937; Peel, 1953; Froyd and Round, 1960; Urquhart, 1961; Vegors and Lucker, 1971). All these reports must be interpreted with caution because field-reared cattle were used in the experiments. In two instances where cattle may have been free from prior exposure (Penfold, 1937; Urquhart, 1961) absolute resistance did not develop with age, but older animals appeared to have an increased proportion of degenerated cysticerci (Penfold, 1937). Infection of field-reared cattle with other parasites, including cestodes such as *T. hydatigena*, cannot be excluded as a source of antigen which could stimulate a level of cross-immunity and yield spurious results. Vegors and Lucker (1971) could find no evidence of age immunity in their experiments with *T. saginata*, but only total numbers of cysticerci were counted. In experiments with outbred animals such as rabbits or cattle the variations in 'take' between individuals are large, which makes the results from small groups of animals difficult to evaluate. No information is available concerning differences in susceptibility between various breeds of domesticated animals.

It has often been supposed that hydatid infection of man is acquired more frequently during childhood. However, Beard (1978) examined data on the incidence of hydatid disease in man during 10 years of the Tasmanian

Control Programme and concluded that adults are relatively susceptible and that the latent period between infection and diagnosis in many cases is only a few years. On the other hand, age resistance has been recorded in secondary echinococcosis of mice due to *E. granulosus* (Schwabe *et al.*, 1959) and *E. multilocularis* (Kamiya, 1972).

C. SEX EFFECTS

There are conflicting reports on differences in the susceptibility of males and females. Curtis *et al.* (1933) and Campbell and Melcher (1940) concluded that male rats were more susceptible than females to infection with *T. taeniaeformis*, but other results have been more equivocal. Some workers have concluded that sex is not important in natural resistance, but examination of their data suggests that, although numbers of cysts have not been affected, adverse effects on the degree of cyst development have been more pronounced in females than in males (Greenfield, 1942; Olivier, 1962a). There appears to be an interaction between sex and strain of mouse. Thus, Dow and Jarrett (1960) found sex differences in only one of three mouse strains infected with *T. taeniaeformis*, and Yamashita *et al.* (1963) reported sex differences in the susceptibility of several strains of mice to infection with *E. multilocularis*.

Mitchell *et al.* (1977a) showed that in the moderately susceptible CBA/H strain of mouse, both the number and degree of development of cysts were less in females than males, but that in the relatively resistant BALB/c strain of mouse differences between males and females were less pronounced. Ambu and Kwa (1980) concluded that sex had no influence on infection with *T. taeniaeformis* in rats but all three of the strains they studied seemed highly susceptible to infection, and no data were given on the viability of the cysts. Bursey (1977) concluded that there was an interaction between sex and age resistance to *T. taeniaeformis* infection in rats; in young rats, males and females appeared equally susceptible to infection, but more degenerated cysticerci were found in older females than in males of the same age.

Male cattle may be more susceptible to naturally acquired infection with *T. saginata* than are females (Ginsberg *et al.*, 1956; Froyd, 1960). Rickard *et al.* (1982) described an experiment in which male cattle that became infected with *T. saginata* by grazing on sewage-irrigated pasture showed a higher rate of infection than females but the difference was not statistically significant. In parallel groups of animals, each of which had been artificially immunized against infection, the differences between males and females became significant.

Some experiments have shown that females are more easily infected. Thus, Esch (1967) found that the infection rate in female mice was higher than in males following oral dosing with *T. multiceps* eggs. Female mice are also

more susceptible to oral infection with eggs of *T. crassiceps* (Freeman, 1962), and intraperitoneally inoculated cysts of this parasite develop more rapidly in females than in males (Chernin, 1975).

D. HOST STRAIN EFFECTS

As could be anticipated, documentation concerning strain variation in resistance to larval taeniids is limited to infections in laboratory rats and mice where various inbred and outbred lines are readily available. There is compelling evidence that variation in strain resistance is an important phenomenon, e.g. *T. taeniaeformis* in rats (Curtis *et al.*, 1933; Olivier, 1962a; Williams *et al.*, 1981); *T. taeniaeformis* in mice (Dow and Jarrett, 1960; Olivier, 1962a; Orihara, 1962; Mitchell *et al.*, 1977a, 1980); and *E. multilocularis* in mice (Yamashita *et al.*, 1958; Lubinsky, 1964). Pennoit-De Cooman and De Rycke (1970) obtained equivocal results concerning strain effects in a study of secondary echinococcosis due to *E. granulosus* in three outbred strains of mice. Although some experiments on strain variation have only involved observations on numbers of cysts present in the animals at a single time after exposure, or on the proportion of animals infected, other workers have presented evidence to show that the survival of larvae that become established also varies from strain to strain (Yamashita *et al.*, 1958; Lubinsky, 1964; Olivier, 1962a; Orihara, 1962; Mitchell *et al.*, 1977a; Williams *et al.*, 1981). In recent experiments Williams *et al.* (1981) investigated the establishment and growth characteristics of *T. taeniaeformis* in different strains of rats and in similar strains obtained from different suppliers. They plotted growth curves for *T. taeniaeformis* larvae from 3 weeks after infection using mean dry weight of larvae dissected from the liver as the index of development. Contrary to the results described by Curtis *et al.* (1933), they found that none of the inbred strains of rats was completely refractory to infection. *T. taeniaeformis* larvae in outbred Sprague–Dawley rats from two different commercial sources had similar growth characteristics, but rats of this type from a third supplier were much more resistant; parasite growth was stunted and there was an increased number of larval deaths later in infection. These experiments emphasize the difficulty in comparing data from experiments on outbred strains of animals from different environments. This must apply particularly to experiments carried out on domesticated animals where there are almost certain to be variations both between breeds and between individuals within a breed.

E. MECHANISMS OF INNATE RESISTANCE

There are probably many physiological and anatomical factors which account for variations in innate resistance of hosts to cestode larvae. For

instance, Musoke *et al.* (1975) suggested that the insusceptibility of young rats (less than 2–3 weeks old) to infection with *T. taeniaeformis* could be due to their having little or no proteolytic enzyme activity in their intestine to assist in hatching of eggs. It has been proposed that the hatching of taeniid eggs (Fig. 1a) may differ between hosts of differing susceptibility (Weinmann, 1970), but there is a lack of data concerning variations within a single host species. The composition of host bile has been shown to have a marked influence on the survival of *E. granulosus* protoscolices (Smyth and Haselwood, 1963).

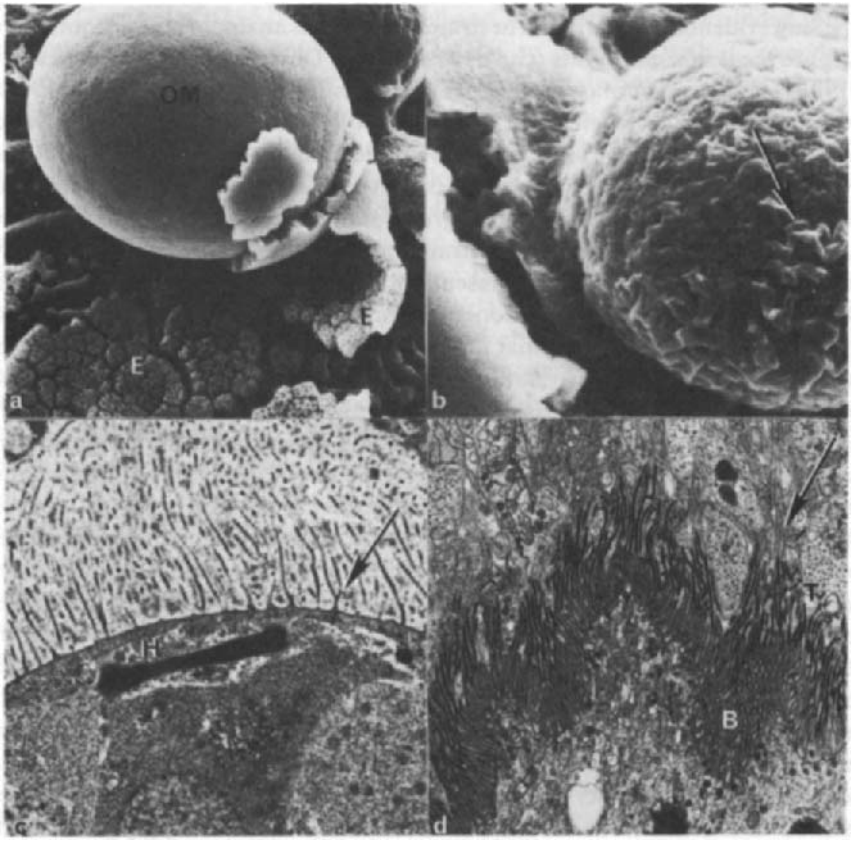

FIG. 1. Scanning and transmission electron micrographs of the surface of early developmental stages of taeniid larvae. (a) Hatching egg of *T. ovis*; magnification ×2800. (b) Activated oncosphere of *T. ovis* showing surface folds in the plasma membrane (arrow). Magnification ×3500. (c) Post oncospheral stage of *T. taeniaeformis* in rat liver 2 days post infection. At this stage the surface has become covered with branching microvilli (arrow). Magnification ×5000. (d) Larva of *T. taeniaeformis* in rat liver 9 days post infection. By this time microvilli have been replaced by microtriches with a base, a whiplike tip and a tenuous streamer-like extension (arrow). Magnification ×9700. Abbreviations: E, embryophore; OM, oncospheral membrane; H, oncospheral hook; B, bases of microtriches; T, tips of microtriches. (a and b were kindly provided by Dr. C. Bursey, and c and d by Dr. P. Engelkirk.)

There are many stages during an infection where natural barriers could influence the outcome. The greater susceptibility of unhealthy animals (Potseleuva, 1958; Williams *et al.*, 1981) may be due in part to altered host physiology, as suggested by Weinmann (1967). Experiments have shown that a high proportion of cestode oncospheres fail to penetrate the gut of innately resistant animals. For example, Heath (1971) observed that oncospheres of *T. pisiformis* did not penetrate the gut of older rabbits. Turner and McKeever (1976) examined the fate of *T. taeniaeformis* oncospheres in the naturally resistant White Swiss Strain of mouse and found that, while there was little difference in the rate of egg hatching in mice of various ages, the numbers of oncospheres penetrating the gut were fewer in older animals. Many of the oncospheres found in the distal half of the intestine appeared to be undergoing digestion, but enhancement of cellular reactivity in the intestinal wall itself was not apparent.

Non-specific cellular events may play a significant part in determining the success or otherwise of infection in an animal of a given age, sex or strain. As discussed more fully later (see Section III), the successful development of cestode larvae is dependent, in the very early stages, on the outcome of a race between the defence mechanisms of the host and the parasite. Turner and McKeever (1976) observed a greatly accelerated cellular response in the livers of older White Swiss mice infected with *T. taeniaeformis*. In their study, a difference in larval development between young and old mice was evident as early as 48 hours post-infection and marked leucotaxis was shown in the older group of mice. This seems too rapid to be an effect of acquired immunity mechanisms but there is no evidence thus far to indicate how normal cells might exert an effect on developing larvae. Furukawa (1974) showed that even though spleen cells from normal mice adhered to *Hymenolepis nana* oncospheres *in vitro*, there was no apparent damage to the organism after 18 hours.

Several workers have recently shown that immunization of animals with BCG has a profound influence on infection with larval cestodes. Thus, Rau and Tanner (1975) and Reuben *et al.* (1978) demonstrated that injection of BCG stimulated almost complete protection of cotton rats against the intraperitoneal inoculation of *E. multilocularis* protoscolices, and Thompson (1976) achieved similar results with *E. granulosus* protoscolices in gerbils. Recently, Thompson *et al.* (1982) showed that BCG influenced the outcome of egg-induced infection with *T. taeniaeformis* in mice. *T. taeniaeformis* larvae in the livers of BCG immunized mice were counted 60 days after infection and both the numbers of live larvae and the total numbers of larvae were significantly less in treated than in control mice, and the effect was, to some extent, dose dependent. However, from the data they obtained, no conclusion can be drawn as to whether oncospheres or early developmental stages were affected, because at 60 days post-infection, lesions produced by death of very young larvae could have resolved.

The effect of BCG may well be the result of enhanced reactivity of non-specific defence mechanisms in the host, e.g. macrophages, although one cannot exclude the possibility of a more rapid specific immune response, e.g. antibody in BCG-injected animals. It would be interesting to compare the efficacy of BCG in strains of mice with known differences in resistance to *T. taeniaeformis* infection and to examine the cellular and antibody responses in these animals. Variations in the innate resistance of animals of varying age, strain and sex may also reflect differences in the responsiveness of their non-specific cellular defence mechanisms; it is known that hormone status and age are closely associated with the preparedness of the body's defence mechanisms (Ingram and Smith, 1965; Solomon, 1969). Olivier (1962b) showed that injections of cortisone 12 days before infection of mice refractory to infection with *T. taeniaeformis* rendered them highly susceptible. He suggested that this effect could be mediated through reduced antibody production in the cortisone treated animals, but it could also have been partly due to the non-specific anti-inflammatory action of this drug.

Specific acquired immunity to invading cestode oncospheres has been shown to be mediated largely by complement fixing antibodies (Musoke and Williams, 1975a, b; Mitchell *et al.*, 1977a). If complement activity in animals were under genetic control, then variations in complement function could play a part in variations in strain resistance. Mitchell *et al.* (1977a) reported that the inbred mouse strains that were most susceptible to *T. taeniaeformis* infection (CBA/H, C3H/He, AKR/J, A/J) were also those known to be deficient in C5 and/or C4. However, they pointed out that there was insufficient genetic evidence to implicate only the loci controlling C5 and C4 levels in resistance, and that multiple genetic factors were likely to be responsible.

There is some evidence to implicate specific immunological responses in innate resistance to infection. Mitchell *et al.* (1980) collected serum from different inbred strains of mice at various times after infection with *T. taeniaeformis* and tested its ability to passively protect BALB/c *nu/nu* mice against infection. The results showed that the antibody response in the more susceptible strain of mice (C3H/He) was slower than that of the more innately resistant strain (C57B1/6). The rate of antibody production could be crucial in the race between host resistance to the parasite and parasite resistance to the host. These authors also studied egg-dose responses in the various mouse strains, and found that in the relatively resistant BALB/c mice, high doses of eggs yielded less parasites than small doses. They suggested that high antigen doses from large numbers of eggs may lead to an accelerated immune response in the more resistant host. It is interesting that in an unrelated parasite/host system, Ito (1980, in press a, b) has also found evidence to suggest that differences in susceptibility between *dd* (susceptible) and BALB/c (resistant)

mice to *H. nana* infection may reflect the rate of onset of immunological responses to the parasite; the more rapid response of BALB/c mice to infection with cysticercoids results in failure of auto-infection whereas this occurs readily in *dd* mice.

Attempted genetic analysis of resistance using backcross mice (Mitchell *et al.*, 1980) was complicated by wide variations between experiments, probably due to lack of standardized parasite material. It was concluded (Mitchell *et al.*, 1977a) that resistance in mice is under polygenic control and is dominant. Further experiments aimed at characterizing the genetic control of resistance to larval cestodes should be carried out.

Whatever the causes of variations in innate resistance to infection, it is obvious that much more research emphasis must be given to this phenomenon because it creates major difficulties in interpretation and comparison of research data. There may also be important practical implications. Recently, Rickard *et al.* (1981b) studied cross-immunity between *T. hydatigena* or *T. pisiformis*, and *T. taeniaeformis* in strains of inbred mice with different innate resistance to infection. They found that there was an interaction between mouse strain and cross-immunity such that the more susceptible strains were more difficult to immunize. This was attributed to a combination of the different rates of antibody response in the various mouse strains together with the production of antibody of lesser avidity for *T. taeniaeformis* antigens being stimulated by the *T. hydatigena* and *T. pisiformis* oncospheres. They suggested that cross-immunity may not be useful as a practical procedure in outbred animals because many individuals, or breeds of animals, may be difficult to protect against infection by this means. Immunity produced by the homologous parasite is more effective, and Mitchell *et al.* (1980) concluded that all strains of mice could be effectively immunized against *T. taeniaeformis* infection. Even so, immunization experiments with outbred animals are notorious for the extreme variability in the numbers of parasites recovered from individuals, and there are many instances in the literature of one or two immunized animals in a group having many more cysticerci than the others. This may pose problems in the practical application of vaccination for the control of larval cestodes, even when homologous antigen preparations are used.

Genetic variation in the host is an important component in many parasitic infections (Wakelin, 1976) and some efforts have been directed recently toward the genetic selection of resistant, or responder, animals (e.g. Windon *et al.*, 1980). This kind of research is tedious and difficult, but important, and none has yet been reported with respect to larval cestode infections.

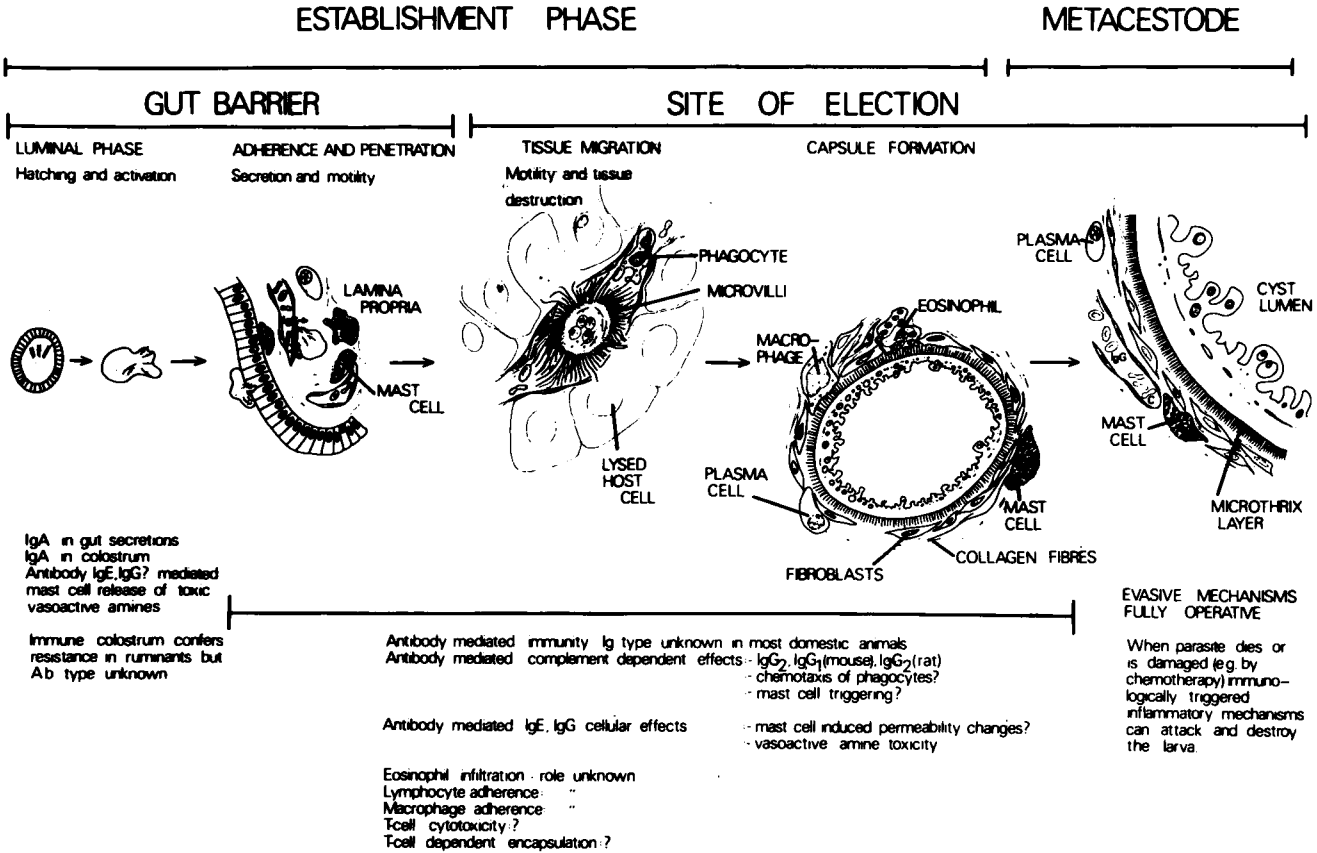

FIG. 2. Diagrammatic illustration of known and postulated immune mechanisms that may operate against larval taeniid cestodes in the intermediate host.

III. Acquired Immunity to Infection

The intermediate hosts of all larval taeniid cestodes thus far examined manifest an impressive level of acquired immunity to reinfection. Evidence for this has been detailed in several reviews of both the early literature (Gemmell and Soulsby, 1968; Gemmell and McNamara, 1972; Gemmell, 1976; Gemmell and Johnstone, 1977) and more recent findings (Flisser *et al.*, 1979; Williams, 1979). What follows is an attempt to synthesize both early and current concepts concerning immunity to larval taeniids into a cohesive picture of the events which may take place in the immune host (Fig. 2), and to identify areas where further research is needed.

We shall consider immunological events in two broad categories: first, immunity directed against the estabishment phase of infection, and second, immune mechanisms that prejudice the survival of established larvae. There has been an unforunate tendency in the literature to apply the terms "early" and "late", or "pre-encystment" and "post-encystment", immunity, first coined by Campbell (1938) in his work on *T. taeniaeformis* infection in rats, to these two phases of immunity. However, an important distinction needs to be made because, in effect, Campbell (1938) did not use the term post-encystment immunity to refer to destruction of fully established metacestodes. On the contrary, all of his observations were made during the establishment phase of infection, and by post-encystment or late immunity he referred to the macroscopically visible degeneration of young post-oncospheral stages. Degeneration of these parasites may very well have resulted from damage inflicted by the host at any point early in development.

A. Immunity to the Establishment Phase of Infection

Experiments to determine the mechanisms of immunity *in vivo* often present considerable difficulties in interpretation. When antibody-mediated immunity is very effective, it is easier to design experiments to show the participation of antibody in resistance than to establish a role for cell-mediated effector mechanisms. Perhaps when *in vitro* culture systems for cestode larvae are sufficiently developed, and substantial progress has been made toward this end (Heath and Smyth, 1970; Heath, 1973c; Heath and Lawrence, 1976; Lawrence *et al.*, 1980), it may be possible to examine various effector systems in isolation or in combination with each other under controlled conditions. This section of the review examines the role of resistance mechanisms which are known to operate in other host–parasite relationships in relation to immunity to larval taeniids; those effector systems which have been shown to be important in other helminthiases, and may affect taeniids, are also discussed.

1. *Antibody*

There is an extensive list of references which attest to a central role for antibody in acquired immunity against the establishment of larval taeniids. Passive transfer of immunity with serum from infected animals has been reported in virtually all laboratory and domesticated animal host–parasite systems, and natural passive protection of neonatal animals via colostral antibody has also been shown to occur in many of them (Flisser *et al.*, 1979; Williams, 1979). Host protective antibody appears very quickly after primary infection and Mitchell *et al.* (1980) have now presented evidence that variations in the resistance of different mouse strains to *T. taeniaeformis* infection could be due to differences in the rate of antibody production. This hypothesis is supported by the observation that temporary B-cell hypofunction induced by cyclophosphamide increases the susceptibility of resistant strains, probably by delaying the onset of antibody synthesis (Mitchell *et al.*, 1977a).

Immune serum has a lethal effect on taeniid oncospheres *in vitro* (Silverman, 1955; Heath, 1970; Rickard and Outteridge, 1974; Heath and Lawrence, 1981) and Heath and Lawrence (1981) showed that lysis of *E. granulosus* oncospheres was especially marked in fresh, non-heated immune serum, suggesting that complement was involved in the process. However, Silverman (1955) and Rickard and Outteridge (1974) found that death of *T. pisiformis* oncospheres occurred in immune sera which had been heated for 30 minutes at 56°C, and in Silverman's (1955) experiments, even some heat-inactivated normal sera were lethal. This emphasizes the care that must be taken in *in vitro* experiments to eliminate the possibility that lytic mechanisms other than antibody and complement may be present in serum. For example, factors cytotoxic to *Trypanosoma brucei* have been described in normal human serum (Rifkin, 1978) and there is no evidence to implicate complement in that phenomenon.

Extensive analyses have been carried out on the antibody classes involved in the passive transfer of immunity via serum in the *T. taeniaeformis*-rodent models. Leid and Williams (1974a) showed that IgG_{2a} antibodies are of primary importance during the first 4 weeks of infection with *T. taeniaeformis* in rats, but later in infection a wide spectrum of antibodies appears which are also effective in passive transfer (Musoke and Williams, 1975a). In mice, both IgG_{2a} and IgG_1 antibodies appear to be involved (Musoke and Williams, 1975b; Mitchell *et al.*, 1977a, 1980). Careful studies with Cobra Venom Factor (CVF) depletion of complement in passively immunized animals suggested that complement fixing antibodies (IgG_{2a} in rats and mice and perhaps IgG_1 in mice) play a major role in the protective activity of immune serum (Musoke and Williams, 1975a), and this conclusion has been supported by Mitchell *et al.* (1977a).

Musoke and Williams (1976) studied immunity stimulated in rats by the

intraperitoneal implantation of *T. taeniaeformis* strobilocerci. Sera from these rats passively immunized recipients, and passive immunity was associated mainly with IgG_1 and IgM antibodies. This was quite different to the findings using serum from rats infected by oral inoculation of eggs, and suggests that the manner of presentation of antigens may be important in determining the nature of the antibody response.

T-cell deficient nude mice are unable to mount an antibody response to *T. taeniaeformis* infection (Mitchell *et al.*, 1977a) but these mice can be immunized using immune serum from normal donors. The implication of this finding is that antibody-mediated immunity to egg challenge with *T. taeniaeformis* is T-cell dependent, but that the effector arm of immunity does not require T-cells. Experiments characterizing the antibodies responsible for passive transfer of immunity are useful, but do not necessarily define precisely the complex events which may take place *in vivo*. Exactly how, or where, the antibody-dependent complement mediated attack on oncospheres takes place is still a matter for conjecture.

The concept of the intestine as a barrier to infection in immune hosts was first advanced by Leonard and Leonard (1941). These authors found that when they bypassed the intestine of passively immunized rabbits by injecting hatched oncospheres into the mesenteric vein, just as many, or even more, larvae developed as in normal rabbits infected in this way. In contrast, far fewer larvae were present after oral infection of passively immunized rabbits. Their results provide little direct evidence for a gut-barrier for several reasons. First, the fact that few 'lesions' were observed in the livers of orally infected passively immunized rabbits 7 days after infection does not necessarily mean that the oncospheres were obstructed by a 'gut-barrier'. They may have reached the liver, and been destroyed without producing macroscopic lesions. This interpretation is more in keeping with the observation by Heath and Pavloff (1975) that when rats were passively immunized against *T. taeniaeformis* just as many oncospheres reached the liver after oral infection as in normal rats, but post-oncospheral re-organization did not occur in 80% of organisms and the majority of developing larvae succumbed by 5–9 days. Musoke and Williams (1975a) also concluded that passive immunization could not be bypassed by avoiding intestinal migration; they examined the livers of passively immunized rats 21 days after oral or intra-mesenteric vein infection and found no cysticerci at all. Leonard and Leonard (1941) gave no indication as to whether there were differences in the characteristics of the lesions observed 7 days post-infection in infected and control animals, although the last line of their discussion implied that degenerating larvae were present. Their observation that more 'lesions' were found in the livers of passively immunized rabbits than in the control rabbits might be interpreted to suggest that more larvae were macroscopically visible because there were more of them undergoing degeneration.

The 'gut-barrier' concept of Leonard and Leonard (1941) seems much more likely to operate in acquired immunity than in animals passively immunized with serum. Lloyd and Soulsby (1978) showed that IgA in the intestinal secretions of infected mice passively protected recipients against *T. taeniaeformis* infection, and that these antibodies were removed by absorption with hatched oncospheres. Banerjee and Singh (1969) found that in previously infected rats, the number of *T. taeniaeformis* oncospheres penetrating the intestine was reduced as well as their rate of penetration, and many oncospheres were retained in the superficial layers of the mucosa. There are no other reports on the intestinal phase of resistance to taeniids, but recent work on *H. nana* may be relevant because passively transferred antibody is also very effective in this system. Bailey (1951) and Miyazato *et al.* (1979) found that *H. nana* oncospheres penetrated the intestine of immune animals. However, Friedberg *et al.* (1979) obtained contrary results and it is difficult to explain this difference. Recently, Furukawa *et al.* (1981) studied the ultrastructural changes in *H. nana* oncospheres penetrating the intestine of immune mice. They found that abnormalities in ultrastructure occurred as early as 8 hours post-infection, and that the damage was confined to the oncospheral membrane. They concluded that the early changes in the epithelium, which consisted of increased density, loss of membrane integrity, reduction in the numbers of mitochondria and formation of large vacuoles, were consistent with antibody-dependent complement-mediated lysis, although completely lysed oncospheres were never seen.

Antibodies involved in colostral transfer of immunity have been examined in detail only in the rodent/*T. taeniaeformis* system. Musoke *et al.* (1975) and Lloyd and Soulsby (1978) showed that IgA immunoglobulins are involved in the passive transfer of immunity via the colostrum of rats and mice, respectively. IgA injected into gut loops reduced the infectivity of freshly hatched oncospheres (Musoke *et al.*, 1975), and Lloyd and Soulsby (1978) were able to absorb out the protective effect of colostrum with hatched oncospheres. Hammerberg *et al.* (1977b) using radiolabelled antibodies, were unable to demonstrate systemic uptake of orally administered IgA in rats, and it seems probable that colostral IgA exerts only a local effect in the intestine of this species. Colostral IgG_2 immunoglobulins in the rat are protective when administered intravenously (Musoke *et al.*, 1975) and their participation in colostral immunization could be significant, a suggestion further substantiated by the results of Lloyd and Soulsby (1978). Neonatal calves have been passively immunized against *T. saginata* infection by oral dosing with serum (Lloyd and Soulsby, 1976) and thus IgG immunoglobulins may play an important part in colostral protection in ruminants also. The many problems associated with experimental work on ruminant parasites, e.g. cost of animals, difficulties in rearing them parasite-free, scarcity of infective material etc.,

make comparable detailed analyses of the immune mechanisms which operate in these systems very difficult.

An association between reaginic (IgE) antibodies and infection with helminth parasites has long been recognized. Leid and Williams (1974b, 1975) described a marked reaginic antibody response in the serum during infection with *T. taeniaeformis* in rats and *T. pisiformis* in rabbits, and found that although IgE was not essential for passive transfer of protection with serum in rats, the presence of this antibody enhanced the degree of protection achieved. These workers postulated that IgE may increase vascular permeability in the immediate vicinity of developing larvae by causing mast-cell degranulation and the release of vasoactive amines. Increased vascular permeability could then assist in destruction of larvae by increasing the availability of antibody at the site, and by stimulating the accumulation of other inflammatory cells. Evidence to support a role for IgE in enhancing the rate of protection was provided by Musoke *et al.* (1978) who showed that when rats were passively immunized with sera containing reagins, almost all *T. taeniaeformis* larvae in the liver were killed within 12 hours of infection, whereas many organisms were still alive after 24 hours when immune serum containing only IgG_{2a} was used (Musoke and Williams, 1975a). These findings contrast with the rapid death of oncospheres exposed to immune serum *in vitro* (Silverman, 1955; Rickard and Outteridge, 1974) and suggest that access of antibody to early oncospheres may in some way be restricted *in vivo*. IgG_1 also can sensitize mast cells in mice, and Mitchell *et al.* (1980) showed that sera from mice infected with *T. taeniaeformis* conferred maximum protection on recipients only when both IgG_{2a} and IgG_1 were present; serum enriched for IgG_{2a} alone was less effective.

Mast cells are known to accumulate at the site of infection with larval cestodes (Coleman and De Salva, 1963; Singh and Rao, 1967; Varute, 1971; Siebert *et al.*, 1979; Lindsay, 1981). Recently, Lindsay (1981) examined in some detail the hepatic and intestinal mast cell response in *T. taeniaeformis* infected rats. She found an increase in the number of mucosal-type mast cells (MMC) around larvae in the liver, with maximum numbers present at 60 days after infection. Interestingly, there was also a persistent increase in MMC in the intestinal mucosa (Cook and Williams, 1981), despite the fact that oncospheres pass rapidly through the intestinal wall during initial infection and subsequently develop at a distant site in the liver. MMC in both sites contained IgE. Intestinal IgA may play an important role in preventing oncospheres from penetrating the intestine of immune animals because passive transfer of immune serum does not affect this (Heath and Pavloff, 1975; Musoke and Williams, 1975a). Nevertheless, a function for MMC at gut level could also be postulated in actively acquired resistance. Lindsay (1981) was unable to show an increase in the number of IgG_{2a} containing plasma cells

in the intestinal mucosa of infected rats. Thus, IgE-mediated release of vasoactive amines may assist in the local accumulation of antibody in the vicinity of oncospheres which have penetrated the mucosa. In addition, a direct effect of the vasoactive amines on the oncospheres themselves cannot be excluded, because an *in vivo* effect of histamine and peritoneal anaphylactic diffusate on the penetration of oncospheres into the intestine of normal rats has been shown (Musoke *et al.*, 1978).

Inflammatory responses have been described in the intestinal mucosa of animals during a challenge infection with oncospheres of taeniids (Bannerjee and Singh, 1969) and hymenolepids (Friedberg *et al.*, 1979; Miyazato *et al.*, 1979), but mast cells were not mentioned in these reports. Furthermore, Furukawa *et al.* (1981) do not describe mast cells in their ultrastructural study of the penetration of *H. nana* oncospheres into the intestine of immunized mice, and no mention was made of tissue changes that might reflect an allergic-type response. Thus, the question of a role for mast cells in the intestinal mucosa during secondary infection with oncospheres must remain open at this time.

A further role for IgE which can only be speculated upon at the present time is in the adherence of macrophages to the parasites. This phenomenon has been shown to occur *in vitro* with *Schistosoma mansoni* (Capron *et al.*, 1975).

Antibody (IgG)-dependent eosinophil-mediated damage to parasites has been described frequently, and probably the best known example is the killing of schistosomula (reviewed by Butterworth *et al.*, 1980). A direct role for eosinophils in the killing of cestode oncospheres, or even later larval stages, has not been convincingly demonstrated. Eosinophils generally accumulate around larval cestodes in tissues (see references in Ansari and Williams, 1976), and Ansari and Williams (1976) described a marked eosinophil response in the peripheral blood of rats following primary exposure to *T. taeniaeformis* which was closely correlated in time with eosinophilic infiltration of the tissue surrounding developing larvae. Rats exposed to a challenge infection with eggs showed a brisk secondary eosinophil response. The accumulation of eosinophils around dying *T. taeniaeformis* larvae in the liver of rats passively immunized with serum suggested a role for antibody in the eosinophil response (Heath and Pavloff, 1975). This was confirmed by Ansari *et al.* (1976) who showed that a fraction of serum enriched for IgG_{2a} played a major part in stimulating eosinophilia, and that reaginic antibody may also be involved. These workers suggested that the eosinophilotactic response may be mediated by complement-derived factors released during reaction of the IgG_{2a} antibody and its corresponding antigen.

The precise role played by eosinophils once they appear is less clearly defined. Although eosinophils accumulate around dying oncospheres in the

intestinal epithelium of mice immune to *H. nana* infection (Friedberg *et al.*, 1979; Furukawa *et al.*, 1981) and are closely applied to the oncospheral epithelium within 24 hours after infection, no evidence of eosinophil degranulation, or changes in the oncospheral epithelium due to eosinophils has been seen (Furukawa *et al.*, 1981). However, an effect of eosinophils cannot be excluded because by 48 hours no oncospheres were present, and there was no record of the events occurring between 24 and 48 hours. Engelkirk and Williams (in press a, b) studied the ultrastructural development of *T. taeniaeformis* larvae in the livers of nonimmune rats. Eosinophils appeared within 6 days after infection, and although they showed close contact with the parasite, there was no evidence of damage to the organisms. It would be interesting to make ultrastructural observations on larvae in the liver after a challenge infection. A role for eosinophils in dampening down the effects of immediate hypersensitivity has been suggested (Butterworth, 1977), and in view of the mast cell responses already described, this cannot be excluded.

There is no information concerning other aspects of antibody function during the establishment phase of infection with larval cestodes, e.g. neutrophil and macrophage functions in relation to antibody-mediated phagocytosis, or neutralization of parasite molecules. Monocytes and polymorphonuclear leucocytes are known to accumulate at reaction sites around larval cestodes but as yet have not been demonstrated to exert any detrimental effects (Furukawa *et al.*, 1981; Engelkirk and Williams, in press a, b). Engelkirk and Williams (in press a, b) showed that phagocytes actively engulf the tips of microvilli from early developing larvae, but these events take place well away from the surface of the larva and do not seem to affect the parasite.

Obviously, there are important deficiencies in our understanding of these antibody-mediated processes *in vivo*. Reinvestigation of questions surrounding the 'gut-barrier' could be rewarding and may provide useful clues to improving vaccination methods because oral immunization using non-living cestode antigens has been shown to be effective (Lloyd, 1979; Ayuya and Williams, 1979; Rickard *et al.*, 1981b). The role of mast cells, eosinophils and all other cells remains to be clarified. Information on the site of antibody attack on cestode oncospheres could give important clues as to the origin and nature of the antigens which stimulate protective immunity.

2. *Antibody-independent cellular aggression*

Direct killing is not documented in larval taeniid infections, but some recent work on *H. nana* may be relevant. Lymphocytes are present near invading oncospheres of *H. nana* in immune mice, but they do not show antiparasite activity, nor are they present in greater numbers than in normal mice during a first infection (Friedberg *et al.*, 1979; Furukawa *et al.*, 1981). Splenic cells from both immune or normal mice adhered to the surface of

H. nana oncospheres *in vitro* but the adherence was greater with cells from immune mice (Furukawa, 1974). The adherent cell population was mainly of small and medium size mononuclear cells and adherence was blocked by rabbit anti-mouse immunoglobulin, iodoacetate and puromycin. There was no evidence that these cells damaged the larvae in any way even after 18 hours, and cells from mesenteric lymph nodes or Peyer's patches did not show marked adherence to the oncospheres. Furukawa (1974) suggested that antibody-producing cells might be responsible for the adherence phenomena observed.

Results of experiments in which neonatal thymectomy or anti-thymocyte serum was used to prevent development of acquired immunity to *H. nana* in mice have been presented as evidence for true lymphocyte-mediated cellular immunity (Okamoto, 1968; Okamoto and Koizumi, 1972). However, many aspects of the host response, for example antibody formation, eosinophilia, mast-cell accumulation and lymphocyte adherence, are likely to be T-cell dependent responses. Adoptive cell-transfer studies have also been unhelpful. Németh (1970) used outbred animals in his study of cell transfer in rabbit cysticercosis, and Kwa and Liew (1975) had no controls for sex and age-matched normal rats in their experiments on transfer of immunity using peritoneal cells from inbred rats infected with *T. taeniaeformis*. *Taenia taeniaeformis* oncospheres survive for up to 48 hours in the livers of rats given doses of immune serum (Musoke and Williams, 1975a), but Cook (1979) showed that the infusion of lymphoid cells from immune donors caused rapid death. Both T-cell and B-cell enriched populations were effective, and thus the question of a direct role for T-lymphocytes cannot be dismissed.

A major problem in cell transfer studies is to prepare 'pure' cell populations. If this were to be achieved, then the T-cell deficient nude mouse should provide a powerful tool for the analysis of T-cell dependent mechanisms. At present there are more questions than answers concerning the role of cell-mediated immunity, and more research effort is required in this area despite the many technical difficulties it poses. Such work may yield important information on more effective techniques for vaccinating against infection with cestode parasites, especially with regard to the role played by adjuvants in enhancing cell-mediated mechanisms.

B. IMMUNITY TO ESTABLISHED METACESTODES

Mature metacestodes have been considered separately from the establishment phase of development for two main reasons. First, these larvae have fully developed their self-protection mechanisms, and therefore, immune mechanisms that affect them are likely to be different to those operating against the early stages. It is important to understand these processes because

the cellular reactions around degenerating cysticerci are often responsible both for the pathogenic effects of infection (e.g. *T. solium*) and for the unsightly lesions which cause economic losses in domesticated animals (e.g. *T. ovis*). Second, most of the research data has been obtained by transplantation of polyembryonic metacestodes from one host to another and often these species are maintained without any recourse to egg-induced infection. Although this is likely to induce a different pattern of immune response, studies of this nature are important because the pathogenesis of a number of larval cestode infections is largely due to unrestrained proliferation of larvae (e.g. *E. multilocularis*).

A protective role of antibody *per se* in immunity to established taeniid metacestodes has not been clearly established. In the earlier part of this review, evidence was given from a number of sources to show that developing larvae become resistant to antibody attack early in their development. The conclusions of Ali-Khan (1974a), with *E. multilocularis* in mice, and Musoke and Williams (1976), with *T. taeniaeformis* in rats, that antibody alone does not have any effect on fully developed larvae are consistent with this concept. Similarly, Varela-Díaz and Marchevsky (1973) were unable to detect any effect of antibodies on *E. granulosus* cysts in persons who were injected repeatedly with hydatid cyst fluid when undergoing the so-called 'biological treatment'.

Passive transfer of immune serum has no influence on the survival of strobilocerci of *T. taeniaeformis* greater than 2·5 months old (Musoke and Williams, 1976). This contrasts with the findings of Kowalski and Thorson (1972) who obtained protection in the *Mesocestoides corti* system against the intraperitoneal inoculation of mice with tetrathyridia using passive transfer of immune serum; however, the immunized mice still had considerable numbers of tetrathyridia present, and mice were killed only 10 days after infection. Partial immunity to *M. corti* infection in rats was also observed following the injection of immune serum intraperitoneally immediately after infection with tetrathyridia (Niederkorn, 1977).

Siebert *et al.* (1978a, b) described a biphasic immune response when *T. crassiceps* larvae were injected intraperitoneally into mice. In the early part of the infection, damage to the larval tegument did not involve cells, and Siebert *et al.* (1978b) attributed the changes they observed to antibody-mediated complement-dependent lysis. Although the differences between immune mice and those infected for the first time were significant, up to 44% of larvae were already adversely affected within 1 week after a primary infection, compared with 78% in the first week after a secondary inoculation. It is possible that the effect observed at 1 week after primary infection of normal mice could be due to a non-specific mechanism of lysis. Chen (1950) described marked changes in the tegument of *T. taeniaeformis* strobilocerci within the

the first 24 hours of incubation in normal rat and guinea-pig serum. Although Engelkirk *et al.* (1981) found no such effect after 60 minutes using 5–6-month-old strobilocerci, Williams *et al.* (1980) found that rat serum did cause some damage to 50-day-old larvae. Cestodes are known to contain anti-complementary substances (Hammerberg *et al.*, 1976), and complement-mediated lysis of *E. granulosus* and *E. multilocularis* protoscolices occurs in normal serum (Herd, 1976; Kassis and Tanner, 1976, 1977; Rickard *et al.*, 1977d) via the indirect pathway of complement activation (Herd, 1976; Rickard *et al.*, 1977d). A similar mechanism may operate to some extent on fully developed metacestode larvae implanted into other hosts, and their survival may depend upon their ability either to prevent, or recover from such an attack. Murrell (1971) described permeability defects in *T. taeniaeformis* larvae incubated in immune serum *in vitro*, and postulated that antibody-dependent complement-mediated effects were involved. His work was later confirmed by Hustead and Williams (1977b) using 42–63-day-old *T. taeniaeformis* larvae and 3–4-month-old *T. crassiceps* larvae. The latter authors found that permeability control was restored when complement in the serum was depleted and suggested that larvae *in vivo* may avoid the adverse reaction by liberating anti-complementary factors to deplete complement levels in their vicinity.

The role of true T-cell mediated cytotoxicity in immunity to mature metacestodes is unclear. Observations have been made on increased blastogenesis of lymphoid cells on exposure to antigen *in vitro*, for example *T. pisiformis* in rabbits (Rickard and Outteridge, 1974) and *E. granulosus* in mice (Araj *et al.*, 1977). Baron and Tanner (1976) showed that thymectomy of mice together with treatment with antithymocyte serum prior to infection with *E. multilocularis* significantly increased the dissemination of the parasite and the total cyst load. In the *M. corti* system, resistance in mice is thymus-dependent, and athymic nude mice are less able to control infection (Mitchell *et al.*, 1977b; Pollacco *et al.*, 1978); this unresponsiveness of nude mice to infection could be restored by transferring syngeneic thymus cells, spleen cells or peritoneal exudate cells. No comparable experiments have been done on taeniids, but recently, Anderson and Griffin (1979a, b) studied the development of resistance to infection with *T. crassiceps* in young AS-strain rats. They found that the onset of resistance was associated with acquisition of responsiveness of host lymphoid cells to the polyclonal T-cell activators concanavalin-A and phytohaemagglutinin. Immunity was adoptively transferred using lymph node cells, whereas serum from the same donors was ineffective. Normal adult rat lymph node cells also conferred immunocompetence on neonatal recipients. Many of these experiments do not exclude the participation of T-cell dependent antibody-mediated cellular processes.

The mechanism of host encapsulation of larval cestodes has not been extensively studied. However, experiments with *M. corti* in T-cell deficient nude mice suggest that encapsulation responses are T-cell dependent (Pollaco *et al.*, 1978). The walling-off of cestode larvae is potentially beneficial to both the host and the parasite. Even though host immunoglobulins can pass through the cyst wall, they are present in cyst fluid in only small amounts, and the parasite may thus be partially protected from the immune system (Varela-Díaz and Coltorti, 1972, 1973; Willms and Arcos, 1977; Kwa and Liew, 1978). On the other hand, the host may be protected from noxious substances produced by the parasite. Engelkirk and Williams (in press b) and Siebert *et al.* (1979) have shown that contact with larval cestodes can be highly toxic to host cells and it has been suggested that mast cells play a part in neutralizing these materials at the time of host capsule formation (Siebert *et al.*, 1979). Indirectly, the long-term protection of the host from noxious parasite agents is beneficial to the parasite by ensuring the host's survival. The T-cell dependence of encapsulation of *M. corti* (Pollaco *et al.*, 1978) is in keeping with the observation that T-cell suppressed mice fail to limit the metastasis and growth of *E. multilocularis* (Baron and Tanner, 1976). Disruption of lymphoreticular tissue and T-cell depletion due to continuous antigenic stimulation have been suggested as a cause for the unrestrained growth of *E. multilocularis* in C57L/J mice (Ali-Khan, 1974b, 1978a, b; Ali-Khan and Siboo, 1980a).

Convincing evidence has been presented that macrophages have a protoscolicidal effect on *E. multilocularis in vitro*, and that antibody probably assists in the adherence of these cells to the parasites (Rau and Tanner, 1976; Baron and Tanner, 1977). These authors argued that macrophages play a key role in limiting dissemination of this parasite *in vivo*. A role for neutrophil attack on antibody coated *E. multilocularis* cysts has also been proposed (Ali-Khan and Siboo, 1980b).

Engelkirk *et al.* (1981) recently examined the effect of rat peritoneal cells on *T. taeniaeformis* larvae incubated *in vitro* for 1 hour. Cells adhered rapidly to the larvae in either normal or immune serum, and caused severe damage to the distal tegument. Fresh serum was essential for adherence to occur, but evidence for involvement of complement was inconclusive. Participation of antibody could not be disregarded on the basis of adherence of cells in normal serum, because antibody may well have already been present on the surface of the larvae. Eosinophils were the most active cells in attacking the tegument and incorporated tegumentary cytoplasm into large phagosomes; although fusion of eosinophil granules with phagosomes occurred, no extracellular release of granules was observed. Mast cells were also present and underwent degranulation. Eosinophils are known to be effective in killing parasites *in vitro* (Butterworth *et al.*, 1980), and it is interesting that Capron *et al.* (1978)

have shown that mast cells may participate in antibody-dependent eosinophil-mediated cytotoxicity to *S. mansoni* schistosomula in the rat. It would be valuable to examine the effects of more prolonged incubation of the *T. taeniaeformis* larvae and to study immune reactions to *T. taeniaeformis* strobilocerci implanted intraperitoneally into rats with hepatic infection. *T. crassiceps* larvae are eventually encapsulated and destroyed when implanted into immune mice (Siebert *et al.*, 1979). These authors showed that eosinophils and macrophages predominated in the early stages of the reaction but that these cells were rapidly destroyed in the vicinity of larvae. When mast cells became apparent, host cells were no longer destroyed and the combined activity of eosinophils, mast cells, macrophages and lymphocytes resulted in death of the encapsulated larva.

There are obviously many aspects of the immune response to mature metacestode larvae that remain controversial. Much more extensive investigation is required along the lines of the work carried out with schistosomula. However, additional work must also be carried out to examine the role of effector systems *in vivo*. Although many resistance mechanisms have been demonstrated in *in vitro* studies on immunity to *S. mansoni*, little work has been directed towards verifying their significance in the infected animal. In order to facilitate this approach, quantitative criteria for assessing the degree of damage to metacestodes must be developed. Up to the present time, morphological observations and dye exclusion methods have been used, but changes in these parameters do not necessarily reflect the true extent of damage to the parasite and they are certainly not precise. The achievement of reliable and reproducible *in vitro* cultivation methods would therefore represent an important advance in dissecting apart the various host mechanisms which operate *in vivo*.

IV. Mechanisms of Evasion of Immunity

The propagation of taeniid cestodes depends on the evolution and persistence of predator–prey relationships between definitive and intermediate hosts, and on the survival of tissue-invading larval stages for the remaining life of infected individuals. It should be clear from the preceding account of the efficacy and duration of acquired immunity to reinfection that the ability of these larvae to evade immune responses is a *sine qua non* for successful transmission. Those metacestodes that die and become encapsulated can only influence the outcome of infection within a population indirectly by their effect on the immune status of hosts. Concomitant immunity is therefore a hallmark of larval cestodiasis, and in few other helminthiases is the pheno-

menon so clearly expressed. Not surprisingly, recent research efforts have begun to probe the mechanisms responsible, and it is becoming apparent that experimental cysticercosis and hydatidosis offer some unique advantages for pursuit of this basic goal in immunoparasitology (Mitchell, 1979).

A. ANTIGENIC SHIFTS

The emerging definition of host-protective effector systems (see Section III) provides us with insights into the kinds of strategies which established larvae may employ in overcoming immune recognition and rejection. For example, the identification of early tissue-invasive stages as the targets for antibody-mediated attack suggests that some form of antigenic variation or shift might occur as a means of evasion by older forms. Post-oncospheral stages rapidly outgrow their susceptibility to antibody (Campbell, 1938; Heath, 1973b; Musoke and Williams, 1975a; Mitchell *et al.*, 1977a), and in primary infections they must do so before the brisk host response identifies and destroys them. Confirmation that this comes about through the expression of antigens in the oncospheral and immediate post-oncospheral stages which are either not displayed or are absent altogether in later forms requires the conclusive demonstration of stage specific or unique determinants as targets for host-protective antibody. There has been no direct evidence for this so far, although some recent data are very supportive.

Direct antibody-mediated protective effects have been shown to occur against oncospheres of *T. pisiformis* (Rickard and Outteridge, 1974), and oncosphere-derived immunogens of *T. saginata*, *T. ovis* and *T. hydatigena* are very potent in stimulating homologous and heterologous protective immunity (Rickard and Adolph, 1976; Rickard and Brumley, 1981). The differing patterns of serological responses to antigenic preparations of oncospheres and cysticerci in sheep infected with *T. ovis* or *T. hydatigena* (Craig and Rickard, 1981a) suggest that there are oncosphere-unique determinants, although the data do not preclude the possibility that these antigens are contained within, but not released by, cystic forms. In fact, the most recent work on this question indicates that many components are indeed shared between oncospheres and other cestode stages (Craig, 1981; see Section VI, p. 277) but more definitive experiments will be necessary to resolve the issue.

Since extracts and *in vitro* culture supernatants of established tissue forms can be used to immunize rats against oral challenge with *T. taeniaeformis* (Campbell, 1936; Kwa and Liew, 1977; Ayuya and Williams, 1979) a case can be made that antigen sharing, at least in this species, includes oncospheral host-protective immunogens; however, it is not known if the resistance which is stimulated operates on the same early stages as that which is provoked by

oncospheral antigens. The tendency for immunized animals to retain small scars on their livers after challenge suggests otherwise (Ayuya and Williams 1979), as does the failure to absorb out protective antibodies from immune (infected) rat serum with strobilocercus-derived antigens (Leid and Williams, 1974a). However, the recognition that host-protective antigens in oncospheres are probably membrane-associated (Rajasekariah *et al.*, 1980a, b) necessitates a re-evaluation of these findings.

There is now ample morphological evidence of profound changes in the characteristics of the limiting membrane of developing metacestodes in mammalian tissues (Engelkirk and Williams, in press, a; Furukawa *et al.*, in 1981). The extensive 'cytoplasmic folds' of the oncospheral surface (Nieland 1968) are rapidly replaced by a dense microvillar coat, which is then abruptly transformed into a microthrix layer (Engelkirk and Williams, in press, b; Fig. 1b, c, d). It remains to be seen if these alterations can be correlated with shifts in the antigenic characteristics of the surface plasma membrane which could be important in evasion. Engelkirk (1980) has recently found that extracts of organisms both before and after the transition from the microvillar to the microthrix surface form can be used very successfully as host-protective immunizing agents in rats; but, again, the result says less about the antigenic nature of the surface projections than it does about the possible internal sharing of antigens over this period.

A vigorous analysis of monoclonal antibody specificity in serological tests (Craig *et al.*, 1980, 1981) as well as in tests of passive protection *in vivo* by cross-absorption with soluble and membrane-associated antigenic preparations of each developmental stage should eventually provide the requisite demonstration of stage specificity. Now that some of the characteristics of host-protective antibodies are known in the laboratory animal systems (Musoke and Williams, 1975a, b; Mitchell *et al.*, 1977a) it should be possible to apply immunohistochemical techniques at the ultrastructural level to localize immunogens in the tissues and on the surface at each phase of development. The *in vitro* production of protective antibodies from hybridomas derived from immune host cells should provide definitive reagents for work of this type.

B. MASKING OF SURFACE ANTIGENS

Although there is morphological evidence that the structure of the tegument of larval cestodes has many features in common with that of blood schistosomes (Lumsden, 1975), there is no evidence, as yet, of the incorporation of host-derived red blood cell glycolipids or histocompatability antigens into metacestodes, as has been shown to occur in *S. mansoni* (Goldring *et al.*,

1976; Sher *et al.*, 1978). The hypothesis that such factors are responsible for immune evasion by masking of the schistosome surface, originally proposed by Smithers and Terry (1969), has received much circumstantial support but no definitive confirmation. Perhaps as our knowledge of the ultrastructural characteristics and biochemical composition of taeniid larval cestode membranes improves, it will be possible to evaluate whether or not this mechanism is a viable explanation for evasion of immunity in cysticercosis and hydatidosis, but at the moment there are very few observations or experiences to draw upon. Red blood cell antigens have been detected in cysts of *E. granulosus* (Feizi and Kabat, 1972; Ben-Ismail *et al.*, 1980) and in some cases their biochemical characteristics have been determined (Cossey *et al.*, 1979). However, their relationship with the surface membrane has not been studied. The glycolipids of larval forms of *T. taeniaeformis* and *T. crassiceps* have been characterized (Mills *et al.*, 1981a, b), and they are quite distinct from those of their rodent hosts.

Assuming that host-derived substances are attached to or incorporated into the tegumental plasma membrane, confirmation of the masking hypothesis would require the demonstration that their presence contributed to immune-evasion. This necessitates an understanding of the mechanism of uptake and turnover of surface antigens, so that experimental manoeuvres either to interfere with their attachment or display, or to provoke specific responses targeted against them, can be devised and shown to affect the survival of larvae. The only investigations of this nature undertaken with taeniid cestodes have emphasized the proposition that host-derived antibody, rather than antigen, may be the effector of masking. This possibility, put forward by Varela-Díaz *et al.* (1972) and Rickard (1974) was tested experimentally by implanting cysticerci of *T. pisiformis* into rabbits after exposure to heterologous anti-rabbit immunoglobulin (Rickard, 1974). The marked decline in survival of treated parasites when compared to controls provided some support for the idea that, normally, a layer of host antibody on the tegumental limiting membrane protects it from recognition and attack. There is now immunohistochemical evidence of the attachment of pig immunoglobulin to the surface of *T. solium in situ* (Willms and Arcos, 1977), and of rat serum proteins on the surface of *T. taeniaeformis* (Kwa and Liew, 1978), but in neither case has there been confirmation that these represent antibody specifically directed against the surface membrane determinants. Immunoglobulins and other serum proteins make their way into the bladder fluids of many species of taeniid larvae (Chordi and Kagan, 1965; Varela-Díaz and Coltorti, 1972; Hustead and Williams, 1977a; Soulé *et al.*, 1979) and may be adsorbed non-specifically onto the surface membranes.

Membrane-bound antibodies have recently been considered to play a slightly different role in masking as it has been demonstrated that specifically

adherent immunoglobulins may be split into fixed Fab and free Fc portions by surface proteases (Eisen and Tallan, 1977; Auriault *et al.*, 1981). This process, termed "Fabulation", could lead to coverage of membrane determinants, but deprive fixed antibody of the opportunity to amplify the immune response via mechanisms such as cell adherence or complement fixation. The demonstration that cysticerci of *T. pisiformis* contain proteases which are not inhibited by parasite-secreted inhibitors of host proteases (Németh and Juhász, 1980) suggests that surface proteolysis of fixed antibody could occur in larval cestodes.

Experimental confirmation that specific antibody masks or blocks immune attack requires first that membrane immunoglobulin be shown to have specificity for parasite determinants. This might be achieved by disassociation of antibody from immune complexes and the demonstration of specific interaction of the purified antibody with antigens of the cestode. If these blocking antibodies are important in survival it should be possible to manipulate the antibody status of the host, either by enhancing or by interfering with the production of the prerequisite antibody type, or even passively transferring antibody so as to affect the persistence of larvae in tissues. This will require a good deal more information about both the pattern of antibody response in cysticercosis and the nature of the membrane antigens or larval forms than is now available.

C. MOLECULAR MIMICRY

Despite a convincing case which can be made for the hypothesis of immune evasion by antigenic convergence of host and parasite (Sprent, 1962), or molecular mimicry (Damian, 1964, 1979) there have been few attempts to generate direct supporting evidence for the synthesis of host-like determinants by parasites. The persistence of host-derived contaminating antigens through extraction or *in vitro* cultivation procedures could account for many of the observations which have been cited in support of the proposal. Recently, however, Willms *et al.* (1980) reported on their efforts to determine the origin of the immunoglobulin (IgG) which they had detected on the surface membrane of *T. solium* by immunoelectronmicroscopy. This IgG was purified but showed no specificity for antigens of the cysticercus, and the possibility that it was synthesized by the organism was tested *in vitro* by translation of parasite-derived RNA. One of the protein products was precipitable with rabbit anti-pig IgG. Unless the RNA preparation was contaminated with nucleic acid extracted from firmly adherent host cells removed from the tissue along with the cysticercus, these results constitute the first direct demonstration of cestode parasite-synthesized host-mimicking antigens.

They open up the possibility of testing whether or not selective inhibition of production of shared components affects larval survival. In conjunction with this, investigation of the effect of enhanced immune responses directed against shared antigens (for example, after transfer of parasites to heterologous hosts) on the establishment of cysticerci, would do much to clarify the question of antigenic convergence in cestodiasis.

D. SUPPRESSION OF IMMUNE RESPONSES

In many tissue helminthiases there is evidence that the immunological responsiveness of the host may become inhibited at various points in the afferent or efferent arms. These effects have been attributed to such mechanisms as antigenic competition, enhanced generation of T-cell suppressor populations, direct inhibition of lymphocyte reactivity by parasite secretions, and defective antigen processing by macrophages. Non-specific immunosuppression seems less tenable as an hypothesis for immune evasion than does the picture of highly selective anti-parasite antigen suppression which is emerging from recent work on the filariases (Piessens *et al.*, 1980). There are few indications that suppressive effects of either type are caused by cysticercosis or hydatidosis, but the occurrence of the phenomenon in other parasitic infections justifies its consideration and experimental analysis.

Certainly one of the most perplexing problems in the serodiagnosis of human cysticercosis and to a lesser extent hydatidosis, is the apparent immunological unreactivity of a significant proportion of infected patients. In a recent report (Flisser *et al.*, 1980) more than half of 54 individuals with symptoms caused by larval *T. solium* showed no detectable responses to antigens of the cysticercus in *in vitro* tests. Patients with pulmonary echinococcosis due to *E. granulosus* are notoriously unreactive in serological assays (Todorov *et al.*, 1979). Whether or not these represent examples of specific suppression remains to be seen, but there is no evidence to date of generalized immunosuppression in larval cestode infections in man.

There are a few instances of unresponsiveness to heterologous antigens *in vivo* in experimental taeniiasis in rodents. Mice infected with *T. crassiceps* showed impaired ability to make antibody against sheep red blood cells (RBC) (Good and Miller, 1976) probably due to the preoccupation of the peritoneal macrophages with the burgeoning population of proliferating cysticerci (Miller *et al.*, 1978). In experimental *E. multilocularis* infections in mice, Ali-Khan (1978a) found that a specific cellular unresponsiveness to *Echinococcus* antigen developed while delayed hypersensitivity reactions to unrelated determinants eventually became normal. He attributed this to massive release of *E. multilocularis* antigen from the enormous burden of

parasites found in the later stages of the disease. Interestingly, at certain stages of the infection the anti-sheep RBC response of infected mice was significantly greater than in the controls (Ali-Khan, 1979), perhaps due to enhancement of macrophage activity and antigen-processing capability. Finally, Leid (1977) has reported that mast cell-mediated, hapten-specific reactions to heterologous antigens were profoundly suppressed *in vivo* in rats infected with *T. taeniaeformis*, possibly as a result of parasite modulation of mast cell functions.

In addition to the possibility that lymphocyte unresponsiveness may be produced by the wholesale production and release of taeniid antigens in overwhelming infections, other parasite-derived factors may result in non-specific lymphocyte exhaustion. Extracts of *T. solium* cause polyclonal B cell activation (Sulivan-Lopez *et al.*, 1980), and normal mouse lymphocytes undergo transformation in the presence of protoscolices of *E. granulosus* (Dixon *et al.*, 1978). Presumably, persistent stimulation by these products could result in elimination of reactive clones. Conversely, there is evidence that factors circulating in the plasma of rabbits heavily infected with *T. pisiformis* can inhibit lymphocyte reactivity in lectin-mediated transformation assays (M. D. Rickard and P. M. Outteridge, unpublished observations). This may explain the anergic status which eventually develops in experimental cysticercosis in rabbits tested for dermal hypersensitivity to cysticercal antigens (Rickard and Outteridge, 1974). Recently, a substance in cyst fluid of *E. granulosus* has also been shown to have suppressive effects on lectin responsiveness of lymphocytes (Annen *et al.*, 1980). Obviously much more information is required about the nature and rate of release of putative non-specific stimulatory or inhibitory factors *in vivo* and *in vitro* in order to evaluate suppression as an evasive mechanism. If it contributes to survival of taeniid larvae then restoration of specific components of the immune response should affect larval persistence in tissues. Furthermore, interference with the expression of suppressive agents should be detrimental to the establishment and/or survival of metacestodes. It may also be possible to exploit genetic differences in susceptibility to suppressive factors to examine their importance in determining the outcome of infection.

E. ANTIGEN MODULATION

The tegumental membrane of established metacestodes must be an extremely active frontier as it not only provides for the uptake of nutrients and the release, by secretion or excretion, of products or wastes, but it is the interface with host defence factors. So little is known about the normal physiological processes of synthesis and turnover of this membrane, and the characteristics

of its transport mechanisms, that it is difficult to assess whether or not modulation of surface antigenicity by internalization or exocytosis could be a part of an evasive strategy. Structural and functional similarities between cestode and trematode teguments have been pointed out elsewhere (Lumsden, 1975), and the emergence of evidence for antigenic modulation by surface membranes of *S. mansoni* (Kemp *et al.*, 1980) raises the possibility that comparable manoeuvres could be undertaken by taeniid larvae. In the schistosome system, ligand binding to membrane determinants quantitatively alters the expression of those antigens by exocytosis of complexes. Internalization of specifically bound ligands occurs in some other host–parasite systems, and would be an equally acceptable strategy for the maintenance of immunological anonymity in cestodes.

It seems unlikely that the adherence of ligands, whether antibody molecules or otherwise, would be entirely without consequences for taeniid larvae, because the phenomenon of movement of membrane constituents (such as patching and capping) in response to receptor bridging appears to be a generalized feature of plasma membrane reactivity. The tegument-free surface at the base of the microthrix layer of larval cestodes appears extremely active (Fig. 1d); the pits and vesicles which are formed could be involved in either uptake or externalization of membrane components complexed with antibody, complement or other humoral factors. More basic work on the physiology of metacestode surfaces, especially of the type undertaken for adult cestodes by Oaks and Lumsden (1971), needs to be done before questions concerning the importance of modulation can be addressed by pharmacologic manipulation of membrane turnover rates or specific inhibition of bound-ligand movements.

F. SEQUESTRATION

After an early, and often brief, period of migration in tissues, taeniid larvae become sessile. The site in which they eventually settle, as well as the nature of their relationship to the encapsulating host response, may contribute to an effective sequestration of the parasites from immune attack. For example, the localization of cysticerci of *T. solium* in the central nervous system usually leads to minimal host cellular reactions as long as the organisms are alive (Showramma and Reddy, 1963; Marquez-Monter, 1971) and, furthermore, they do not often provoke serologically detectable antibody responses (Flisser *et al.*, 1980). The central nervous system may therefore be an immunologically privileged site for this organism. However, pulmonary cysts of *E. granulosus* in human patients are also often undetectable immunologically, especially if the parasite is alive and the germinal and laminated membranes are smooth and intact, resulting in a so-called 'hyaline' appearance radio-

graphically (Yarzábal *et al.*, 1974). Hyaline cysts in other tissues are frequently associated with minimal host antibody responses and it seems likely that the larvae are effectively sequestered by the intact outer laminations and host capsule so that very little immunological stimulation occurs.

The firm, fibrous encapsulation which often surrounds taeniid metacestodes *in vivo* may represent a physical barrier to the influx of host cells, although there is ample evidence that humoral factors do gain access to the tegumental membrane and even to the internal fluids of many cestode larvae (Coltorti and Varela-Díaz, 1972; Varela-Díaz and Coltorti, 1973; Hustead and Williams, 1977a; Willms and Arcos, 1977; Kwa and Liew, 1978). Moreover, the immediate onset of intense inflammatory infiltration of larval cestodes once they are chemotherapeutically damaged or die from other causes (Showramma and Reddy, 1963; Verheyen *et al.*, 1978) suggests that, given the opportunity, effector cells have little trouble negotiating their way through the fibrous layers.

Nevertheless, the relationship between the sessile cystic larva and the surrounding fibrous host response may contribute to enhanced survival by means of the enforced physical separation of the foreign antigenic mass and the blood-borne elements of defence. Humoral and cellular components must extravasate and diffuse towards the parasite or migrate along a gradient of chemoattractants, respectively, in order to encounter the target. If other evasive mechanisms are at play which constantly limit the generation of chemotactic stimuli or vascular permeability factors, then the balance *in situ* may favour the parasite. Correspondingly, deprived of their anatomically advantageous setting, metacestodes would become vulnerable to unrestrained host defences if this hypothesis were valid.

Some favourable evidence comes from observations on the susceptibility of taeniid larvae to attack *in vitro*. Metacestodes of *E. granulosus*, *E. multilocularis* and *T. taeniaeformis* are extremely susceptible to complement-mediated lysis when exposed to normal serum (Herd, 1976; Kassis and Tanner, 1976; Rickard *et al.*, 1977d; Williams *et al.*, 1980). Peripheral blood leucocytes from infected patients adhere to the surface of protoscolices of *E. granulosus in vitro* (Dumon *et al.*, 1976), and strobilocerci of *T. taeniaeformis* are attacked by adherent peritoneal cells, especially eosinophils, when incubated *in vitro* for only 60 minutes (Engelkirk *et al.*, 1981). Furthermore, metacestodes of *T. taeniaeformis* do not survive when implanted into the peritoneal cavity of infected rats but live for many weeks in normal animals (Musoke and Williams, 1976). Similarly, strobilocerci removed from their capsules and allowed to move out freely into the peritoneal cavity of the same host do not survive (J. F. Williams and A. J. Musoke, unpublished observations). These kinds of experiences indicate that established larvae have advantages at their site of predilection, which probably supplement the

efficacy of other evasive tactics. The availability of the nude mouse and its susceptibility to *T. taeniaeformis* (Mitchell *et al.*, 1977a) offers an excellent opportunity for the analysis of the immunology of host–parasite relationships under circumstances where the effects of sequestration are likely to be abrogated by the failure of T-cell dependent encapsulation (Pollaco *et al.*, 1978).

G. DIRECT INTERFERENCE WITH HOST DEFENCE SYSTEMS

The chain of events which leads to immunologically mediated rejection of invading organisms involves a series of complicated and co-ordinated steps, beginning with recognition and processing of antigens; stimulation of specifically reactive cells and their eventual proliferation; the extravasation and interaction of mobilized cells or their products with the target in the tissues, and, often, an amplification mechanism whereby all the forces of non-specific inflammation are brought to bear on the offending parasite. The concept of direct interference by bacterial products with steps in the pathway has been supported for some years by evidence for so-called "agressins" which can adversely affect the behaviour, secretory functions, motility or viability of host cells, or neutralize antimicrobial humoral factors (Glynn, 1972). Less attention has been afforded to the occurrence of comparable reactions in helminthiasis, although a number of clinical and experimental observations suggest that they may be equally important in parasitic infections (for review see Leid and Williams, 1979).

Specifically in the case of cysticercosis and hydatidosis, there are now data which indicate that parasite products may: inhibit host enzymes (Németh and Juhász, 1980); affect host cell motility (Goetzl and Austen, 1977); limit responsiveness to mitogens (Annen *et al.*, 1980); reduce cell viability (C. Coley, G. Mills and J. F. Williams, unpublished observations); influence host cell mobilization and differentiation (Cook *et al.*, 1981); reduce the efficacy of phagocytic degradation of substrates in granulocytes (Grills and Hammerberg, 1979); and cause non-specific consumption of complement and inhibition of coagulation (Hammerberg *et al.*, 1977a; Hammerberg and Williams, 1978a, b). There is no direct evidence, in any instance, that these factors are crucial for survival in tissues, but their characteristics and mechanisms of action are very worthy topics for further research.

Host enzyme inhibition is a recognized component of defence of adult cestodes against degradation by intestinal secretions in the gut lumen (Pappas and Read, 1972; Matskazi and Juhász, 1977). The recent observation that cysticerci of *T. pisiformis* maintained *in vitro* release a potent, low molecular weight inhibitor of both trypsin and chymotrypsin (Németh and Juhász, 1980) raises the possibility that antienzyme tactics may also be employed by larval taeniids in avoiding adverse secretions from host inflammatory cells.

The results of Goetzl and Austen (1977) show that larvae of *T. taeniaeformis* contain chemoattractants which enhance the directed migration of eosinophils and neutrophils; this, combined with evidence that strobilocerci can induce the secretion of inhibitors of slow reacting substance of anaphylaxis (SRSA) from eosinophils (Signs *et al.*, 1978), suggests that there may even be a parasite-controlled modulation of local inflammatory events. Annen *et al.* (1980) have shown that substances in *E. granulosus* inhibit host lymphocyte responsiveness to non-specific transformational stimuli. They attributed this to cytotoxicity, although no data were given on cell viability at the end of the experiments; however, one of the five glycolipids purified from *T. taeniaeformis* (Mills *et al.*, 1981b) has been shown to be highly cytotoxic to a wide variety of mammalian cell types, including granulocytes (C. Coley, G. Mills and J. F. Williams, unpublished observations). The glycolipid responsible is also present in *T. crassiceps* and *E. granulosus*, and may represent another component of the armamentarium of anti-host mechanisms available to established taeniid larvae.

Mast cells increase in numbers in the lamina propria of the intestine in experimental taeniiasis in rats (Cook and Williams, 1981) and accumulate around the parasites in the liver (Lindsay, 1981). Their functions at these sites are unclear, but the central role of mast cells in the regulation of inflammatory responses, histamine-mediated vascular permeability changes and angiogenesis, suggests thay they could be modulating the local environment to the advantage of the parasite. The appearance of mast cells in increased numbers in the tissues of uninfected parabionts (Cook *et al.*, 1981) indicates that some control is being exercised over the distribution and/or differentiation of mast cells as a result of the development of metacestodes in the liver.

Finally, there is now good evidence for the production and release of complement-consuming factors by the larval stages of *T. taeniaeformis* (Hammerberg and Williams, 1978a). Similar activity, quite separate from the insoluble complement-fixing activity shown by calcareous corpuscles of *E. multilocularis* (Kassis and Tanner, 1976), *T. crassiceps* and *T. taeniaeformis* (J. F. Williams, unpublished observation), can be demonstrated in cyst fluid of *E. granulosus* (Hammerberg *et al.*, 1977a). The soluble complement-fixing agent of *T. taeniaeformis* triggers alternative pathway activation, and is a highly sulphated acidic proteoglycan (Hammerberg and Williams, 1978b). It has anticoagulant properties (Hammerberg *et al.*, 1980) and inhibits granulocyte phagosomal enzyme function (Grills and Hammerberg, 1979). The constant release of a substance with such a wide variety of biological activities may constitute an important evasive mechanism *in vivo*.

A recurrent theme in accounts of the pathology of larval taeniiasis is that host reactions seem to be held in abeyance while parasites live, but that immediate inflammatory and infiltrative changes occur whenever the organ-

isms are damaged or die. This observation invites explanations based on the synthesis and surface expression or release of factors which actively interfere with host effector systems. Clearly, there is now no shortage of candidates for these roles but the experimental challenge which must be faced is to devise means of inhibiting production of such factors, or neutralizing their activities, so as to be able to measure their various contributions to immune evasion *in vivo*. Substantially more needs to be known about the biochemistry and physiology of larval cestodes before questions such as these can be addressed.

V. Immunopathology

On the whole it is not in the best interests of the transmission of taeniid cestodes for severe, incapacitating disease to result from metacestode infection and, in fact, most intermediate hosts accommodate their parasite burden for extended periods with minimal effects on health. When pathological complications do override this benign tolerance on the part of the host, they often involve acute inflammatory episodes, specifically triggered by the release or exposure of antigens to which the individual has previously become sensitized. While much of the information about these pathological events in larval taeniiasis is still descriptive, the increasing sophistication of investigative clinicopathological techniques and the use of experimental models has allowed the definition of a number of the underlying pathogenetic processes in recent years. The comments that follow relate specifically to instances where immune events have been incriminated in the development of symptoms or lesions.

In human hydatidosis caused by *E. granulosus*, infection is generally manifested clinically after a long latent period during which the parasites not only grow large enough to impinge upon local tissues and vessels, but also stimulate antibody and cell-mediated hypersensitivity responses. Reaginic antibodies are produced against potent allergens within the cyst (Dessaint *et al.*, 1975; Rickard *et al.*, 1977c), and many patients show type I cutaneous immediate hypersensitivity to these components (Williams, 1972). Traumatic rupture of cysts results in systemic reactions typical of those associated with anaphylactic shock in atopic individuals. This syndrome may also occur spontaneously in patients in whom there is no evidence of trauma or cyst damage at post-mortem (Deenchin *et al.*, 1976). Others suffer repeated allergic episodes which resemble those of angioneurotic oedema (Werczberger *et al.*, 1979). It is possible that some of these allergic signs are exacerbated by the biological activities of peptide fragments generated through the release of complement-fixing factors from within hydatid cyst fluid (Hammerberg *et al.*, 1977a; Perricone *et al.*, 1980).

Echinococcus granulosus patients may show severe systemic hypersensitivity symptoms after intradermal inoculations of microgram quantities of antigen for immunodiagnostic purposes (J. F. Williams, personal observation), and the onset of hypotensive shock, with peribronchial oedema and blood coagulation deficits, follows almost immediately after intravenous administration of hydatid fluid in sensitized sheep (Schantz, 1977). Immunoglobulin E (IgE) levels in the serum of infected human beings rise markedly (Dessaint *et al.*, 1975; Richard-Lenoble *et al.*, 1978), but there are no indications, as yet, that a non-specific potentiation of reaginic antibody production to heterologous antigens occurs in any larval cestodiasis, comparable to that which characterizes experimental trematode and nematode infections (Jarrett, 1973). The extreme sensitivity of hydatidosis patients to parasite allergens has given rise to questions about the possible life-threatening consequences of allergen release following chemotherapy with mebendazole. Kern *et al.* (1979) have described allergic reactions in *E. granulosus* patients receiving the drug, but, as a general rule, this does not seem to be a common sequel to the initiation of benzimidazole treatment (Kammerer, 1979).

Many other kinds of parasite antigens are recognized by the immune system of hydatidosis patients, and high levels of antibodies of various immunoglobulin classes are found (Richard-Lenoble *et al.*, 1978). There is evidence that circulating immune-complexes appear in the plasma (Richard-Lenoble *et al.*, 1978), and occasionally become deposited in renal glomeruli, resulting in classical membranous immune-complex nephropathy as a pathological feature of chronic echinococcosis in man (Vialtel *et al.*, 1981). Ali-Khan (1978a) has proposed that high levels of circulating antigens of *E. multilocularis* probably account for the anergic state which develops in chronic experimental infections in rodents, and clinical similarities between this disseminated form and the terminal stages of inoperable human multilocular disease make it likely that comparable pathogenetic mechanisms are at play.

It is clear from the extensive series of reports on the pathology of alveolar echinococcosis in mice by Ali-Khan and his collaborators that immunological events have a major role in shaping the progress of the disease and the growth and extension of the parasite (Ali-Khan, 1974a, b, 1978a, b, c, 1979; Ali-Khan and Siboo, 1980a, b). Early in infection there are intense local histiocytic and neutrophilic infiltrates (Ali-Khan and Siboo, 1980a) but important changes in the immune system quickly follow. There is disorganization of the paracortical areas of draining lymph nodes (Ali-Khan and Siboo, 1980a) and severe T-cell depletion, associated with thymic involution (Ali-Khan, 1978b). B-lymphocytes proliferate and significant cell-mediated immunological defects develop which may be responsible for continued dissemination and growth of the metacestodes (Ali-Khan, 1978a, b, c). There are many analogies with the immune phenomena which characterize syngeneic

tumour transfer and growth, and this, coupled with clinical similarities between rapidly progressive visceral cancer and multilocular hydatidosis in man, makes the continued dissection of the immunopathogenesis of marine echinococcosis especially relevant to the human disease.

It appears that there may be factors other than antigens produced by *Echinococcus* which result in non-specific B-cell transformation (Dixon *et al.*, 1978). The occurrence of similar stimulation of B-lymphocytes by extracts of *T. solium* (Sulivan-Lopez *et al.*, 1980) has led to speculation that the large number of plasma cells around the cysticerci *in situ* may be the result of polyclonal B-cell activation and subsequent plasmacytosis (Willms and Merchant, 1980). Plasma cells containing several different immunoglobulin types also assemble in the host capsule surrounding *T. taeniaeformis* in the rat liver (Lindsay, 1981). The extent to which these cells are involved in parasite-specific antibody production is not known, but the marked elevations in circulating immunoglobulin (IgG_1) levels which occur in *T. taeniaeformis* infected mice (Chapman *et al.*, 1979a) apparently result from chronic parasite-antigenic stimulation rather than non-specific clonal activation (Chapman *et al.*, 1979b).

The abrupt onset of clinical signs in human neurocysticercosis, often after latent periods of many years, coincides with intense pathological reactions to degenerating metacestodes in the brain (Marquez-Monter, 1971). These reactions, in their intensity and composition, appear to have an immunological origin, but this is a very difficult hypothesis to confirm in human patients, especially when high-level corticosteroid therapy may be instituted in an effort to ameliorate the severity of the disease. Arthus-like reactions have been described in some cases (Gerez-Greiser, 1961), and the fact that at least some of the locally accumulated plasma cells seem to contain anti-parasite antibodies (Willms *et al.*, 1980) supports the notion that antigen–antibody reactions could be responsible for the initiation of inflammatory foci.

The release of internal components from the degenerating parasite which have potent chemotactic effects, such as those in *T. taeniaeformis* (Goetzl and Austen, 1977), or lymphocyte-stimulating capacities (Sulivan-Lopez *et al.*, 1980), may also serve to exacerbate the process. The observation that viable cysticerci of *T. solium* implanted intraperitoneally into mice release factors which depress, rather than enhance, lymphocyte reactivity (Willms *et al.*, 1980) is consistent with the picture of unresponsiveness as long as the larvae remain alive. Unresolved for the moment is the question of which factors precipitate the degeneration of cysticerci in the first place, although the seriousness of the consequences is sufficient to raise concern about the possible hazards of chemotherapy, now that some drugs which are effective against metacestodes have been identified (e.g. praziquantel, albendazole). There seems to be doubt that the racemose forms of *T. solium* in man are more

pathogenic, and it would be valuable to know if there were antigenic differences between this type and the normally formed cysticercus. So far, efforts to detect antigenic differences between geographically separated "strains" of *T. solium* have been unsuccessful (Flisser, 1980).

Some of the answers to these questions may come from studies on pathological events occurring in larval taeniid infections in natural host–parasite relationships in domestic animals; however, given the peculiarities of cellular reactivity in each species, extrapolations to man must be made with caution. In most infections in domesticated animals there is pathological evidence of a substantial amount of degeneration of fully established metacestodes at intervals after exposure (Slais, 1970) and in infections caused by *T. saginata* in cattle and *T. ovis* in sheep most of the cysticerci in chronic cases may be dead or degenerating. Even in echinococcosis there is significant attrition of quite large cysts of *E. granulosus* after experimental exposure of pigs (Slais and Vanek, 1980), and the fate of individual cysts appears to be related to the aggressiveness of cellular infiltration at the host–parasite interface. Possibly there is heterogeneity in metacestode populations in the extent to which they are able to hold immunological defences at bay, with the balance tipping in favour of the host around those that are less successful. Obviously, defining mechanisms of immune evasion (see Section IV) would do much to enhance our understanding of the immunopathology of larval taeniiasis.

In view of the remarkable consistency with which intermediate hosts of metacestodes can be shown to develop IgE-mediated hypersensitivity to parasite allergens it is very tempting to ascribe some role to the mast cell triggered system in acute tissue responses to taeniid larvae. Reaginic antibodies have been demonstrated in the serum, or immediate skin reactivity detected, in virtually every instance which has been examined, including *T. solium* in pigs (Marler, 1978), *T. saginata* in cattle (Moreira *et al.*, 1978), *T. multiceps* in sheep (Williams, 1975), *T. pisiformis* in rabbits (Leid and Williams, 1975), *T. taeniaeformis* in rats (Leid and Williams, 1974b), *E. granulosus* in sheep (Schantz, 1973) and, of course, echinococcosis and cysticercosis in man. We are, perhaps, too ready to focus on the ability of IgE to bind to mast cells, and overlook the implications of recent evidence that reagins may confer specific reactivity on macrophages (Capron *et al.*, 1975) and eosinophils (Capron *et al.*, 1978) in some circumstances. However, evidence that mast cells accumulate around larval cestodes in tissues (Coleman and De Salva, 1963; Varute, 1971; Lindsay, 1981), and appear to influence the effectiveness of antiparasitic reactions (Siebert *et al.*, 1979), points to their having some controlling influence on the outcome of confrontations between host responses and taeniid larvae. Some of the possible mechanisms involved have been reviewed by Leid and Williams (1979).

Further research on mast cell origins and functions in cysticercosis and

hydatidosis should be rewarding, especially in view of the observation that two distinct mast cell populations occur in hepatic tissue of rats infected with *T. taeniaeformis* (Lindsay, 1981). With this kind of information in hand it may eventually be possible to devise rational means of medical intervention aimed at preventing the onset and expansion of immunopathologic inflammatory foci, whether these result from the natural course of larval cestode diseases or to anthelmintic therapy in human patients.

VI. Immunization

There have been several reviews describing the early work on immunization against larval taeniid cestode infections, (e.g. Gemmell and Soulsby, 1968; Gemmell and MacNamara, 1972; Gemmell, 1976; Gemmell and Johnstone, 1977) and Clegg and Smith (1978) and Williams (1979) have discussed some of the most recent developments. In the first part of this section, we will attempt to relate the available data on immunization to some of the practical questions raised by Urquhart (1980) in his comments on the prospects for vaccines in hydatidosis/cysticercosis control, and in the latter part discuss the results of current experimental approaches to determine the nature and source of functional antigens in these parasites.

A. General considerations

1. *Is there a need for a vaccine?*

Are these parasites of sufficient importance to warrant the research endeavour required to develop vaccines; is there evidence to show that immunity plays a regulatory role in the natural transmission patterns, and are other measures for control adequate?

The importance of larval taeniid cestodes is twofold. First, some are health hazards to the public, and these include *E. granulosus*, *E. multilocularis*, *E. vogeli*, *T. solium* and to a lesser extent *T. saginata;* and second, others such as *E. granulosus*, *T. solium*, *T. saginata*, *T. ovis*, *T. hydatigena* and *T. multiceps* are a socio-economic burden on livestock industries. Detailed consideration of these aspects is not within the scope of this review, but they have been examined at length by Arundel (1972), Pawlowski and Schultz (1972), Matossian *et al.* (1977), Gemmell (1978) and Flisser (1980). Since 1964, United Nations agencies such as the Food and Agriculture Organization, World Health Organization and the United Nations Environmental Programme have sponsored a series of seminars and both formal and informal meetings on the

subject (see review by Gemmell, 1978), the most recent being in Geneva from 29th April to 1st May, 1981. Research priorities arising from these meetings are usually published.

One of the continuing frustrations is that there have been few detailed estimates concerning the cost of cysticercosis and hydatidosis in endemic areas because it is so difficult to obtain accurate data. Apart from the economic consequences of the public health importance of cestodes, improved standards of hygiene imposed by meat importing countries, which affect the acceptability of infected carcases, may act as barriers to the expansion and development of international trade, and cause stagnation of pastoral industries in developing countries. Since cysticercosis/hydatidosis is endemic in many areas, direct losses due to condemnation of carcass meat or offal must be substantial on a global basis. Howkins (1975) estimated that in New South Wales, Australia, alone, annual losses due to condemnation of offal infected with *T. hydatigena* and *E. granulosus* were $1·25 million. Grindle (1978) suggested that the overall loss to beef farmers in Kenya and Botswana due to *T. saginata* infection was of the order of $2 per head on cattle supplied to the abattoir, and Pawlowski and Schultz (1972) estimated that losses due to cysticercosis in cattle could be as high as $25 per infected animal in developing countries and $75 per animal in industrialized countries. There have also been reports of heavy losses due to infection with *T. saginata* in feedlot cattle (Schultz *et al.*, 1969; McAninch, 1974; Slonka *et al.*, 1975, 1978) or where sewage has been used for irrigating pasture (Rickard and Adolph, 1977a).

The significance of hydatid disease, in particular, is attested to by the many national control programmes which have been implemented in the past (Gemmell and Varela-Díaz, 1980). Recent reviews (Gemmell and MacNamara, 1972; Gemmell, 1976; Gemmell and Johnstone, 1977) have highlighted the role played by acquired immunity in the epidemiology of these diseases. Gemmell (1978) provided elegant flow-chart diagrams showing the factors which influence patterns of dispersion of cestodes, and it is apparent that the successful establishment and survival of larvae in the intermediate host is a key factor in regulating the epizootiology of taeniiasis.

The third aspect, and perhaps the most controversial, is the question of whether or not other methods of control currently available are adequate. Various forms of chemotherapy for adult tapeworm infection in the definitive host have been used for many years, and with the release of praziquantel, there is now a highly effective taeniacide and echinococcocide available. Gemmell (1978) suggested that a parasite like *E. granulosus*, in which the adult worm has limited biotic potential, responds very quickly to standard control measures applied through the definitive host. On the other hand, adult *Taenia* spp. have a very high rate of egg production (Coman and

Rickard, 1975), and experience in New Zealand in the Styx Valley trial (Gemmell, 1978) showed that even after 22 years of strict treatment of dogs, 'epidemic' outbreaks ("cysticercosis storms") occurred. More recently, M. A. Gemmell (personal communication) found that in the Otago-Southland area of New Zealand where 6-weekly dosing with praziquantel is being carried out, cysticercosis storms in sheep were frequent, and eggs from a single focus could infect sheep within an area of 30 000 ha. One of the natural outcomes of a successful control programme is to create a population of highly susceptible 'at risk' intermediate hosts. If a single tapeworm were resistant to the drug being used, and that risk must be increased with a highly efficient compound such as praziquantel, it could create an extensive focus of new infection.

A new factor in the control equation is the advent of drugs effective against the larval stages of tapeworms. None of these compounds has reached an economically practical stage of development, but this seems likely to change in the future. Even were drugs available, a strong case could still be made for research work into the development of vaccines against the larval taeniid cestodes as an adjunct to control, because prophylaxis is preferable to the chemical destruction of established larvae. Of all the parasitic helminths, the natural history of larval cestodes in their mammalian hosts holds out the greatest promise for development of a practical vaccine.

2. *Defining the objectives of vaccination*

It is important to define clearly what the objectives of a vaccination procedure are; for example, is 100% protection essential or will control of reproduction alone be adequate? Urquhart (1980) cites the canine hookworm vaccine developed by Miller (1978) as a classic example of commercial failure of an anti-parasite vaccine despite the fact that it was a very effective tool for preventing 'hookworm disease' in dogs. Its failure was due to factors quite unrelated to this objective and concerned the continued susceptibility of vaccinated dogs to infection rather than disease. With larval cestodes, failure of a vaccine to produce 100% immunity is going to be very visible to the farmer, meat inspector, etc., because the lesions are clear to see in the carcase or viscera, especially with *E. granulosus*. It would certainly be desirable for reasons of acceptance of the product if complete resistance were produced, but this is unlikely to come about in the field.

Experiments on immunization against larval taeniid cestodes have frequently given results close to 100% efficiency against experimental infection in sheep, cattle and rodents (Rickard and Bell, 1971a, b; Wikerhauser *et al.*, 1971; Gaillie and Sewell, 1976; Rickard and Adolph, 1976, 1977b; Heath, 1976; Kwa and Liew, 1977; Ayuya and Williams, 1979; Lloyd, 1979; Rajasekariah *et al.*, 1980a, b; Heath *et al.*, in press; Osborn *et al.*, in press). However,

there have been two recent reports of failure to immunize calves against experimental infection with *T. saginata* using *in vitro* culture antigens (Wikerhauser *et al.*, 1978; Mitchell and Armour, 1980). In the first section of this review, emphasis was placed on factors that influence innate resistance to infection, and this concept can be extended to explain variations in the level of acquired resistance achieved by vaccination. Thus, Rickard *et al.* (1982) showed that there were differences in the efficacy of immunization of male and female calves against naturally acquired infection with *T. saginata*. Variations may occur because of age and strain (breed) of animal as well as differences between individuals. Problems associated with differences in parasite material were also discussed earlier. Factors such as these probably help to explain why, in the few experiments so far conducted in which 'non-viable' parasite material has been used to immunize animals against naturally acquired infection, a much lesser degree of protection has been achieved than in laboratory experiments (Rickard *et al.*, 1976; Rickard *et al.*, 1981a, 1982).

Another question that has to be addressed concerns the level of vaccination efficiency which will yield satisfactory results in terms of parasite control. It might be supposed that if vaccination were to reduce both the numbers of infected animals, as well as the numbers of cysts in those animals, then transmission would be drastically curtailed. However, the situation may be more complicated. Cysticerci of *T. ovis* in the muscle of a lamb (cysts are more likely to be degenerate in old sheep) are much less likely to be ingested by a dog if few are present. On the other hand, mature *E. granulosus* cysts are found principally in older animals, and a single viable cyst can give rise to enormous numbers of adult worms in a dog. The overall effect of reducing the prevalence and intensity of infection in sheep can therefore be undone more quickly.

An attractive role for vaccination would be not only to reduce the numbers of cysts present, but to cause degeneration of any that become established. However, none of the immunization experiments using non-viable oncospheral antigens (Rickard and Bell, 1971a; Rickard and Outteridge, 1974; Lloyd, 1979; Onawunmi and Coles, 1980; Rajasekariah *et al.*, 1980a, b; Osborn *et al.*, in press) has shown much effect on survival of larvae that evade the early immune response and become established in the host. Nevertheless, experiments on immunization by injection of living oncospheres prior to challenge infection (Gemmell, 1970, 1972) have shown that the long-term survival of larvae can be affected. Furthermore, when naturally infected sheep are reinfected, the long-term survival of larvae from the challenge infection is prejudiced (Rickard *et al.*, 1976). It is highly likely that different host responses may be detrimental to larvae at successive stages in their development, and the issue of a retrospective effect of immunization needs further study. When a vaccination programme is initiated on a property,

there will be a large reservoir of infected animals containing viable cysticerci. Thus, unless a 'cocktail' vaccine can be developed that not only prevents infection but also causes death of these cysts, control measures will be prolonged. The suggestion by Harris *et al.* (1980) that chemotherapy of all intermediate hosts may be a useful first step at the commencement of a vaccination programme is likely to assume great importance if suitable effects on established cysts cannot be produced biologically.

Clearly, we do not know at present what is needed in terms of efficacy of vaccination to achieve adequate control in the field. Harris *et al.* (1980) used a computer model to simulate various control strategies. Lack of basic data in many areas makes acceptance of their model difficult at this stage, but studies such as these using appropriate data as it becomes available would be a valuable approach.

3. *Practical considerations for vaccination*

(a) *Protection of the young.* Under conditions of high environmental contamination, young animals can become infected before it is practically feasible to immunize them. Research work demonstrating natural transfer of resistance to young animals via the colostrum of their mothers has been reviewed in Section III. There have been several reports showing that vaccination of sheep and cattle with oncospheral antigens prior to parturition confers passive protection on their offspring (Rickard *et al.*, 1977a, b; Heath, 1978; Lloyd, 1979; Sutton, 1979). The duration of immunity is in the region of 6 to 9 weeks (Rickard and Arundel, 1974; Heath *et al.*, 1979b; Sutton, 1979) and the presence of colostral antibody does not interfere with immunization of the young animal (Rickard *et al.*, 1977a; Heath, 1978). Sutton (1979) vaccinated pregnant ewes from a "*T. ovis*-free" farm with activated oncospheres 1 month prior to parturition and found that immunity was not transferred passively to their lambs. On the other hand, good colostral transfer of immunity was achieved by inoculating pregnant sheep or cattle with oncospheral antigens emulsified in adjuvant (Rickard *et al.*, 1977a, b, 1981a), and in one of these experiments (Rickard *et al.*, 1977b) the sheep were also from a "*T. ovis*-free" farm. Thus, the method of administration of antigen may be critical in obtaining an antibody response of sufficient duration and/or magnitude in 'susceptible' animals to ensure satisfactory transfer of protection to the newborn, and more experimental work is necessary to clarify this important point.

(b) *Duration of immunity.* Annual immunization of animals is generally the most acceptable practical routine. In sheep for instance, the incorporation of larval cestode antigens into the clostridial vaccines used to inoculate ewes prior to parturition and lambs at castration would be an important practical

advantage. The normally transient nature of anti-oncospheral antibody responses in the sera of infected or challenged animals has been demonstrated by testing the lethal effect of immune sera on oncospheres *in vitro* (Rickard and Outteridge, 1974; Heath and Lawerence, 1981) or by measuring the binding of antibody to oncospheral antigens in an enzyme-linked immunosorbent assay (Craig and Rickard, 1981a). Rickard *et al.* (1976) found that sheep which were infected with *T. ovis*, but which had not been exposed to futher infection for at least 5 months, became heavily infected when grazing pasture contaminated with *T. ovis* eggs, although the developing larvae were rapidly killed. When sheep were immunized against *T. hydatigena* infection by the parenteral injection of hatched and activated oncospheres, the level of immunity was waning by 6 months (Gemmell and Johnstone, 1981). On the other hand, immunity in lambs given injections of *T. ovis* oncospheral antigens in incomplete Freund's adjuvant (IFA) persisted for at least 12 months without booster injections (Rickard *et al.*, 1977b). When the lambs were autopsied after the final challenge infection there were still substantial vaccination lesions present at the inoculation site, and this may have helped to prolong the immune response. Rabbits that were immunized against *T. pisiformis* infection by oral inoculation with eggs and termination of the infection with mebendazole therapy retained their immunity for at least 12 months (Heath and Chevis, 1978), but this also could have been due to prolonged antigen release from degenerating larvae.

These studies emphasize what is likely to be an important role for adjuvant in making immunization a practical procedure, and attention should be devoted to testing safe and commercially acceptable adjuvants in future experiments.

(c) *Protection against naturally acquired infection.* Few experiments on immunization of sheep and cattle against infection acquired by grazing on infected pasture have been reported. Lambs immunized with antigens from *T. ovis* oncospheres cultured *in vitro* have been shown to be highly resistant to infection with *T. ovis* when grazed on pasture heavily contaminated with eggs, and lambs grazing lightly contaminated pasture were also highly resistant (Rickard *et al.*, 1976). However, in none of the groups was resistance absolute, and one immunized animal exposed to the higher level of contamination had 27 viable cysticerci at autopsy.

A preliminary field trial on vaccination against infection with *T. saginata* was carried out on a farm which uses sewage effluent to irrigate pasture grazed by cattle (Rickard *et al.*, 1981a). It was shown that vaccination of cows 1 to 2 months prior to calving, and vaccination of their calves at 3 months of age with *T. saginata* culture antigens caused a 78% reduction in infection rate, by comparison with controls, in calves killed and examined at 13 to 14 months of age. A similar vaccination regime using *T. hydatigena* culture antigens, but

with one extra vaccination of calves at 4 months of age, decreased infection by 64%. A more extensive trial, involving groups of 250 cattle each, examined more fully the use of *T. hydatigena* culture antigen to immunize calves against *T. saginata* infection (Rickard *et al.*, 1982). Cows were immunized 1 to 2 months prior to calving, and calves were immunized when 2, 4 and 10 months old. When calves were killed at 20–21 months of age it was found that the vaccination regime had lowered the infection rate by 51% compared with the controls. Not only was the proportion of infected animals reduced, but significant reductions in the mean numbers of cysticerci and the number of animals with multiple infections of organs suggested that the overall level of infection was decreased.

These experiments clearly demonstrate that immunization methods can be applied to field situations, but the level of protection obtained thus far with cross-immunizing antigens is far from satisfactory.

(d) *Supply of antigens.* The most consistent results on immunization have been obtained using oncospheral antigens, but other antigens have also been used. In the *T. taeniaeformis*-rodent system, strobilocercus antigens are highly efficient in immunizing against a challenge infection with eggs (see review by Gemmell and Soulsby, 1968; Kwa and Liew, 1977; Ayuya and Williams, 1979; Rajasekariah *et al.*, 1980b) and 'somatic' antigens have also been used effectively against *T. pisiformis* infection in rabbits (see review by Gemmell and Soulsby, 1968; Heath, 1973a). Gallie and Sewell (1976) were able to immunize calves against *T. saginata* infection using an extensive series of injections of adult tapeworm homogenate emulsified in adjuvant, but this experiment is difficult to interpret because the method described does not preclude the presence of *T. saginata* eggs in the worm material.

Despite the success of immunization experiments, supply of antigen for any large-scale application remains a problem. Dose rates of antigen that have been used to immunize sheep and cattle successfully are equivalent to 20 000 to 30 000 oncospheres per animal (Rickard and Bell, 1971a; Rickard and Adolph, 1976; Rickard and Brumley, 1981). Assuming a figure of 2×10^6 eggs from an adult taeniid (such as *T. saginata*), one tapeworm could be used to immunize 100 animals. *T. solium* and *T. saginata* are parasites of man, and collecting adequate material for large-scale immunization would be impractical. Furthermore, oncospheres of *T. solium* and *E. granulosus* pose problems because of the danger of infection of personnel required to handle them.

Because of these difficulties in availability, extensive investigations have been carried out to produce cross-immunity by innoculating oncospheral antigens from cestodes obtained more easily from animal sources, e.g. *T. ovis* vs *T. hydatigena* vs *E. granulosus* vs *T. pisiformis* in sheep (Gemmell, 1964, 1965, 1969; Rickard and Bell, 1971a; Varela-Díaz *et al.*, 1972; Heath *et al.*, 1979a), *T. ovis*, *T. hydatigena* and *T. taeniaeformis* vs *T. saginata* in cattle

(Wikerhauser *et al.*, 1971; Rickard and Adolph, 1976; Lloyd, 1979; Rickard and Brumley, 1981; Gallie and Sewell, in press), *T. pisiformis* and *T. hydatigena* vs *T. taeniaeformis* in mice (Rickard *et al.*, 1981b) and *T. ovis* and *T. hydatigena* vs *T. pisiformis* in rabbits (Gemmell, 1966; Ermalova *et al.*, 1968; Rickard and Coman, 1977). Experiments on cross-immunity have rarely given results as good as those achieved with homologous antigen preparations, and Rickard *et al.* (1981b) suggested that the greater the innate susceptibility of an animal, or breed of animal, the less likely it is that cross-immunity will give a satisfactory level of protection. Therefore, cross-protection is likely to fail in protecting a number of individuals in an outbred population of animals in the field.

Material derived directly from animal sources is not likely to be an attractive proposition for large-scale antigen preparation. Thus, a satisfactory supply of antigen may ultimately depend on the development of methods such as the *in vitro* culture of organisms or cell-lines, or production of purified antigens by recombinant DNA techniques. The point was made earlier that approximately 20 000 oncospheres were needed to immunize a calf against infection with *T. saginata*. The protein content of these oncospheres is only in the order of 10 μg. It would seem that *in vitro* methods could quite easily provide amounts of antigen adequate for large scale use.

(e) *Storage and shelf life problems.* This is an aspect about which there is no defined experimental information available with respect to larval cestode antigens although it has obvious practical implications, especially if vaccine is to be used in areas where refrigeration and storage facilities are inadequate. Rickard and Brumley (1981) stored *in vitro* culture antigens of *T. saginata* oncospheres homogenized in incomplete Freund's adjuvant for 3 months at 4°C and still obtained significant protection in cattle. Also, *T. taeniaeformis* oncospheres can be frozen, thawed, sonicated, solubilized in sodium deoxycholate and fractionated using high-performance liquid chromatography without losing their biological activity (Rajasekariah *et al.*, 1982). These findings suggest that oncospheral antigens are quite robust.

(f) *Cost-effectiveness.* There is no way at this time in which a value can be placed upon the likely benefits of vaccination, but they are unlikely to be enjoyed directly by the agricultural producers in most situations.

(g) *Practical implementation of vaccination.* If the ideal cheap, safe, effective vaccine were available, the problems would still be far from over. How and where would such a vaccine be used? How would use of the vaccine be encouraged (enforced?)? Is it possible to persuade farmers to use a vaccine, at their expense, to control a public health problem of man? How would a vaccine be applied in underdeveloped countries where there is likely to be the most need? Are there particular situations which lend themselves to use of a vaccine, e.g. feedlot enterprises, sewage irrigation farms, control of 'cysticer-

cosis storms' in the late stages of control programmes, immunization of 'clean' animals entering contaminated areas, etc?

If vaccines are to be produced for use in man, then the ethics of immunization of people presents a major problem. In Mexico where 2% of persons have cysticercosis (Flisser, 1980) vaccination of man may be a justifiable goal, but testing the efficacy and safety of such a vaccine will be very difficult. Perhaps in some circumstances it may be possible to carry out pilot immunization schemes, but human experimentation is an increasingly controversial problem in most countries of the world; vaccination against neurocysticercosis or hydatidosis is not likely to be undertaken without long and weighty discussion of the risks and potential benefits involved.

B. THE NATURE OF ANTIGENS STIMULATING PROTECTIVE IMMUNITY

As discussed in Section VI A, antigens from various stages of larval cestode parasites have been used successfully to immunize animals against infection. However, reports of critical studies on the nature of these antigens are few.

Oncospheres have been a consistent source of potent immunizing antigens. The early work (reviewed by Gemmell and Soulsby, 1968; Gemmell and MacNamara, 1972) strongly suggested that living oncospheres were necessary to stimulate a high level of resistance to infection. Subsequently, a series of publications by Rickard and co-workers (reviewed by Williams, 1979) demonstrated the immunogenicity of antigens collected during *in vitro* cultivation or maintenance of taeniid oncospheres, and more recent reports have corroborated these findings (Lloyd, 1979; Onawunmi and Coles, 1980; Osborn *et al.*, in press). It has now been shown that *in vitro* cultivation of oncospheres is not necessary to collect immunizing ("functional") antigens, and that oncospheres killed by freezing, thawing and ultrasonic disintegration are equally effective (Rajasekariah *et al.*, 1980a, b). Furthermore, sonicated eggs without any prior treatment have been used successfully to immunize mice against *T. taeniaeformis* infection (Rajasekariah *et al.*, 1980b) and lambs against infection with *T. ovis* (Osborn *et al.*, 1981). It appears, therefore, that the functional antigens are present in oncospheres prior to their activation and are not produced *de novo* by metabolically active parasites. All of these experiments were qualitative only, and it is possible that a period of *in vitro* cultivation may increase the yield of antigen obtained from a given number of oncospheres.

When oncospheres are cultured *in vitro* and the supernatant media collected, or when they are disrupted by ultrasonic disintegration, the amount of antigen present in the supernatants is reduced by high-speed centrifugation (Rajasekariah *et al.*, 1980a, b; Rickard and Brumley, 1981). This is persuasive

evidence that the functional antigens in oncospheres are particulate or are bound to membranes, and this would help to explain why purification of soluble antigens by methods such as gel filtration have not been successful (Rickard and Katiyar, 1976). Recent experiments have shown that the oncospheral antigens can be solubilized using sodium deoxycholate (DOC) without losing their biological activity (Rajasekariah *et al.*, 1982), and purification studies using DOC solubilized antigens should now be possible. Rajasekariah *et al.* (1980a, b) speculated that the secretion granules contained in 'blebs' produced from the so-called penetration glands of activated oncospheres may be a source of the particulate functional antigen(s) (Heath and Smyth, 1970; Lethbridge and Gijsbers, 1974; Miyazato *et al.*, 1977). However, in a recent ultrastructural study, Furukawa *et al.* (1981) showed that the antibody-mediated complement-dependent attack on the oncosphere first occurs in the surface membrane and was apparent as early as 8 hours after infection. This result makes it less likely that the secretion granules are the primary source of functional antigen, and previous findings concerning the removal of antigen by high-speed centrifugation of oncosphere supernatants could equally well substantiate a case for the epithelial membrane being directly involved. It is highly likely that more than one source of functional antigen(s) is present and the initial antibody attack on the membrane of the oncosphere may increase its permeability to antibody and expose targets inside it.

The outer membranes of freshly hatched oncospheres of *T. taeniaeformis* (Nieland, 1968), *H. nana* (Furukawa *et al.*, 1977) and *T. ovis* (Fig. 1b) are thrown into a series of folds, but by 24 hours after infection the surface structure undergoes a marked transformation. Long microvillar protrusions develop with a series of electron-lucid vesicles stacked along their distal portion (Fig. 1c) (Engelkirk and Williams, in press, a; Furukawa *et al.*, 1981). It has been suggested that these microvilli may be involved in stimulating protective immunity (Engelkirk and Williams, in press, a), especially as they are actively ingested by the host's phagocytic cells. Antigens which immunize animals are present in oncospheres before they develop microvilli or microvillar vesicles, but that does not necessarily preclude them as a further source of antigen. Heath (1973a, 1976) has presented evidence that antigen from *T. pisiformis* larvae up to 2 weeks old can be used to immunize rabbits against infection.

Many early experiments showed that the injection of homogenates or extracts of parasite material from metacestode stages stimulated a degree of immunity to cysticercosis in rats and rabbits (reviewed by Gemmell and Soulsby, 1968; Flisser *et al.*, 1979). More recently, antigens from homogenates or *in vitro* culture supernatants of strobilocerci of *T. taeniaeformis* have been shown to be highly immunogenic in rats and mice (Kwa and Liew, 1977; Ayuya and Williams, 1979; Rajasekariah *et al.*, 1980b). Kwa and Liew (1977)

described a process for the purification of functional antigen from homogenates and *in vitro* supernatants of *T. taeniaeformis* metacestodes. Using a combination of gel filtration and polyacrylamide-gel electrophoresis, these workers prepared a protein with molecular weight 140 000 daltons which stimulated strong immunity in rats. However, their initial selection of a fraction from Sephadex G200 for further testing was based on its reaction in a double-immunodiffusion technique *in vitro*, and not on *in vivo* immunization assays. Thus, other fractions not detected by the *in vitro* test may also have had immunizing activity. J. F. Williams (unpublished observations) has shown that several gel filtration fractions of *in vitro* culture supernatants from strobilocerci of *T. taeniaeformis* stimulate immunity in the host, and that macromolecular polysaccharide antigens which are not precipitated by trichloracetic acid are also effective immunogens. Similar investigations should be carried out using metacestode stages of ruminant taeniids although with *T. ovis* and *T. saginata* it is difficult to obtain an adequate source of material; *T. hydatigena* could be a useful model for study because metacestodes of this parasite are easier to obtain.

There has been little experimental work which examines the relationship between antigens found in the different stages of larval development. Heath (1973a) concluded that mainly the early stages of *T. pisiformis* larvae stimulated immunity against infection, although this is contrary to evidence in the *T. taeniaeformis*/rodent systems. In some recent experiments, Craig and Rickard (1981a) used 500g supernatants of media collected during *in vitro* incubation of *T. ovis* and *T. hydatigena* oncospheres as antigen in an enzyme-linked immunosorbent assay to measure antibody responses in sheep during primary or challenge infections with these parasites. In both cases, antibody responses were shown to be transient, peaking rapidly and then returning quickly to background levels. On the other hand, with antigens prepared from homogenates of metacestode or adult tapeworm stages the antibody response was of long duration. Oncospheral antigens solubilized in DOC also detected prolonged antibody production (Craig, 1981). On the basis of these findings, Craig and Rickard (1981a) speculated that the oncosphere possesses unique stage-specific antigens not shared by later developmental stages. Although this may be so, an alternative explanation for their data is possible (see Section IV, p. 253).

Clarification of this point may finally depend on isolation and purification of the antigens concerned. In the absence of that, it would be interesting to immunize rodents with oncospheral or strobilocercus antigens and kill them sequentially after challenge to determine whether different stages of the parasites are affected by immune responses to antigens from these two sources.

VII. Prospects and Perspectives

Despite significant increases in the amount of research in helminth immunoparasitology in recent years, on the whole few practical tools have been put into the hands of clinicians, pathologists, epidemiologists and others who directly confront parasitic diseases in man and animals. In cestodiasis, the present-day success of serodiagnostic methods in human hydatidosis represents a remarkable, and very welcome, exception to this rule. Nevertheless, the scene has been set by much of the work described in this review for a period of great expansion of the importance of immunology and immune mechanisms in the prevention, diagnosis and treatment of cysticercosis/hydatidosis in the future. Moreover, taeniid metacestode infections have shown themselves to be worthy models for basic investigations on the immunology of host–parasite relationships which have relevance in the broader context of helminthic diseases in general.

Vaccination, as we have pointed out in Section VI, is likely to become a practical proposition for larval taeniiasis far more quickly than for other parasitic infections; the effectiveness of the protective mechanisms discussed in Section III makes that clear. Nevertheless, some breakthroughs will be needed in areas of antigen purification and production before the potential is realized. Fortunately, technological advances in the last few years on several fronts seem likely to make valuable contributions to this effort. For example, methods for the establishment and maintenance of invertebrate cell lines have improved to the point where the *in vitro* cultivation of antigen-producing cells from cestodes is a realistic goal. Sakamoto (1978) has reported modest success in this direction for *E. multilocularis*. Identifying the antigens that are necessary would be an important step in the selection of cell lines, and here the development of hybridoma technology can be profitably exploited. Monoclonal products can be used to detect and identify cestode antigens (Craig *et al.*, 1980, 1981), and once their protective value in passive immunization studies is established, specific antigen types can be purified by means of monoclonal antibody immunoabsorbent techniques (Craig and Rickard, 1980) and their source in the organism identified using immunoperoxidase labelling methods.

If the antigens prove to be, as currently appears likely, membrane-bound (Rajasekariah *et al.*, 1980a, b, 1982) then contemporary techniques for membrane isolation and characterization can certainly be applied. Cestode tegumental plasma membranes have already been separated successfully and their constituents partially identified (Knowles and Oaks, 1979), and Rajasekariah *et al.* (1982) have been able to solubilize the oncospheral antigens with deoxycholate. It is not too early to imagine that, once the crucial antigens could be specifically identified and purified, bacterial organ-

isms bearing enzymatically inserted parasite gene material could be screened using hybridoma antibody and cloned, if productive. An important determinant of the likelihood of this, of course, is the nature of the protective immunogens; if they are non-proteinaceous to a significant degree, then advances in the recombinant DNA/cloning system itself will be necessary before these steps can be taken.

Monoclonal products promise to have as much impact on diagnosis as they do on immunization, and through their use it seems likely that vastly improved specificity and sensitivity of immunodiagnostic procedures can be achieved. This may enable clinicians to overcome the difficulties of detecting low-level antibody responses in problem cases of cysticercosis and hydatidosis in man. It may also make feasible the specific serologic diagnosis of cysticercosis/hydatidosis ante-mortem in domestic animal hosts (Craig *et al.*, 1980, 1981), and also opens up the possibility of detecting minute quantities of antigen in the circulation or cerebrospinal fluid. This could prove to be a valuable adjunct to diagnosis in man and animals.

Narrowly defined monoclonal antibody probes could be useful in characterizing the so-called "strains" which appear to be responsible for such marked differences in biological behaviour within cestode species in various parts of the world. This may enable researchers to identify more clearly the organisms present in an area and thereby standardize many of the experimental procedures which appear presently to give conflicting results in terms of innate and acquired resistance. It is worth noting here, however, that a potential shortcoming of monoclonal antibodies in all these applications may be their very specificity. Antigens necessary for diagnosis or immunoprophylaxis may need to be more complex than those that can be selected on the basis of a single antigenic determinant, so that batteries of monoclonal antibodies may be needed to select appropriate spectra of antigenic activity.

Innate resistance mechanisms will probably assume greater importance as it becomes increasingly likely that highly selective genetic improvements will be possible in farm livestock through procedures such as embryo transfer and mammalian cell cloning. The development of reliable *in vitro* correlates of innate resistance would clearly be of value in contributing information on, for example, predisposition or resistance to *Echinococcus* infection in the process of genetic improvement of sheep.

In man, where genetic manipulations are unlikely to have any place in infectious disease resistance in the near future, there are, nevertheless, indications that immunological advances could influence therapeutic approaches. Skromne-Kadlubik *et al.* (1977) have shown that intravenously administered, isotopically labelled antibodies can be used as a specific detector system for cranial cysticerci of *T. solium;* subsequent scintilligraphic scanning reveals the radioactive foci caused by parasite-bound antibody.

Appropriately conjugated antibodies against cestode larvae could be used as carriers of chemotherapeutic agents designed to attack the parasite or to ameliorate local inflammatory processes. Again the specificity of hybridoma products and their potential for large-scale production will do much to bring this new approach to fruition.

Obviously the prospects are not only scientifically challenging, but also offer the promise of having a very rewarding and important impact on a group of diseases of global significance. Cysticercosis and hydatidosis are especially prevalent amongst the rural poor of the world and attention to them will be demanded as a critical component of the development process. This is certainly an area where immunoparasitologic practical tools are within our sight and grasp; attaining that goal deserves continued priority in biomedical and agricultural research programmes in many nations.

Acknowledgements

We are grateful to Drs. T. Furukawa, M. A. Gemmell, D. D. Heath, A. Ito, M. M. H. Sewell and R. C. A. Thompson for providing us with unpublished data or manuscripts in press. The unenviable job of preparing the manuscript was carried out expertly by Mrs. Barbara Chambers. We would also like to acknowledge financial support provided for work carried out in our own laboratories by: National Health and Medical Research Council of Australia; Australian Meat Research Committee; Ministry of Water Resources of Victoria; Melbourne and Metropolitan Board of Works; U.S. National Institute of Health, Grant No. AI-10842; Michigan Agricultural Experiment Station.

References

Ali-Khan, Z. (1974a). Host–parasite relationship in *Echinococcosis*. I. Parasite biomass and antibody response in three strains of inbred mice against graded doses of *Echinococcus multilocularis*. *Journal of Parasitology* **60**, 231–235.

Ali-Khan, Z. (1974b). Host–parasite relationship in *Echinococcosis*. II. Cyst weight, haematologic alterations, and gross changes in the spleen and lymph nodes of C57L mice against graded doses of *Echinococcus multilocularis* cysts. *Journal of Parasitology* **60**, 236–242.

Ali-Khan, Z. (1978a). *Echinococcus multilocularis:* cell mediated immune response in early and chronic alveolar murine hydatidosis. *Experimental Parasitology* **46**, 157–165.

Ali-Khan, Z. (1978b). Cellular changes in the lymphoreticular tissues of C57 L/J mice infected with *Echinococcus multilocularis* cysts. *Immunology* **34**, 831–839.

Ali-Khan, Z. (1978c). Pathological changes in the lymphoreticular tissues of Swiss mice infected with *Echinococcus granulosus* cysts. *Zeitschrift für Parasitenkunde* **58**, 47–54.

Ali-Khan, Z. (1979). Humoral response to sheep red blood cells in C57 L/J mice during early and chronic stages of infection with *Echinococcus multilocularis* cysts. *Zeitschrift für Parasitenkunde* **59**, 259–265.

Ali-Khan, Z. and Siboo, R. (1980a). Pathogenesis and host response in subcutaneous alveolar hydatidosis. II. Intense plasmacellular infiltration in the paracortex of draining lymph nodes. *Zeitschrift für Parasitenkunde* **62**, 255–265.

Ali-Khan, Z. and Siboo, R. (1980b). Pathogenesis and host response in subcutaneous alveolar hydatidosis. 1. Histogenesis of alveolar cyst and a qualitative analysis of the inflammatory infiltrates. *Zeitschrift für Parasitenkunde* **62**, 241–254.

Ambu, S. and Kwa, B. H. (1980). Susceptibility of rats to *Taenia taeniaeformis* infection. *Journal of Helminthology* **54**, 43–44.

Anderson, M. J. D. and Griffin, J. F. T. (1979a). *Taenia crassiceps* in the rat. I. Differences in susceptibility to infection and development of immunocompetence in relation to age and host strain. *International Journal for Parasitology* **9**, 229–233.

Anderson, M. J. D. and Griffin, J. F. T. (1979b). *Taenia crassiceps* in the rat. II. Transfer of immunity and immunocompetence with lymph node cells. *International Journal for Parasitology* **9**, 235–239.

Annen, J., Kohler, P., Eckert, J. and Speck, S. (1980). Cytotoxic properties of *Echinococcus granulosus* cyst fluid. *In* "The Host-Invader Interplay" (H. Van den Bossche, ed.), pp. 339–342. Elsevier/North-Holland Biomedical Press, Amsterdam.

Ansari, A. and Williams, J. F. (1976). The eosinophilic response of the rat to infection with *Taenia taeniaeformis*. *Journal of Parasitology* **62**, 728–736.

Ansari, A., Williams, J. F. and Musoke, A. J. (1976). Antibody mediated secondary eosinophilic response to *Taenia taeniaeformis* in the rat. *Journal of Parasitology* **62**, 737–740.

Araj, G. F., Matossian, R. M. and Malakian, A. H. (1977). The host response in secondary hydatidosis of mice. II. Cell mediated immunity. *Zeitschrift für Parasitenkunde* **52**, 31–38.

Arundel, J. H. (1972). A review of cysticercoses of sheep and cattle in Australia. *Australian Veterinary Journal* **48**, 140–155.

Auriault, C., Quaiss, M. A., Torpier, G., Eisen, H. and Capron, A. (1981). Proteolytic cleavage of IgG bound to the Fc receptor of *Schistosoma mansoni* schistosomula. *Parasite Immunology* **3**, 33–44.

Ayuya, J. M. and Williams, J. F. (1979). Immunological response of the rat to infection with *Taenia taeniaeformis*. VII. Oral and parenteral immunization with parasite antigens. *Immunology* **36**, 825–834.

Bailey, W. S. (1951). Host-tissue reactions to initial and superimposed infections with *Hymenolepis nana* var. *fraterna*. *Journal of Parasitology* **37**, 440–444.

Bannerjee, D. and Singh, K. S. (1969). Studies on *Cysticercus fasciolaris*. IV. Immunity to *Cysticercus fasciolaris* in rat. *Indian Journal of Animal Science* **39**, 250–253.

Baron, R. W. and Tanner, C. E. (1976). The effect of immunosuppression on secondary *Echinococcus multilocularis* infections in mice. *International Journal for Parasitology* **6**, 31–42.

Baron, R. W. and Tanner, C. E. (1977). *Echinococcus multilocularis* in the mouse; the *in vitro* protoscolicidal activity of peritoneal macrophages. *International Journal for Parasitology* **7**, 489–495.

Beard, T. C. (1978). Evidence that a hydatid cyst is seldom "as old as the patient". *Lancet* **2**, 30–32.

Ben-Ismail, R., Carme, B., Niel, G. and Gentilini, M. (1980). Non-specific serological reactions with *Echinococcus granulosus* antigens. Role of anti P_1-antibodies. *American Journal of Tropical Medicine and Hygiene* **19**, 239–245.

Bullock, F. D. and Curtis, M. R. (1920). The experimental production of sarcoma of the liver of rats. *Proceedings of the New York Pathological Society* **20,** 149–175.

Bursey, C. C. (1977). "The Biology and Experimental Systematics of Cestodes in the Genus *Taenia* L., 1758 S. Str." Ph.D. Thesis, University of New Brunswick.

Butterworth, A. E. (1977). The eosinophil and its role in immunity to helminth infection. *Current Topics in Microbiology and Immunology* **77,** 127–168.

Butterworth, A. E., Vadis, M. A. and David, J. R. (1980). Mechanisms of eosinophil-mediated helminthotoxicity. *In* "The Eosinophil in Health and Disease" (A. A. F. Mahmoud and K. F. Austen, eds), pp. 253–273. Grune and Stratton, New York.

Campbell, D. H. (1936). Active immunization of albino rats with protein fractions from *Taenia taeniaeformis* and its larval form *Cysticercus fasciolaris. American Journal of Hygiene* **23,** 104–113.

Campbell, D. H. (1938). The specific protective property of serum from rats infected with *Cysticercus crassicollis. Journal of Immunology* **35,** 195–204.

Campbell, D. H. and Melcher, L. R. (1940). Relationship of sex factors to resistance against *Cysticercus crassicollis* in rats. *Journal of Infectious Diseases* **66,** 184–188.

Capron, A., Dessaint, J. P., Capron, M. and Bazin, H. (1975). Specific IgE antibodies in immune adherence of normal macrophages to *Schistosoma mansoni* schistosomules. *Nature* (*London*) **253,** 474–475.

Capron, M., Capron, A., Torpier, G., Bazin, H., Bout, D. and Joseph, M. (1978). Eosinophil-dependent cytotoxicity in rat schistosomiasis. Involvement of IgG_{2a} antibody and the role of mast cells. *European Journal of Immunology* **8,** 127–133.

Chapman, C. B., Knopf, P., Hicks, J. D. and Mitchell, G. F. (1979a). IgG_1 hypergammaglobulinemia in chronic parasitic infections in mice: magnitude of the response in mice infected with various parasites. *Australian Journal of Experimental Biology and Medical Science* **57,** 369–387.

Chapman, C. B., Knopf, P., Anders, R. F. and Mitchell, G. F. (1979b). IgG_1 hypergammaglobulinemia in chronic parasitic infection in mice: evidence that the response reflects chronicity of antigen exposure. *Australian Journal of Experimental Biology and Medical Science* **57,** 389–400.

Chen, H. T. (1950). The *in vitro* action of rat immune serum on the larvae of *Taenia taeniaeformis. Journal of Infectious Diseases* **86,** 205–213.

Chernin, J. (1975). The growth of the metacestodes of *Taenia crassiceps* in white mice. *Journal of Helminthology* **49,** 297–300.

Chordi, A. and Kagan, I. G. (1965). Identification and characterization of antigenic components of sheep hydatid fluid by immunoelectrophoresis. *Journal of Parasitology* **51,** 63–71.

Clegg, J. A. and Smith, M. A. (1978). Prospects for the development of dead vaccines against helminths. *Advances in Parasitology* **16,** 165–218.

Coleman, E. J. and De Salva, J. J. (1963). Mast cell responses to cestode infection. *Proceedings of the Society for Experimental Biology and Medicine* **112,** 432–434.

Coltorti, E. A. and Varela-Díaz, V. M. (1972). IgG levels and host specificity in hydatid cyst fluid. *Journal of Parasitology* **58,** 753–756.

Coman, B. J. and Rickard, M. D. (1975). The location of *Taenia pisiformis, Taenia ovis* and *Taenia hydatigena* in the gut of the dog and its effect on net environmental contamination with ova. *Zeitschrift für Parasitenkunde* **47,** 237–248.

Cook, R. W. (1979). "Pathology of *Taenia taeniaeformis* infection in the rat". Ph.D. Thesis, Michigan State University.

Cook, R. W. and Williams, J. F. (1981). Pathology of *Taenia taeniaeformis* infection in the rat: gastrointestinal changes. *Journal of Comparative Pathology,* **91,** 205–217.

Cook, R. W., Williams, J. F. and Lichtenberger, L. M. (1981). Hyperplastic gastropathy in the rat due to *Taenia taeniaeformis* infection: parabiotic transfer and hypergastrinemia. *Gastroenterology* **80,** 728–734.

Cossey, A., Dimichiel, A. and Dunstone, J. (1979). The use of equilibrium density-gradient ultracentrifugation in the isolation and characterisation of glycoproteins with blood group P1 activity from sheep hydatid cyst fluid. *European Journal of Biochemistry* **98,** 53–60.

Craig, P. S. (1981). "Studies on the serological diagnosis of infection with larval taeniid cestodes in sheep and cattle". Ph.D. Thesis, University of Melbourne.

Craig, P. S. and Rickard, M. D. (1981a). Anti-oncospheral antibodies in the serum of lambs experimentally infected with either *Taenia ovis* or *Taenia hydatigena*. *Zeitschrift für Parasitenkunde* **64,** 169–177.

Craig, P. S. and Rickard, M. D. (1981b). Studies on the specific immunodiagnosis of larval cestode infections of cattle and sheep using antigens purified by affinity chromatography in an enzyme-linked immunosorbent assay (ELISA). *International Journal for Parasitology* **11,** 441–449.

Craig, P. S., Mitchell, G. F., Cruise, K. M. and Rickard, M. D. (1980). Hybridoma antibody immunoassays for the detection of parasitic infection: attempts to produce an immunodiagnostic reagent for a larval taeniid cestode infection. *Australian Journal of Experimental Biology and Medical Science* **58,** 339–350.

Craig, P. S., Hocking, R. E., Mitchell, G. F. and Rickard, M. D. (1981). Murine hybridoma-derived antibodies in the processing of antigens for the immunodiagnosis of hydatid (*Echinococcus granulosus*) infection in sheep. *Parasitology* **83,** 303–317.

Curtis, M. R., Dunning, W. F. and Bullock, F. D. (1933). Genetic factors in relation to the etiology of malignant tumours. *American Journal of Cancer* **17,** 894–923.

Damian, R. T. (1964). Molecular mimicry: Antigen sharing by parasite and host and its consequences. *American Naturalist* **98,** 129–149.

Damian, R. T. (1979). Molecular mimicry in biological adaptation. *In* "Host Parasite Interfaces" (B. B. Nickel, ed.), pp. 103–126. Academic Press, New York.

Deenchin, P., Milenkov, K. H., Tevziev, G. and Tashkova, M. (1976). Hydatidosis in autopsy data. *S'Vremennaya Meditsina* **27,** 28–32.

Dessaint, J. P., Bout, D., Wattre, P. and Capron, A. (1975). Quantitative determination of specific IgE antibodies to *Echinococcus granulosus* and IgE levels in sera from patients with hydatid disease. *Immunology* **29,** 813–823.

Dixon, J. B., Jenkins, P., Allan, D. and Connor, R. J. (1978). Blastic stimulation of unprimed mouse lymphocytes by living protoscolices of *Echinococcus granulosus:* a possible connection with transplant immunity. *Journal of Parasitology* **64,** 949–950.

Dow, C. and Jarrett, W. F. H. (1960). Age, strain and sex differences in susceptibility to *Cysticercus fasciolaris* in the mouse. *Experimental Parasitology* **10,** 72–74.

Dumon, H., Veruloet, D. and Quilici, M. (1976). Adherence *in vitro* des leukocytes circulants humains aux scolex d'*Echinococcus granulosus*. *Compte rendu des séances de la Société de biologie* **170,** 639–641.

Eisen, H. and Tallan, I. (1977). *Tetrahymena pyriformis* recovers from antibody immobilisation producing univalent antibody fragments. *Nature (London)* **270,** 514.

Engelkirk, P. G. (1980). "Ultrastructural and immunological studies on the development of *Taenia taeniaeformis* in the rat liver". Ph.D. Thesis, Michigan State University.

Engelkirk, P. G. and Williams, J. F. (In press, a). *Taenia taeniaeformis* in the rat: ultrastructure of the host–parasite interface on days 1–7 post infection. *Journal of Parasitology.*

Engelkirk, P. G. and Williams, J. F. (In press, b). *Taenia taeniaeformis* in the rat: ultrastructure of the host–parasite interface on days 8–22 post infection. *Journal of Parasitology*.

Engelkirk, P. G., Williams, J. F. and Signs, M. M. (1981). Interactions between *Taenia taeniaeformis* and host cells *in vitro*. I. Rapid adherence of peritoneal cells to strobilocerci. *International Journal for Parasitology* **11**, 463–474.

Ermalova, Y. N., Moiseyenko, K. G., Balandin, E. G. and Ivershin, Y. M. (1968). Immunological and pathological changes in rabbits infected with *Taenia hydatigena* eggs. *Trudy Kazakhskogo Nauchno-Issledovatel'skogo Veterinarnogo Instituta USSR* **13**, 104–108.

Esch, G. W. (1967). Some effects of cortisone and sex on the biology of coenuriasis in laboratory mice and jackrabbits. *Parasitology* **57**, 175–179.

Feizi, T. and Kabat, E. A. (1972). Immunochemical studies on blood groups. LIV. Classification of anti-I and anti-i sera into groups based on reactivity patterns with various antigens related to the blood groups A, B, H, Le^a, Le^b and precursor substances. *Journal of Experimental Medicine* **135**, 1247–1258.

Flisser, A. (1980). The immunology of human cysticercosis. *In* "Molecules, Cells and Parasites in Immunology" (C. Larralde, K. Willms, L. Ortiz-Ortiz, M. Sela, eds), pp. 125–143. Academic Press, New York.

Flisser, A., Pérez-Montfort, R. and Larralde, C. (1979). The immunology of human and animal cysticercosis: a review. *Bulletin of the World Health Organization* **57**, 839–856.

Flisser, A., Woodhouse, E. and Larralde, C. (1980). Human cysticercosis: antigens, antibodies and non-responders. *Clinical and Experimental Immunology* **39**, 27–37.

Freeman, R. S. (1962). Studies on the biology of *Taenia crassiceps* (Zeder, 1800) Rudolpi 1810 (Cestoda). *Canadian Journal of Zoology* **40**, 969–990.

Friedberg, W., Neas, B. R., Faulkner, D. N. and Congdon, C. C. (1979). *Hymenolepis nana:* Intestinal tissue phase in actively immunized mice. *Journal of Parasitology* **65**, 61–64.

Froyd, G. (1960). Cysticercosis and hydatid disease of cattle in Kenya. *Journal of Parasitology* **46**, 491–496.

Froyd, G. and Round, M. C. (1960). The artificial infection of adult cattle with *Cysticercus bovis*. *Research in Veterinary Science* **1**, 275–282.

Furukawa, T. (1974). Adherence reactions with mouse lymphoid cells against the oncosphere larvae of *Hymenolepis nana*. *Japanese Journal of Parasitology* **23**, 236–249.

Furukawa, T., Miyazato, T., Okamoto, K. and Nakai, Y. (1977). The fine structure of the hatched oncospheres of *Hymenolepis nana*. *Japanese Journal of Parasitology* **26**, 49–62.

Furukawa, T., Niwa, A. and Miyazato, T. (1981). Ultrastructural aspects of immune damage to *Hymenolepis nana* oncospheres in mice. *International Journal for Parasitology* **11**, 287–300.

Gallie, G. J. and Sewell, M. M. H. (1976). Experimental immunization of six-month old calves against infection with the cysticercus stage of *Taenia saginata*. *Tropical Animal Health and Production* **8**, 233–242.

Gallie, G. J. and Sewell, M. M. H. (In press). Attempted immunization of calves with heterologous species against infection with the cysticercus stage of *Taenia saginata*. *Tropical Animal Health and Production*.

Gemmell, M. A. (1964). Immunological responses of the mammalian host against tapeworm infections 1. Species specificity of hexacanth embryos in protecting sheep against *Taenia hydatigena*. *Immunology* **7**, 489–499.

Gemmell, M. A. (1965). Immunological responses of the mammalian host against tapeworm infections III. Species specificity of hexacanth embryos in protecting sheep against *Taenia ovis*. *Immunology* **8**, 281–290.

Gemmell, M. A. (1966). Immunological responses of the mammalian host against tapeworm infections IV. Species specificity of hexacanth embryos in protecting rabbits against *Taenia pisiformis*. *Immunology* **8**, 270–280.

Gemmell, M. A. (1969). Immunological responses of the mammalian host against tapeworm infections XI. Antigen sharing among *Taenia pisiformis*, *T. hydatigena* and *T. ovis*. *Experimental Parasitology* **26**, 67–72.

Gemmell, M. A. (1970). Hydatidosis and cysticercosis. 3. Induced resistance to the larval phase. *Australian Veterinary Journal* **46**, 366–369.

Gemmell, M. A. (1972). Some problems of inducing resistance to *Taenia hydatigena* under conditions of a strong infection pressure. *Australian Veterinary Journal* **48**, 29–31.

Gemmell, M. A. (1976). Immunological responses and regulation of the cestode zoonoses. *In* "Immunology of Human Parasitic Infections" (S. Cohen and E. Sadun, eds), pp. 333–358. Blackwell Scientific Publications, Oxford.

Gemmell, M. A. (1978). Perspectives on options for hydatidosis and cysticercosis control. *Veterinary Medical Review* **1**, 3–48.

Gemmell, M. A. and Johnstone, P. D. (1977). Experimental epidemiology of hydatidosis and cysticercosis. *Advances in Parasitology* **15**, 311–369.

Gemmell, M. A. and Johnstone, P. D. (1981). Factors regulating tapeworm populations: estimations of the duration of acquired immunity by sheep to *Taenia hydatigena*. *Research in Veterinary Science* **30**, 53–56.

Gemmell, M. A. and Macnamara, F. N. (1972). Immune responses to tissue parasites II. Cestodes. *In* "Immunity to Animal Parasites" (E. J. L. Soulsby, ed.), pp. 235–272. Academic Press, London and New York.

Gemmell, M. A. and Soulsby, E. J. L. (1968). The development of acquired immunity to tapeworms and progress towards active immunization, with special reference to *Echinococcus* spp. *Bulletin of the World Health Organization* **39**, 45–55.

Gemmell, M. A. and Varela-Díaz, V. M. (1980). Review of programs for the control of hydatidosis/cysticercosis. *Series of Scientific and Technical Monographs, No. C.P.Z. 8*, 99 pp. PAHO/WHO, Ramos Mejia, Argentina.

Gerez-Greiser, C. (1961). "Meningitis aséptica en cisticercosis cerebrale". Thesis, Facultad de Medicina, Universidad de Mexico. Cited by Biagi, F. and Willms, K. (1974) *Annales de Parasitologie Humaine et Comparée* **49**, 509–513.

Ginsberg, A., Cammeron, J., Goddard, W. B. and Grieve, J. M. (1956). Bovine cysticercosis with particular reference to East Africa. *East Africa Medical Journal* **33**, 495–505.

Glynn, A. A. (1972). Bacterial factors inhibiting host defence mechanisms. *Symposia of the Society of General Microbiology* **22**, 75–112.

Goetzl, E. J. and Austen, K. F. A. (1977). Cellular characteristics of the eosinophil compatible with a dual role in host defence in parasitic infections. *American Journal of Tropical Medicine and Hygiene* **26**, 142–150.

Goldring, O. L., Clegg, J. A., Smithers, S. R. and Terry, R. J. (1976). Acquisition of human blood group antigens by *Schistosoma mansoni*. *Clinical and Experimental Immunology* **26**, 181–187.

Good, A. H. and Miller, K. L. (1976). Depression of the immune response to sheep erythrocytes in mice infected with *Taenia crassiceps* larvae. *Infection and Immunity* **14**, 449–456.

Greenfield, S. H. (1942). Age resistance of the albino rat to *Cysticercus fasciolaris*. *Journal of Parasitology* **28**, 207–211.

Grills, C. C. and Hammerberg, B. (1979). Inhibition of leukocyte lysosomal enzymes by surface extracts of larval *Taenia taeniaeformis*. Abstract in *Proceedings of the 54th Annual Meeting of the American Society for Parasitology, Minneapolis, Minnesota*, p. 32.

Grindle, R. J. (1978). Economic losses resulting from bovine cysticercosis with special reference to Botswana and Kenya. *Tropical Animal Health and Production* **10**, 127–140.

Hammerberg, B. and Williams, J. F. (1978a). Interaction between *Taenia taeniaeformis* and the complement system. *Journal of Immunology* **120**, 1033–1037.

Hammerberg, B. and Williams, J. F. (1978b). Physico-chemical characterization of complement-interacting factors from *Taenia taeniaeformis*. *Journal of Immunology* **120**, 1039–1045.

Hammerberg, B., Musoke, A. J., Hunstead, S. T. and Williams, J. F. (1976). Anticomplementary substances associated with taeniid metacestodes. *In* "Pathophysiology of Parasitic Infection" (E. J. L. Soulsby, ed.), pp. 233–240. Academic Press, New York and London.

Hammerberg, B., Musoke, A. J. and Williams, J. F. (1977a). Activation of complement by hydatid cyst fluid of *Echinococcus granulosus*. *Journal of Parasitology* **63**, 327–331.

Hammerberg, B., Musoke, A. J., Williams, J. F. and Leid, R. W. (1977b). Uptake of colostral immunoglobulins by the suckling rat. *Laboratory Animal Science* **27**, 50–53.

Hammerberg, B., Dangler, C. and Williams, J. F. (1980). *Taenia taeniaeformis:* Chemical composition of parasite factors affecting coagulation and complement cascades. *Journal of Parasitology* **66**, 569–576.

Harris, R. E., Revfeim, K. J. A. and Heath, D. D. (1980). Simulating strategies for control of *Echinococcus granulosus*, *Taenia hydatigena* and *T. ovis*. *Journal of Hygiene, Cambridge* **84**, 389–404.

Heath, D. D. (1970). "The developmental biology of larval cyclophyllidean cestodes in mammals". Ph.D. Thesis, Australian National University.

Heath, D. D. (1971). The migration of oncospheres of *Taenia pisiformis*, *T. serialis* and *Echinococcus granulosus* within the intermediate host. *International Journal for Parasitology* **1**, 145–152.

Heath, D. D. (1973a). Resistance to *Taenia pisiformis* larvae in rabbits I. Examination of the antigenically protective phase of larval development. *International Journal for Parasitology* **3**, 485–489.

Heath, D. D. (1973b). Resistance to *Taenia pisiformis* larvae in rabbits II. Temporal relationships and the development phase affected. *International Journal for Parasitology* **3**, 491–498.

Heath, D. D. (1973c). An improved technique for the *in vitro* culture of taeniid larvae. *International Journal for Parasitology* **3**, 481–484.

Heath, D. D. (1976). Resistance to *Taenia pisiformis* larvae in rabbits: Immunization against infection using non-living antigens from *in vitro* culture. *International Journal for Parasitology* **6**, 19–24.

Heath, D. D. (1978). Immunization of neonatal lambs against the larvae of *Taenia hydatigena*, using viable eggs followed by chemotherapy. *Veterinary Parasitology* **4**, 11–19.

Heath, D. D. and Chevis, R. A. F. (1978). Duration of immunity to *Taenia pisiformis* larvae in rabbits. *Journal of Parasitology* **64**, 252.

Heath, D. D. and Elsdon-Dew, R. (1972). The *in vitro* culture of *Taenia saginata* and *Taenia taeniaeformis* larvae from the oncosphere, with observations on the role of serum for *in vitro* culture of larval cestodes. *International Journal for* Parasitology **2**, 119–130.

Heath, D. D. and Lawrence, S. B. (1976). *Echinococcus granulosus:* development *in vitro* from oncosphere to immature hydatid cyst. *Parasitology* **73**, 417–423.

Heath, D. D. and Lawrence, S. B. (1981). *Echinococcus granulosus* cysts: Early development *in vitro* in the presence of serum from infected sheep. *International Journal for Parasitology* **11**, 261–266.

Heath, D. D. and Pavloff, P. (1975). The fate of *Taenia taeniaeformis* oncospheres in normal and passively protected rats. *International Journal for Parasitology* **5**, 83–88.

Heath, D. D. and Smyth, J. D. (1970). *In vitro* cultivation of *Echinococcus granulosus, Taenia hydatigena, T. ovis, T. pisiformis* and *T. serialis* from oncosphere to cystic larva. *Parasitology* **61**, 329–343.

Heath, D. D., Lawrence, S. B. and Yong, W. K. (1979a). Cross-protection between the cysts of *Echinococcus granulosus, Taenia hydatigena* and *T. ovis* in lambs. *Research in Veterinary Science* **27**, 210–212.

Heath, D. D., Yong, W. K., Osborn, P. J., Parmeter, S. N., Lawrence, S. B. and Twaalfhoven, H. (1979b). The duration of passive protection against *Taenia ovis* larvae in lambs. *Parasitology* **79**, 177–182.

Heath, D. D., Parmeter, S. N., Osborn, P. J. and Lawrence, S. B. (in press). *Journal of Parasitology.* Resistance to *Echinococcus granulosus* infection in lambs.

Herd, R. P. (1976). The cestocidal effect of complement in normal and immune sera *in vitro. Parasitology* **72**, 325–334.

Howkins, A. B. (1975). The control of cysticercosis in New South Wales. *Science and Technology* **13**, 11–14.

Hustead, S. T. and Williams, J. F. (1977a). Permeability studies on taeniid metacestodes: I. Uptake of proteins by larval stages of *Taenia taeniaeformis, T. crassiceps* and *Echinococcus granulosus. Journal of Parasitology* **63**, 314–321.

Hustead, S. T. and Williams, J. F. (1977b). Permeability studies on taeniid metacestodes: II. Antibody-mediated effects on membrane permeability in larvae of *Taenia taeniaeformis* and *Taenia crassiceps. Journal of Parasitology* **63**, 322–326.

Ingram, D. G. and Smith, A. N. (1965). Immunological responses of young animals. I. Review of the literature. *Canadian Veterinary Journal* **61**, 194–203.

Ito, A. (1980). *Hymenolepis nana:* Survival in the immunized mouse. *Experimental Parasitology* **49**, 248–257.

Ito, A. (In press a). *Hymenolepis nana:* Immunogenicity of a lumen phase of the direct cycle and failure of autoinfection in BALB/c mice. *Experimental Parasitology.*

Ito, A. (In press b). Comparison of the kinetics of infection with *Hymenolepis nana* between BALB/c and dd strains of mice. *Japanese Journal of Parasitology.*

Jarrett, E. E. E. (1973). Reaginic antibodies and helminth infection. *Veterinary Record* **93**, 480–483.

Kamiya, H. (1972). Studies on *Echinococcus.* XXIV. Age differences in resistance to infection with *Echinococcus multilocularis* in AKR strain of mouse. *Japanese Journal of Veterinary Research* **20**, 69.

Kammerer, W. (1979). Case 25—1979. Medical treatment of echinococcosis. *New England Journal of Medicine* **301**, 727.

Kassis, A. I. and Tanner, C. E. (1976). The role of complement in hydatid disease: *in vitro* studies. *International Journal for Parasitology* **6**, 25–35.

Kassis, A. I. and Tanner, C. E. (1977). Host serum proteins in *Echinococcus multilocularis:* complement activation via the classical pathway. *Immunology* **33,** 1–9.

Kemp, W. M., Brown, P. R., Merritt, S. C. and Miller, R. E. (1980). Tegument associated antigen modulation by adult male *Schistosoma mansoni. Journal of Immunology* **124,** 806–811.

Kern, P., Dietrich, M. and Volkmer, K. J. (1979). Chemotherapy of *Echinococcus* with mebendazole—clinical observations of seven patients. *Tropenmedizin und Parasitologie* **30,** 65–72.

Knowles, W. J. and Oaks, J. A. (1979). Isolation and partial biochemical characterization of the brush border plasma membrane from the cestode *Hymenolepis diminuta. Journal of Parasitology* **65,** 715–731.

Kowalski, J. C. and Thorson, R. E. (1972). Protective immunity against tetrathyridia of *Mesocestoides corti* by passive transfer of serum in mice. *Journal of Parasitology* **58,** 244–246.

Kwa, B. H. and Liew, F. Y. (1975). The role of cell-mediated immunity in *Taenia taeniaeformis* infections. *South East Asian Journal of Tropical Medicine and Public Health* **6,** 483–487.

Kwa, B. H. and Liew, F. Y. (1977). Immunity in taeniasis-cysticercosis. I. Vaccination against *Taenia taeniaeformis* in rats using purified antigen. *Journal of Experimental Medicine* **146,** 118–131.

Kwa, B. H. and Liew, F. Y. (1978). Studies on the mechanism of long-term survival of *Taenia taeniaeformis* in rats. *Journal of Helminthology* **52,** 1–6.

Lawrence, S. B., Heath, D. D., Parmeter, S. N. and Osborn, P. J. (1980). Development of early larval stages of *Taenia ovis in vitro* using a cell monolayer. *Parasitology* **81,** 35–40.

Leid, R. W. (1977). Immunity to the metacestode of *Taenia taeniaeformis* in the laboratory rat. *American Journal of Tropical Medicine and Hygiene* **26,** 54–60.

Leid, R. W. and Williams, J. F. (1974a). The immunological response of the rat to infection with *Taenia taeniaeformis* I. Immunoglobulin classes involved in passive transfer of resistance. *Immunology* **27,** 195–208.

Leid, R. W. and Williams, J. F. (1974b). The immunological response of the rat to infection with *Taenia taeniaeformis.* II. Characterization of reaginic antibody and an allergen associated with the larval stage. *Immunology* **27,** 209–226.

Leid, R. W. and Williams, J. F. (1975). Reaginic antibody response in rabbits infected with *Taenia pisiformis. International Journal for Parasitology* **5,** 203–208.

Leid, R. W. and Williams, J. F. (1979). Helminth parasites and the host inflammatory system. *Chemical Zoology* **11,** 229–271.

Leonard, A. B. and Leonard, A. E. (1941). The intestinal phase of the resistance of rabbits to the larvae of *Taenia pisiformis. Journal of Parasitology* **27,** 375–378.

Lethbridge, R. C. and Gijsbers, M. F. (1974). Penetration gland secretion by hexacanths of *Hymenolepis diminuta. Parasitology* **68,** 303–311.

Lindsay, M. (1981). "The localisation and identification of antibody-forming cells in the tissues of rats infected with *Taenia taeniaeformis*". M. S. Thesis, Michigan State University.

Lloyd, S. (1979). Homologous and heterologous immunization against the metacestodes of *Taenia saginata* and *Taenia taeniaeformis* in cattle and mice. *Zeitschrift für Parasitenkunde* **60,** 87–96.

Lloyd, S. and Soulsby, E. J. L. (1976). Passive transfer of immunity to neonatal calves against the metacestodes of *Taenia saginata. Veterinary Parasitology* **2,** 355–362.

Lloyd, S. and Soulsby, E. J. L. (1978). The role of IgA immunoglobulins in the passive transfer of protection to *Taenia taeniaeformis* in the mouse. *Immunology* **34**, 939–945.

Lubinsky, G. (1964). Growth of the vegetatively propagated strain of larval *Echinococcus multilocularis* in some strains of Jackson mice and in their hybrids. *Canadian Journal of Zoology* **42**, 1099–1103.

Lumsden, R. D. (1975). Surface ultrastructure and cytochemistry of parasitic helminths. *Experimental Parasitology* **37**, 267–339.

Marler, J. M. (1978). Antígeno intradermico para diagnóstico de cysticercose. *Revista Saude Publica* **12**, 23–25.

Marquez-Monter, H. (1971). Cysticercosis. *In* "Pathology of Protozoal and Helminthic Diseases" (R. A. Marcial-Rojas, ed.), pp. 592–617. Williams and Wilkins, Baltimore, U.S.A.

Matossian, R. M., Rickard, M. D. and Smyth, J. D. (1977). Hydatidosis: A global problem of increasing importance. *Bulletin of the World Health Organization* **55**, 499–501.

Matskazi, J. and Juhász, S. (1977). *Ligula intestinalis* (L. 1785): investigation of plerocercoids and adults for protease and protease inhibitor activities. *Parasitologia Hungarica* **10**, 51–60.

McAninch, N. H. (1974). An outbreak of cysticercosis in feedlot cattle. *Canadian Veterinary Journal* **15**, 120–122.

Miller, T. A. (1978). Industrial development and field use of the canine hookworm vaccine. *Advances in Parasitology* **16**, 333–342.

Miller, K. L., Good, A. H. and Mishell, R. I. (1978). Immunodepression in *Taenia crassiceps* infection: restoration of the *in vitro* response to sheep erythrocytes by activated peritoneal cells. *Infection and Immunity* **22**, 365–370.

Mills, G. L., Taylor, D. C. and Williams, J. F. (1981a). Lipid composition of metacestodes of *Taenia crassiceps*. *Comparative Physiology and Biochemistry* **69B**, 553–557.

Mills, G. L., Taylor, D. C. and Williams, J. F. (1981b). Lipid composition and changes during development of larvae of *Taenia taeniaeformis*. *Molecular and Biochemical Parasitology* **3**, 301–318.

Mitchell, G. F. (1979). Effector cells, molecules and mechanisms in host-protective immunity to parasites. *Immunology* **38**, 209–223.

Mitchell, G. B. B. and Armour, J. (1980). Failure to protect calves against *Taenia saginata* using antigens prepared from *in vitro* cultivation of the larval stage. *Research in Veterinary Science* **29**, 373–377.

Mitchell, G. F., Goding, J. W. and Rickard, M. D. (1977a). Studies on the immune response to larval cestodes in mice. I. Increased susceptibility of certain mouse strains and hypothymic mice to *Taenia taeniaeformis* and analysis of passive transfer of resistance with serum. *Australian Journal of Experimental Biology and Medical Science* **55**, 165–186.

Mitchell, G. F., Marchalonis, J. J., Smith, P. M., Nicholas, W. L. and Warner, N. L. (1977b). Studies on immune responses to larval cestodes in mice: Immunoglobulins associated with the larvae of *Mesocestoides corti*. *Australian Journal of Experimental Biology and Medical Science* **55**, 187–211.

Mitchell, G. F., Rajasekariah, G. R. and Rickard, M. D. (1980). A mechanism to account for mouse strain variation in resistance to the larval cestode, *Taenia taeniaeformis*. *Immunology* **39**, 481–489.

Miyazato, T., Furukawa, T., Inoue, M., Niwa, A., Inoue, T. and Shimoda, K. (1977). Electron microscopic observations on the penetration of oncospheres of *Hymenolepis nana* into the intestine of the mouse. *Acta Medica Kinki University* **2**, 1–18.

Miyazato, T., Furukawa, T. and Inoue, T. (1979). Intestinal pathology associated with primary and secondary infections of *Hymenolepis nana* in mice. *Japanese Journal of Parasitology* **28**, 185–195.

Moreira, W. S., Santos, A. F. Dos and Oliveira, Q. C. (1978). A intradermo-reacão de Casoni no diagnostico da hidatidose de bovinos. *Revista do Centro De Ciencias Rurais* **8**, 97–103.

Murrell, K. D. (1971). The effect of antibody on the permeability control of larval *Taenia taeniaeformis*. *Journal of Parasitology* **57**, 875–880.

Musoke, A. J. and Williams, J. F. (1975a). The immunological response of the rat to infection with *Taenia taeniaeformis*. V. Sequence of appearance of protective immunoglobulins and the mechanism of action of 7Sγ2a antibodies. *Immunology* **29**, 855–866.

Musoke, A. J. and Williams, J. F. (1975b). Immunoglobulins associated with passive transfer of resistance to *Taenia taeniaeformis* in the mouse. *Immunology* **28**, 97–102.

Musoke, A. J. and Williams, J. F. (1976). Immunological response of the rat to infection with *Taenia taeniaeformis:* protective antibody response to implanted parasites. *International Journal for Parasitology* **6**, 265–269.

Musoke, A. J., Williams, J. F., Leid, R. W. and Williams, C. S. F. (1975). The immunological response of the rat to infection with *Taenia taeniaeformis*. IV. Immunoglobulins involved in passive transfer of resistance from mother to offspring. *Immunology* **29**, 845–853.

Musoke, A. J., Williams, J. F. and Leid, R. W. (1978). Immunological response of the rat to infection with *Taenia taeniaeformis:* VI. The role of immediate hypersensitivity in resistance to reinfection. *Immunology* **34**, 565–570.

Németh, I. (1970). Immunological study of rabbit cysticercosis. II. Transfer of immunity to *Cysticercus pisiformis* (Bloch, 1780) with parenterally administered immune serum or lymphoid cells. *Acta Veterinaria Academiae Scientarium Hungaricae* **20**, 69–79.

Németh, I. and Juhász, S. (1980). A trypsin and chymotrypsin inhibitor from the metacestodes of *Taenia pisiformis*. *Parasitology* **80**, 433–446.

Niederkorn, J. Y. (1977). Immunization of rats against *Mesocestoides corti* (Cestoda) by a subcutaneous vaccination of living tetrathyridia and by passive transfer with serum. *Proceedings of the Arkansas Academy of Science* **31**, 79–80.

Nieland, M. L. (1968). Electron microscopic observations on the egg of *Taenia taeniaeformis*. *Journal of Parasitology* **54**, 957–969.

Oaks, J. A. and Lumsden, R. D. (1971). Cytological studies on the absorptive surfaces of cestodes. V. Incorporation of carbohydrate-containing macromolecules into tegument membranes. *Journal of Parasitology* **57**, 1256–1268.

Okamoto, K. (1968). Effect of neonatal thymectomy on acquired resistance to *Hymenolepis nana* in mice. *Japanese Journal of Parasitology* **17**, 53–59.

Okamoto, K. and Koizumi, M. (1972). *Hymenolepis nana:* effect of antithymocyte serum on acquired immunity in mice. *Experimental Parasitology* **32**, 56–61.

Olivier, L. (1962a). Natural resistance to *Taenia taeniaeformis* I. Strain differences in susceptibility of rodents. *Journal of Parasitology* **48**, 373–378.

Olivier, L. (1962b). Studies on natural resistance to *Taenia taeniaeformis* in mice. II. The effect of cortisone. *Journal of Parasitology* **48**, 758–762.

Onawunmi, O. A. and Coles, G. C. (1980). Attempts to immunise sheep with culture antigens of *Taenia hydatigena*. *Research in Veterinary Science* **29**, 122–123.

Orihara, M. (1962). Studies on *Cysticercus fasciolaris*, especially on differences of susceptibility among uniform strains of the mouse. *Japanese Journal of Veterinary Research* **10**, 37–56.

Osborn, P. J., Heath, D. D. and Parmeter, S. N. (1981). Immunization of lambs against infection with *Taenia ovis* using an extract of *T. ovis* eggs. *Research in Veterinary Science* **31**, 90–92

Osborn, P. J., Heath, D. D. and Roberts, M. G. (In press). Vaccination of sheep against *Taenia ovis*—The respnse to various dose rates of antigens obtained by incubation of oncospheres *in vitro*. *Research in Veterinary Science*.

Pappas, P. W. and Read, C. P. (1972). Trypsin inactivation by intact *Hymenolepis diminuta*. *Journal of Parasitology* **58**, 864–871.

Pawlowski, Z. and Schultz, M. G. (1972). Taeniasis and cysticercosis. *Advances in Parasitology* **10**, 269–343.

Peel, C. (1953). Apparent acquired immunity to *Cysticercus bovis* in certain age groups of N'dama cattle of Sierra Leone. *Veterinary Record* **65**, 244–247.

Penfold, H. B. (1937). The life history of *Cysticercus bovis* in the tissues of the ox. *Medical Journal of Australia* **1**, 579–583.

Pennoit-De Cooman, E. and De Rycke, P. H. (1970). Experimental secondary echinococcosis of *Echinococcus granulosus* I. Development in different strains of mice. *Zeitschrift für Parasitenkunde* **34**, 362–370.

Perricone, R., Fontana, L., De Carolis, C. and Ottaviani, P. (1980). Activation of alternate complement pathway by fluid from hydatid cysts. *New England Journal of Medicine* **302**, 808–809.

Piessens, W. F., Ratiwayanto, S., Tuti, S., Palmieri, J., Piessens, P., Koiman, I. and Dennis, D. (1980). Antigen specific suppressor cells and suppressor factors in human filariasis with *Brugia malayi*. *New England Journal of Medicine* **302**, 833–837.

Pollacco, S., Nicholas, W. L., Mitchell, G. F. and Stewart, A. C. (1978). T-cell dependent collagenous encapsulating response in the mouse liver to *Mesocestodes corti*. *International Journal for Parasitology* **8**, 457–467.

Potseleuva, V. A. (1958). Superinvasiveness and age immunity to cysticercosis in rabbits. *In* "Contributions to Helminthology", pp. 304–310. Israel Program for Scientific Translations, Jerusalem, 1968.

Rajasekariah, G. R., Mitchell, G. F. and Rickard, M. D. (1980a). *Taenia taeniaeformis* in mice: protective immunisation with oncospheres and their products. *International Journal for Parasitology* **10**, 155–160.

Rajasekariah, G. R., Rickard, M. D. and Mitchell, G. F. (1980b). Immunization of mice against infection with *Taenia taeniaeformis* using various antigens prepared from eggs, oncospheres, developing larvae and strobilocerci. *International Journal for Parasitology* **10**, 315–324.

Rajasekariah, G. R., Rickard, M. D., Mitchell, G. F. and Anders, R. F. (1982). Immunization of mice against *Taenia taeniaeformis* using solubilized oncospheral antigens. *International Journal for Parasitology* **12**, 111–116.

Rau, M. E. and Tanner, C. E. (1975). BCG suppresses growth and metastasis of hydatid infections. *Nature* (*London*) **256**, 318–319.

Rau, M. E. and Tanner, C. E. (1976). *Echinococcus multilocularis* in the cotton rat: the *in vitro* protoscolicidal activity of peritoneal cells. *International Journal for Parasitology* **6**, 195–198.

Reuben, J. M., Tanner, E. E. and Rau, M. E. (1978). Immunoprophylaxis with BCG of experimental *Echinococcus multilocularis* infection. *Infection and Immunity* **21**, 135–139.

Richard-Lenoble, D., Smith, M. D., Loisy, M. and Verroust, P. J. (1978). Human hydatidosis: evaluation of three serodiagnostic methods, the principal subclass of specific immunoglobulin and the detection of circulating immune complexes. *Annals of Tropical Medicine and Parasitology* **72**, 553–560.

Rickard, M. D. (1974). Hypothesis for the long term survival of *Taenia pisiformis* cysticerci in rabbits. *Zeitschrift für Parasitenkunde* **44**, 203–209.

Rickard, M. D. and Adolph, A. J. (1976). Vaccination of calves against *Taenia saginata* infection using a "parasite-free" vaccine. *Veterinary Parasitology* **1**, 389–392.

Rickard, M. D. and Adolph, A. J. (1977a). The prevalence of cysticerci of *Taenia saginata* in cattle reared on sewage-irrigated pasture. *Medical Journal of Australia* **1**, 525–527.

Rickard, M. D. and Adolph, A. J. (1977b). Vaccination of lambs against infection with *Taenia ovis* using antigens collected during short-term *in vitro* incubation of activated *T. ovis* oncospheres. *Parasitology* **75**, 183–188.

Rickard, M. D. and Arundel, J. H. (1974). Passive protection of lambs against infection with *Taenia ovis* via colostrum. *Australian Veterinary Journal* **50**, 22–24.

Rickard, M. D. and Bell, K. J. (1971a). Successful vaccination of lambs against infection with *Taenia ovis* using antigens produced during *in vitro* cultivation of the larval stages. *Research in Veterinary Science* **12**, 401–402.

Rickard, M. D. and Bell, K. J. (1971b). Immunity produced against *Taenia ovis* and *T. taeniaeformis* infection in lambs and rats following *in vivo* growth of their larvae in filtration membrane diffusion chambers. *Journal of Parasitology* **57**, 571–575.

Rickard, M. D. and Brumley, J. L. (1981). Immunisation of calves against *Taenia saginata* infection using antigens collected by *in vitro* incubation of *T. saginata* oncospheres or ultrasonic disintegration of *T. saginata* and *T. hydatigena* oncospheres. *Research in Veterinary Science* **30**, 99–103.

Rickard, M. D. and Coman, B. J. (1977). Studies on the fate of *Taenia hydatigena* and *Taenia ovis* larvae in rabbits, and cross-immunity with *Taenia pisiformis* larvae. *International Journal for Parasitology* **7**, 257–267.

Rickard, M. D. and Katiyar, J. C. (1976). Partial purification of antigens collected during *in vitro* cultivation of the larval stages of *Taenia pisiformis*. *Parasitology* **72**, 269–279.

Rickard, M. D. and Outteridge, P. M. (1974). Antibody and cell-mediated immunity in rabbits infected with the larval stages of *Taenia pisiformis*. *Zeitschrift für Parasitenkunde* **44**, 187–201.

Rickard, M. D., White, J. B. and Boddington, E. B. (1976). Vaccination of lambs against infection with *Taenia ovis*. *Australian Veterinary Journal* **52**, 209–214.

Rickard, M. D., Adolph, A. J. and Arundel, J. H. (1977a). Vaccination of calves against *Taenia saginata* infection using antigens collected during *in vitro* cultivation of larvae: passive protection via colostrum from vaccinated cows and vaccination of calves protected by maternal antibody. *Research in Veterinary Science* **23**, 365–367.

Rickard, M. D., Boddington, E. B. and McQuade, N. (1977b). Vaccination of lambs against *Taenia ovis* infection using antigens collected during *in vitro* cultivation of larvae: passive protection via colostrum from vaccinated ewes and the duration of immunity from a single vaccination. *Research in Veterinary Science* **23**, 368–371.

Rickard, M. D., Davies, C., Bout, D. T. and Smyth, J. D. (1977c). Immunohistological localisation of two hydatid antigens (antigen 5 and antigen B) in the cyst wall, brood capsules and protoscoleces of *Echinococcus granulosus* (ovine and equine) and *E. multilocularis* using immunoperoxidase methods. *Journal of Helminthology* **51**, 359–364.

Rickard, M. D., Mackinlay, L. M., Kane, G. J., Matossian, R. M. and Smyth, J. D. (1977d). Studies on the mechanism of lysis of *Echinococcus granulosus* protoscoleces incubated in normal serum. *Journal of Helminthology* **51**, 221–228.

Rickard, M. D., Arundel, J. H. and Adolph, A. J. (1981a). A preliminary field trial to evaluate the use of immunisation for the control of naturally acquired *Taenia saginata* infection in cattle. *Research in Veterinary Science* **30**, 104–108.

Rickard, M. D., Brumley, J. L. and Anderson, G. A. (1982). A field trial to evaluate the use of antigens from *Taenia hydatigena* oncospheres to prevent infection with *T. saginata* in cattle grazing on sewage-irrigated pasture. *Research in Veterinary Science*, **32**, 189–193.

Rickard, M. D., Rajasekariah, G. R. and Mitchell, G. F. (1981b). Immunisation of mice against *Taenia taeniaeformis* using antigens prepared from *T. pisiformis* and *T. hydatigena* eggs or oncospheres. *Zeitschrift für Parasitenkunde* **66**, 49–56.

Rifkin, M. R. (1978). *Trypanosoma brucei:* some properties of the cytotoxic reaction induced by normal human serum. *Experimental Parasitology* **46**, 189–206.

Rohde, K. (1960). Quantitative Infektion von weissen Ratten mit Eiern des Katzenbandwurmes (*T. taeniaeformis*) und deren Entwicklung zur Finne (*Cysticercus fasciolaris*). *Zeitschrift für Tropenmedizin und Parasitologie* **11**, 43–50.

Sakamoto, T. (1978). Development of echinococcal tissue cultured *in vitro* and *in vivo*. *Memoirs of the Faculty of Agriculture, Kagomisha University* **14**, 109–115.

Schantz, P. M. (1973). Homocytotropic antibody to *Echinococcus* antigen in sheep with homologous and heterologous larval cestode infection. *American Journal of Veterinary Research* **34**, 1179–1181.

Schantz, P. M. (1977). *Echinococcus granulosus*: acute systemic allergic reactions to hydatid cyst fluid in infected sheep. *Experimental Parasitology* **43**, 268–285.

Schultz, M. G., Halterman, L. G., Rich, A. B. and Martin, G. A. (1969). An epizootic of bovine cysticercosis. *Journal of the American Veterinary Medical Association* **155**, 1708–1717.

Schwabe, C. W., Schinazi, L. A. and Kilejian, A. (1959). Host–parasite relationships in echinococcosis. II. Age resistance to secondary echinococcosis in the white mouse. *American Journal of Tropical Medicine and Hygiene* **8**, 29–36.

Sher, A., Hall, B. F. and Vadas, M. A. (1978). Acquisition of murine major histocompatibility complex gene products by schistosomula of *Schistosoma mansoni*. *Journal of Experimental Medicine* **148**, 46–57.

Showramma, A. and Reddy, D. B. (1963). Silent cysticercosis of the brain. An analysis of five cases with special reference to histopathology. *Indian Journal of Pathology and Bacteriology* **6**, 142–147.

Siebert, A. E. Jr., Good, A. H. and Simmons, J. E. (1978a). Kinetics of primary and secondary infections with *Taenia crassiceps* metacestodes (Zeder, 1800) Rudolphi, 1810 (Cestoda: Cyclophyllidea). *International Journal for Parasitology* **8**, 39–43.

Siebert, A. E. Jr., Good, A. H. and Simmons, J. E. (1978b). Ultrastructural aspects of early immune damage to *Taenia crassiceps* metacestodes. *International Journal for Parasitology* **8**, 45–53.

Siebert, A. E. Jr., Good, A. H. and Simons, J. E. (1979). Ultrastructural aspects of the host cellular immune response to *Taenia crassiceps* metacestodes. *International Journal for Parasitology* **9**, 323–331.

Signs, M., Leid, R. W. and Williams, J. F. (1978). Aryl sulfatase release from eosinophils in the presence of *Taenia taeniaeformis*. Abstract in *Proceedings of the 53rd Annual Meeting of the American Society for Parasitology, Chicago, Illinois*, p. 67.

Silverman, P. H. (1955). A technique for studying the *in vitro* effect of serum on activated taeniid hexacanth embryos. *Nature (London)* **176,** 598–599.

Silverman, P. H. (1956). The infectivity of the hexacanth embryo of *Taenia pisiformis*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **50,** 7.

Singh, B. B. and Rao, B. V. (1967). On the development of *Cysticercus fasciolaris* in albino rat liver and its reaction on the host tissue. *Ceylon Veterinary Journal* **15,** 121–129.

Skromne-Kadlubik, G., Celis, C. and Ferez, A. (1977). Cysticercosis of the nervous system: diagnosis by means of specific radioimmunoscan. *Annals of Neurology* **2,** 343–344.

Slais, J. (1970). "The Morphology and Pathogenicity of the Bladder Worms, *Cysticercus cellulosae* and *Cysticercus bovis*". W. Junk, N.V., The Hague, Netherlands.

Slais, J. and Vanek, M. (1980). Tissue reaction to spherical and lobular hydatid cysts of *Echinococcus granulosus*. *Folia Parasitologica* **27,** 135–143.

Slonka, G. F., Moulthrop, J. I., Dewhirst, L. W., Hotchkiss, P. M., Vallaza, B. and Schultz, M. G. (1975). An epizootic of bovine cysticercosis. *Journal of the American Veterinary Medical Association* **166,** 678–681.

Slonka, G. F., Matulich, W., Morphet, E., Miller, C. W. and Bayer, E. (1978). An outbreak of bovine cysticercosis in California. *American Journal of Tropical Medicine and Hygiene* **27,** 101–105.

Smithers, S. R. and Terry, R. J. (1969). Immunology of schistosomiasis. *Advances in Parasitology* **7,** 41–93.

Smyth, J. D. and Haselwood, G. A. D. (1963). The biochemistry of bile as a factor in determining host specificity in intestinal parasites, with particular reference to *Echinococcus granulosus*. *Annals of the New York Academy of Sciences* **113,** 234–260.

Solomon, G. B. (1969). Host hormones and parasitic infection. *In* "International Review of Tropical Medicine" (D. R. Lincicome and A. W. Woodruff, eds), Vol. 3, pp. 101–158. Academic Press, New York and London.

Soulé, C., Remond, M. and Chevrier, L. (1979). *Cysticercus bovis*: antigenes issus du cysticerque en survie. *Recueil de Médecine Veterinaire* **155,** 889–894.

Sprent, J. F. A. (1962). Parasitism, immunity and evolution. *In* "The Evolution of Living Organisms" (J. N. Leeper, ed.), pp. 149–165. Melbourne University Press.

Sulivan-Lopez, J., Sealey, M., Ramos, C., Melendro, E. J., Willms, K. and Ortiz-Ortiz, L. (1980). B lymphocyte stimulation by parasitic organisms. *In* "Molecules, Cells and Parasites in Immunology" (C. Larralde, K. Willms, L. Ortiz-Ortiz and M. Sela, eds), pp. 113–124. Academic Press, New York.

Sutton, R. J. (1979). The passive transfer of immunity to *Taenia ovis* in lambs via colostrum. *Research in Veterinary Science* **27,** 197–199.

Thompson, R. C. A. (1976). Inhibitory effect of BCG on development of secondary hydatid cysts of *Echinococcus granulosus*. *Veterinary Record* **99,** 273.

Thompson, R. C. A., Penhale, W. J., White, T. R. and Pass, D. A. (1982) BCG induced inhibition and destruction of *Taenia taeniaeformis* in mice. *Parasite Immunology* **4,** 93–99.

Todorov, T., Dakov, I., Kosturkova, M., Tenev, S. and Dimitrov, A. (1979). Immunoreactivity of pulmonary echinococcosis. 1. A comparative study of immunodiagnostic tests. *Bulletin of the World Health Organization* **57,** 735–740.

Turner, H. M. and McKeever, S. (1976). The refractory responses of the White Swiss strain of *Mus musculus* to infection with *Taenia taeniaeformis*. *International Journal for Parasitology* **6,** 483–487.

Urquhart, G. M. (1961). Epizootiological and experimental studies on bovine cysticercosis in East Africa. *Journal of Parasitology* **47**, 857–869.

Urquhart, G. M. (1980). Immunity to cestodes. *In* "Vaccines Against Parasites" (A. E. R. Taylor and R. Muller, eds), pp. 107–114. Blackwell Scientific Publictions, Oxford, London, Edinburgh, Boston and Melbourne.

Varela-Díaz, V. M. and Coltorti, E. A. (1972). Further evidence of the passage of host immunoglobulins into hydatid cysts. *Journal of Parasitology* **58**, 1015–1016.

Varela-Díaz, V. M. and Coltorti, E. A. (1973). The presence of host immunoglobulins in hydatid cyst membranes. *Journal of Parasitology* **59**, 484–488.

Varela-Díaz, V. M. and Marchevsky, N. (1973). Aspectos inmunológicos del tratamiento biológico de la hidatidosis. *Zoonosis* **15**, 20–39.

Varela-Díaz, V. M., Gemmell, M. A. and Williams, J. F. (1972). Immunological responses of the mammalian host against tapeworm infections. XII. Observations on antigenic sharing between *Taenia hydatigena* and *Taenia ovis*. *Experimental Parasitology* **32**, 96–101.

Varute, A. T. (1971). Mast cells in cyst-wall of hydatid cyst of *Taenia taeniaeformis* (Batsch). *Indian Journal of Experimental Biology* **9**, 200–203.

Vegors, H. H. and Lucker, J. T. (1971). Age and susceptibility of cattle to initial infection with *Cysticercus bovis*. *Proceedings of the Helminthological Society of Washington* **38**, 122–127.

Verheyen, A., Vanparijs, O., Borgers, M. and Thienpont, D. (1978). Scanning electron microscopic observations of *Cysticercus fasciolaris* (=*Taenia taeniaeormis*) after treatment of mice with mebendazole. *Journal of Parasitology* **64**, 411–425.

Vialtel, P., Chenais, F., Desgeorges, P., Couderc, P., Micouin, C. and Cordonnier, D. (1981). Membranous nephropathy associated with hydatid disease. *New England Journal of Medicine* **304**, 610–611.

Wakelin, D. (1976). Genetic control of susceptibility and resistance. *Advances in Parasitology* **16**, 217–308.

Weinmann, C. J. (1967). Effects of stress upon acquired immunity to the dwarf tapeworm *Hymenolepis nana*. *Experimental Parasitology* **21**, 61–67.

Weinmann, C. J. (1970). Cestodes and Acanthocephala. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds), Vol. 2, pp. 1021–1059. Appleton-Century-Crofts, New York.

Werczberger, A., Golhman, J., Wertheim, G., Gunders, A. E. and Chowers, I. (1979). Disseminated echinococcosis with repeated anaphylactic shock treated with mebendazole. *Chest* **76**, 482–484.

Wikerhauser, T., Žuković, M. and Džakula, N. (1971). *Taenia saginata* and *T. hydatigena*: Intramuscular vaccination of calves with oncospheres. *Experimental Parasitology* **30**, 36–40.

Wikerhauser, T., Brglez, J., Džakula, N., Asaj, R. and Matíc-Piantanida, D. (1978). Experimental immunization of calves against cysticercosis, using a vaccine prepared from metabolic products of activated *Taenia saginata* oncospheres. *Acta Parasitologica Jugoslavica* **9**, 51–56.

Williams, B. M. (1975). Homocytotropic antibodies to *Coenurus cerebralis* antigen in sheep. *British Veterinary Journal* **131**, 361–363.

Williams, J. F. (1972). An evaluation of the Casoni test in human hydatidosis using an antigen solution of low nitrogen concentration. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **66**, 160–164.

Williams, J. F. (1979). Recent advances in the immunology of cestode infections. *Journal of Parasitology* **65**, 337–349.

Williams, J. F., Picone, J. and Engelkirk, P. (1980). Evasion of immunity by cestodes. *In* "The Host-Invader Interplay" (H. Van den Bossche, ed.), pp. 205–216. Elsevier/North Holland Biomedical Press, Amsterdam.

Williams, J. F., Shearer, A. M. and Ravitch, M. M. (1981). Differences in susceptibility of rat strains to experimental infection with *Taenia taeniaeformis*. *Journal of Parasitology* **67**, 540–547.

Willms, K. and Arcos, L. (1977). *Taenia solium*: host serum proteins on the cysticercus surface identified by an ultrastructural immunoenzyme technique. *Experimental Parasitology* **43**, 396–406.

Willms, K. and Merchant, M. T. (1980). The inflammatory reaction surrounding *Taenia solium* larvae in pig muscle: ultrastructural and light microscopic observations. *Parasite Immunology* **2**, 261–275.

Willms, K., Merchant, M. T., Arcos, L., Sealey, M., Diaz, S. and de Leon, L. D. (1980). Immunopathology of cysticercosis. *In* Molecules, Cells and Parasites in Immunology" (C. Larralde, K. Willms, L. Ortiz-Ortiz, M. Sela, eds) pp. 145–162. Academic Press, New York.

Windon, R. G., Dineen, J. K. and Kelly, J. D. (1980). The segregation of lambs into "responders" and "non-responders": response to vaccination with irradiated *Trichostrongylus colubriformis* larvae before weaning. *International Journal for Parasitology* **10**, 65–73.

Yamashita, J., Ohbayashi, M., Kitamura, Y., Suzuki, K. and Okugi, M. (1958). Studies on *Echinococcosis*. VIII. Experimental *Echinococcosis multilocularis* in various rodents; especially on the difference of susceptibility among uniform strains of the mouse. *Japanese Journal of Veterinary Research* **6**, 135–156.

Yamashita, J., Ohbayashi, M., Sakamoto, T., Suzuki, K. and Okugi, M. (1963). Studies on *Echinococcosis* XIV. Further observations on the difference of susceptibility to *Echinococcus multilocularis* among uniform strains of the mouse. *Japanese Journal of Veterinary Research* **11**, 50–54.

Yarzábal, L. A., Leiton, J. and López-Lemes, M. H. (1974). The diagnosis of human pulmonary hydatidosis by the immunoelectrophoresis test. *American Journal of Tropical Medicine and Hygiene* **23**, 662–666.

Subject Index

This Page Intentionally Left Blank