FIRST U.C.L.A. CONFERENCE ON RADIOBIOLOGY

Proceedings of a Conference on

RADIOBIOLOGY AT THE INTRA-CELLULAR LEVEL

Held at Catalina Island, September 9–12, 1957

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PREFACE

In the spring of 1956 a group of investigators working in radiobiology and related fields of research at the Atomic Energy Project (now Department of Nuclear Medicine and Radiation Biology) School of Medicine, U.C.L.A., first entertained the notion that it might be possible, with a pooling of knowledge from different disciplines, to narrow some of the gaps in understanding which persist between radiation effects on macromolecules in the test tube and those on similar molecules in the living organism. After considerable reflection, it was decided that the filling of those gaps might be initiated by a meeting of scientists with different training and general interests but all anxious to explore the totality of phenomena which take place in irradiated cells. The organizing committee, set up to examine the ways and means of accomplishing this, decided that the best way to get the most out of such an assembly of experts would be to have as informal a conference as possible or, in other words, a free discussion group, large enough to include individuals of considerably varied backgrounds and yet small enough for unlimited participation. It was hoped that such an arrangement would lead to much speculation, new generalizations and new approaches, both experimental and theoretical, to radiobiology. The pleasant, palm-fronded island of Santa Catalina appeared to be a suitable setting for the fruition of such aims.

A ready ally was found in the Department of Zoology, U.C.L.A., which contributed a member to the organizing committee. The Departments of Radiology and Physiological Chemistry of the School of Medicine, U.C.L.A., also proved to be willing co-sponsors of such a meeting. Support from the Atomic Energy Commission came not only in the form of active participation but as indispensable financial assistance as well.

The selection of participants, always a task fraught with troubles for conference organizers, was made especially difficult by the fact that to retain the desired informal atmosphere, the meeting had to be restricted to a total of approximately thirty individuals. Since the members of the organizing committee were automatically included among the conferees, this meant that just over twenty scientists could be selected from among the many who no doubt could have made valuable contributions. Thus,

the selections had to be made largely by lot from a long list of eminent workers. As can be seen from the roster of those attending, participants were drawn from all parts of the country and three members of the conference were from England.

Thanks to the extremely effective work of Mrs. Betty Minifie of Medical Extension, U.C.L.A., the conferees all arrived on the island on time and, after a short-lived initial pause following introductory comments, entered the fray with considerable spirit, even having to be restrained from time to time by the co-chairmen, whose ostensible function was subtly to maintain the tenor of the discussions within the vague confines of a pre-prepared outline. Needless to say, the outline of problems was practically ignored. This was because the outline was logical and sequential, although perhaps somewhat extensive, whereas the participants were not always logical, rarely sequential and prone to select certain questions for especially critical scrutiny. Add to these tendencies the fact that several participants had prepared 'statements' which were to be inserted into the proceedings at any cost, and it will not surprise the reader that the titles of the various sessions and the contents thereof bear scant relationship. If the reader should desire the participants' remarks on a specific subject, he had best look in the index. If he prefers to be pleasantly entertained and intellectually nourished by brilliant conversation on many matters of fundamental scientific importance, he can start anywhere and read in any direction!

The consensus of the participants was that the aims of the conference were well met. New concepts were synthesized through discussion by representatives of various disciplines, new research projects were suggested, and the hiatus separating purely biochemical from cellular research was at once both narrowed and emphasized. It is possibly unfortunate that all the anticipated topics could not be discussed. However, the conference was stimulating to all, instructive to many, and inspirational to some. If the publication of these proceedings results in similar reactions among the larger group of reading participants, it will have accomplished its purpose.

The organizing and editorial committee wishes to thank the conferees, Miss Elaine Millar of Pergamon Press, and Mrs. Betty Minifie of Medical Extension, U.C.L.A., for their friendly cooperation during both the conference and the publishing of this book.

FIRST U.C.L.A. CONFERENCE ON RADIOBIOLOGY

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SESSION I

RADIOSENSITIVITY OF THE MODEL CELL

Introductory Speaker: O. A. SCHJEIDE

ONE morning, early this summer, our three-year-old was pacing the beach. Since he obviously was at a loss as to how to entertain himself, a sympathetic sunbather proposed that he gather a few of the pelican plumes strewn along the strand. This our son proceeded to do. With much delight he brought feather after feather to the blanket of the sunbather, demanding and receiving enthusiastic praise. Finally, a great heap of plumes lay by the side of a, by now, very much bored sunbather. When there were no more feathers in the near vicinity, our son confronted the sunbather and demanded instructions as to what he should do *next*. The sunbather contemplated both the pile of feathers and our son at length. Finally he said: 'Take these feathers, go home, and make yourself a bird!'

If we apply this allegory to the present conference, it is apparent that what we, too, are trying to do is make ourselves a bird. By now you are all aware that a variety of disciplines are represented within this assemblage. These include mathematics, statistics, physics, engineering, chemistry, biochemistry, genetics, cytology, physiology, embryology, and medicine. Our discussions will be so oriented that each participant will be able to contribute from his unique views and experiences not only once but several times and at points in the discussion where they will meld with the views and experiences of others who have different perspectives of the same problem.

Despite such an impressive array of talent, this bird that we are going to make may not cut much of a figure as birds go. Indeed, some would suggest that it would be best to forgo any attempt at a synthetic conference at this time, pointing out that by so doing we are committing the age-old folly of placing the cart before the horse. They are of the opinion that the very foundation for an attempt to correlate knowledge bearing on the nature of the sensitivity of cells to ionizing radiation is lacking. Specifically, they point to our limited knowledge of *normal* cells, a subject which they opine can only be properly elucidated by prodigious efforts on the part of teams of investigators whose members are trained in

different disciplines but who are all working at the same time on the same mechanisms of the same normal cell. I appreciate and agree with this view.

I suggest, however, that since considerable confusion would arise if we should seek, all at once, to leave this island, that we proceed with the conference as planned. Let us determine just what kind of bird can be synthesized from our pooled efforts. From this result we may be able to evaluate from a new perspective. Perhaps the present review of radiation studies will eventually assist in a more complete description of the *normal* cell.

Now I am told that the nature of the introductory address should be such as to provoke everyone and, failing this, to stimulate general discussion.

Toward this end I am first going to introduce a concept which I hope you will accept as an aid in these discussions, namely, the 'model cell'. The 'model cell' is intended to be an abstraction which has embodied within it those morphological, chemical, and metabolic features which seem to exist to varying degrees in the majority of cells we are likely to discuss. It is thus, primarily, a term which groups into a not too compact package these features and allows us to minimize differences between cells so that we can speak in general terms. If we assign the 'model cell' a value of 1, it can be expressed algebraically as $Z = aA + bB + cC + dD \ldots$ Here Z is the model cell; the capital letters are the component systems of the cell; and the small letters are numerical coefficients defining the fraction of Z which that system comprises. (We ignore for the present minor qualitative differences between component systems.) For any given instance the small letters will have different values. If one or more of these values is permanently reduced so that the sum of the fractional values no longer equals unity, the cell no longer exists.

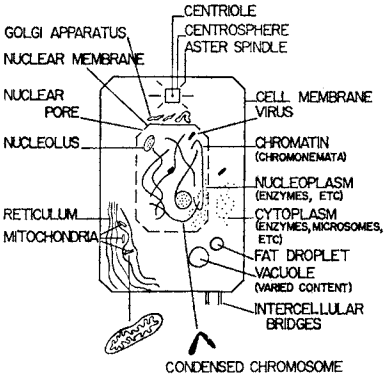

Fig. 1.1. Model cell.

We will, in practice, generally observe the following systems in cells which are to be regarded in their morphological, chemical, and metabolic aspects all at once and at all times: a limiting or *plasma membrane*, appearing to be composed of bimolecular layers of lipid oriented perpendicularly to the surface, between which are found flat layers of protein molecules of an extended shape; *cytoplasm*, a heterogeneous colloidal system consisting of a framework of filaments, membranes, and micelles which enter into viscosity changes according to local metabolic events; included in this thixotropic milieu, among other things, water, the radicals OH, HO_2 and H, molecular oxygen, hydrogen and H_2O_2, glucose, fructose, and other carbohydrates, ADP, ATP, glutathione, amino acids, peptides, soluble proteins including enzymes and enzyme systems, fatty acids, carotinoids, fat droplets, and vacuoles of various types and dimensions; *mitochondria*, rod-shaped bodies, just visible under the light microscope, shown by electron microscopy to be sponge-like internally, containing the bulk of oxidative type enzymes and about 10–20 per cent lipid, mainly phospholipid; *microsomes*, of submicroscopic dimensions, isolated by ultracentrifugation, containing RNA, some enzyme systems which will function under anaerobic conditions and about 10–20 per cent lipid, mainly phospholipid; *Golgi apparati*, irregular reticulums, demonstrated by osmic acid impregnation, hence containing much lipid, observed to be largest in cells of secretion during active secretion; *cell centers*, consisting of one or two little bodies (centrioles) in the midst of a spherical mass referred to as the microcentrum (during division the centrioles move to opposite ends of the cell), about the microcentrum a clear gel-like zone from which aster spindles radiate (the spindles have been shown to consist of sulfhydryl containing proteins giving negative birefringence—upon oxidation of the sulfhydryl groups the aster filaments contract); the *nucleus* with its porous nuclear membrane, a relatively large cellular inclusion containing besides the nuclear sap (which must contain most of the diffusible elements of cytoplasm) a series of twisted and interlaced filaments, the *chromonemata* which are the *chromosomes* in their non-contracted form, consisting in contracted form of a *centromere* (or kinetochore) the clear region to which the spindles attach in pulling the chromosomes apart, the *arms of the chromosome*, which consist of twisted fibres of DNA and both basic and acid proteins as well as lipid and salts of Na, K, etc.; the *nucleoli*, spherical areas usually present on two chromosomes of a nucleus with which is associated most of the nuclear ribose nucleic acid as well as several types of proteins; *Genes*, configurational and ionic orientations on the arms of the chromosomes which are able to serve

as templates for self, enzyme and, perhaps, other syntheses and, finally, *viruses*, which may or may not be present in every normal cell but which we will include in our concept of the model cell. (The relationship between the genes and DNA of the chromosomes and RNA of the differentiating cytoplasm may be as follows: (1) the genes, consisting in part of DNA, participate in formation of nucleolar RNA; (2) nucleolar RNA is released to the cytoplasm where it is present in microsomes; (3) oxidative synthesis processes proceed within the mitochondria and provide the energy in protein synthesis which is correlated with the microsomal portion of the cell.)

The above outline is wholly inadequate both in terms of known and unknown systems of the cell. Especially regrettable is the probability that certain systems, by virtue of having no readily detectable morphological features, may be unknown, but may be present and may be critical for the radiation sensitivity of cells. The outline is, however, a starting point of sorts and regarded in this light, may prove helpful.

In fact, considering the effect of irradiation on such systems as those presented in our diagram, I am led to several notions, which I should like to present as such, but which I must, my colleagues tell me, present as my own fast and hard conclusions, so that the discussants will attack them.

First, I should like to discuss that site in many animal cells which is most critical with regard to attack by radiation. If we can visualize the cell as being in essence a collection of virus-like particles which during a long period of time have elaborated about them not only protective envelopes, the cell nucleus, and cytoplasm, but complete organisms composed of such cells, it is not difficult to imagine further that these modified viruses, the chromosomes, have been subjected to many thousands of roentgens during their existence. As a result of such exposure certain border defenses must have been erected. Also, the surviving chromosomes would, by virtue of survival of the fittest, have themselves assumed a genic structure which would be at least partially resistant to oxidizing radicals.

Granted, if a gene (or set of genes) is affected by irradiation, a drastic effect should soon follow if that cell is in any way dependent upon nuclear synthesis. However, we are looking for the most sensitive site and although WHITING and others have shown that the nucleus is generally more radiosensitive than the cytoplasm as far as cell death is concerned, this nuclear site may be some other than the chromosomes or their genes.

Arguments that have been issued in favor of the theory of the gene as being the most sensitive site for irradiation effects include: (1) the

evidence of increasing resistance to radiation with increasing ploidy of the cell, (2) the fact that many irradiated cells seem to be quite normal until they begin to divide.

These arguments support almost equally well the idea that some other critical system which varies in direct proportion to the chromosome number, and is necessary to prepare the cell for division, is the one affected by radiation.

In view of the fact that recovery can take place in irradiated cells as evidenced by dose rate studies, mitotic inhibition, temperature experiments and administration of nutrients and growth-promoting extracts, it seems indeed quite possible that a nuclear system other than the genes is the most sensitive to radiations and produces the most consistent endpoint. It is expected that a genic change or mutation would be reversed very quickly, if at all, as it would apparently require a primary template, namely its intact self, to repair extensive damage. On the other hand, the laws of evolution could permit non-self-replicating enzyme systems to be relatively radiosensitive since the dose rate experienced in nature rarely approaches a magnitude which would erase the entire complement of such enzymes. We know of many radiosensitive enzymes, especially those bearing sulfhydryl groups, and we are also aware that reducing agents such as cysteine may protect these against radiation (by forming temporary disulfide linkages, among other mechanisms). Sulfhydryl proteins and enzymes have been identified in the nucleus but sulfhydryl groupings are not known to be significant components of the chromosomes. Thus these most sensitive sites are possibly mainly present in soluble enzymes at relative liberty in the nuclear sap. By virtue of their sulfhydryl groupings these would have excellent reducing capacities, almost as effective as cysteine, and could indeed be considered to be agents which would protect the genes from radiation damage. Recent studies in our laboratories by DR. GINOZA indicate that sulfhydryl agents added to viruses, before irradiation in the *dry* state, have the capacity to protect. Perhaps some of you have some ideas on the modes of protection of cysteine. To this date, three mechanisms of action have been proposed, (1) competitive oxidation of cysteine sulfhydryl; (2) masking effect—formation of disulfide linkage between cysteine and enzyme; (3) energy transfer from enzyme to sulfhydryl in adjacent cysteine molecule. Do you have any thoughts on these possible mechanisms?

While I have been talking about the possible site of primary damage in the cell, I have been assuming that most of the disarrangements have been imposed by strongly oxidizing radicals derived from irradiated

intracellular water. I have two main reasons for making this assumption: (1) the first is the success of reducing agents in protecting against radiation damage; (2) the second is the fact that application of strongly oxidizing radicals to cells produces the same types of change as have been observed with ionizing radiation. It is probably worthwhile, however, to consider in our discussions the ratio of direct actions of irradiation to indirect actions on the sensitive system, especially since the critical site may exist in a milieu which is much less watery than has usually been assumed. We should also consider the possibilities of protection from direct action of radiations by such agents as cysteine.

In concluding, I wish to reiterate an issue on which I have expressed myself rather strongly on several occasions. Much of the literature in the field of radiobiology has been contributed by physicists and chemists who have written on the interaction of rays and radicals with molecules large and small, but with no reference to the eventual effect on the cell. In fact, the solvents used in these experiments and the concentrations employed rarely simulate biological conditions. This is probably because no one knows what the biological conditions are.

Another great sector of the literature has been oriented toward the death, genetics, repair and modification of cells, with only cursory reference to the molecular systems which must be involved in these processes. Will this conference achieve one of the hopes of its originators—that of relating the molecular level to the physiological and morphological levels? Or will it serve best by emphasizing such large gaps in our knowledge as that which separates the effects of irradiation on nucleoproteins in the test tube from repair of chromosomes in the living cell?

DISCUSSION

MYERS: I hope that everyone will not at this moment lose the volubility that was so evident after dinner last night. Perhaps we ought to go back and talk a little about Arne's model cell. He has proposed that the model cell be the subject of our discussion and that we come to some agreement as to whether this is the kind of cell we want to talk about.

LESSLER: I think that it would be very wise at the onset to divide the morphological aspect of the model cell into three levels at least—perhaps more will come out of the discussion. There is a great deal of confusion as we swing from the light microscope to electron microscope to the intramolecular level. In many discussions of the morphology of typical cells, this adds to the confusion. I think we ought to decide which *level of confusion* we are discussing; the one that you can see through a light microscope—the typical picture of the cell; real and artificial material as observed in the electron microscope; or physical interactions in cells between molecules that participate in the biochemical systems.

SCHJEIDE: I think it is a fallacy to separate distinctly the chemical and metabolic

features of the cell in one's thinking. In speaking of any one of these artificial categories we should be speaking of all three. When I use the word 'mitochondria', for example, there is in my mind a haze about the word which embodies all sorts of metabolic events, and chemical orientations. Thus, when I henceforth speak of 'mitochondria' in this session I would like everyone to know that I am keeping in mind all of their known properties, morphological, chemical, and metabolic.

LESSLER: I agree heartily with what you say. I have just returned from the Cell Biology Congress in Scotland, where Prof. M. CHÈVREMONT, of Liège, showed some remarkable motion pictures of normal mitochondria in living cells. These films are a morphological study of mitochondrial changes as seen with the phase microscope. Some of you have seen these motion pictures. When you are speaking of the effects of radiation on the morphological appearance of mitochondria, are you also including all of these other things that you mentioned at the same time?

SCHJEIDE: I hate to dominate this conversation and I will soon cease to do so. However, when one views cell structures alive under the microscope one sees the movements and morphological changes which are occurring because of metabolic reactions. These, in turn, are due to chemical changes. In the introductory talk I pointed out that there are critics who feel that what is really required in order to interpret any of these phenomena are teams of workers, some of whom (all looking at the same mitochondria under the microscope) are focussing attention on the chemical aspects, others on metabolic aspects. I feel that the main purpose of this conference is to provide such a team in a single place; to pool knowledge from different disciplines and geographically separated laboratories toward elucidation of a common phenomenon. When we look at this mitochondrion on the board or at living structures under the microscope, let us each apply the views that we have as a result of our specific discipline, but listen to the views of others so that we can take home a fuller concept of what actually exists.

O'BRIEN: I would like to suggest that while I recognize how complicated this is, it should be yet more complicated because a model cell does not exist in a vacuum. You have said nothing, Dr. Schjeide, as far as I can remember, at least very little, about the environment in which this cell exists. This cannot be. We cannot talk about the effects of radiation on a cell alone. In other words, what's around it is just as important as the cell itself; and that doesn't seem to be included in this model set-up.

MYERS: I find, Arne, that while I agree with much of what you say, I'm now also somewhat on Lessler's and O'Brien's side. I would like to continue by phrasing a comment in the form of a question which I think maybe Jim Mead could answer for us. This cell that you have proposed is an anatomical diagram as far as I can see. Now, to a mere physical chemist who knows practically nothing about the cell, I wonder if we couldn't equally well discuss a model cell based on the biochemical reactions which go on in it. We might find, for example, that certain structures within one cell carry on certain kinds of synthesis and in another cell don't.

MEAD: As a chemist, I suppose I ought to rise to the bait. I think that Ralph McKee would be much better equipped to do that and perhaps he can comment later. And indeed, later, I may have something to say about this subject also. However, I think that as a biochemist I prefer to disregard that whole system, to forget that the cell is a morphological entity, and to regard just certain phases of it. Then when I'm finished with this sort of treatment I apologize for having been too specialized and say that other effects may exist also. I have a theory that I'd like to air when the time is proper, but perhaps people are being too polite now for me to bother.

MYERS: You rather pulled the rug out from under us, Jim—you'll have either to say more or retract everything you said.

MEAD: Let me say a few words: Perhaps I led into this in a way. As I say, I know nothing whatever about the makeup of the cell except what I've seen in textbooks, and even then not very specialized textbooks. Perhaps some of the other chemists here are in the same fix. However, I have concerned myself with certain aspects of the cell—mainly some of the enzymes. Now, lately, some of the enzymes that have, I think, been most interesting from this point of view have been the cytochrome oxidase-cytochrome reductase systems. As a lipid chemist, I was very much interested in finding that cytochrome oxidase consists of about 30 to 35 per cent lipid. Incidentally, that lipid is of the type that I've been most interested in: that which contains the highly unsaturated, or poly-unsaturated fatty acids. Everybody knows that one of the main properties of these fatty acids is to absorb oxygen. As a matter of fact, they represent one of the few systems in the body that can absorb oxygen spontaneously and form peroxides. Cytochrome reductase, we'll say DPN-cytochrome reductase, and certain others also contain large amounts of lipid. In both cases, the removal of a small amount of this lipid inactivates the enzyme. The cytochrome reductase contains lipids of an antioxidant nature. It may be that tocopherol is not the main anti-oxidant in the cytochrome reductase. On the other hand, removal of a small amount of the tocopherol inactivates the enzyme. Now, these are the facts. What about their meaning? Having also come back from England just recently and learned a few things at the fountainhead, we'll say, I find that a very reasonable theory of how these lipids may act is that the cytochrome oxidase is activated by small amounts of lipid peroxides. This may be the reason for the existence of such lipids in the cytochrome oxidase system. On the other hand, the cytochrome reductase containing an anti-oxidant which destroys the peroxide may have exactly the opposite effect.

Now, I wonder if I can mention a few other facts—and perhaps build a very small structure of conjecture on these. I am very much intrigued by an article in *Radiation Research* which just appeared. This is by the University of Washington Group, which indicated—and I suppose many of you disagree—that amounts of radiation of 1 r per day or greater are detrimental to the animals. Amounts of radiation of less than 1 r per day (the actual amount used was $\frac{1}{10}$ r per hour per eight hours of the day) while perhaps not being beneficial, at least lengthened the life of the animals by 20 per cent. Now, even more interesting than that was the fact that these small amounts of radiation increased the resting oxygen consumption of these animals, and increased the food consumption which would go along with that. Now, the question is, how does this tie up with the fact that we noticed before for the cytochrome oxidase and cytochrome reductase systems? Well, the cytochromes have the function of reducing oxygen. This may not be a function that is generally considered for them, but at any rate considering the nature of the cytochromes, they probably reduce oxygen stepwise and in doing so make it pass through oxidation stages which are identical or similar to those that are obtained on the oxidation of water by irradiation. If this is true (and it must be) we can conclude from our knowledge of how damaging these radicals are that the mitochondria which contain the cytochromes must be well protected against such radicals. One would say that small amounts of radiation, even if they form peroxides or conduct some oxidizing action, are not too damaging to the mitochondria. Then the question is—what does radiation do to the mitochondria, or mitochondrial enzymes, if it does anything? Here I have to start with an apology that this is only one system of the cell and I'm disregarding

all the rest of it. Other questions would then come up: How does radiation affect the cytoplasm, or nuclear elements of the cell? How is it, if it is really true, that radiation can shorten the life of the cell in the animal in large doses and increase the life of the animal or cell in small doses? I think we might be able to answer, at least hypothetically, some of these questions.

For instance, if radiation catalyzes formation of peroxide in the living animals (and I think there's a good deal of evidence for this) and if fatty acid peroxides are a part of the cytochrome oxidase system, then you might say that small amounts of radiation will catalyze oxidative reactions in mitochondria, perhaps even under conditions in which oxidative reactions could not normally occur. This would tie in very well with the increase in oxygen consumption of animals with daily small doses of radiation, and perhaps also with the increase in food intake and life expectancy. However, I think we would have to say that this same amount of radiation may also be initiating some structure problem in the cell, probably in the nucleus, and that we may have to consider two actions of radiation. Small amounts of radiation may very well carry on reactions that are ordinarily carried on in the cell and may actually increase their efficiency. At the same time, they may be destroying other elements in the cell and other parts of the cell and gradually increase their effect to the point where radiation is completely damaging. I suspect that this is not a new idea to anybody and it may also be one that makes little sense—but to me, at the moment, it makes a great deal of sense and I would like to hear if there is anyone else who could comment on that subject.

TOTTER: I would like to suggest that it might be an appropriate time to consider the photosynthetic mechanism since Dr. Mead has raised the question of radicals and their relation to radiation damage. The chloroplast, I think, can be considered to be a modified mitochondrion, and the photosynthetic mechanism almost surely may involve the production of oxidizing lethal radicals at a much greater rate than most animal tissues are called upon to deal with. Photosynthesis is highly resistant to gamma radiation damage. Therefore, the photosynthetic apparatus does have a mechanism capable of dealing with large numbers of oxidizing radicals.

BOND: I wonder. I'm not a chemist, so I can speak freely on this. It seems to me that normally those oxidative mechanisms are kept well in hand by the cell and that they are orderly processes. On the other hand, in the course of irradiation the events that occur in tissue would be completely random. Under these conditions it is difficult for me to see how the normal processes could be augmented. Would you care to comment on this?

MEAD: I'd have to agree with you definitely and that's why I mentioned that anywhere else but in the mitochondria this should be a very damaging process.

BOND: But even in the mitochondria are not the mechanisms, again, well in hand? Does not the cell, so to speak, know where these oxidative mechanisms are in progress? They are initiated when needed and are under control even in mitochondria, while with radiation this is not the case. Processes may be initiated by radiation in the mitochondria where the cell may not want them, and cannot handle them. I think it rather odd that low doses would be stimulating, as you say. It seems to be that a random process like this could not contribute to the normal orderly processes.

MEAD: Perhaps, hence, the very large amount of lipid in the mitochondrial system. Regardless of where the radiation hits the mitochondrion, if it ultimately affects one of the lipids, it may result in peroxide formation and this can be taken care of by some normal function.

BOND: Independent of where it occurs? And when it occurs?

MEAD: That's the idea.

BOND: It's an idea!

KELLY: It seems to me rather dangerous, at the moment at least, to assume that mitochondria are not affected by radiation. One of the few biochemical changes which has been found shortly after radiation at rather low dosage is the inhibition of oxidative phosphorylation.

MYERS: At the moment there are five people wanting to speak, so I hope the four of you that I don't call on will forgive me. I think Dr. Rustad was first.

RUSTAD: What I was going to say—Dr. Kelly almost said it—was that phosphorylation appears to be uncoupled in situations where there is very definitely no effect on respiration as you'd measure it in a Warburg. This is rather suggestive, and I wonder what you think of it.

MCKEE: Perhaps I could speak to this last question very briefly and to some of the points Dr. Mead has raised. I'm not too keen on a model cell, I'm afraid, and speaking of the Ehrlich ascites tumor, I don't know whether we have a model cell. Nevertheless, with this tumor in radiation studies we see at 500 r a small drop in oxidative metabolism. At 2000 r, there is a stimulation to something above the controlled non-irradiated cell. I would ask the same question that everybody else is asking, namely, is this an indiscriminatory oxidation—uptake of oxygen, or perhaps specific action on certain parts of the cell? We have no evidence to point in that direction, but the fact that we get increases above the control at higher levels of irradiation would, perhaps, indicate uncoupling, and certainly would indicate there might be some disturbance in metabolism, maybe of a different nature than we usually consider. Along with that, there is complete destruction of the cell as far as viability is concerned. In some studies made in our laboratory by GARCIA there is an indication of breakdown of nucleic acid to nucleotide. This is very preliminary and we don't know the nature or extent of it. There is strong indication of nuclear destruction of the cell.

BOND: What dose?

MCKEE: Good point. At about 1500 r there is complete destruction of viability.

BOND: And how about the destruction of nucleic acids?

MCKEE: Nucleic acid destruction occurs at all levels of radiation studied, but of course is much greater at higher doses, such as 1500 r. This is very preliminary information and we can't give a quantitative answer on it at this point. But there is destruction from 500 r up to 2000 r.

KELLY: Is this *in vitro*?

MCKEE: This is *in vitro*.

KELLY: And how long do you keep the cells *in vitro*?

MCKEE: Studies have been made, following irradiation, for up to two hours in half-hour intervals.

TOTTER: A question, Dr. McKee: How do you determine viability?

MCKEE: Viability of the cells is tested by injection into the mice and determining the time of death.

TOTTER: The cells break up?

MCKEE: Yes, the cells become pretty badly disrupted by the irradiation.

POWERS: I am wondering what process follows what process in this series of events. As the cells die the nucleic acid changes might be the result of some kind of autolytic process, only secondarily, then, associated with the radiation.

MCKEE: It is very hard to say—mitochondria seem reasonably intact but they are somewhat changed. Certainly the nuclear material is pretty badly scrambled. This is apparent immediately after irradiation.

MYERS: I've been holding Schjeide off ever since the middle of Mead's speech, and I think we should allow him to defend himself.

SCHJEIDE: Dr. Mead has described the effects of radiation on a system which I feel, from morphological and other evidence, to be rather unrelated to the critical events which occur in many, if not most, cells. This is a system in the cytoplasm, and the cytoplasm has been shown by various experiments not to be the system most critically damaged by radiation. It is true that this system is affected, but in a way which is in no way harmful to the cells as far as I can see. The second comment that I have to make is that nearly all the work done heretofore on enzymes following irradiation is on shaky ground. When we irradiate a cell and then look at almost any enzyme system, we will usually observe an effect on that enzyme system. But sometimes the enzymes are increased in activity, and sometimes they are decreased in activity. There is no evidence anywhere that tells us that this or that enzyme is the one which is primarily affected by irradiation, which I think is our real reason for looking at it. Many enzyme changes seem, in fact, to be purely secondary changes.

MEAD: Secondary to what?

SCHJEIDE: Secondary to an essential primary enzymatic change that we're looking for.

MYERS: Ducoff is next, I believe.

DUCOFF: It seems to me that this calls attention to a deficiency in the model cell as given, in that it is too static a description. In addition to all the structures shown, which I will buy, the important factor is the interrelationships between and amongst them. Most certainly the nucleus may be the critically damaged entity, but it has also been shown that the nucleus can be damaged by the events in cytoplasm.

SCHJEIDE: How?

DUCOFF: It has been illustrated, for example, in the experiments of ORD and DANIELLI on nuclear transplantation, with irradiated amoeba. In addition, there is the work of WOLFF at Oak Ridge, showing that an energy source may be vital in reconstitution of chromosomal damage. And it seems quite possible that uncoupling of phosphorylation would interfere with the availability of energy for repairing nuclear damage. Now I don't know what the mechanism of the immediate chromosomal damage would be, but I don't think we can afford to lose sight of the effect on nuclear damage by cytoplasmic events, including effects on enzyme systems such as those Mead has suggested.

KELLY: I second Dr. Ducoff. It seems to me that if the decrease in oxidative phosphorylation is true, then the cell is essentially dead. Regardless of what happens to the chromosomes, if the cell no longer has an energy supply, then it's gone. The cells which I would consider good candidates for that type of damage are lymphatic cells, while in others, in liver cells, for example, this does not seem to be the limiting step. Which, in turn, brings up the question of whether it is valid to consider one primary type of radiation damage for a model cell. Whether perhaps we don't have to be quite specific in saying we'll discuss immediate cell death, and then we'll discuss chromosome breakage, and then we'll discuss gene mutation, and probably a whole host of other things, which may be of importance to one cell and not to another.

SPARROW: I don't know how many of you here are geneticists. There is one point that hasn't been brought up in the discussion yet, as far as I can remember, and that is the difference in the location of the original site of damage and the position of the actual breakdown of the cell process in question. I think it has been shown very clearly by a number of workers and perhaps the best example I can think of is the work with *Zygnema*, which happens to be an alga, in which the radiation dose to the cytoplasm required to kill the cell was 700 times as great as that required to kill the cell when the nucleus was irradiated, too. Obviously,

in a situation like this, the cytoplasm as such cannot have a high degree of radiosensitivity. It's not possible, of course, to irradiate a cell in most cases without irradiating some cytoplasm, but I think ZIRKLE's work also shows fairly clearly that you can irradiate the cytoplasm with fairly large doses of radiation without affecting the nucleus. Now when you do irradiate the nucleus . . .

POWERS: Excuse me. In ZIRKLE's experiment, though, a large dose is highly localized in the cytoplasm. There are large areas of the cytoplasm which are not irradiated.

BOND: And, also, he's looking at a criterion of damage that may not be pertinent in other situations.

SPARROW: As I was saying; I think, therefore, the nucleus must be a primary site of damage. But I don't see that this necessarily means that the final biochemical processes that result in cell death must also occur in the nucleus. It is obvious, of course, that the nucleus acts as a control center—this is the way the genes work. For instance, if there is a primary site of damage in the chromosomes, the lethal process I think could very well occur, at least in part, in the cytoplasm at some remote point.

LESSLER: Some of this discussion reminds me of a paper that one of my colleagues wrote on the 'Effect of Decapitation on Egg Laying in Chickens'. The point involved is: if we're going to study processes, we have to keep the cell alive. If we're going to study dead material, well then it is a rather different story. It would seem to me that the process itself may not be a critical system as Schjeide suggests. If we consider, for example, the work of VAN R. POTTER of Wisconsin, there may be dozens of critical systems, or pathways. These may vary considerably from cell to cell. May I give a little bit of evidence—before E. S. GUSMAN BARRON died, he and I had an extensive conversation on his earlier work with Arbacia eggs and sperms, much of which was never published. The reason being that he found that at low levels of radiation the cells either increased their respiration or decreased their respiration (this is the level of about 10 roentgens). Since these data never made any statistical sense, he just put it down to inefficiency in his radiation systems. Later, working with nucleated erythrocytes of amphibians, we found much the same thing. In our own laboratory, for example, we find that 60 roentgens given to bullfrog erythrocytes decreases the respiration of these cells in an *in vitro* system (Fig. 1.2.) The exact dosage given to amphiuma erythrocytes, a very similar type of cell, causes an increase in respiration.[1,2] It leads one to the thought that even in the same type of cell we may have different interactions. Not only the mitochondria participate in respiratory

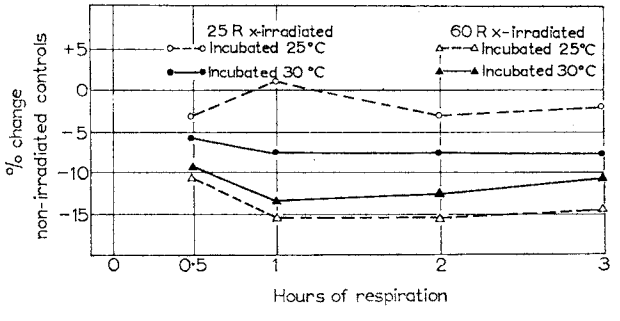

Fig. 1.2. Effect of low-level X-irradiation on the oxygen consumption of frog R.B.C. at 25°C and 30°C.

activity. There may be many undiscovered components in the respiratory chain when we adequately describe it at the cellular level.

CASARETT: I want to use Dr. Schjeide's model cell. I'm sure he knows that it is only a model. It is not dynamic as it is presented and it doesn't apply to every individual type of cell. I want to point out that increase in oxygen tension has been shown to produce changes in cells, similar to radiation-induced changes, including mutations, i.e. changes in chromosomes, and that whatever is in the cytoplasm of the cell, this action has to occur from the environment, through the membrane, through the cytoplasm, past whatever barriers or defenses exist there in some form or another in some metabolic or biochemical change, to produce changes in the nucleus. Perhaps this is an indication that, although Dr. Schjeide may be correct in indicating that the cytoplasm may not be especially radiosensitive, it may be of great importance in terms of defense of the nucleus against presentation of oxidants, and that the constitution of the cytoplasm, the number of mitochondria, for example, somehow may be important in terms of relative radiosensitivity and radio resistance of different kinds of cells. I would like to point out also that there is some pertinent work by DURYEA, who irradiated cytoplasm, nuclei, and cytoplasm and nuclei together, in various combinations. In his work he irradiated all of the cytoplasm that was used in the tests. His results seemed to indicate that the cytoplasm was important in the production of nuclear damage caused by radiation.

POWERS: One more item concerning the radiation of the nucleus and cytoplasm. DANIELS[3] (to be distinguished from DANIELLI who was quoted a little earlier here) has shown in pelomyxa, one of the large amoebae, that an irradiated organism (nucleus and cytoplasm in their normal relations) will die generally from a dose of about 20,000 r. However, a large number of these very cells will survive, if they receive an injection of a small amount of unirradiated cytoplasm. This cytoplasm is effective when centrifuged free of the nuclei. This is very easy to do because these cells are very large.

SCHJEIDE: I think there will be a penalty imposed for everyone who mentions an irradiation dose over 600 r.

POWERS: Many cells don't die at 600 r. What are you going to do about those? Are these non-model cells?

LESSLER: That introduces a very important point that I think should be considered. This level of radiation will wipe out these cells that do not die at low levels of radiation. In a sense the key question to be asked is: Is cytoplasm always the same substance? Is the cytoplasm of the amoeba and a paramecium strictly comparable to that of an ascites tumor cell or an embryonic cell?

POWERS: One cannot assume that it is not.

LESSLER: I'm not assuming anything.

POWERS: All of these cells are living cells, and I prefer to include them in the discussion.

FLANDERS: I think the points that have been raised about the amoeba are interesting. Some varieties of it have an astonishing resistance to radiation. It can be shown, by enucleating a cell and then irradiating the cytoplasm before putting a nucleus back into it, that the principal sensitivity is cytoplasmic. In this sense, it is an exceptional organism. It may remind us that there is a weakness in trying to streamline one's thoughts too much and in considering only one particular kind of cell.

POWERS: Why do you say the amoeba is exceptional?

FLANDERS: In being in some cases rather multinucleate. This gives it unusual resistance to radiation.

POWERS: There are uninucleate amoebae.
SCHJEIDE: But we're not studying the balance of nature.
POWERS: We're studying nature, though. We study the cell. One cannot modify the cell to suit a particular hypothesis, or special doses of radiation. The cell is there and we have to deal with it. An amoeba is a cell; it lives. And one of the basic assumptions that we all make when we approach any biological object is that there is uniformity. There are rules which apply across the board.
LESSLER: This was my question—whether or not the rules about protoplasm shouldn't be reconsidered.
POWERS: The mammalian biologist has to go off by himself and the man who works on the yeast cell has nothing to say to the mammalian biologist.
WARREN: Aren't there special features that have enabled these cells to differentiate themselves from their neighbors? They have different functions in certain environments. This is what makes the difference in the vegetable cell and the motile cell.
PERSON: I think the question that has just been raised, of a functional cell, is important. I think in what follows we might better talk about functional cells. This cell that Dr. Schjeide has on the board really looks more like a static cell, especially by the representation of aA + dD. This indicates that the components function independently of each other.
SCHJEIDE: There are several ways of talking about cells. There is only one good way. That's to sit down for several weeks and go through all the morphological, chemical, and metabolic features. We didn't have time for that this morning; but I repeat that this little haze that I have talked about, surrounding each of these terms, includes not only the word itself but a conception of the structure, and not only what we see but the chemical and the metabolic interactions within that structure and from structure to structure.
DUCOFF: Should they all be plus signs in that equation? I think it's an important question.
SCHJEIDE: Perhaps—they can't be all times.
DUCOFF: It is quite conceivable that your sensitivity in this cell is a function of $A \times D$, rather than $A + D$.
SCHJEIDE: Why don't we assign someone the task of rewriting that algebraic question?

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BREAK

MYERS: It seemed to me this morning that we had an excellent example of the kind of discussions and writing that we've had so often in the field of radiobiology. We talked a lot about Schjeide's model cell and we talked all around the question of nuclear versus cytoplasmic sensitivity. We even hinted at the importance of end points and the dose leading to the end points. Now, perhaps because of a background in thermodynamics and physical chemistry, I'd like to

see the discussion a little better organized around these points. I wonder if we couldn't recognize first of all that there are a number of different end points. We may be talking about pyknosis or some interference with the mitotic apparatus. We might be talking about lysis; we might be talking about the rate of change of some enzymatic process following irradiation. It might even be that for a given cell we get a different end point depending on the total dose to which the cell is exposed. A very low dose might cause a relatively minor gene mutation, for example; a high dose could conceivably cause lysis in the same cell. Furthermore, the response of different cells to the same radiation dose is very important. Sparrow pointed out to me, during the intermission, that he has to give some of his cells half a million roentgens to get an appreciable effect. On the other hand, there are some cells that are killed by as little as 10 r. By considering these points explicitly, could we clear up some of the confusion? Would anyone care to comment?

KELLY: I think you've said it very well. As I was trying to say earlier, I don't think there's any point in arguing about nuclear versus cytoplasmic effects until we agree on which end point we're talking about. It seems fairly obvious that for a gene mutation caused by a direct hit it is the chromosome which is important. On the other hand, in chromosome breaks there is some evidence that rejoining is a metabolic event and there something else may be of importance. I think it would be very nice to specify which end point we want to discuss and then go ahead and discuss that particular one, recognizing that it may not be the important one for every cell. To give an example, lymphocytes for some reason die rather rapidly after low doses, whereas liver or nerve cells do not. On the other hand, cells which are dividing rapidly are strongly affected by mitotic inhibition, by chromosome breakage or by mutation. I just don't see how one can talk about a 'model', and lump all discussion together.

BOND: In addition to the factors which Lola brought out, there is one other important factor and that is the time at which observations are made. It makes a very critical difference. You mention liver cells versus the lymphocyte. It may take the liver cell longer to die than the lymphocyte. It may take longer for the liver cell to manifest any kind of damage. If observation is limited to a period of time shorter than that required for a liver cell to show damage, we are not comparing the same criteria of effect in the different cells.

SCHJEIDE: I would like to say this with regard to the model cell. We have already defined the systems within the model cell as varying in proportion and their relationship to each other. So it really should embrace almost all the end points that we're going to talk about. In different discussions about the model cell we will merely be focussing our attention on components of it which could be affected by irradiation. Now I will say this in addition: my own interests have centered about the most sensitive site of rather sensitive cells. And I do feel I've put in a strong plea to consider the site or sites which may be most critical in the cell, most easily affected by radiation, and those which will induce the biggest change. If practical things are to come out of this conference and out of radiation studies, we should have a knowledge of these systems so that they can be protected, modified, circumvented, etc.

JAMES: I for one would support the concept of the model cell. But I think that the main difficulty here is that we aren't considering the fact that we all do believe in evolution, and that the cells are, at least basically, alike. Now the difficulty in my mind would be to pin down precisely what systems that are common to all cells are affected. I think probably the best way of looking at this would be to consider the fact that the cell is a very complex feed-back system, with the chemical

energies obviously controlling each other. You run into a logical difficulty in any feed-back system the minute that you try to pin down what is the cause and what is the effect. I think we can get lost between these two. There's the problem of the thermostat, the room, the radiator, the furnace. You stop any one of them or, I should say, cause a defect in any one of them, and the same thing happens. This, I think, is probably what we see here in so many of these cases. Irradiate the cell and something happens; and then you try to figure out which one of a whole series in a cycle has been affected, which is the first—and this is the problem with the nucleus versus the cytoplasm.

If you receive *Endeavor*, there's a neat little article by KREBS in the last issue in which he speaks of metabolic cycles and the importance of the feed-back notion in metabolic cycles. Obviously the tricarboxylic acid cycle is a feed-back system. People don't say it in many instances because this implies that you're being a little bit—oh, a little bit fancy. But, nevertheless, there is this control in a cell that seems to compensate when any particular distortion presents itself. I think that as far as radiation is concerned, you can throw everything but the kitchen sink in—you hit everything. Then you ask, 'well, what happens?' These compensations occur due to the fact that this system is regulating itself.

KELLY: If something breaks down in your cell, it would be all right because if your feed-back works perfectly we wouldn't see any radiation effect.

JAMES: Obviously, it doesn't work perfectly.

O'BRIEN: Do you think it would be fair to remind some of the physicists and chemists here—perhaps they don't know—that a living system can exist apart from cellular construction? That the cell is not necessarily a fundamental unit? I thought it worth mentioning.

SCHJEIDE: Do you care to enlarge?

O'BRIEN: I'm thinking of syncytial systems, for example, where it seems that cellulation is something that has been added later on. In these systems nuclear material and cytoplasm have been developed in a certain ratio, but there is no cell wall. I'm thinking of SACH'S concept of the energy as being more fundamental than the concept of the cell.

MEAD: Perhaps I'm not speaking for all the biochemists, but it seems to me that our mutual difficulty is in having to be reminded that there is such a thing as a cell. Actually, my own interests lie in a field that has perhaps little to do with the cell. We know something about the nature of the rays that we're dealing with, we know something about the nature of their effect on water, and we also know something about what happens to the cell after it has been irradiated—perhaps not a great deal, but something. But we know nothing whatever about the material that is first hit—either by the activated water or by the radiation in the cell. In other words, we don't know what chemical changes take place immediately after absorption of the radiation. I think this is something that a biochemist, or chemist might feel apologetic about—this complete lack of information in this field.

MYERS: Jim, do you think the same initial reactions would be important irrespective of the end point, the radiation dose or the time of observation?

MEAD: I think the time of observation is of primary importance in the first place. If you're going to find out what's going on chemically in the initial reaction in radiation of the cells, you're going to have to make your observations immediately afterward, because secondary things, which may be noticed later, may have little to do directly with the initial step. I really agree with Schjeide's earlier comment that most of the observations that have been made on cells, especially cells of living animals, have been made too late to tell us anything about the initial reaction.

LEVEDAHL: Would it be fair to restate Mead's position as follows: What we really need is a different definition of end point. The end point that Jim is talking about is essentially the end point observed at a molecular level, the first interaction. Hence, there is no confusion in end points. There is no problem of observing at a later time, or of a secondary step. Really what Mead is saying —and I'm in the same position that Dr. Myers is in, I don't really know very much about the cell—is that if you could find the interaction of the radiation with the chemical substance in the first step, then you would have eliminated all discussion about end points.

FLANDERS: I do not agree with Dr. Mead in his statement that it is impossible to give any information about early steps in radiation action. On the contrary, much has been learned about the mechanism of radiation injury from the study of effects on microorganisms. Of course, it is hard to deduce anything about the mechanism of radiation injury at the sub-cellular level by experiment carried out on the whole animal.

BERNHEIM: How soon after irradiating the cell can one observe chromosome breakage?

SPARROW: This depends on a variety of factors—first of all it depends on the stage of the nuclear cycle that is irradiated, then on how fast the cells are dividing. In the best situation, a cell growing at normal room temperature, irradiated in late prophase, will show aberrations in a matter of minutes. However, in certain other cases, there is apparently a time lag of many days to many weeks during which irradiated chromosomes can be seen going through at least one cell division without any morphological breakdown. After they've gone through an interphase, they will produce a whole family of chromosome breaks and rearrangements. There really seem to be two kinds of breaks: one kind that you might call immediate or almost immediate, a matter of a few minutes; and another kind that is a delayed break. The length of the delay depends on the physiological conditions in the cell, the stage irradiated, and a number of other factors.

BERNHEIM: But in that second case, you probably have immediate damage which doesn't manifest itself—breaking a hydrogen bond or the like—and this might be the first stage in radiation damage. The rest is, you might say, dependent upon the subsequent history of the chromosome.

LEVEDAHL: I was simply going to ask whether the second case (the late effect) was not just our failure to observe until a later time, not a failure of the radiation to interact until that later time. Granted you have to continue the observations to see that you've picked up a point, it seems rather difficult for me, at least, to conceive of interaction of radiation with some complement of a cell—a model cell or not—that does not reflect itself for some length of time when the half-life of interaction that we're attributing action to is so very short.

SCHJEIDE: I would like to interject at this point that we are now discussing the first issues brought up in the introductory talk. The last few comments have focussed on the radiosensitive or critical site. I move that we continue this trend for a moment so as to obtain something fairly constructive out of this first session. I move specifically that we consider three sites as being perhaps critical, namely, chromosome and its genes, the enzymes present in the nucleus which are not part of the chromosomes or genes, and finally the cytoplasm. I wonder whether we could focus our discussion for a few moments about this point.

RUSTAD: I wonder whether I could bring in a couple of possible systems which people often overlook. Dr. Schjeide has introduced the concept that we have the chromosomes as a primary self-duplicating unit. We have a few other primary self-duplicating units associated rather intimately with the nucleus. One is the

kinetochore which is associated with the chromosomes, another is the centriole. These are self-duplicating and might also be connected with radiation sensitivity.

O'BRIEN: I want to ask Dr. Schjeide why he insists on leaving out the environment. You want us to concentrate on the nucleus and the cytoplasm and certain parts of them, and then you just forget about this part that I brought up earlier —and as I said to someone, I might as well have read the weather report and nobody would make a comment on that.

SCHJEIDE: Dr. O'Brien, we accept the notion that environment in some situations may be the critical factor. If the cell exists in a situation where it is not affected directly by irradiation, it is still possible that toxic products, or other things in the environment other than oxidizing radicals, will kill the cell.

GLASSER: In this rather abstract consideration of the model cell, I submit that the cytoplasm is the vital environment to the nucleus, and in each and every situation we're considering on this cellular level, a complete system with, probably, an integral homeostatic mechanism. So we have not disallowed the environment. To continue Dr. Schjeide's suggestion: I think to search for the critical site or the primary critical site of radiation damage you propose two rather diverse situations; first, the chromosomal breakage phenomenon—where we are particularly looking for something rather acute; secondly, the searching for a biochemical aberration, which I think may be, in the long run, a more fruitful approach. We are looking for aberrations which may be latent in terms of our present ability to search them out. A rather long period of observation may be fruitful in picking up small aberrations, perhaps indicative of alternate metabolic pathways which contribute to the success of an animal to survive irradiation, albeit for a span life less than that expected of a given species. Any information pertaining to the physiology of survival is of prime importance in the analysis of radiation damage and/or death.

SCHJEIDE: I wonder, since we're having difficulty with end points, if we could for the next few minutes arbitrarily take cell death as the end point.

KELLY: That's still not an end point. You have to define whether you mean cell death within a couple of hours after irradiation or cell death after five or six divisions or cell death after the first division.

HOWTON: For my own edification I'd like to ask if there is any distinction being made here between 'end point', which is a term being used frequently, and 'effect'?

BOND: What do you mean?

HOWTON: I think if we're using 'end point' as synonymous with 'effect', then using the words 'end point' is misleading and should be avoided.

BOND: Don't you have to have an end point to measure an effect of radiation?

HOWTON: Not if you know there was an effect.

BOND: How would you know there was an effect if you didn't have an end point with which to observe this effect?

LESSLER: In an observance of continuous processes there is no end point. One can observe the respiration of the cell for an hour, a day, a week, or a month. You don't wait for the cell to die, you want to observe this continuously. There are many radiation effects which have definite continuous functions which can be observed over dozens of generations—genetic effects, for example.

BOND: I think you're bringing in the time factor again. It is partly a matter of semantics but we could avoid the difficulty by using 'criterion of effect' rather than 'end point'. Now I'd like to rise to the defense of cell death as a criterion of effect. I think it's a very old and venerable criterion in radiobiology. Under some conditions it is definitely observable; one can tell when a cell dies. I think the chief difficulty involved in comparing cell populations is, again, time. Other

conditions in addition must be specified, rather than just whether a cell dies or not. You've got to specify the dose employed when the cell dies, and a number of other variables.

CASSEN: I think we're all committed, until it is proved otherwise, to consider cellular units as physical systems and, as a matter of convenience, as isolated physical systems. Now, in physics, you are dealing with much less complicated systems, but you get a similar problem, which has led to a concept used in physics and engineering—the black box. You have a box into which you can pass certain input stimulations and each input stimulation gives an output effect. If you start shooting holes into this black box at random, you're going to injure some of the unknown internal mechanisms. You might kill the black box and no input signals will give any output signals at all, or it might change the action or stimulation which in our simplified model can be either chemical, physical, or radiation stimulation. Therefore, from the point of view of the physical model there is no such thing as a sharp and defined end point except complete disorganization and disruption of the function of the box—killing the black box. However, you can have any number of quantitative effects by observing how the output changes for certain types of input signals as influenced by mistreatment of the black box. In our case, this would be changing the chemical environment or producing other types of physical stimulants.

MYERS: It seems to me, Ben, that your remarks are very closely related to those that James was making about feed-back systems.

CASSEN: Except that in the black box concept you regard the system as a functional system, and say nothing specifically about what goes on inside the black box, such as whether or not it involves a feed-back mechanism. We know that it involves amplifications, negative and positive feed-back and storage of information, and means of transmitting information. I don't think this is the place but later on I would like to make some remarks about information theory as applied to a cell.

DOWDY: I'd like to rise to the defense of death. I think it is a very good end point; once something is dead it seems to me that it has changed permanently. What criteria are you going to use to establish that it is dead? With reference to radiation on cancer, epidermal cancers, the pathologist frequently finds cells among the non-viable cells which seem to be viable. When he says non-viable, he means that his cell has differentiated and it is able to go ahead and carry on all of its functions with the exception of reproduction. Dr. McKee a moment ago spoke of his criteria of injecting these cells into another animal and getting another tumor. That does not necessarily mean death. It may take 500,000 or a million cells injected into the organism before you can obtain this tumor. Some of you others may want to speak upon the cessation of respiration. So what criteria are you going to use to determine death?

BERNHEIM: I would like to put this a little more on a chemical basis. I think the experiments of BRACHET are pertinent here. He shows that after removal of the nucleus from a unicellular organism the cell can still synthesize protein for quite a long time. In other words, the cytoplasm has all the very complicated mechanism for synthesizing protein and eventually it stops because the template, the RNA gives out. Now if the radiation hits the nucleus first, this complicated synthesis of protein will go on in different cells for different lengths of time depending upon the autonomy of the microsomal mechanism. One end point that might be very critical would be the time when the protein synthesis no longer takes place in the cell; and this might explain the differences in sensitivity.

FLANDERS: I think that's a very useful lead. Of course, it may be that the cell dies as the result of the lack of a particular protein, or a particular enzyme. Radiation injury may have interrupted the manufacture of one such molecule. It is not necessary that they should all be interrupted at once.

BERNHEIM: I agree. I mean there might be one critical enzyme in the protein synthesis that is interrupted.

SCHJEIDE: May I again suggest that we go ahead now and consider several so-called sensitive systems, leading perhaps to different types of death.

MYERS: Arne, I think Dr. Kelly wanted to say something.

KELLY: I just want to say that what Dr. Bernheim has mentioned is like the way I've pictured the mechanism involved in killing, as defined by the inability to form a macrocolony. This would apply to microorganisms or PUCK's mammalian cultures. At the right dose many of the cells divide several times and then magically they all die or lyse. The way one might explain this is to say that some enzyme-synthesizing template is missing in these cells and they go ahead and make more cells until they've used up the enzyme that was present, or something of the sort, and then they all die.

MYERS: You mean a gene mutation?

KELLY: Yes.

MYERS: Necessarily?

KELLY: Not necessarily, but possibly, since genes are supposed to control the synthesis of enzymes. Any one of the essential enzymes could be involved and it could be a different one in each irradiated cell.

DOWDY: I think that Dr. Bernheim and Dr. Kelly are not talking about death now—they're talking about the causes of death. This was the part of my statement that I failed to make a while ago. We must define what is death. We accept that it is an end point. What are the criteria, and then the causes? We accept death. Now, what are the criteria of death? Then, we go back and say, 'What caused death?'

BOND: Have we accepted death?

DOWDY: I did.

DUCOFF: Arguing against use of death as an end point is the fact that we're going to talk about the time of death. We have to decide just when these cells die. Many microorganisms will exhibit unimpaired motility, respiration, and synthetic capacity for hours, or even days, after irradiation. Fibroblasts in tissue culture may behave similarly and may even divide once or twice. Nevertheless, their capacity for successive divisions has been lost. By physiological criteria, these cells go on living for extended periods of time. On the other hand, some workers employ ability to reproduce as the sole criterion for viability. So, just when do you score a doomed cell (or its progeny) as dead?

JAMES: I would like to ask Dr. Schjeide what are some of the criteria of sensitivity which he would consider as being good.

SCHJEIDE: James, I thank you for this opportunity, but I'm going to be a turncoat. I propose that we go ahead and consider some sensitive systems. Let's define the system as we talk about it, mention the end point if we so desire, and we will, perhaps, be able to hold forth some old and new concepts of critical systems for scrutiny. We've already had suggestions from Rustad regarding two centers which might be critical. He mentioned the centriole and kinetochore. Perhaps someone would like to attack him on this or help him out. Perhaps someone would like to attack my concept (not a personal one) of sulfhydryl enzymes in the nucleus as being very sensitive in certain cells.

DUCOFF: I wonder if it would be out of place to raise one additional objection to

this type of cell model. It may be undesirable to confine our thoughts to specific structures as sites of damage. We might better think of a cell as performing certain operations. These could be considered to include replication of the whole and its parts. Also, the cell has to maintain its integrity as a discontinuity in the environment. It has to maintain internal functions, and possibly external functions. And it has to have the energy supply for all these things. Perhaps, instead of worrying as to which particular structure or which geographic compartment is most sensitive, we could talk about which of these operations is critically involved in radiation damage.

SCHJEIDE: Would you like to enlarge on that, Dr. Ducoff?

DUCOFF: In talking of cell death we've already raised the question of whether the elimination of replication is the critical thing, or whether it is the elimination of the cell's maintenance or discontinuity that is critical. For some studies, I have found the acetate flagellates eminently suitable, because they lyse so readily after treatments, which would lead to satisfaction of most other criteria of death. You simply lose the cell and get lysis, so some energetic or maintenance activity must be damaged. Other workers have suggested that there may be an uncoupling of phosphorylation, which implies a certain loss of an ability to marshall energy. This would possibly, but not necessarily, be a critical type of event in cell death or other loss of function. A large number of biochemical events can be described as following radiation. And similarly, a large number of biological end points or effects can be described. Our problem is to see which of the biochemical events and physico-chemical events which we detect are the critical ones in achieving biological effect and which chemical events are occurrences which are rather superfluous in terms of biological effect.

SCHJEIDE: You vote for uncoupling of oxidation and phosphorylation?

DUCOFF: Personally, no.

FLANDERS: Perhaps it would be interesting to go back a year or two to some work which DOUGLAS LEA and his collaborators did and which was reported in his book, *The Actions of Radiation on Living Cells*. The effects of radiation on *E. Coli* as judged by cell death may be summarized simply. The survival curves were exponential. The sensitivity appeared to be independent of dose rate and temperature. In experiments on the effects of different radiations he found that the mean lethal dose increased with increasing density of ionization. Now, all these facts, when he placed them together, led him to suppose that cell death in *Coli* could result from a single ionization in the right place within the cell. This seems to me important and I think his conclusions are as valid today as they were when they were put forward. It seems to me we can still conclude that under aerobic conditions a single ionization in the right place in the cell suffices to kill it. The point in mentioning this now is that if, in fact, the single ionization can lead to cell death, there must be quite a simple mechanism. It can only survive one molecule initially. It may be of interest to consider some of the less complicated mechanisms before going on to some of the higher cells where I'm sure the same does not apply.

MYERS: I would like to generalize for just a moment on what Howard-Flanders said, by way of pointing out the real significance of this concept of the model cell. What we're really trying to do here is to generalize to a rather large degree, to establish what might be called a standard cell in a standard system which would behave in a standard way. Now, we would admit from the beginning that no other cell, no living or no actual cell, has the properties of our model cell. However, if we could come to some conclusions on how this model cell should behave, we could then talk about the behaviour of real cells in terms of differences from

the model cell. This would enable us to generalize and we would hope to organize the information about the cellular aspects of radiobiology. Now, I might point out that this approach is nothing new. This is what has been done in chemistry and it has been done in physics from time immemorial. I wonder, now, if the real result that we have come to here isn't that perhaps it's too early to do this sort of thing. Perhaps we are either not ready to make such generalizations and to try to organize the fundamental facts of radiobiology or perhaps this is something we can't do—that we never will be able to do.

SPARROW: I like the suggestion that has been made here repeatedly—to try to focus our level of attention on some particular position or process in the cell. And after the several comments that have just been made, it would seem that a very appropriate place to begin would be on a DNA molecule. It has interest for the cytologists, for the biochemists, the enzymologists, and the geneticists. Now, we all have our own peculiar ideas about how damage to this particular molecule can affect the system we are studying, but I would like to take the liberty of asking Dr. Scholes if he would say a few words about radiation work that he has been doing on irradiation of DNA or DNA components and then perhaps this will stimulate some of the biochemists or geneticists to take part in some comments on how the effects he has observed can produce whatever end point they are concerned with, whether this be mutation, chromosome breakage, mitotic inhibition, or cell death.

SCHOLES: I certainly will go into this later.

FLANDERS: May I ask Dr. Sparrow if he would like to put forward evidence connecting the injury with DNA

SPARROW: Well, that's a rather large order. I think we could go on for hours trying to show relationships between the presumed chemical damage to the DNA molecule and observable biological effects. For instance, there must be some relationship between the primary chemical events that occur in or near DNA molecules and the chromosome break as we observe it. The relationship, however, is not clear.

FLANDERS: The kind of experiments in which P^{32} is incorporated into the nucleic acid of bacteria is, perhaps, near the point. I don't know whether you'd like to comment on it.

SPARROW: As you have brought up the subject, perhaps you would comment. I think this is probably pertinent, but I don't think it proves the points perhaps any more than the fact that irradiation of DNA produces a degredation of the DNA molecule is pertinent.

FLANDERS: Dr. STENT studied the death of bacteria resulting from the disintegration of P^{32}. He put bacteria into a special medium which was low in stable phosphorus, but to which carrier-free radioactive phosphorus had been added. The bacteria held in this medium, at about zero degrees C., were subject to the beta rays from the phosphorus. He studied the survival rate of these bacteria. He also took some bacteria and incubated them in this medium for a cell generation and then held them at the same temperature, close to zero degrees C., so he could compare the rate of survival of the cells which had had time to incorporate phosphorus with those which had not had this time. He found a very large difference in the effectiveness of the radioactive decay. Those cells in which phosphorus had been incorporated showed a much greater rate of cell death than those merely in the hot medium. This he attributed to a specific effect on the DNA.

SPARROW: Was this radiosensitivity from ionization, or P^{32} recoil, or what?

FLANDERS: It certainly was the result of the disintegration of the P^{32} atom, presumably incorporated into DNA. The disintegrating P^{32} atom will have a recoil

energy as the result of the beta ray. He showed by putting the bacteria into a hot medium that the ejected recoil electrons did not kill many cells at the doses commonly used. It was the disintegration of the incorporated P^{32} atom in the DNA which appeared to be effective.

POWERS: Didn't he use the thymine-less strain, 15T⁻? This is a mutant which will not synthesize DNA unless thymine is present. When these cells were incubated with P^{32} without thymine, they did not show this high rate of death, whereas those which are provided with thymine and in which, presumably, DNA synthesis is proceeding more or less, do show an increased rate of cell death. This experiment is a further development of one done quite a while ago by RUBIN,* who showed that the mutation to streptomyacin resistance in *E. Coli* can be brought about, apparently, by P^{32} disintegrations if DNA synthesis is not impeded. In all these cases, however, there is the problem of energy transfer. While the evidence looks very good at the present time, one still has to hold his mind open just a little bit concerning what is happening to the energy as it is degraded in these large molecules. It is not necessarily the site of absorption which is being measured when we measure an effect within the molecule. With this qualification, I think that STENT probably is correct.

FLANDERS: P^{32} decay is a little more specific than the general ionization due to P^{32} in the rest of the cell and surroundings. The result of the P^{32} decay is transmutation to sulphur, thus providing a specific chemical change.

POWERS: Yes, that's correct. It is sulphur at that point, but we must not assume that its becoming sulphur at that point is critical.

FLANDERS: Perhaps the chemists present would like to comment upon the effects of a change from phosphorus to sulphur in the DNA molecule.

PERSON: I would just like to add one thing that has not been brought out on STENT's work yet, and that is that the efficiency for killing is not one but one-tenth. For every ten decays, he gets an inactivation.

FLANDERS: The one in ten applies to liquid nitrogen temperatures. There was a higher efficiency at zero degrees or thereabouts.

POWERS: It is the kind of temperature dependence that ALEXANDER and CHARLESBY saw in their plastic experiments.

FLANDERS: Yes, a similar temperature dependence is shown by bacteria exposed to ionizing radiation.

POWERS: And, as we observed, by dry bacterial spores,† as well as (as we observed quite a while back) by dry T1 bacteriophage.‡

MEAD: Do we accept the idea that suicide of one of these molecules results in its complete destruction, but that it takes ten of them to destroy the cell? Then where are we with regard to our one ionization from external radiation resulting in destruction of the cell?

FLANDERS: This is a very pertinent question, I think. It seems to me that there is a rather similar situation. At liquid air temperatures a number of disintegrations of P^{32} occur without cell death resulting. It has been suggested that out of ten P^{32} atoms nine either are a non-essential part of the cell or the damage is somehow insignificant. The effect of an ionizing radiation may be similar. A mean lethal dose of radiation may produce a thousand ionizations within the volume of the cell. A few of these, perhaps 10 or 50, would be in the nucleus of the

*RUBIN, B. A., PERRY, M. F. C., and THANASSI, F. Z., *Bacteriol. Proc.* 32 (1953).

†WEBB, R. B., EHRET, C. F., and POWERS, E. L., *Radiation Research* 7, 459 (1957).

‡BACHOFER, C. S., EHRET, C. F., MAYER, S., and POWERS, E. L., *Proc. Nat. Acad. Sci.* 39, 744–750 (1953).

bacterial cell, or enter into the nuclear material. Again, much of the ionization is ineffective. It is just one lucky ionization which may kill the cell.

SCHJEIDE: Could I have an opinion from one of the chemists present as to how the introduction of sulphur into the DNA molecule would affect its predicted sensitivity to ionizations?

KELLY: Dr. Scholes tells me that the recoil from the transmutation is almost certainly strong enough so that the sulphur isn't in the DNA chain any more.

POWERS: No, I don't think that's the point, Lola. Actually the sulphur wouldn't move very far. The chemistry is different. One replaces phosphate with sulphate.

MYERS: Wasn't your point that the bonds would be broken by the recoil and the sulphur would not even be involved?

LEVEDAHL: Yes, this is the point.

POWERS: I dispute that, Blaine.

SCHOLES: I think that many of the sulphurs will be there.

BOND: I'd like to comment on a point that Dr. Person made. Is it one out of ten of these recoils that are effective?

PERSON: Every ten decays gives rise to the inactivation of one phage particle, but from the shape of the survival curve it does not appear necessary to inactivate ten different structures. When inactivation occurs, it occurs because of the one ionization.

BOND: I was wondering about the mechanism. Recoil energies will vary with different events, and with some it may be very small. Could it be that an effect occurs only when the recoil energy is sufficient to dislodge an atom from the molecule?

PERSON: Personally, I would guess that every time there is a decay the backbone chain of DNA is broken, because you have a different number of valence electrons in sulphur than in phosphorus. If these bonds are quite specific, then I don't see how they could exist with sulphur, with a different number of electrons.

SPARROW: Is this, Stan, one in ten in the DNA, or one in ten of those that were taken up in the whole cell?

PERSON: As I understand it, for every ten disintegrations of P^{32}.

SCHJEIDE: In what?

SPARROW: At what site? In the whole cell or in the nucleus only?

PERSON: I'm speaking of the experiment on bacteriophage, which STENT has done.

KELLY: As you just said, I think the one in ten was in phage, where essentially all the disintegrations are in the DNA. As I understand it, STENT's interpretation is that the inactivation occurs only if the break is opposite an existing break in the double helix structure of WATSON and CRICK.

PERSON: Yes, and in connection with this, ZAMENHOFF'S work with DNA also indicates that you can have breakages of the backbone chain—not both chains, but of one chain—without inactivation of the molecule.

SPARROW: Why is it they don't get a two-hit curve, then? This doesn't make sense.

KELLY: SCHACHMAN says that the chains are normally discontinuous, but a complete break of the molecule occurs only if a new break is created opposite an existing one.

HOWTON: A few comments back it was implied that, even supposing sulphur has remained in place, having risen from the phosphorus atom, the resulting sulphate ester would be very unstable. Can I ask what the evidence on this point is? That the sulphate ester would be any more readily hydrolyzed, for example, than the corresponding phosphate ester?

POWERS: I was the one who made this suggestion. My evidence is simple: is there such a thing as a ribose sulphate? I know of none.

HOWTON: Well, that's rather tenuous evidence. Whether there is or not, I think is beside the point. One must also consider the mechanism by which such a hydrolysis might occur, and the fact that, in order for such an ester to hydrolyze it has to be accessible to a water molecule, for example. There may well be certain steric requirements involved in the reaction of the ester moiety with a water molecule which are not easily satisfied in the intact DNA molecule.

POWERS: But they might be.

FLANDERS: Whatever interpretation is put on the experiments, the same finding of about one in ten disintegrations being effective applies to bacteriophage, as well as bacteria.

TOTTER: I recently had the privilege of reading a manuscript that Dr. BERNARD STRAUSS had submitted for publication, in which this whole question had been re-examined with the help of Dr. FAILLA's group, so that the radiation doses could be worked out with an expert at hand to help do the calculations. I believe the conclusion of the paper was that it was difficult on the basis of any experiment that had been performed to distinguish between a transmutation effect and a recoil effect. I believe they concluded that P^{32} is the only radioactive element with which this could ever be done. Dr. STRAUSS has performed an experiment with radioactive sulphur, in which he found on the basis of the then calculated amount of beta radiation that sulphur^{35} was more effective in producing suicide than was phosphorus.

POWERS: Say it again.

TOTTER: When he incubated at liquid air temperatures with incorporated carrier-free sulphate (or as near as he could get to it), the total beta effect was greater when compared with the phosphorus experiment at the same radiation dose. At any rate, he got rather strong inactivation when radioactive sulphur was incorporated into the phage instead of radioactive phosphorus.

KELLY: Is this not a radiation effect?

TOTTER: Well, the experiment was an attempt to distinguish between the recoil and the transmutation effects. And if the sulphur was not incorporated in the material of the phage, he got a similar differential effect to that when the exposure was to phosphorus incorporated into DNA.

SCHJEIDE: I wonder whether the participants wish to continue this line of discussion. Recognizing that genes, when affected by irradiation can be critical for the life of the cell or for other end points as well, can we pass on now to discussion of another system in the cell which might be critical for some other end point?

MYERS: Arne, we have just two minutes before we break up. I doubt whether we could get very far on another subject.

DOWDY: Well, we might have time for one more question. I noticed that a number of people said they didn't know anything about cells: some said it facetiously; others, I thought, said it contemptuously. I say it humbly and I'd like to direct this question to Dr. Rustad. What is a kinetochore, where is it, and what is its function?

RUSTAD: It is a little object on the chromosome—I believe it contains protein and possibly RNA—to which the spindle fibre attaches in mitosis when the chromosomes go apart. This is the actual point of attachment to the mechanism that pulls the chromosomes apart.

SCHJEIDE: I had considered that since it was part of the chromosome, it was included within it and gave it no particular significance. I ask this question: Why do you give it particular significance?

RUSTAD: One example of its importance has come out of ZIRKLE'S work. If you irradiate the kinetochore, you'll get a chromosome which does not migrate to the proper site. These, of course, are with very massive doses.

BOND: Are those massive doses really massive? I thought that he was able to observe some effect with only a few protons.

PERSON: There are a few particles per square micron, and a lot of particles per square centimeter.

CASSEN: I would like to ask whether the people working on chromosome break phenomena regard the break to be disruption of a nucleic acid molecule or disruption of some sort of linkage between nucleic acid molecules?

MYERS: Would anyone care to answer that?

SPARROW: I haven't any experiments of my own to report, but it is my impression that both of these have been considered by different investigators to be possibilities. In my opinion it has not yet been determined unequivocally which occurs. In fact, both may occur.

CASSEN: I failed to express that you need such a large dosage to affect the nucleic acid, while the fact that you get the chromosome breaks with such small dosage supports the concept that what is really breaking is something like a histone linkage with a radical that requires very little energy.

SPARROW: If we assume that a chromosome break results from damage to a few DNA or nucleoprotein molecules, then the magnifying power of the chromosome-break method of studying radiation damage is very great indeed, whereas the chemical or physical measures of breakage or degradation of DNA molecules probably do not approach this sensitivity by several orders of magnitude. Since only a few ionizations are required to produce a chromosome break, it would seem that a lesion in one or at most a few DNA or nucleoprotein molecules can produce a chromosome break. There are probably many, many thousands, if not hundreds of thousands of such fibers in a single chromatid, and all you need to have is one or a few of these very large number of strands or fibers damaged to initiate a chromosome break. The chances of detecting such a small percentage of damaged molecules by standard chemical or physical methods are at present almost nil.

FLANDERS: Just to reinforce Sparrow's point. The yield of chain breaks in DNA irradiated wet or dry is quite high. For example, light-scattering methods give evidence of one break for every 200 electron Volts.

DUCOFF: Doesn't BUTLER have experiments in which DNA irradiated in the dry state show much less effect if it is irradiated as the nucleoprotein and then separated?

BERNHEIM: Yes, I think I've heard that DNA is more sensitive in irradiation.

TOTTER: There also are some experiments that I have not heard referred to here that are pertinent to this point. MONTY and DOUNCE have separated nucleoprotein from tissue and found that one gets, with 500 r exposure, a change in viscosity that would have required 20,000 r with a purified DNA. This may support the idea of first breaking off the protein from the DNA rather than the chain.

MEAD: I was going to refer to those experiments of MONTY and DOUNCE, too, and was going to ask if anybody from Rochester can tell me whether MONTY still believes (he isn't at Rochester any more, I guess) that the nucleic acid and the protein are held together by a covalent bond which is very sensitive to radiation. He stated before that this is the bond that he thinks is broken during the irradiation of nucleoprotein.

GLASSER: Let's say this remains his working hypothesis. The nucleoprotein is more sensitive to X-irradiation than either nucleic acid or protein. The dilution necessary to break gels formed from nuclei decreases in a linear fashion as the dose increases and is described by MONTY in his thesis (University of Rochester, 1955).

SPARROW: There are two things I'd like to say. One is that STEFFENSEN at Brookhaven has been working on this approach for several years. He has grown plants deficient in calcium or magnesium and then studied the chromosome breakage radiosensitivity. He has found that for both spontaneous breakage and for radiation-induced breakage there is an increase in amount of breakage in plants grown in a severe deficiency of calcium or magnesium. This obviously has a bearing on the mechanism of the breakage.

Now, I want also to refer to the earlier discussion of the kinetochores. Some of you seem to be a little confused as to why this was brought up and what possible relationship this has to radiosensitivity. There is a chromosomal condition which occurs in both animals and plants which is different from the usual in that the kinetochore or centromere is referred to as diffuse. Now, this simply means that when such a chromosome is fragmented, the fragment doesn't lag at anaphase but has the ability to move to the poles along with the rest of the chromosomes. In other words, it is not possible to produce lagging acentric fragments in this material. Now, I've been investigating the plant genus *Luzula*, all species of which have this diffuse kinetochore type of chromosome. On the basis of what we know about radiosensitivity of other genera, this genus has an unexpectedly high tolerance for radiation. We cannot be certain whether this high tolerance is due directly to the type of kinetochore present or whether it is due to the fact that there is no loss of genetic material (chromatin) due to lagging.

BOND: What do you call an unexpectedly high figure?

SPARROW: I was rightly quoted, but it gave the wrong impression earlier in the day when Dr. Myers said the material I worked with would tolerate one-half million roentgens. This is the upper limit for some species, but there are other species of plants that are more sensitive even that the average mammal. We have investigated the tolerance of perhaps 200 different species of plants and we think we know where a given species should fall in relationship to chromosome size and chromosome number, which are two of the major factors which determine the radiosensitivity of a plant. These *Luzula* plants do not fall in line. And we assume the reason they don't fall in line, on a basis of chromosome size and chromosome number, is the presence of the diffuse kinetochore. Now, we cannot prove this unequivocally, since there is no species in this genus which does not have the diffuse kinetochore.

BOND: Related question. It seems to me incredible that the chromosome breaks occurred at very low doses. Could you elucidate that a little more? Just how low? At what order of magnitude of dose did you see this?

SPARROW: A simple type of chromosome break, which is simply breakage and not reunion, varies directly in proportion to dose. This, of course, is a function of the radiosensitivity of the species. In some plants with large chromosomes and, hence, a high radiosensitivity, a significant number of breaks is produced at very low doses. Now, by very low doses, I mean between 1 and 15 roentgens. At certain stages of meiosis in *Trillium*, for instance, a dose of 50 roentgens is completely lethal as far as the further development of those cells is concerned. At a slightly lower dose, say 25 roentgens, you still get 100 per cent of the cells showing chromosome breakage of a kind presumed to lead to cell lethality. I'm sure that if you were industrious enough to give a couple of roentgens and then look for chromosome breaks, you still would find quite a few chromosome breaks in terms of percentage of cells. I think the situation here is really comparable to mutation, i.e. there is no threshold value. The number of simple one-hit chromosome breaks is strictly proportional to dose. Two-hit aberrations increase at a rate which is a function of dose, usually between $(\text{dose})^{1.5}$ and $(\text{dose})^{2}$.

SCHJEIDE: Isn't it quite clear from what Dr. Sparrow has been saying that it is not necessarily the effect of radiation on the chromosome *per se* which causes breakage of that chromosome? The action would have to be magnified somehow if a dose of, say, 1 to 10 r will produce a chromosome break.
SPARROW: I'm not sure I understood the question.
SCHJEIDE: What I'm asking is: When you get chromosome breakage with such a low dose of radiation, does this not imply that the break is not induced directly by the local ionizations but that there has to be some magnifying system to produce enough energy at a site to make this break?
SPARROW: I don't think so. I don't think a segment of a chromosome can see what is happening around it. It does not matter whether there are a million ion pairs per cubic micron or 50 ion pairs, as long as there is a high enough concentration of ionization in the immediate vicinity of the chromatid. LEA has calculated that 17 ion pairs are required in a given volume of chromosome or DNA. As long as that one localized area has this minimum number, it does not matter whether a single proton produced them or whether there were a million protons in the rest of the cell. The breaks are independent random events.
FLANDERS: There are some algae which have a similar multi-centromere structure in the chromosomes. Dr. GODWARD, in London, has results which I think perhaps would be essentially similar to yours. These algae will stand doses of hundreds of thousands of roentgens of X-rays, following which they divide with chromosomes torn to pieces just moving apart to the poles and leaving no debris in the middle.
SPARROW: Yes, this seems to be parallel. I'm very much interested.
MYERS: I hate to break the discussion off at this point. But I've just been told that we will go without lunch if I don't.

JAHN (*written comment*): Much of the discussion this morning has centered around the mechanism and timing of radiation effects, but there is one series of relationship which seems to need to be emphasized.

Let us consider the DNA-RNA-enzyme relationship. There is only one locus on a chromosome which is assumed to be a primary template for formation of a certain enzyme. This primary DNA template may form numerous RNA templates, each of which, in turn, may form hundreds of enzyme molecules. Any of these may be affected by radiation.

If the radiation affects only the enzyme molecules, the effect of a small to moderate dose is probably partial, because all of the molecules presumably will not be modified. The effect on the cell as a whole will be immediate but temporary, because the enzymes can be replaced.

If the radiation effect is on the RNA, the effect on the cell will be delayed until more enzyme is needed, and will presumably be partial and temporary, unless all of the RNA loci of a given type are affected, in which case it would be total but temporary, an unlikely possibility.

If the radiation effect is on the DNA, the effect is total but delayed until the enzyme needs to be replaced through new and defective RNA.

For these reasons, the question of what is *the* mechanism of the effect of radiation on enzyme action is a meaningless one. There does not seem to be only one possible mechanism but at least several.

Similar logic could be applied to the effect of mitochondria, chloroplasts, centrioles, etc., with allowances for which locus of each particle, if any, is considered to be self-perpetuating.

END OF SESSION I

SESSION II

RADIOSENSITIVITY OF THE MODEL CELL

(*continued*)

Introductory Speaker: G. SCHOLES

THE CHEMICAL ACTION OF IONIZING RADIATIONS AND RADIOBIOLOGICAL EFFECTS

IN this introductory talk I would like to confine my remarks to certain aspects of the chemical changes which follow the absorption of ionizing radiations, since it is here that the radiation chemist is able to make his more significant contribution towards the understanding of radiobiological effects.

It is not unreasonable to suppose that most of the energy absorbed by the living organism will be taken up by water, since this is the chief component of living matter. To a smaller extent, direct action of the radiation on the cellular components will also take place. The relative extents of these so-called indirect and direct effects may, of course, vary in different parts of the cell and it is probably fair to say that both mechanisms of energy dissipation will be responsible for the final biological damage.

Indirect Action

It is known[1,2] that water itself is decomposed by ionizing radiations to produce H· atoms, OH· radicals, molecular hydrogen and hydrogen peroxide, viz.:

$$H_2O \sim\!\!\sim\!\!\sim H\cdot,\ OH\cdot,\ H_2,\ H_2O_2 \qquad (1)$$

It is believed that H· and OH· are formed in spurs in the path of the ionizing particle and that they then diffuse out into the bulk of the liquid. During this diffusion process radical interactions can yield H_2 and H_2O_2, and the extent to which these molecular products are formed will depend upon the ionization density of the incident radiation.

A little more is now known of the manner in which these radicals react with organic compounds in aqueous solution. Studies with relatively simple organic solutes give a starting point from which to speculate about the effects *in vivo*, although one, of course, must be very careful in any extrapolation of the data obtained in aqueous systems.

I would like, first of all, to discuss briefly certain aspects of what is known about the influence of oxygen in the radiolysis of organic solutes, since this would appear to have some important bearing on the well-known 'oxygen-effect' *in vivo*. Usually the presence of oxygen increases the extent of oxidation of the solute, this being due to a number of factors. The initial reaction with OH· radicals can, in many cases, be represented as a dehydrogenation step of the form:

$$RH_2 + OH\cdot \rightarrow RH\cdot + H_2O \qquad (2)$$

In the absence of dissolved oxygen some possible 'back' reactions are as follows:

$$RH\cdot + H\cdot \rightarrow RH_2 \qquad (3)$$

$$RH\cdot + RH\cdot \begin{cases} \nearrow (RH)_2 \text{ (dimerization)} \\ \searrow RH_2 + R \text{ (dismutation)} \end{cases} \qquad (4)$$

In cells, where many solutes are present, it is possible that, in the absence of available oxygen, there is a reaction of the type

$$AH\cdot + XH_2 \rightarrow AH_2 + XH\cdot \qquad (5)$$

(If AH_2 is an essential cell constituent and XH_2 an unessential one, reaction (5) will provide a means of natural self-protection.)

Oxygen can influence the course of the radiolytic decomposition because of its reaction both with hydrogen atoms,

$$H\cdot + O_2 \rightarrow HO_2\cdot \qquad (6)$$

and with organic radicals,

$$RH\cdot \begin{cases} \nearrow R + HO_2\cdot \\ \searrow RHO_2\cdot \end{cases} \qquad (7)$$

In this manner the 'back' reactions (2)–(4) can be suppressed. The inhibition of dimer formation by molecular oxygen, for example, has been clearly demonstrated in aqueous solutions of acetic acid[3] and also of ethanol.[4]

The first point I want to emphasize here concerns the role of the $HO_2\cdot$ radicals. From the chemical point of view, these radicals are somewhat less oxidizing than OH· radicals; in fact, in neutral solution the $HO_2\cdot$ may act as a reducing agent because of the dissociation equilibrium $HO_2\cdot \rightleftharpoons H^+ + O_2^-$. The fate of the hydroperoxy radicals in most cases appears to be in reaction with the organic peroxy-radicals ($RHO_2\cdot$) and/or dismutation to form hydrogen peroxide. Hence, it would seem

that undue emphasis has been given to the part played by $HO_2^{\bullet}$ in radiobiological effects. Some authors, for example, have suggested that the $HO_2^{\bullet}$ radical is *the* important one in the induction of mutations and chromosome breakages. The importance of molecular oxygen in *indirect* action probably lies in the fact that once it had reacted with an organic radical that particular entity has no chance whatsoever of restitution.

The second point I wish to make is that the presence of oxygen in aqueous systems can have a marked influence on the *nature* of the radiation products. To mention just a few specific examples, oxygen is necessary for the formation of acetyl phosphates from ethyl phospate, for formyl kynurenine from tryptophan, pyruvic acid from propionic acid and also for the formation of hydroperoxides and peroxides. All these reactions seem to be bound up in some way with the reaction of molecular O_2 with the initially formed organic radicals. On this basis, it would not be impossible to imagine that certain biological effects could perhaps be specific to oxygen although no such effects appear to have been reported.

The question of the biological importance of radiation-produced hydrogen peroxide, organic hydroperoxides and organic peroxides has long been of concern to the radiobiologists. With regard to hydrogen peroxide it has often been asserted that this substance will be rapidly rendered ineffective by catalase. KIMBALL[5] has provided rather convincing proof that, at least in the case of X-ray mutagenesis, hydrogen peroxide cannot contribute significantly to the biological effect; he believes the same argument also applies to organic peroxides and hydroperoxides. However, it may be of interest, in this respect, to mention some experiments recently carried out with deoxyribonucleic acid.[6] If this substance is irradiated in aqueous solutions containing oxygen, what appear to be hydroperoxides are produced; hydroperoxide formation is not an insignificant process, at least in radiation-chemical terms, since it occurs with a G-value of the order of 1 (i.e. 1 molecule is formed per 100 eV absorbed by the system). It seems that these hydroperoxides are associated with the pyrimidine components of the nucleic acids. In neutral solution, the hydroperoxides decompose slowly, the post-irradiation decay going according to first-order kinetics. There is, of course, no direct evidence that hydroperoxides are formed on the nucleo-proteins *in vivo*; nevertheless, it would be perhaps interesting to speculate about the possible biological implications of the formation of such compounds in the genetic material.

What I have talked about so far is what one may call the oxidation theory of indirect action, in which the biological events are considered to

be the results of oxidation processes. About the only definite case where reduction apparently plays a predominant role is the inactivation of bacteriophage S 13 in aqueous suspensions, as studied in great detail by ALPER.[7] She has concluded that the phage is not inactivated by OH· or HO_2· but by the H· atom and in certain circumstances by O_2^-. STEIN and SWALLOW,[8] however, have suggested that reduction phenomena may generally play some part in the biological effects of ionizing radiations. They arrive at this conclusion from experiments with model compounds such as diphosphopyridine nucleotide. Broadly, the idea is that the OH· radical, say, can react with a comparatively unessential cell component to give an organic radical which may then *reduce* an essential constituent. Such induced reductions certainly occur *in vitro* in de-aerated systems but seemingly not in the presence of oxygen. To account for the oxygen-effect *in vivo*, these authors have invoked LASER'S theory on the influence of oxygen on the enzymatic oxidation-reduction systems, the theory that these are predominantly in the oxidized form and thus can be more easily reduced. Apart from this, however, it is somewhat difficult to imagine that under aerobic conditions an organic radical will react, say, with enzymes (which are presumably present in low concentrations) rather than with oxygen; this competition will, of course, depend upon the oxygen tension and for the whole thing to work out STEIN and SWALLOW have assumed that biological systems are virtually oxygen-free. In one or two respects the theory sounds plausible enough but presupposes so many conditions within the cell.

It seems that, on the whole, little is known about the biological effects, if any, which can be attributable to H· atoms. In aqueous systems, as well as being a reducing agent, the H· atoms may also affect oxidation by dehydrogenation processes; such a reaction with formic acid, for example, is a well-known case.[9] With aromatic compounds, such as benzene, there is a tendency to take up hydrogen atoms leading to saturation of the ring. In acid solution, because of the equilibrium $H\cdot + H^+ \rightleftharpoons H_2^+$, H· atoms can become quite powerful oxidizing agents, rather comparable to OH· radicals;[10] hence in cellular regions of low pH and low oxygen tension, reactions of this type will presumably take place to a considerable extent.

Direct Action

There has, in the past, been much discussion about the relative merits of indirect and direct action *in vivo*, but much of this particular aspect of the subject has been plagued with dogmatisms. A decade or so ago,

the target theory held the fort. Then, the rather close parallelism between certain features of radiobiological action and those of the radiolysis of dilute aqueous systems led to a rather weighty emphasis on exclusively indirect action. More recently, however, the target theory of biological action has again received considerable attention, since it has become increasingly clear that the nature of the environment may, in certain cases, also affect the final results of direct action. It has been shown by ALEXANDER,[11] for example, that the presence of oxygen influences the extent of inactivation of dry trypsin by γ-rays and high energy electrons (2 MeV) but not by α-particles. Oxygen can also influence the direct effects of radiation on polymers, proteins and several other organic substances. With regard to protective agents, besides a role of radical removal in the ambient cell fluids, there have been some data presented which show that certain protective agents, e.g. cysteine, cysteamine, can possibly protect by inducing a state of anoxia in the cell. Protection from direct action is probable and could, for example, involve some selective energy absorption mechanism. There have also been observations in the case of seeds, that the water content appears, at least within certain limits, to be more important in setting the pace of physiological development than in supplying radicals. Yet again, cases have also appeared where it seems that the role of oxygen is, at least in part, metabolic. All these observations indicate that some caution must be exercised in interpreting the effects of these various modifying factors, particularly in suggesting that such-and-such a result points to a particular mode of radiation action.

In isolated chemical systems, for example in macromolecules of biological importance, the detailed chemistry of the changes undergone on direct absorption of radiation is still very imperfectly understood, although progress is gradually being made in this direction. However, what appears to be a very tangible and perhaps a very significant development in this field of direct action is the observation that free radicals are produced on irradiation of dry materials. These radicals can be detected by microwave spectroscopy. GORDY and his associates[12] have demonstrated radical formation on irradiation of several compounds of biological interest, e.g. amino acids, proteins or nucleic acids. In some instances, the radicals appear to be stable, particularly when kept under oxygen-free conditions. The magnetic centers induced in certain proteins and in nucleic acid can remain for quite long periods *in vacuo*, but on introduction of oxygen there is a quick damping of the resonance. ZIMMER and EHRENBERG[13] have detected paramagnetism in irradiated dry barley embryos and found, moreover, that the magnetic centers decay with time;

they point out the possible connection with radiobiological after-effects. Some very interesting results in this respect have been obtained recently by CURTIS, DELIHAS, CALDECOTT and KONZAK,[14] who have studied after-effects in irradiated dormant barley seeds. These authors interpret their results on the assumption that irradiation sensitizes certain sites within the cell and that destruction is completed by chemical action involving atmospheric oxygen; if this is a diffusion-controlled process an after-effect could be accounted for. These particular sites, incidentally, can be desensitized by interaction with deoxygenated water. These seed experiments parallel quite closely some work carried out by GLEGG[15] on the degradation of cellulose by irradiation in the dry state; here, it was shown that an after-effect can take place and that this can be initiated by oxygen and terminated by water. If, indeed, organic radicals are produced in these systems it appears that interaction with oxygen is of great importance for biological, as well as for chemical damage. It has been shown in certain cases that, in the presence of oxygen, direct action can lead to products of a peroxidic nature; for example, it has been found that irradiated serum albumin can initiate the polymerization of methyl acrylic acid.[16] ALPER and HOWARD-FLANDERS[17] have already discussed what they have called 'metionic reactions', that is to say, reactions following ionization of the target and it would be interesting to hear if Dr. Howard-Flanders has any further comments to make on this subject.

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DISCUSSION

MEAD: Do you want to rise to that bait, Howard-Flanders?

FLANDERS: May I ask a question first? You discussed the formation of the peroxide radical, but not its fate. What happens to it?

SCHOLES: It can go by various processes. The hydroperoxy radical, $RHO_2\cdot$, can be reduced by a simple electron addition as follows:

$$RHO_2\cdot + \epsilon^- \rightarrow RHO_2^-$$

or it could possibly dismutate to give alkoxy radicals—

$$2RHO_2\cdot \rightarrow O_2 + 2RHO\cdot$$

which could then undergo further reaction by known processes.

MEAD: Once you have oxygen in the molecule, then the oxygen will remain there under all ordinary circumstances.

SCHOLES: Yes, once the oxygen has gone in you can't get it out; and I think you have there the importance of oxygen in radiobiology.

BERNHEIM: You made one point about the oxygen decay of the free radicals in the dry material. How does that fit in with the general oxygen effect? You have the dry material which you irradiated to get the free radical. Now you admit oxygen and this free radical reacts?

SCHOLES: Yes, but this reaction could be given perhaps by the peroxy radical. Suppose, for simplicity, that it would decompose in the following manner:

$$RHO_2\cdot \rightarrow R + HO_2\cdot$$

BERNHEIM: In order words, R is no longer a free radical.

FLANDERS: In relation to Dr. Scholes' remarks, it may be of interest to mention some recent experiments on the effect of oxygen on the radiosensitivity of bacteria. These experiments were carried out in collaboration with Miss ALPER. An important feature of these experiments is that great care was taken to insure that the oxygen concentration was controlled with precision. Bacteria are a favorable material for such a study because they are small, a matter of some importance for the rapid and accurate control of oxygen tension. Also the sensitive structures within the organism are smaller and less complex than those in the cells of higher organisms. The bacteria were held in suspension which was vigorously stirred with a gas mixture during irradiation so that the oxygen concentration was accurately controlled. The gases used ranged from oxygen-free nitrogen, mixtures containing a tenth of 1 per cent oxygen—and so on—up to pure oxygen. At each oxygen concentration we obtained survival curves with X-irradiation and from the slope of these curves we deduced the relation between radiation sensitivity and oxygen concentration. In these experiments the proportion of bacteria surviving irradiation was determined from the colony counts following incubation on nutrient agar. We found that the sensitivity rises with increasing oxygen

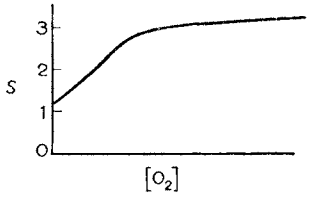

Fig. 2.1. The Relationship between irradiation sensitivity and oxygen concentration for bacteria.

concentration, at first very steeply indeed, and then it levels off at about three times the sensitivity under anoxia (see Fig. 2.1). If oxygen is absent and a particular dose is given it may, on an average, score, say, one hit in the bacterium. Had oxygen been present, there would have been an average of three hits. This suggests that there is some radiation-induced change in the cell which becomes lethal only on reaction with oxygen. In some ways this parallels the action of oxygen in radiation chemistry mentioned by Dr. Scholes a few minutes ago. I would like to discuss now the nature of this radiation-induced change which requires oxygen to become lethal. Suppose that in the cell there are molecules —call them R—which are essential if the cell is to retain the capacity for multiplication. The action of the ionizing radiation is to change R to some short-lived state which we will call R′. If no oxygen is present, R′ may be restored possibly to the original molecules R. If oxygen is present, it may meet with R′ to produce some permanent and lethal change. Dr. Scholes has mentioned two possible reactions with oxygen. Either a peroxide is formed or the oxygen removes yet another hydrogen atom from R′, so that the oxygen becomes HO_2·. In either case the injury is permanent and results in the observed cell death or failure to form a colony. This then describes the oxygen-dependent reactions leading to injury in bacteria. If oxygen is not present, injury still occurs. There must be some other type of injury, so that even under nitrogen, or vacuum, or helium, another type of injury—yielding R″—may occur to the vital molecule, which is lethal whether oxygen is present or not.

We know several things about R′. The first thing is that it reacts with oxygen. Secondly, it appears to be the result of a single ionization. We may follow the argument used by LEA, who found that vegetative bacteria, *Bact. Coli*, gave exponential survival curves. Moreover, the slope of the survival curves was independent of temperature between 0 and 37°C. Finally, it was found that the mean lethal dose required to inactivate the bacteria increased with increasing density of the ionization when comparing different radiations. These findings fulfill the criteria which LEA put forward for the injury being the result of a single ionization. Bearing in mind the effect of oxygen on radiosensitivity, we may conclude that R′ appears to be the result of a single ionization.

We may ask how long R′ remains reactive to oxygen. Recently, Mr. MOORE and I tried to set a time limit to the existence of R′. Bacteria were exposed to a short pulse of irradiation while under anoxia and were then transferred as rapidly as possible into oxygen. This procedure was carried out in 20 milliseconds. There was no difference in the survival of the bacteria according to whether the bacteria were plunged rapidly into oxygen or they were left in nitrogen. We interpret these results as implying that the reaction between R′ and oxygen must be complete in a time which is appreciably shorter than 20 milliseconds. The molecular weight of all the target molecules R may be calculated from the mean lethal dose and is somewhat larger than 10^8. This would no doubt be divided amongst many different molecules, each one of which is essential to the cell for cell multiplication. But still is seems very reasonable to assume that R is a large molecule. So now we have the concept that R′ is the result of a single ionization affecting a rather large molecule; that it is highly reactive towards oxygen, that it lasts for less than 20 milliseconds. The most likely explanation of these observations is that R′ is a radical. As oxygen is very reactive towards carbon radicals, we may postulate further that R′ is a carbon radical somewhere in one of the large essential molecules R in the cell. It has been found in experiments in the gas phase that the affinity of oxygen for carbon radicals is shared by nitric oxide. In common with oxygen it is paramagnetic.

Recently I've been trying to establish whether nitric oxide behaves in an oxygen-like way on the radiosensitivity of bacteria. Nitric oxide is stable only in the absence of oxygen and so it must be used under conditions of complete anoxia. It turns out that it does in fact increase the radiosensitivity in much the same way that oxygen does, and presumably reacts with R′ as predicted.

CASSEN: From this point of view is there a natural explanation for the 3 to 1 enhancement of the oxygen effect?

FLANDERS: There is no special reason to expect exactly 3 to 1; and, indeed, there are variations; for example, bakers' yeast shows an enhancement of just over 2 to 1. There are certain genetic reversions which show a ratio much less than 2 to 1.

MEAD: It seems to me that perhaps with this information we are in a position to speculate a little further as to the nature of the molecule, and you have probably made some pretty good guesses as to what it is.

FLANDERS: One can guess, but I would be very happy if somebody has an idea as to how one could obtain evidence as to which molecule it is.

POWERS: Before you speculate too much on this sort of thing, may I ask, Dr. Flanders, if it is essential that the response to radiation be independent of dose rate?

FLANDERS: If the sensitivity is dependent on dose rate, the lethal effect must be the result of the action of more than one ionization. With a multiple ionization effect you may have similar reactions with oxygen, but they will be more complicated and they may be more difficult to study for that reason.

POWERS: You were careful to say vegetative bacteria.

FLANDERS: Yes, because, as you have shown, bacterial spores behave very differently from bacteria.

POWERS: We have been working with dry spores—*Bacillus megaterium*. The response is not that which will lead one to believe that this reaction takes place, if I understood you correctly. In the first place, the radiation sensitivity appears to be dose rate dependent and, in the second place, with changes in wave lengths of the X-rays (a lengthening) there is an increase in efficiency—just opposite to expectation on the 'one-hit' basis. That is, the more densely ionizing radiations cause an increase in radiation sensitivity of the bacterial spores.

I just wanted to introduce this to impress upon everyone that there is a complication and the bacterial picture is not straightforward and altogether clear.

FLANDERS: Bacterial spores are very different from the vegetative bacteria. This is a point of considerable radiobiological interest.

POWERS: For the purposes of this evening's conversation, I'll agree with you. I just haven't measured the vegetative bacteria.

TOTTER: In the spores you have tested, is there a discontinuity at 0°C in the sensitivity?

POWERS: No. There isn't any that is recognizable at the present time. If there is any fluttering at this point we can't see it.

FLANDERS: In vegetative bacteria there is a marked temperature effect below the point at which water crystallizes in the cell. The cell is less sensitive at very low temperatures, but STAPLETON and EDINGTON have shown that there is still some oxygen effect. Perhaps the low temperature may interfere with some of the processes of formation of the carbon radical, R′.

TOTTER: I would like to ask Dr. Howard-Flanders why he has an R′ and an R″ there? Why do you prefer this way to look at it rather than the other possible ways?

FLANDERS: I think that R′ and R″ have distinct properties. For example, R′

seems the result of single ionization; R″ behaves as if it is the result of multiple ionizations. The damage R″ is of such a kind that oxygen can't influence it. There has been sufficient ionization to make sure of chemical change without the presence of oxygen when testing the vegetative bacteria with different radiations. The mean lethal dose in the absence of oxygen decreases with increasing density of ionization. This indicates that the densely ionizing radiation of fast neutrons is more efficient than X-rays; a situation which is in contrast to that found in the presence of oxygen. Those are the two main properties that I associate with R′. There is another reason for supposing that R″ and R′ are different entities arising from the exact manner in which the radiosensitivity varies with oxygen concentration.

TOTTER: I'd like to hear it but I don't know about the rest of you people.

MYERS: If I could throw in a word of encouragement, I'd like to hear that too.

FLANDERS: In the experiments which I spoke about before, we obtained survival curves with several microorganisms at a series of different oxygen concentrations, ranging from no oxygen up to 100 per cent oxygen and intermediate percentages. It is interesting that as little as one-third per cent of oxygen is sufficient to raise the radiosensitivity by approximately half the total amount found at the high oxygen concentrations. When we drew a smooth line through the experimental points, it looked very much as though oxygen took part in a first order competitive reaction. Assuming this to be so, we wrote down the appropriate equation and obtained a very satisfactory fit to the data. The equation for the relative radiosensitivity is:

$$\frac{S}{S_N} = \frac{K + m[O_2]}{K + [O_2]} \quad \begin{matrix} (K = \text{constant}) \\ (m = \text{constant}) \end{matrix}$$

Now this quantity, S/S_N, has the value one for zero oxygen concentration while for high oxygen concentration the quantity K becomes negligible, and the relative radiosensitivity has the value m. With bacteria, then, m is approximately 3. K proved to be one-third per cent by partial pressure in a gas mixture at 1 atmosphere pressure, or in chemists' language, 4 micromolar. This formula can be derived in the following way. Supposing you say that R′ is a short-lived molecule which restores to the original form at a rate K_1 but reacts with oxygen at the rate $K_2[O_2]$. Reaction with oxygen is necessary to convert R′ to a lethal injury. The proportion of R′ which will be converted to lethal injuries and so become manifest will depend upon the reaction rates of the two possible reactions, and will be given by:

$$P = \frac{K_2[O_2]}{K_1 + K_2[O_2]} = \frac{[O_2]}{K + [O_2]}$$

Suppose we also assume that there is the second kind of injury R″ and moreover that the radiation produces (m − 1) times as many R′ as R″ in a population of cells. Then the total number of injuries will be proportional to (m − 1) p + 1. The ratio of the numbers of injuries with and without oxygen will then be:

$$\frac{K + m[O_2]}{K + [O_2]} = \frac{S}{S_N}$$

This function is also equal to the sensitivities. It is unity in the absence of oxygen and has the value m at high oxygen concentrations. We have attained data on various microorganisms and found that for the *E. Coli B/r*, *E. Coli B*, *Shigella Flexneri* and haploid yeast the agreement with this kind of formula is surprisingly good. GRAY and DESCHNER have obtained data on chromosome aberrations in the Ehrlich mouse ascites tumor which fit the same relation very closely.

TOTTER: Would you not get approximately the same kinetics if you substituted an H· atom for the R′ there?

FLANDERS: You can obtain a similar reaction scheme for the oxygen sensitivity injury on the assumption that reaction between H· and O_2 is involved.

TOTTER: Yes.

FLANDERS: The kinetics do not provide any reason for rejecting the H· atom hypothesis. A widely discussed hypothesis is that H· atoms act by combining with O_2 to produce HO_2· and that this then reacts with the target and induces the injury. But HO_2· is not a very powerful oxidizing agent and there is little evidence of its attacking organic compounds. For example, it doesn't attack acetic acid. There is another piece of evidence which is suggestive. In experiments with nitric acid in place of oxygen, the sensitivity of bacteria may also reach the aerobic level. Yet in this case no HO_2· is formed. In its place HNO will be formed, which is not even a radical. The chemistry of this substance is not well understood, but it appears that it is not long-lived and has a tendency to disproportionate, forming N_2O and water. HNO could hardly be a candidate for the intermediate responsible for oxygen-dependent radiation damage. Another point to consider is that by using densely ionizing radiation you can crowd together the radiation products so that radical concentrations up to a few millimolar may be obtained instantaneously on the track. We have compared the effects of densely ionizing radiations, such as fast neutrons, with those of X-rays on bacteria. The concentration of oxygen required to raise the radiosensitivity by half the maximum increase was the same for the two kinds of radiations. In other words, crowding together the H· atoms and the other radicals which compete for H· atoms does not appear to alter the competition of oxygen for the particular radical concerned in the oxygen-dependent radiation injury.

TOTTER: This is of interest in biological material where actually there is very little opportunity for H· atoms to recombine, although in pure water this may be an important mechanism.

FLANDERS: If you increase the concentration of radicals in the tracks, the number that recombined will increase.

TOTTER: Even in biological material?

FLANDERS: I imagine so. EBERT showed that the hydrogen peroxide yield in a 10 per cent solution of alcohol is the same as in water, indicating that the OH· radicals do combine to form peroxide in spite of the alcohol. A more active way to approach the HO_2· hypothesis is from the study of the radiation chemistry of HO_2· So far HO_2· has been found to play a part in reactions in pure water, or in oxidation reduction of ferrous sulphate which is particularly easy to reduce and oxidize.

TOTTER: Of course, HO_2· approximately accounts for the change in yield; and as you mentioned, this other theory—there is no direct way of accounting for the $2\frac{1}{2}$, 3-fold increase in the oxygen. You could have a maximum of a 4-fold change, I believe, with HO_2·.

FLANDERS: Yes, I think the R″ and R′ are produced independently by the radiation. If the quality of the radiation is varied, then so is the ratio of R′ and R″. This concept, I think, does give a more unified picture than the radical HO_2· hypothesis. I have already mentioned that R′ appears to be the result of a single ionization. Under aerobic conditions, however, X-rays are more efficient than neutrons in their bactericidal action. In contrast, it is found that, in the absence of oxygen, fast neutrons are more efficient that X-rays. Crowding the ionizations together renders them more effective, suggesting that R′ is caused by multiple ionization.

SCHOLES: There's just one point. Would you not also explain this kinetic equation on the idea that this doesn't involve two separate entities but involves a particular reaction of the R′. In this sort of restorational effect, does R′ go back to the original, plus another compound X? As I mentioned before, once the oxygen has reacted, there is not the injury.

FLANDERS: An indication that this is not the case is to be found in the fact I have already mentioned, namely, that you can alter the amount of injury which is oxygen sensitive and the proportion of injury which is oxygen independent, by using a different radiation. This would not be the case if the oxygen effect were determined only by the chemical nature of the targets in the cell.

SCHOLES: But the reaction might be dependent on radiation ion density too.

FLANDERS: If it is dependent on radiation density, it must depend in the observed fashion which points to a multiple ionization effect.

TOTTER: Is there not one more experiment that we need and has perhaps already been done? Or perhaps you know that you don't have to do it; that is the direct and indirect effect in the same material.

FLANDERS: Could you suggest a way of doing it?

SCHJEIDE: In that regard, I read recently in *Science*, although the names of the authors escape me for the moment, that $HO_2\cdot$ applied in solution to certain cells did produce the same type and ratio of chromosome breakage as does radiation.

SCHOLES: How was the $HO_2\cdot$ produced, may I ask?

FLANDERS: Was this with PACKOWITZ—using peroxide and ferrous ions?

SCHJEIDE: Yes.

FLANDERS: I was puzzled by this paper because it is hard to get ferrous ions into solution in the biological range of pH.

HOWTON: It has been mentioned that $HO_2\cdot$ can oxidize ferrous ions but cannot oxidize acetic acids and in view of the fact that acetic acid is probably the most difficult of all organic compounds to oxidize, I think that this leaves a tremendous range if we're trying to pin down just how good an oxidizing agent $HO_2\cdot$ is. Can anyone pin it down more definitely than that? Maybe it's potent enough.

SCHOLES: The point is that it's less potent than $OH\cdot$ radical.

MYERS: I actually intended to go back, but I should mention in answer to Dave's question that the oxidation potential of $HO_2\cdot$ is known. I can't quote it to you—apparently no one else can at the moment—but there's no doubt that it is lower than that of the $OH\cdot$ radical.

The thing I do want to say, though, is that I've been very impressed with this discussion which attributes a lesser role to the $HO_2\cdot$ radical because it helps me to explain some results which I have obtained in strictly *in vitro* systems which have puzzled me quite a bit. We have been irradiating several different porphyrin compounds in alkaline solutions (0.1 normal sodium hydroxide) and we have found if oxygen is present we get breakage of the porphyrin ring. If oxygen is absent, this porphyrin ring apparently is completely stable. Now it has always seemed particularly unreasonable to attribute this ring breakage to the $HO_2\cdot$ radical in these alkaline solutions, because there the $HO_2\cdot$ is largely ionized to O_2^-, and the O_2^- ion is a pretty good reducing agent. You would hardly expect an oxidative reaction and you would not expect ring breakage to occur from reduction. And so in this one system, at least, it seems to me that it makes much more sense to say the oxygen is reacting with organic radicals which are presumably produced by the $OH\cdot$ radicals.

SCHJEIDE: Could I ask the chemists what the relative oxidative potentials of some of the organic radicals might be, as compared, shall we say, to $HO_2\cdot$?

MYERS: Well, Arne—I think the point here is that the organic radical is already

oxidized and the oxygen unites with it to form the peroxy compound. Oxygen, here, is the oxidizing agent.

DANIELS: I'd like to add that some work of the last year and a half or so in inorganic systems has shown that we often get induced oxidation reactions occurring when the solute is ionized. This has been shown in some French work on phosphites and with some work on arsenites. When you move into alkaline pH you get chain reactions with oxygen. I'd also like to point out again that it has been mentioned that $HO_2\cdot$ is a radical which ionizes and becomes a reducing agent. The same sort of thing no doubt could happen to the $RO_2\cdot$, but this depends on the potential of it about which nothing is known. We must go on to remark, however, the same sort of effect can take place. You can get an O_2^- again from the $RO_2\cdot$.

BACHOFER: What is the reaction of NO is aqueous solution under irradiation? Would that tend to enhance the output of hydrogen atoms?

FLANDERS: Of course, when you're testing a biological system under NO you can't have any $HO_2\cdot$ present, as HNO would be formed. This would tend to reduce the number of hydrogen atoms in solution.

BACHOFER: One might look into this.

FLANDERS: Yes. It would be valuable to look further into the properties of $HO_2\cdot$ and HNO.

BACHOFER: One might be tempted to think that a higher yield of $H\cdot$ atoms might indicate a sensitivity to a reducing agent, rather than something that you indicated up there a little while ago.

FLANDERS: Higher yield?

BACHOFER: Of hydrogen atoms. In an aqueous system with NO present, on irradiation—this would tend to give a higher yield of hydrogen atoms and this would fit into the general idea, for example, that phage are more sensitive to reduction. You might say the hydrogen atoms are actually reducing the phage.

FLANDERS: Yes, I appreciate that.

BACHOFER: Of course, I don't believe that is what happens, but it would seem to point this way.

FLANDERS: And I think that the case you're talking about is phage in high dilution.

BACHOFER: Yes, I realize that you're talking about that, too.

FLANDERS: The situation is very different in a suspension of bacteria.

BACHOFER: Well at least it points up some differences in another system. The point is—if one were dealing with phage in high dilution with a higher yield of hydrogen atoms, one might be tempted, at first glance, to say the phage would then turn into reducing compounds. But you can twist this thing around a little and, for example, add nitrogen—well, first of all you could add hydrogen, molecular hydrogen, which would in turn increase the yield of hydrogen atoms. The phage is more sensitive in this solution than it is in an oxygen-saturated aqueous solution. That will again tend to point to the fact that the phage is sensitive to reduction because of the presence of hydrogen atoms. But you can also then put nitrogen into the solution, following the curve at exactly the same place. Which would seem to indicate that it is not the hydrogen atoms acting as the reducers, which are causing inactivation of the phage, because the nitrogen will not increase the yield of hydrogen atoms.

FLANDERS: You mean, then, irradiation under hydrogen gives the same results as irradiation under nitrogen?

BACHOFER: Right. I should specify this is with phage T1.

FLANDERS: Miss ALPER found that irradiation under hydrogen indicated a greater sensitivity than was the case for irradiation under nitrogen.

BACHOFER: I think that's what she says. Although on that point she's a little bit hazy, I think—but I studied her stuff very, very carefully and on this point it's not too clear. We've run this thing over and over and over again and found that nitrogen and hydrogen points come out the same. You can't distinguish between them. If we throw CO in that, of course, will increase the H· atom yield. The CO survival goes way down but this throws in another complicating factor. The phage themselves are quite sensitive to CO, independent of any irradiation, so there you have the phage being partly knocked out by CO—you come along and irradiate—then it goes down very fast. But the phage is quite stable in a solution saturated with molecular hydrogen. So that you can compare hydrogen and nitrogen in activation curves and they're identical—that is, they're essentially the same. This, then, would argue against the reducing action of the hydrogen atom under irradiation. It looks from this as though you'd have to say that oxygen protects and I have not been able to detect any chemical equation which will show how it does this. I would like to ask this: Do you or Dr. Scholes know any kind of reaction with oxygen and hydrogen peroxide which would in some way neutralize the hydrogen peroxide? The point is simply that phage in an oxygen-saturated solution to which hydrogen peroxide is added comes out much better, or the survival is much higher than in nitrogen to which hydrogen peroxide is added. I'm not aware of any reactions you could write out involving oxygen and hydrogen peroxide which would tend to neutralize the hydrogen peroxide. Do you know of anything of this sort?

FLANDERS: I suppose oxygen would in fact tend to stabilize hydrogen peroxide against decomposition by chain reactions involving H· and OH·.

BACHOFER: You think the oxygen might stabilize hydrogen under the hydrogen peroxide?

SCHOLES: This is the way irradiation reacts on hydrogen peroxide in solutions in the absence of oxygen. It is very rapidly decomposed by irradiation.

BACHOFER: Leave radiation out of the picture—just a simple aqueous solution with oxygen present and, of course, the phage in these two solutions. Add hydrogen peroxide to one and then to the other in equal quantities. Would there be any decomposition of hydrogen peroxide in the presence of oxygen independently of any radiation? This I've not been able to find or establish.

DANIELS: There is a normal catalytical decomposition of hydrogen peroxide, but it is usually not significant in concentrations below 10^{-3} molar, and it is quite stable if the peroxide is at least pure to start with. There should be no difference whatsoever with oxygen or nitrogen present.

BACHOFER: Right! This is what the radiochemists say. But in this set-up the virus (T1 I should say) is considerably more stable if the oxygen is present—it is more stable with oxygen than with nitrogen. Take this in the light of the other picture, namely, that the viruses are more stable, the survival curve is higher, when they are present in an oxygen-saturated solution which is irradiated, than in a hydrogen- or nitrogen-saturated solution which is irradiated. It all points to this—that you can't explain this thing on strict radiation chemistry, in terms of the radicals, as far as I can see. But the fact is there, and the only solution I can offer is that somehow the oxygen reacts with the phage—somehow oxidizes it or at least puts it in some kind of stable form. Perhaps it stabilizes it during a time that the phage particle in itself might be considered a radical, and then lets the energy pass off and then it comes out all right. Here are two cases where the results do not fit in clearly with what one would expect from radiation chemistry, presumably owing to the complexity of biological material.

DANIELS: I would just like to remark here—we found that these reactions with

gases are very sensitive to the purity of the gases, and that normal commercial gases always contain hydrocarbon impurities which are always interfering in reactions. That comes from the simple compression machinery used in shoving the gas into the cylinders.

BACHOFER: Well, we have tried with helium and with nitrogen and find that with a couple of different sources including some specially purified nitrogen—the picture seems to come out about the same. The curves for nitrogen and oxygen are quite widely separated. Another point is that the viruses are more sensitive in an acid medium, say, saturated with oxygen, in which one might expect HO_2· to be produced—both OH· and HO_2·. This could be, I think, called upon as an argument against the reducing power of the radicals. Or, to say it differently, it seems to point toward, or still point toward, the conventional oxidizing ability of the radicals formed upon irradiation which, if only in the presence of hydrogen, might tend to give the opposite view. I don't know if you follow this or not. It is most complex. The fact is that in an acid solution with oxygen present (only oxygen, as far as gases are concerned), the phage is more sensitive than in an alkaline solution.

SCHOLES: Is this to radiation?

BACHOFER: Quite right! This is somewhat consoling because it shows that the viruses apparently are like most biological systems—I should say T1, is like most biological systems—in that it is sensitive to oxidation. More so than to reduction. Which would be a different conclusion from that which Miss ALPER arrived at.

SCHOLES: How do these phages differ?

FLANDERS: T1 is larger than S1.

BACHOFER: Fifty millimicrons in diameter, has a tail, probably a little more nucleoprotein around the DNA core.

FLANDERS: I think S1 is about 25 millimicrons.

BACHOFER: That's essentially correct. I've been tempted very much to try what we did with R1 and S13 but I'm drifting away from this type of work. The question in my mind is—how would S13 perform under the conditions we employed? We can't generalize if we have something different as in the case of T1. I have come to the conclusion that T1 is not particularly sensitive to reducing action during irradiation in aqueous medium. I don't say that it is not sensitive to reduction, but I think it is more sensitive to oxidation. That is the general conclusion, plus the fact that the oxygen seems to do something to phage. I think if one takes that as an assumption, and looks at the reaction between the phage and oxygen, one can come up with a more sensible, more rational picture than if one looks only at the reaction of oxygen with the products of the irradiated water.

MEAD: I think perhaps that we're in a better position than when we started this last session. We're certainly in a better position than when I made the remark this morning that we didn't know anything about the type of molecule that might be affected in the cell by irradiation. As we begin this second half of this session, I think I may be correct in saying that we know something about the size of the molecule (that it's a very large molecule) that is affected. We know something about the type of substances that must be formed by irradiation and we won't say at the moment whether that radiation is a direct or an indirect effect, whether it is even mediated by some organic peroxide or peroxy radical. At any rate we know that a radical is formed and probably a carbon radical. We know that the substance is of utmost importance to the cell, that is, the substance that is ultimately affected. Now is there anything else that I've left out that is very important?

BACHOFER: I'd like to correct one statement or, rather, add observations. You say we don't know as yet whether it's direct or indirect. The last discussion that we were having here is definitely concerned with indirect effects, at least as commonly defined. Because if one adds as much as 10^{-2} by weight concentration of any kind of proteinatious material, such as broth or gelatin, all these things we've been talking about simply don't take place. The survival curve jumps way up, indicating that we are dealing here definitely with indirect effects. And immediate results were all with the solid material at the level of about 10^{-7} or 10^{-8} concentration. So these are definitely indirect effects, and not direct effects.

MEAD: What I meant actually—perhaps I should have been more explicit—was, I suppose, not direct effect and indirect effect in the usual sense of the words, but perhaps that some other molecule might have mediated in the reaction rather than the $OH^{\cdot}$ radical, or something of that sort—some other radical. We couldn't eliminate that on the basis of this discussion, could we?

BACHOFER: I don't get the point here.

MEAD: In other words, some other organic radical could have mediated between the initial effect of the radiation and the large molecule that we're talking about.

DANIELS: You mean an indirect and direct reaction and not an indirect ionization.

MEAD: Right!

BACHOFER: This is somewhat less probable, I think, when you're dealing with a fairly simple structure like a phage particle. It becomes more probable, I should think, in the case of bacteria. I mean your statement would become more probable.

MEAD: I certainly agree with you there. We do know that this molecule, which I said is ultimately concerned, is of utmost importance to the cell. Now I think perhaps we're in a position to say something about the actual nature of this molecule—to say something about its location in the cell, and to go on from there back to where we left off this morning, and to say something about the types of cells that we might expect to be most sensitive to radiation. I hope someone else will start off a discussion of this type. I'm hoping that Dr. Bernheim, for instance, will contribute to this, and a number of others. It seems to me a number of these considerations are perhaps obvious.

BERNHEIM: Some of the effects of ionizing radiation may be the result of lipid peroxide formation in the cell. Lipid peroxides are toxic. When injected into or fed to animals, death may occur in one to five days, depending on the dose. Histological examination of the tissues shows some hemorrhage and cell damage in the bone marrow, intestinal mucosa and spleen, but these lesions are not extensive enough to account for death, the cause of which remains unknown. Under normal conditions, therefore, peroxides of unsaturated lipids must not accumulate in the body. Lipids are closely associated with chromosomes and are present in mitochondria and microsomes, and catalysts for the peroxidation, such as cytochrome, other iron complexes, and ascorbic acid are widely distributed in the cell. These catalysts may be prevented from acting because they are sequestered in such a way that they do not come in contact with the unsaturated lipid, or by the presence of anti-oxidants, substances which can trap free radicals or inactivate catalysts.

Such anti-oxidants are present in the body. Adrenaline and serotonin and vitamins A and E when added to unsaturated lipids *in vitro* effectively prevent peroxide formation. *In vivo*, however, these compounds apparently play a minor role in this respect because it is possible to deplete the animal of them and still have a little or no peroxide formed. In the case of vitamin E deficiency, small amounts of peroxide are present but mostly in the adipose tissue and not in the metabolically active organs.

The major organs of the body may be divided into two classes. The first includes the brain, liver and kidney in which, normally, no cell division is occurring and which are relatively resistant to ionizing radiations. In the second, which comprises the bone marrow, intestinal mucosa and testis, cell division occurs continuously and sensitivity to radiation is high.

When organs of the first class are homogenized and incubated in air, lipid peroxides are rapidly formed. This suggests that breaking the cell structure allows catalysts to come in contact with unsaturated lipids and that the cells of these organs contain little anti-oxidant. On the other hand, similar treatment of the organs of the second class causes no lipid peroxide formation although it can be shown that unsaturated lipids are present. Evidently these cells contain a powerful anti-oxidant, the existence of which can be further demonstrated by adding extracts of bone marrow or intestinal mucosa to liver homogenates and preventing peroxide formation when the mixture is incubated. Tumor cells and cells from regenerating liver also show high anti-oxidant activity.

When an animal is exposed to whole body radiation with X-rays the picture changes. Incubation of intestinal mucosa or bone marrow homogenates now results in lipid peroxide formation and when extracts are added to liver homogenates the anti-oxidant activity, based on dry weight, wet weight, nitrogen or DNA content, is greatly increased. (A similar effect occurs in testis but only a few experiments have been done with this organ.) It seems, therefore, that the radiation has either destroyed the anti-oxidant or released a powerful catalyst. These effects can only be definitely demonstrated 24 hours after exposure.

To attempt to characterize the anti-oxidant, intestinal mucosa was used since it is easier to work with than bone marrow and contains a more uniform cell population. Differential centrifugation shows that there is some in the nucleus and in the mitochondria, but that most of it remains in the supernate associated with the microsomes. It is thermolabile, sensitive to acid, non-dialyzable, and can be precipitated with streptomycin. This last suggests that RNA is present and this can be demonstrated. Incubation with RNAase or DNAase and subsequent dialysis has no effect on the activity. Up to this point the distribution and properties of the anti-oxidant from mucosa are identical with those of a factor from tumors obtained by COHEN and MONTALCINI (*Proc. Nat. Acad. Sci.* **42,** 571, 695 (1956)) which causes proliferation of nerve fibers in chick embryos. Thus the anti-oxidant effect may be only one facet of the activity of this factor. Whether the two activities will remain parallel with further purification remains to be seen.

Ionizing radiation thus directly or indirectly affects a factor which is present in dividing cells and one function of which appears to be the inhibition of lipid peroxide formation. It may do this by chelating potential catalysts. Once the factor is altered by radiation, lipid peroxides can form and, since these are toxic to the whole animal and *in vitro* inhibit cell division, inhibit certain enzymes, and depolymerize DNA, some of the overall effects of radiation may be the result of the actions of these peroxides. The pertinence of this discussion may be that a group of molecules of this type, which seem to be essential to the life of the organism, may be similar to the R′ that Dr. Howard-Flanders has been talking about even though changes can be demonstrated only 24 hours post-irradiation.

MEAD: Perhaps what I meant by the discussion we were having earlier might be in here somewhere. Could you accept something like that?

FLANDERS: Yes, but there may be other materials besides oxygen and nitric acid which can enhance radiation injury.

MEAD: This is just the question I was about to ask you, whether R″ couldn't

indeed by a polymer of one sort or another, and the other question was on the dependence or, I should say, the independence of this reaction on dose rate. Now dependence on dose rate means, in the first place, multiple hit—in the second place, chain reaction. This reaction would apparently be neither. Hence, the other point that I tried to make, about the importance of this molecule to the cell. One hit on the molecule will not only inactivate the molecule but apparently will destroy the cell.

DOWDY: I would like to ask Dr. Bernheim—we know that the mucosa of the small intestine has quite a lot of unsaturated fatty acids. It also has this anti-oxidant you speak of. How do you explain the extreme sensitivity of the mucosa of the small intestine to radiation?

BERNHEIM: I don't explain it except that apparently the cell function in the normal intestinal mucosa depends on the integrity of this complex associated with microsomes.

DOWDY: The irradiation would be inactivating your anti-oxidant, wouldn't it?

FLANDERS: Do you know whether this anti-oxidant complex is only the result of whole body irradiation or whether local irradiation to the gut will produce the change?

BERNHEIM: No, I don't. I know you can inactivate it with ultra violet light, *in vitro*. There's just one more point I might add. If you allow the intestinal mucosa to recover morphologically after six or seven days, it looks very normal; but this presumably morphologically normal mucosa hasn't got this anti-oxidant in it. In other words these new cells are not normal, and I believe that it has been shown that they are abnormal in other respects as well.

MYERS: I would first like to ask a question and then, depending on your answer, maybe another. You refer to a 24-hour period. Now what was the nature of this period?

BERNHEIM: Post radiation.

MYERS: Do you have to wait that long?

BERNHEIM: This is where we get the first statistically significant difference in the anti-oxidant activity.

MYERS: Now, isn't that in itself evidence that what you're reporting here could not be anything such as Howard-Flanders has been talking about? As I understand his scheme, he's talking about the first or the second thing that happens when a cell absorbs radiation. Now, it if takes 24 hours for the anti-oxidant to become apparent, this must mean than an infinitude of chemical reactions have intervened between the time that the radiation is first absorbed and the time that you can begin to observe it. Of course, there is a further point—that presumably you're measuring an effect on very large numbers of molecules. I would imagine that by this time a much larger number of molecules have been affected than could possibly have been initially affected by irradiation.

BERNHEIM: Yes, I think that's perfectly true, if you simply take the anti-oxidant activity into account. But we believe that this anti-oxidant activity is only one of the activities of this particular complex we're dealing with, and if we could test this with COHEN'S technique we might find that the increase in the neutral growth of the chick embryo might be affected sooner, but this is purely hypothetical. I realize that this is a discrepancy—this 24 hours is a discrepancy.

MYERS: Isn't it actually more likely that you detected a synthetic process, which synthesizes your anti-oxidant?

BERNHEIM: That's a possibility.

BACHOFER: This is a simple question, really, on the technique—or rather what you're doing. I think you said that apparently the anti-oxidant activity of the

guts had decreased in this period after irradiation. Have you actually, say, made extracts from the gut 24 hours later—and found that it does have a lower anti-oxidant activity?

BERNHEIM: Yes.

BACHOFER: It does experimentally?

BERNHEIM: Yes.

BACHOFER: You don't need to say apparently then. I mean, I thought maybe you were theorizing it—you've demonstrated this.

BERNHEIM: I meant apparently—as it is apparent.

BACHOFER: I'd like to ask another question or two. They're simple questions on technique. This is direct to Dr. Howard-Flanders. You mentioned restoration and injury. You apparently have to measure in order to get figures in the equation, degree of restoration and degree of injury. What is the basic assumption here, that all of the cells are injured? Then you simply check the number, say, that come through uninjured, as opposed to those that are damaged? How do you establish the number that are restored? What's bothering me is that you say that these are restored and those go through to injury. Maybe the first ones weren't damaged in the first place. I'm bothered by this restoration bit.

FLANDERS: The restored cells are among those surviving an irradiation under anoxia.

BACHOFER: But maybe they've never been R′ in the first place. Maybe they were just straight normal cells all along. What evidence is there that they went through this process and then were restored.

FLANDERS: There's the difference between the proportions of cells surviving a given dose when delivered either in the presence of oxygen or in its absence.

BACHOFER: Okeh.

FLANDERS: And I attribute this difference to whether or not oxygen is present to react with R′ and so complete the process of injuring the cell.

BACHOFER: Okeh. Just to interject a point. I think Dr. Scholes said that you added oxygen 20 milliseconds after irradiation; is that correct?

FLANDERS: I'd be happy to discuss this, but are we not discussing Dr. Bernheim's paper at the moment. Should we not stick to that for the time being? But I'd be happy to show you afterwards.

HALEY: I'd like to direct a question to Dr. Bernheim concerning unsaturated fatty acids. Five or six years ago it was shown that an unsaturated fatty acid in the brain, if given intravenously to a normal animal caused a rapid hemolysis. Now there wasn't any discussion in the paper concerning the other areas that would have been affected by the same blood supply. I just wonder whether peroxide really had anything to do with the effect in those animals where you gave the organic peroxide; or whether the action itself was predicted on the acid without having had the peroxide group in it.

BERNHEIM: No, if you inject a similar amount of unoxidized fatty acid, you get no effect at all.

HALEY: Not even in the mouse?

BERNHEIM: You don't inject intravenously, you inject intraperitoneally.

GLASSER: It occurred to me that if we characterized this anti-oxidant, and if there were a relationship between the anti-oxidant and R′, we may come a bit closer. Now how specific is this anti-oxidant? In other words, are there other biological anti-oxidants which can, in part, substitute for your microsomal fraction?

BERNHEIM: Yes. I mean vitamin E, vitamin K——

GLASSER: To what extent?

BERNHEIM: You can't really compare them because we don't know the molecular weight of our anti-oxidant.

GLASSER: Well, in terms of characteristics. Are there any differences between the tocopherols and your fraction in rate of response?

BERNHEIM: No. The technique is this: You take mitochondria, which contain 30 per cent of fat, a good deal of it unsaturated, and you incubate them with either iron or a correct amount of ascorbic acid. When you do that you get rapid peroxide formation in the mitochondria. Then you take your fractions and add them to the mitochondria before you incubate and you find out how much inhibition of peroxide formation there is. Now, small amounts of tocopherol, or vitamin K will do this, but we don't know what the relative effectiveness of the known anti-oxidants is to this complex that we are dealing with simply because we don't know what the molecular weight of the component of the complex is. It's not a question of rates, it's a question of how much you have to add to cause complete inhibition of peroxide formation in the system.

SCHJEIDE: The objection was raised, I believe by Dr. Myers, that a significant amount of this peroxide could only be detected after 24 hours post-irradiation. I wonder if we can, however, consider this possibility—it would seem that the existence of an anti-oxidant system would indicate that there is, in these particular cells, a significant tendency towards peroxide formation. At all times there may be a minute quantity of peroxide present; and this might be enough to enter into such a reaction as proposed by Howard-Flanders.

CASARETT: I will ask some questions designed to see if this is related to the acquired radio-resistance of the intestinal mucosa in repeated daily doses as shown by BLOOM. What are the doses that you used to reduce the anti-oxidant?

BERNHEIM: I would guess 600.

CASARETT: You measured this reduction in the anti-oxidant. At this point did you try to abuse the tissue to see whether lipid peroxides were then formed?

BERNHEIM: Yes.

CASARETT: And were they in a stable state?

BERNHEIM: Yes. You abuse the tissue after irradiation. It now produces peroxide.

MEAD: I would like to report some work that has to do with this. This is work by PHILPOT at Harwell. You might say at first glance that it is whole body work of the grossest sort. PHILPOT irradiates mice and then takes the whole animal and dumps it into a Waring blender and extracts it with butanol. Then he titrates the butanol for peroxide; and according to his method of titration he finds a certain amount of peroxide in the butanol extract of the irradiated animal which is considerably in excess of the peroxide of the control. The interesting thing about this is that he claims that the amount of peroxide he gets in a mouse from an LD-50 dose of X-irradiation is equivalent to the amount of lipid peroxide which on intraperitoneal injections is also an LD-50. I do not think it logical that these two effects could be as closely related as these results would indicate, but, on the other hand, when I had recently reported some work that we had been doing which indicated that it was not the peroxide formation that had been induced by radiation but anti-oxidant destruction, he performed his experiments under nitrogen and he found that under these conditions he got little or no peroxide formed in the irradiated mouse. It was only after he exposed this irradiated mouse, or the butanol solution, to air that he got this large amount of peroxide. So the theory now, I think, is more a destruction of anti-oxidant than a formation of peroxide in tissues of this sort.

FLANDERS: So the high yield he reported for peroxide was spurious.

MEAD: Well, not exactly—because, you see, if, after having done everything anaerobically, then he exposed it to air he did get the high peroxide in the irradiated mouse and not in the control.

DANIELS: How long does the mouse live in the absence of oxygen in the first place?
MEAD: This is after the mouse is dead.
DANIELS: Is catalase present in these tissues? Catalase is essentially universal.
MEAD: Catalase doesn't attack these organic peroxides.
DOWDY: Jim, didn't you do some of your experiments with linoleic acid *in vitro* in which I assume you had an anti-oxidant, and formed peroxides. There is a difference between doing it with and without oxygen. There, you had no anti-oxidant present, did you?
MEAD: You can prevent this oxidation reaction. The question was didn't we do experiments of this sort with or without anti-oxidant, and with or without oxygen.
DOWDY: Well, you did it *in vitro* and I assume you did it without anti-oxidants.
MEAD: Yes.
DOWDY: Didn't you produce organic peroxides, the only difference in this production being the amount of oxygen you had present? Now, how does that tie up with the theory that you really aren't forming them *in vivo*, but you're only destroying the anti-oxidant?
MEAD: Suppose you do the experiment *in vitro* with anti-oxidant present. Then you can irradiate until the anti-oxidant is destroyed, and at that point you get peroxide formed.
LESSLER: Would you clarify for us again what you are referring to as anti-oxidant—are you referring specifically to cytochrome reductase which you mentioned this morning, or do you mean other types of anti-oxidant materials?
MEAD: Unknown anti-oxidants—I should say known or unknown.
LESSLER: Can you give us some ideas of these other ones, other than the reductase system?
MEAD: Well, the anti-oxidant in the reductase system was tocopherol, apparently. I suppose there is a number of anti-oxidants known to be used in the cell, such as some of the other lipid anti-oxidants. Vitamin A is an example, and did you say vitamin K worked well?
BERNHEIM: It does not. It works, but not too well.
MEAD: Neither A nor K works as well as tocopherol. There are also aqueous anti-oxidants such as the SH materials—I think we could probably think of some others.
FLANDERS: Can Dr. Bernheim give some idea of the level of toxicity to be found in these peroxides?
BERNHEIM: I wouldn't like to state if off-hand. I have the data, but momentarily I don't recall it. They're very toxic in very small amounts.
DUCOFF: I just wanted to ask Dr. Bernheim whether he had ever looked to the appearance of the anti-oxidant in regenerated liver, and if so what its prime course of appearance would be in comparison.
BERNHEIM: In regenerated liver you find that the anti-oxidant increases when mitotic activity is at maximum. Thus you take the partially heptectomized rat and you find that 10 hours afterwards it has pretty well normal anti-oxidant activity. Then, at 48 hours (or was it 24?) you have a period when there is maximum mitotic activity. Then you have increased anti-oxidant activity. And then as the mitotic activity falls off the anti-oxidant activity approaches normal.
DUCOFF: Could this perhaps be interpreted as a sort of adaptive enzyme?
BERNHEIM: Yes. I'd like to think of it as a protection against peroxide formation, since peroxides are very toxic to cellular division; and if you want to look at it that way, I don't see why not.
DUCOFF: Is an increase of anti-oxidant brought about by administration of small quantities of peroxide?

BERNHEIM: We haven't tried that.

SCHJEIDE: Would it be correct to say that the theoretical significance of your work is not in the amount of peroxide formed after any certain period, as you detect it. That's not the important point, though you take this material and inject it into animals or into tissues or tissue cultures and observe effects from it. The latter experiment is merely to show that the material is toxic. Don't you feel that the real significance of your experiment is that peroxide accumulation occurs (continuously) within cells in minute quantities and perhaps there is a slow peroxide accumulation after irradiation due to breakdown of the anti-oxidant system? Minute amounts of peroxide in a specific site may be more damaging than more peroxide applied randomly.

BACHOFER: Did you find that tocopherol protected the animal against irradiation?

BERNHEIM: No.

BACHOFER: Did you use it?

BERNHEIM: It has been reported that it doesn't.

BACHOFER: How was it administered?

HALEY: It was administered intraperitoneally. I did the work, and this was a water-soluble tocopherol. Dr. Mead has done the oil-soluble tocopherol, and somebody else also did oil-soluble tocopherol, and it doesn't work. I don't think it gets into the cell.

BERNHEIM: Does vitamin K? Have you tried that?

HALEY: No. Lately ELLINGER claims that it has no effect in protection against irradiation, but there are about four papers over the last ten years that show it has a radiomimetic effect, so you flip your coin and take your choice.

MEAD: Has anyone done a tocopherol-deficient animal?

GLASSER: They're being done now in our laboratory, but no results are available yet.

BACHOFER: We irradiated some tocopherol-deficient, we think probably they were tocopherol deficient, but these were not mammals, these were flour beetles, *Tribolium Confusum*. There are several views on this, but actually we got highly purified patent flour in which the tocopherol content was very low. We fed these insects on this for quite a while, then irradiated them. Then we used the same patent flour, with varied quantities of tocopherol added, and saw no difference. Then we thought maybe we just needed more, so we tried feeding the insects on straight tocopherol—pure tocopherol. They ate it and liked it—thrived on it. There was no difference in the radiosensitivity, all the way from purely highly refined patent flour to pure tocopherol. We could see no difference.

SCHJEIDE: There may actually be critical systems within cells which require the interaction of peroxides. But in other parts of the cell there may be critical systems such as the sulfhydryl enzymes, which may be attacked with harmful results by organic peroxides as well as by HO_2 radicals.

MEAD: I wonder if, before we end this session tonight, we could return to something that I tried to bring up at the very start, and that is to end on a note in which we at least have eliminated chemical substances of the cell which are not directly affected by radiation, and I can start this discussion off by saying that I think we can eliminate the very small molecules, and we can eliminate the molecules of which there is an abundance in the cell. For instance, I think we could say that the destruction of formed enzymes is not going to be the destruction that kills the cell. You can say that the destruction of carbohydrate *per se* is not going to do it and the destruction of fat is not going to do it, although any of these could be indirect. Perhaps we're going to have to limit ourselves to a compound of which a single molecule destroyed, will destroy the cell, and the question is

what type of molecule is this? It seems obvious at the moment, to me at any rate, that it must be a template in order that this destruction can be multiplied many-fold in the cell and result in the cell's death. I wonder if this is a statement of fact and whether we can go on from there and discuss the effect on the cell, or if this statement has to be modified.

SCHJEIDE: I would certainly like to restate the possibility which was brought up this morning that sulfhydryl enzymes, in a location where they are not very well protected by other anti-oxidants, can be inactivated by very low doses of radiation. Presumably they have some purpose in a nucleus or they wouldn't be there. Their continued presence in full concentration could be required for viability in some cells.

MEAD: But if you irradiate the cell and then examine these enzymes, they may have increased.

LESSLER: To come down to a template type of molecule (I frankly don't see how you arrive there) what about a molecule of the type that sets off intracellular triggers? These intracellular triggers are numerous in type. They are part of what we've earlier been hinting at as feed-back mechanisms. Once these particular triggers are set—and the cell has a large number of these triggers I think—then a chain reaction can sweep on. This is not a template type of molecule. This is a disruptive series that occurs as a type of self destruction of the cell in the sense that there are parts of certain organisms that are shot out like trichocysts and so on by a single stimulation. Isn't it conceivable that within the cytoplasm, or nucleus, or both, we have a series of such triggers, and may hit one or more of these? Is this not a separate type of molecule, or molecular system, from the one mentioned?

MEAD: Could you give a concrete example of such a molecule?

JAMES: This is a poor example. Considering down the chain from glycolysis you approach one critical spot—co-enzyme A. Here is a sulfhydryl-bearing system that obviously controls a whole set of different reactions. It is in a sense a pace maker; the system will go in the direction of the KREBS cycle, it may go toward fat formation, or a myriad of other reactions. Obviously, here is a point where the system is poised and anything that would tend to disrupt the equilibrium would tend to push one of these syntheses off in another direction and consequently a build-up of a product which would in a sense be autocatalytic. This is something like the examples Dr. Lessler was talking about. It may trigger the sweeping of the whole system out of existence.

MYERS: At the Ciba Conference on radiation biology, KREBS made some remarks that are very similar to these which we have just heard. He considered the various enzyme systems and pointed out that for most of them, the enzyme is not the rate-controlling factor at all, but that a substrate is. He did select a half dozen enzyme systems in which he felt that the enzyme concentration itself was the rate-controlling factor, and he suggested that if these enzymes were knocked out, or if their synthetic mechanisms were knocked out, that this would be disastrous to the cell. In this connection I would like to object to something else which you have said, Jim, and this refers to eliminating small molecules. In the first place I haven't the least idea where you draw the line on the small molecule. But I can see that in some of the enzymes the co-factor might be a small molecule such as a porphyrin or some other molecule which many of us would consider small and which could be very important if these pace-maker reactions are important.

HALEY: Jim, I would like to bring out the point that you and I have discussed many times in the past—that it may not be the enzyme but it may be this substrate that Larry is talking about. We all know about the original sulfanilamide

work. Now you can take sulfanilamide in the presence of paraminobenzoic acid and practically titrate out the effect of sulfanilamide. It is acting somewhat as a template or, in this particular case, a substrate for the enzyme system. It is possible that in the production of this R′ we've spent so much time on tonight, we make an aberrant molecule which is only changed slightly. It isn't changed by a large order of magnitude, so that the cell may not die in the first generation. It's changed just enough that we can have three situations occur. We can either force the reaction at a much greater rate than normally, or it will have no effect on it. Now, with those organisms we discussed this morning, where the death didn't occur until the fifth generation, I would like to think that the R′ just gradually exhausted the material that was essential with each cell division, and that the reason we may not be able to find out what this is, and also the manner in which is disarranges the system into which it goes, is due to rather minor changes in the molecule.

TOTTER: I don't disagree with your reaching the conclusion you were trying to reach a while ago, but I agree with the discussant over here that you got there awfully quickly.

MEAD: Well, I thought we'd been getting there this entire session, and just to defend myself against some of these remarks that have been made recently, I wonder if confusion isn't existing here between one molecule and one substance. It doesn't seem to me that the destruction of one molecule of an enzyme is going to affect the cell at all.

TOTTER: There is a system which, if one could produce some evidence for it, would be exactly what you're speaking about. Now suppose you had in a cell a sack of RNAase held together with RNA, and the ionization released one bound RNAase molecule outside the sack. You would have a single hit-killing curve, it would be acting upon an enzyme and the results would be capable of destroying the whole cell very readily.

LEVEDAHL: I think in keeping with a few of these last remarks, one is driven almost to the conclusion that it must be a template type of structure that is involved. In the last case that Dr. Totter was mentioning, it seemed to me that the mere destruction of an RNA molecule *per se* is of little or no consequence. The problem, it seems to me, is that only if RNA acts as a template, which is what was originally suggested, does the feed-back mechanism and the response of the system to low level irradiation make sense. Let's say that we use a very low level of whole body radiation and, from that, attempt to calculate the number of molecules of fat or enzyme, or what you would, that will be affected. It seems that the number is very, very small and cells would be able to recover simply by replacement of these molecules, through, if you would, synthesis involving an RNA or a DNA template. It doesn't seem to me that irradiation effects, particularly at low dose levels, can be explained by the destruction of such things as the sulfhydryl enzyme or unsaturated fat or some other such thing. Rather, information must be fed back to the template mechanism and I, for one, would like to defend the statement that I don't think Dr. Mead jumped to this conclusion. I think it was obvious from the knowledge of the fact that low levels of radiation will effect biological systems.

LESSLER: Ever since you posed the idea that we come up with a couple of trigger mechanisms, I've been thinking what I could throw into the hopper here. Two things come to mind to produce very rapid cell death, in the absence of radiation in living cells, and in all living cells in which we've ever tested it. One is to put a micro needle into the cytoplasm and allow the tip of the needle to create a very small vibration. Usually this vibration is hardly enough to see with the higher

powers of the microscope. A wave of cytolysis sweeps through the entire cell and the cell dies. Another simple trigger which you can pull, is to take a micro needle and play with the nuclear membrane of the resting cell, and again you can create this wave of cytolysis which will cause the death of the entire cell. Now, there also have been experiments on the injection of fantastically small amounts of materials into cells, but it's a little late at night and I can't quite think of the names of these things. MIKE KOPAK did some of them and ROBERT CHAMBERS did some of them, that also triggered some peculiar mechanism that the cell has for self destruction. Certainly the area of injury that we're speaking of is in the realm of almost sub-microscopic proportions. You don't damage more than a tenth of a micron of a total cell.

JAMES: I, for one, don't see any reason why one can't consider the template important in this situation and as important as you've stated. In fact it may be the template which actually provides for the production of this so-called pace-maker. What do you see first if you look at metabolic activity? You're going to see a change in the metabolic activity which may be trivial; whereas if you wait and let the effect on the template take its course you will see a pronounced change in metabolic activity.

GLASSER: Well, I must agree that the consideration of the so-called feed-back approach to the problem does not exclude considerations of template, but considerations of the feed-back opens up the entirely new approach which we haven't considered yet, and that is the residuum of radiation injury, which we appreciate in the mammalian organisms as perhaps life-shortening or early geriatric changes. It is conceivable that a disruption in some integral system of the cell or cells or organ systems can seek perhaps a less efficient but alternate metabolic pathway which in irradiation injury does not necessarily mean death. I appreciate that this is not the tack we've been taking but something which we must come to consider in the influences of sub-lethal radiation.

MYERS: I want to throw a left hook into the proceedings, Jim—and you may not appreciate this, but I'm afraid that for lack of exercising our critical faculties here, we may have come to the conclusion that we're making progress. And I don't believe that we are. Here we're talking about templates and what molecule is initiating damage and once again we don't know what end-point we're talking about. We don't know what radiation dose we're talking about. We don't know anything about the time period after the irradiation; and I want to remind you that with the same cell you can have different things occurring. You can have lysis of the cell if you give it a great radiation dose, or you can get as small a change as a gene mutation. In the first case I would doubt if templates have anything to do with it. In the second case, perhaps they do.

MEAD: Well, I'd like to put the situation up to you; it is now 10·25. At the beginning of this session I asked Dr. Bernheim if he could possibly, some time during the session, try to summarize in two or three minutes what we have accomplished, if anything. According to Dr. Myers we haven't accomplished anything during the session, and I wonder if we will accomplish anything more during the next five minutes or so and, if not, do you, Bernheim, have anything you can say about this session?

BERNHEIM: I have some notes of what has been said. They are subject to somewhat violent correction because some of the material is unfamiliar to me. The session began with a consideration of the radicals formed and apparently the HO_2· radical is fading from the picture, as being the most important. Instead, the OH· radical oxidizes some organic material in the cell which now is able to accept oxygen and cause peroxide formation. This is an irreversible phenomenon and

there are other reversible possibilities here which I won't go into. This peroxide can be formed on pyrimidine parts of the DNA molecule and peroxides can be formed on unsaturated fats. We don't know what damage peroxide on the pyrimidine molecules does to the organism but probably it does considerable. In the case of the fat peroxides, we know that they're highly toxic. The reason why the $HO_2\cdot$ radical is suspect, in spite of the fact that it hasn't got a high oxidizing ability, is that it may break down into H^+ and O_2^- ions which are highly reducing. And the reduction doesn't play apparently a very important part in radiation damage. The bacteriophage is apparently now suspect but Bachofer believes that reduction is not as important as oxidation. And when you talk about protection from radiation you're talking about materials that will (a) mop up these oxidizing radicals, or (b) produce an anaerobic environment in the cell, or (c) act as anti-oxidants. All these sulfhydryl groups or substances are fairly good anti-oxidants.

In the bacterial cell, it is postulated that a substance is formed as a result of a hit on a cell, presumably at a critical point in the cell. A one hit phenomenon produces a substance which has a life of less than 20 milliseconds, apparently a large molecule and this R′, as Dr. Howard-Flanders called it, has two possible fates. One, under an anaerobic condition, it may be restored to its original condition or it may go on to an R″ and this second reaction is a result of a multi-hit phenomenon. In oxygen, however, the R′ goes presumably to some type of peroxide or an oxidation product. This picture is somewhat confused by the fact that in spores the radiation effect is dependent on the dose rate and consequently the greater the radiation density, the greater the radiosensitivity. Also the sensitivity to radiation goes up with temperature. This picture as outlined for a bacterial cell does not hold for spores of this particular megeterium. The fact that the $OH\cdot$ radical is important in the original production of an organic radical clears up the situation for the oxidation of porphyrins which apparently are oxidized by the $OH\cdot$ radical and then are further oxidized in the presence of oxygen. That is, a radical is formed as a result of radiation which then adds on oxygen and this breaks the rings. I don't think it's necessary to summarize the general discussion as to whether a template, or what might be called a critical molecule, is being hit by irradiation or whether the essential, or key, enzymes are being hit.

BACHOFER: This is an excellent summary. I don't think it necessary right now to go into any particulars, but as a kind of generalized point of interest, the importance of $HO_2\cdot$, recalling the question, I believe you said $HO_2\cdot$ breaks down to H^+ and O_2^-, which is highly reducing. And I believe that in an acid medium it is oxidizing. So this would be an erroneous note, for the $HO_2\cdot$ breaks down to $H^+ + O_2^-$ and O_2^- is oxidizing in the acid medium, is that correct?

SCHOLES: No. $HO_2\cdot$ ionizes in alkaline solution. $HO_2\cdot$ is an oxidizing agent in acid solution and a reducing agent in alkaline solution.

MEAD: Well, if the summary is accepted with that correction, then I propose we adjourn now and convene tomorrow morning to go into something complicated.

END OF SESSION II

SESSION III

SENSITIVITY OF DIFFERENT CELLS IN THE SAME ORGANISM

Introductory Speaker: V. P. BOND

IN yesterday's sessions some possible mechanisms to explain the radiosensitivity of a 'model' cell were discussed. Although mechanisms remain elusive, it is clear that all living cells are 'radiosensitive' in that serious biochemical or morphological changes, and even death, will result if the dose of ionizing radiation is sufficiently high. This morning we begin to consider the sensitivity of different cells in the same organism. Here the situation becomes more complex, and I shall be quite pleased if we can agree on outlining the problems involved, let alone solving them.

Yesterday we dealt with individual cells or essentially uniform cell populations; today we deal with cell populations in a multi-cellular organism such as the mammal. In considering differences in sensitivity among the different cells, we are concerned primarily with different cel' populations within a normal individual. Are there true differences in radiosensitivity and, if so, why? It is important to distinguish between *cell* and *tissue* sensitivity. With cell sensitivity, we refer to cell populations that are considered to be homogeneous morphologically and functionally, such as mature erythrocytes, hepatic cells, erythroblasts, small lymphocytes, etc. The population is considered independent of the degree of maturity—independent of its origin or its destination. With tissue or organ sensitivity, we refer to the entire sequence of developing cells in a tissue or organ from the most primitive normal progenitor cells to the mature functioning cell. For instance, erythroid tissue consists of several cell populations including erythroblasts, normoblasts, reticulocytes and mature erythrocytes (and each apparently homogeneous subpopulation undoubtedly can be broken down into further subdivisions depending on age, stage of mitotic cycle, etc.). We are thus dealing with compartments, which may include generative, maturation, storage and functional compartments. These compartments are morphologically distinct in some tissues, not apparent in others.

With either cell or tissue sensitivity, we are dealing with cell populations, and usually deal with averages or means in comparing the behavior

of cell types. And like an 'effective energy' to represent an X-ray spectrum; so this may only poorly reflect the behavior of each constituent of the population. We have the advantage that presumably all cells in the populations we consider are homogeneous genetically; yet, certainly, under some conditions at least, damage to one cell population (or organ) in an animal may result in damage to or affect others. This interaction may occur in populations both of which are irradiated, or irradiation of one population (organ) may result in changes in distant populations not exposed to radiation (the 'abscopal' effect of MOLE[1]). With single cells or isolated cell populations, frequently wide variations in oxygen tension, temperature, pH, etc., can be accomplished in order to study the mechanisms of differences in sensitivity; these approaches are limited in scope in the mammal. With isolated populations or clones one can be certain of a homogeneous population; in the mammal it is frequently difficult to be sure, even in a group of cells considered to be identical by morphological criteria, that one is dealing with a uniform population.

It must be stated what is meant by the rather confusing term 'radiosensitivity', and the all-important factors of dose, criterion of effect, and time at which the observation is made must be considered carefully. Sensitivity can be defined in terms of different apparent degrees of response for the same radiation dose. A comparison of the doses required to produce the same degree of biological effect, however, allows more quantitative treatment. The criterion of effect chosen obviously must be potentially manifest in the populations studied in a reasonable period of time, and it must be amenable to quantitative treatment. Even in attempting to compare only two cell populations such as mature heterophil granulocytes and erythrocytes, it is difficult to choose criteria that are observable, are common to both, will appear within a reasonable period of time, and are free of 'abscopal' effects. When attempting to compare a number of populations in the same organism these problems become extremely difficult. Because of the difficulties of considering adequately the many possible variables, the literature on the relative 'sensitivity' of different cells and cell systems has become confused indeed. In the usual study, some criterion of effect (cell death, mitotic inhibition, biochemical changes, etc.) has been observed in two or more populations. An attempt has been made to correlate the degree of effect with some property of the normal population. No small problem, with this approach, is that cell populations change in composition after irradiation, and thus observations on a population may bear little relationship to those of the unirradiated population. Frequently, the sensitivity of cell

populations or organs have been compared in relation to their contribution to mortality in the irradiated animal. Although of considerable importance, this criterion of effect has only limited usefulness in attempting to understand the differences in radiosensitivity of cell populations in the same organism. Confusion has arisen in differentiating between organ and cell sensitivity. Not only is the distinction important in considering the disappearance of cells after irradiation, but also in studying tissues at a time after radiation such that some 'recovery' has taken place. It is highly unlikely that any significant 'recovery' of an irradiated cell takes place. The damage probably is permanent. However, a tissue can regenerate, although the source of the new cells is open to some question. Presumably, especially at lower doses, more 'resistant' cells of the population in question could serve as a nucleus for repopulation. It is thought by many that some cells in the body have the capacity to become transformed under some circumstances and thus serve as a nucleus for regeneration of a depleted cell population. The organ can thus 'recover'. However, this is not recovery in the true sense; rather it is regeneration involving replacement and thus new population(s) of cells. It is possible, indeed probable, that a radiation-induced defect may be present in the regenerated population.

In view of the above, it is seen that 'sensitivity' to radiation, like the relative biological effectiveness (RBE) of different radiations,[2] is meaningless without a complete description of the conditions employed. Similarly, just as the RBE of different radiations may have many values depending on the biological criterion of effect used, there can be no single 'relative sensitivities' of different organs to a given radiation—the ratios will depend on the criterion of effect employed and the precise conditions of study.

Apparent differences in radiosensitivity of different cell populations in the same organism were recognized early in radiobiology. Like a surprising number of radiobiological concepts that appeared first around 1905 in the German or French literature, radiosensitivity of different tissues was considered in a definite 'law' formulated by two Frenchmen in 1904. This 'law' of BERGONIÉ and TRIBONDEAU was derived from studies of the effects of X-rays on spermatogenesis in the rat and has had a profound influence in radiology and radiobiology. Actually the law was stated briefly in three parts, and it will probably suffer now in translation as it has in the past. The first part states that X-rays are more effective on cells with greater reproductive activity, meaning apparently that cells in more active mitosis are more sensitive. The second part refers to cells

having a 'longer dividing or mitotic future' as being more sensitive, indicating apparently that cells requiring the greater number of divisions before maturity is reached are more sensitive. The third part states that cells are more resistant in proportion to the degree of morphologic and physiologic differentiation. There is no reference to sensitivity related to the phase of mitosis, or mitosis versus the resting stage. Tumor cells were considered to be relatively radiosensitive because of their 'great mitotic future'—the large number of divisions required before maturity. They noted that radiation produces abnormally dividing cells, and that these atypical cells might be avoided by means of fractionation. The law contains three variables, the values of which are not known with any degree of certainty for most cell populations. Because of the three conditions of the law, it is not difficult to mold almost any situation into conformity.

With regard to cell sensitivities, the law holds in a very general way, although exceptions are not difficult to find. By any criterion of effect (e.g. pyknosis, cell disappearance) there is no doubt that, in general, younger or generative cells of a given tissue are more sensitive than the mature functioning cells. Also, there is no doubt, for instance, that the rapidly-dividing bone marrow precursor cells are more sensitive than the adult liver cells which rarely divide. An outstanding exception, which I am sure will receive much discussion today, is the small lymphocyte. This cell is extremely sensitive in that it becomes pyknotic and dies at relatively low doses *in vivo* or *in vitro*, even though mitosis is extremely rare indeed.

A basic difficulty arises in carrying through the law of BERGONIÉ and TRIBONDEAU to its logical conclusions. If the more primitive cells are more radiosensitive, it is hard to reconcile this with the fact that, at least with renewal tissues such as the blood cells, regeneration occurs after essentially complete destruction of the generative populations by radiation. By the law, the young stem cells should have been demolished if the entire population is destroyed. With tissues in which the most primitive precursor is known to disappear at relatively low doses, the problem arises as to the source of cells that repopulate the tissue. It seems evident that the most primitive cell in the generative compartment must either survive (be resistant), or in some way be made available in order for regeneration to take place. If logarithmic growth occurs, only a relatively few cells in the generative compartment would be required for a nucleus. If a wide spectrum of 'sensitivities' exist in a population of generative cells, some would escape serious damage even at relatively high doses. It is also possible that wide differences in sensitivity of cells

in an apparently homogeneous generative compartment might result from differences in vulnerability at different stages of the mitotic cycle, or to local differences in oxygen or sulfhydryl concentration. An alternate explanation to survival of a few primitive cells in the generative compartment of interest would be the possible transformation of cells of a different type into the required primitive cells. In this regard, the large lymphocyte[3] and small lymphocyte[4] have been implicated. It appears likely that 'reticulum' cells—primitive mesenchymal, or reticuloendothelial cells may have the capability of transformation when required. It is known that with some tissues (testis, ovaries, small bowel), at doses sufficient to deplete the tissue, the younger cells do survive and allow repopulation.

With regard to tissue sensitivity, the law does have interesting implications. For example, consider the behavior of the peripheral erythrocyte population, reflecting the activity of the bone marrow progenitor pool, which has been well studied following irradiation. The red cells themselves are highly resistant to radiation; red cell precursors in the marrow are 'sensitive' to radiation in that additional division in inhibited and cell pyknosis and death occur. After a dose of radiation sufficient to inhibit or destroy the precursors, yet insufficient to cause thrombocytopenia bleeding, the rate of disappearance of red cells can be predicted for a time on the basis of the normal rate of disappearance of the mature erythrocyte. That is, the red cells disappear at a rate as though the supply were cut off and normal destruction without replacement were progressing. In this situation, gross evidence of damage (disappearance of cells) is apparent in the mature functioning cells of a tissue, even though the mature elements are quite radioresistant. This part of the law has attractive implications. If it held fairly generally, it would not be necessary to postulate a direct radiation death of mature, or even immature cells at doses in the lethal range, nor a difference in radiosensitivity of mature cells. A temporary cessation of activity in the generative compartments would explain the observed effects. (There is, of course, no doubt that essentially immediate death and destruction of any cell will result if the dose is sufficiently high.) If a vital mature functioning cell population reaches low levels before sufficient regeneration in the generative compartment can occur, the animal would die. There might thus be no 'true' difference in radiosensitivity of different tissues at these dose levels—apparent differences would only reflect differences in average life span of the mature cells, and 'regenerative capacity' of the precursor cells in the generative compartments.

This concept is obviously too simple—it is known that death of both mature and immature cells does result from radiation in the lethal range. Yet it may be profitable to see what degree of generalizations may apply. Although mitotic activity and estimates of the average life span for a number of cell populations in the mammalian body have been determined, it is difficult to find a large series in which a number of tissues in the same organism has been examined by the same group of investigators under the same conditions.[5] One such group of data is that of KNOWLTON and WIDNER.[6]

These authors used whole-body X-irradiation to inhibit cell division, from which mitotic times for various tissues were estimated. The mitotic time thus determined was divided by the normal mitotic index of each tissue to yield 'intermitotic time' (actually the generation time or mean life span under the conditions used). The generation times thus obtained are shown in Table III(1). The data were collected carefully, and the results, in general, bear out the thesis that the degree of mitotic activity of the various tissues falls in line with classical ideas regarding their relative sensitivity. The outstanding exception, again, is the lymphocyte. The generation times obtained, however, must be regarded as rough approximations only. The reasons for this will be brought out in the following discussion.

Table III(1)

Mitotic index, mitotic time and generation time of mouse tissues (*from ref.* 4)

Tissue	Mitotic time (minutes)	Mitotic index	Generation time (hours)
Jejunum	24	9	43
Nucleated Red Blood Cells	30	5	99
Myelocytic Series	35	4	155
Ovary	21	3	123
Lymph Node	23	7	580
Epidermis	30	8	670
Adrenal Gland	14	2	1090

Many authors have used mitotic index alone as some sort of index of the proliferative activity of tissues. The mitotic index, however, is a static parameter that has limited usefulness by itself in a dynamic situation. In addition, it is a ratio of two values (number of mitotic figures/total cells in the population), and thus it alone does not give absolute values. There are real technical difficulties in determining accurately the number of mitotic figures present in a tissue, and a greater difficulty usually in

determining which cells belong to a single population. The ratio itself tells nothing of turnover rate, average life span or turnover time in the population.

If we consider what is known to be a mature (non-dividing) population such as the erythrocytes, if the 'supply' is cut off completely (assume no 'recovery' or regeneration) the cells will disappear in proportion to the average life span of the adult cells. Mitosis is not involved directly. If the generative and maturation compartments, responsible for the production rate of mature cells, is considered, the following pertains. A crude estimate of an average life span (generation time) can be obtained if both the mitotic index and the mitotic duration (mean life = mitotic duration/mitotic index) are known for a uniform population. However, this equation holds only if the life cycles of all cells in a uniform population are essentially equal in length (no cell death in generative compartment), and if the population is stable with regard to the numbers in a given age group. If this is not true, large errors may result. In probably all cell types, or tissues, we are dealing to some degree with several compartments during the life cycle. This is clear-cut in some tissues such as the blood, in which progenitor, maturation, storage, and functional compartments are distinguishable. Frequently, the compartments are not anatomically or morphologically distinct, and more than one compartment (including that representing loss to tissues) may be included in the experimental data. Probably different cell systems follow different pathways in the process of cell generation and maturation. Generation time alone gives no information on the rapidity of cell production in the generative compartment; the size of the compartment must be known also. In addition, the formulae apply to populations in which all cells in the generative compartment have the potential to divide. It is by no means clear to what degree this applies in most tissues. Obviously, the already too simple mathematics break down completely under these circumstances. What I am getting at is that both the experimental data and the mathematics applied to the observations have been greatly oversimplified, and we are at present in no position to make definitive statements regarding the life span of most cells and, therefore, are in no position to say to what degree the consequences of the law of Bergonié and Tribondeau may apply with regard to different tissue sensitivities. It would appear that agreement should have been reached by now on the life span of such much-studied cells as the lymphocyte and the heterophil leukocyte; however, this is not the case.[7–13] It appears that a critical reappraisal of the experimental and mathematical techniques used in this field of endeavor is needed badly.

The organs of the body, in general, fall into three categories. Tissues such as the skin, blood cells, and gastro intestinal epithelium show daily losses and renewal. Mitoses are frequent and the entire cell population presumably is turned over fairly frequently. A generative compartment is usually distinguishable. Other tissues such as the adrenal and the liver show only rare mitosis, and division occurs presumably only to replace an occasional cell that dies. A third class of tissues includes the nervous system, in which the cells presumably are incapable normally of mitotic division. It is conceivable that this difference may be one of degree rather than kind, and a wide spectrum of life spans may encompass all mature cell types. All tissues may have a 'generative compartment' although not morphologically apparent. It appears reasonable, when sufficient data are available, that the rate of disappearance initially of many mature cell populations following irradiation may be related closely to the normal life span. Repopulation of mature cells in an organ will depend upon the time interval before regeneration can begin in the generative compartment, and the rate of regeneration once it has begun. It becomes difficult to test the hypothesis in adult cell populations with a long life span, since 'recovery' or regeneration in the generative compartment would ensue before the adult population has become sufficiently depleted to allow detection of cell loss. The degree to which the law operates under these conditions, then, can be studied only under conditions in which the dynamics of the cell populations, both in the generative and adult compartments, and in the 'degenerative' and 'regenerative' phases can be studied.

It appears certain, then, that a mechanism implicit in the law of BERGONIÉ and TRIBONDEAU—the shutting off of the source of supply—will not explain entirely the differences in 'sensitivity' of different cell systems in the mammalian body. Direct non-specific cell death does occur to a significant degree at doses in the lethal range. It is equally clear, however, that such a mechanism as that described in the 'law' is operative in the lethal range and below, and is extremely important in accounting for the rate of depletion and of 'recovery' or regeneration of tissues, at least of the renewal tissues. To determine the degree to which this mechanism is operative, considerable more quantitative information on the dynamics of cell proliferation is necessary. The static indices used heretofore are inadequate for the purpose. It is possible that labelling of cells with tritiated thymidine may provide the necessary answers.[14]

In this introductory discussion I have dwelt on proliferative activity of cells and tissues as a partial explanation for differing radiosensitivity in the same organism, to the exclusion of other possible explanations. Other

factors that have been mentioned as a possible basis for the observed differences include differing oxygen tensions,[15] membrane permeability,[16] differing nucleus to cytoplasm ratios,[17] and differing nucleus to cytoplasmic nucleic acid ratios.[18] Time did not permit a discussion of these factors, and I am sure they will be brought up in the succeeding discussions. It is my opinion that most of the qualities represented in each factor would, in general, show little difference among different tissues in the same organism, and that any perturbation in radiosensitivity brought about by those factors would be second order in nature except, perhaps, in a few special instances.

It seems unlikely that a general theory to explain differences in sensitivity within the same organism will evolve from a consideration of these factors.

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DISCUSSION

HENNESSY: One point which Dr. Bond mentioned that we might start with was this rather peculiar radiosensitivity of the lymphocyte, and I wonder if Dr. Kelly would possibly have anything to contribute on this.

KELLY: Well, I wish I did. I know it's peculiar, but I don't know why. I would like to say first that I believe that KNOWLTON'S lymph node turnover time is too long. In the rat, using DNA incorporation as a criterion for cell turnover time, which is I think as adequate as this one, the thymus has a turnover time of the order of 48 hours, and the remaining pooled lymph nodes of the order of 60 hours.

BOND: May I say again, I put these data on—not that I endorse them, but they are as uniform a set of data as is available and they are useful in bringing out points—to air the problems involved. Actually, what he did was count mixed populations—for instance, he counted the large and small lymphocytes together.

KELLY: Well, my data would include this also. When we study the rate of DNA formation we can't distinguish DNA from the mature lymphocytes and from the immature ones—so that the turnover time which we get is for the total population; just as it is in his case if he includes all of the cells of the lymph node. In fact, to be comparable to our data he has to include not only the lymphocytes but all the stroma cells. There is a footnote in one of WIDNER'S later papers which changes it to 100 hours—which is much closer to what we find in the rat. Regardless of what the true time is, however, I think that TROWELL'S and PATT'S experiments leave no doubt that the death of the lymphocyte has nothing to do with mitotic inhibition, or a cell attempting mitosis or anything of the sort. It seems to be a very immediate reaction and to my mind is very different from what one sees in most other cells that in general break down after cell division or at least after an attempted division. I think the lymphocyte death should be discussed quite separately from most other things, such as genetic and chromosome breakage effects.

POWERS: Some microorganisms die immediately. I would suspect also that some lymphocytes in lymph nodes die after cell division too. Wouldn't that be allowed?

KELLY: I think so, but the death of the majority of the cells must involve some more direct mechanism. According to TROWELL, after 400 r something like 80 or 90 per cent of lymph node lymphocytes are pyknotic within 4 or 5 hours. Most of these are cells which would probably never undergo another division. Possibly an interference with the cell's energy supply or some other acute reaction must be involved.

BOND: In mature small lymphocytes, mitoses are virtually absent and mitotic inhibition clearly would not account for any percentage of the apparent deaths.

MEAD: What is the effect of total dose on the type of death of the cell? As you increase the dose, do you get more of this direct death, and less of the death following an attempted division?

BOND: This has been studied qualitatively and quantitatively in the lymphocyte.[1]

CRONKITE: Dr. Bond has just said what I was going to say. There's very clearly a dose dependence phenomenon of an exponential nature—as the dose goes up there is an increase in the pyknotic nuclei. There's another general comment for which I'll possibly be accused of chiding the biochemists a little bit. I think it is quite evident from what Dr. Bond has said that in normal tissues—take, for example, the gastro-intestinal tract, namely the small intestine—there is both a functional compartment and a generative compartment. And when one irradiates one the proportion of one compartment to another changes. If the dose of radiation at 600 r that Dr. Bernheim used in the results we discussed last night is sufficient for a period of time to stop all new cell division, one then has an ageing

population in the functional compartment. As regeneration commences the size of the generative compartment becomes much greater. I think that the biochemists who stew up a tissue and make a measurement of some enzyme have to normalize it to some compartment because there is an entirely changing population of cells. I don't think that you can do this sort of thing without risking the chance of being seriously misled, as happened in the case of ATPase. One must make the biochemical measurement on a single cell population or prove the identity of enzymes, etc., in each cell population.

BOND: I should like to comment on the difficulty which arises when one attempts to use differences in the irradiated tissue to explain a difference in radiosensitivity between two tissues. As an example of the difficulty, I'd like to cite Dr. Kelly's work on DNA content in different irradiated tissues. In passing, I might say she started out with a whole mouse and wound up working with an ascites tumor, probably because she encountered difficulties with mixed populations of cells. I think HARVEY PATT went to thymocytes *in vitro* for the same reason. When one irradiates an organ, as Dr. Cronkite indicated, a number of things happen within that organ. For most biochemical procedures you are working with a block of tissue. You are usually working with the whole organ such as the spleen or the liver. These do not represent a homogeneous cell population. There is a variety of cell populations, and when one irradiates, one gets disappearance of cells, and they disappear at different rates. And when one irradiates, one also has dead cells. So when one takes the tissue after irradiation, one has a proportion of dead to live cells that varies as a function of time following irradiation. In addition to this one gets nuclear changes. One may get polyploidy, enlargement of the nucleus, and so forth; so that the cell population is changing in this fashion. Take the spleen for instance. When one irradiates these disappear at different rates, and one no longer has the tissue that one started with. Also, when the tissue begins to regenerate, from KAPLAN'S data on the thymus, a new population appears. One population of thymocytes is there normally. During regeneration there is clearly a morphologically different cell present. So, in any situation of this type, where one irradiates and so changes the cell population, any relationships between what you get there and what might be the situation in the normal tissue that one started out to study, are, I think, rather coincidental.

BERNHEIM: Well, the biochemists might as well shut up shop—lest we take this too seriously—because there is no way of comparing the preradiation and post-radiation data biochemicalogically.

CRONKITE: Unless you can get comparable cell populations in respect to age and enzyme constitution you cannot interpret your data. You should not shut up shop but sharpen your cell separation techniques or work with single cells.

BERNHEIM: I have just about decided that the microsome should be considered a possibility in terms of radiosensitivity, because when a cell is dividing or about to divide, the microsome, being the center of protein substances, has to be much more active. It would seem to me that it is theoretically possible to stop a cell division by knocking out the microsomes, and leaving the nucleus intact. I don't think this actually ever happens, but at any rate you have a greater difference in microsomal activity in the dividing cell than in the non-dividing cell and this might change radiosensitivity and explain why in certain cases there is increased sensitivity preceding or during mitosis.

CRONKITE: Apropos of what Dr. Bernheim is commenting on, I'd like to point out that in the testicle there is both mitotic and meiotic division; mitotic division is very sensitive to radiation. Doses of 200–300 r will practically eradicate it for a prolonged period of time, whereas meiotic division continues after doses as

large as 2000 r without any apparent interruption in hamster testicle. And though I do not claim to know all the details of the comparison of meiotic and mitotic division, I believe, so far as the centrosomes and so on are concerned, that all of these are functioning in both in roughly the same fashion. Whereas the vast difference between the two is the new DNA synthesis preceding mitotic division. These comments are based on unpublished observations of DR. BRECHER at NIH, Bethesda, Md.

CASARETT: I'd like to add to that; hyperplasia—erythropoietic hyperplasia—is brought about by various means and also myelocytic hyperplasia, brought about for instance by estrogen treatment, has increased the resistance of these tissues to radiation. I think this is along the same general line that Dr. Cronkite is arguing about.

LESSLER: I'd like to correct what may seem like an erroneous impression. I don't think that any of us conceive of cell division as a synthetic process. I think this was inherent in one of the remarks made. The synthetic processes take place before cell division, and cell division in itself—in many cells—occurs completely in the absence of any evidence of any type of synthesis. It's a physical process and this physical process in itself is double and this should be recognized. There is a nuclear division which is quite independent of cytoplasmic division, and these mechanisms, I think, should be separated in our minds. Synthesis and nuclear division and the cytoplasmic division may each have an entirely different radiosensitivity.

CASSEN: Wouldn't an alternative interpretation—except in special cases of the general correlation of turnover time and radiosensitivity—be that you are producing a latent damage to the nucleus, or perhaps the mitotic apparatus of the nucleus, with equal probability in all nuclei, and that this latent damage only shows up or is developable, as you might say, in those cells that have a quick turnover time. Instead of saying that the mitotic cells are more radiosensitive.

BOND: I am not sure I heard all that you said, but it sounded equivalent to what we have been saying. I'm not quite sure I see the distinction.

CASSEN: Suppose that you say that a nerve cell would cease to be very radioresistant—it only appears to be so, but it never divides.

BOND: Well, that's exactly what I was getting at. This would be a very nice explanation for the whole difference in radiosensitivity. That the differences may be only apparent and that there really is no true difference. It is simply a time phenomenon and that if you could wait long enough for a criterion of effect to manifest itself, all of the cells would have equal radiosensitivity. However, this seems clearly not to be the entire picture because of many exceptions, notable among which is the lymphocyte.

HENNESSY: We've been hearing about the turnover time, mitosis, etc., and I think it would be nice at this point if we could correlate the biochemical events that occur with the processes, if there is anyone here who can do that.

SCHJEIDE: Let me precede such a statement with the comment that although I agree it's very desirable in these studies to work with uniform cell populations we should not be excessively discriminating with regard to the many pertinent studies already carried out on non-uniform cell populations. Much of the data must be valid for our discussions here today. My second comment is the one that you really wanted, I think, and it pertains largely to our discussion of lymphocytes. This cell is not alone in being very sensitive to X-irradiation. There are some other cells such as developing ova, developing sperm cells, abnormal lymphocytes as well as normal lymphocytes, differentiating embryonic cells of several types, proliferating cells of the gut epithelium, etc., which are nearly

equivalent in sensitivity. These cells have some morphological features in common. They all have a very large ratio of nucleus to cytoplasm and they all have relatively few cytoplasmic particles such as mitochondria. But this in itself might not seem important if we could not relate the large nucleus in each of these different types of cells to extensive anaerobic synthesis under aerobic conditions. I want to cite one of these cell types in particular, namely the differentiating embryonic cell. Prior to differentiation, prior to the enlargement of its nucleus and prior to a period of specialized nuclear synthesis, there is often extensive division. However, cells in these division states are *not as sensitive* to irradiation as are those in states in which differentiation is actually taking place. This indicates a high degree of susceptibility at a time when there is a high rate of anaerobic synthesis taking place. I suggest that we look further at these cells and consider the effect of irradiation on this anaerobic synthesizing system.

BOND: Do you feel that this may apply to all of the different types of cells—tissues with different turnover times and with individuals of different ages?

SCHJEIDE: In the developing organism all of the various organs have, prior to their ultimate adult form, passed through a period during which they were very sensitive to irradiation. For example, if the rat foetus is exposed to irradiation at any time between eight days to thirteen days of gestation, you will, depending upon the exact time of irradiation, produce a number of specific abnormalities. This, because the specific organ systems which are undergoing differentiation at that time are very sensitive to irradiation. Within a given period these tissues might include the nervous system, the eyes, the skeleton, etc.

BOND: The reason I asked—I'm not too sure how pertinent this is—was that we know there are differences in sensitivity among species that are of the order of magnitude you were talking about. Now, I wonder if you are still talking about differences in sensitivity in the same organism, or if you might almost consider the young embryo to be a different organism from the adult, and that differences between the sensitivity of embryonic and adult cells might not apply to differences in sensitivity within the same organism.

CASARETT: My impression regarding these periods of differentiation of the embryo is that, during these periods of organic genesis, the characteristic feature of the actual cells that is related to their sensitivity is a high proliferation rate rather than a mitotic rate. In other words, the proliferated neuroblasts in the process of differentiation of the nervous system become sensitive and then nervous system abnormalities are produced, when the irradiation occurs at that time.

SCHJEIDE: The first comment was that there might be a considerable difference in the organisms at one time as compared to another time, and I say that for our purpose it does not make any difference. What we are interested in is a difference in cells, and if we can find out what the difference is, then we've made progress. In answer to the second comment, I can only repeat that apparently it is not the rate of proliferation that makes these cells so sensitive, it is something concerned with the process of differentiation at these particular stages.

KELLY: Dr. Schjeide is referring to HICKS' work on neuroblast sensitivity, are you not? Are not these neuroblasts, which are so very highly sensitive, an exception among embryonic cells? He says that they are no longer dividing cells and yet are killed by very low doses, something like the lymphocyte. Certainly from his paper I gather that he considers these rather an exception. He can correlate many of the neurological abnormalities with the stage of development at the time of irradiation by tracing which neuroblasts were killed.

SCHJEIDE: Most embryologists are of the opinion that the sensitivity of the neuroblast in the newborn rat is due to processes similar to those which most tissues

of the embryo go through. For example, HICKS has shown that neuroblasts, although undifferentiated, will tolerate anoxia much better than adult-type cells.

GLASSER: This may be a very good time to come specifically to terms with the general thesis advanced by BERGONIÉ and TRIBONDEAU because I think that right now we have a number of very outstanding exceptions to the general thesis. First we discussed the small lymphocyte which no longer has a reproductive future and also has a high rate of sensitivity. I want to ask Dr. Schjeide to compare the rat embryo and the chick embryo in these areas of differentiation. The pecularity in the chick embryo is—as the embryo becomes older, as it becomes more differentiated, as its mitotic activity increases—there are areas of relatively low radiosensitivity, and as the embryo goes through these situations it becomes increasingly radiosensitive. This is contrary to what we find in the mammalian foetus, which is very sensitive in its early stages and becomes increasingly radioresistant. In this particular stage of high differentiation, these two forms are divergent and I wonder if Dr. Schjeide, through his experience in this field, may be able to compare them and to point out what may be the differences.

SCHJEIDE: I think this belongs to tomorrow's session, at which time it would be very pertinent. Very briefly, the increased sensitivity of the chicken embryo in later stages may be due to 'toxins' produced by cells which are not present at earlier stages, or to an increased dependence of all cells on a particularly radiosensitive group of cells.

DUCOFF: I have an impression that some of the talk of the lymphocyte indicated perhaps a qualitative rather than a quantitative difference between it and most other mammalian cells. You get this death by an almost immediate pyknotic type death; is not that correct, Dr. Bond?

BOND: Yes.

DUCOFF: Now you also mentioned that you can get an early pyknotic type death in other cells at higher doses, and whereas the dose response of division block appears rather linear, the dose response in a pyknotic type death appears to be an exponential function dose. And how about the death of the lymphocyte—is that a linear type response or exponential type?

KELLY: It's more nearly exponential according to the measurements of TROWELL and of PATT.

BOND: I don't know the data on other cells.

JONES: I would like to discuss pyknotic change in the lymphocyte. If we talk about these exponential effects, and if we plot per cent of survival on a log scale, the ordinate scale might be 100 per cent, 10 per cent and 1 per cent, as in Fig. 3.1, and the dose along the horizontal axis—then we will get some function such as line 'A' which is what we've been talking about. But we have to define dose with respect to time of observation. Because 'A' represents the data one hour after exposure, then if we look at the survival after two hours, we may get line 'B'; at three hours, we may get line 'C'; at four hours, 'D'; five hours—six hours—you get a family of curves like this; so that time varies from curve to curve. But if we look at any one of these curves, it tells us how the fraction of the pyknotic cells varies with the dose if we keep time constant. On the other hand, the data might be just as well represented if we look at it with respect to time. We'll keep this scale of per cent survival; now, if we look at survival in time for a defined dose, we will get some line like D_1. In other words, for this particular dose there will be a fixed rate of decay of cells that are surviving at any time; so that a cell surviving in this system will always maintain about the same chance that it will become pyknotic. If, then, we plot the data for stepwise increases of dose, we get curves D_2, D_3, and so on. Neither of these two representations quite

fits the observations; but it would look as though, for the first time interval—that is, the early time in which pyknotic changes can occur—which is somewhere about the first 6 to 8 hours, that the chance of pyknosis . . .

CRONKITE: May I interrupt? Did you say you don't see pyknosis before 6 to 8 hours?

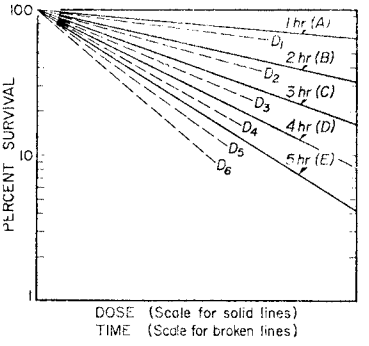

Fig. 3.1. Pyknotic change in the lymphocyte.

JONES: No, I say that these changes are taking place in the first 6 to 8 hours. After that, mechanisms of recovery come in so that, if the cell hasn't become pyknotic by that time, it probably won't become pyknotic; but this particular model only fits the first 6 to 8 hours. So that you have roughly, then, a constant chance for pyknosis, which is approximately 10^{-4} per roentgen per hour. This, you see, is quite a different interpretation from a strict adherence to HICKS' concept—it doesn't seem to work out that way. In other words, the cell has a fixed chance of becoming pyknotic. If you want to increase the probability that the cell will become pyknotized in a given time, you have to double the dose for a two-fold increase. And if, for the same dose, you want to increase the probability by a factor of two that the cell will become pyknotized, you have to increase the time under observation by two.

POWERS: Is this the same thing as saying that these cells are dying anyway—is that right?

JONES: Well, these events are happening much faster, or with a much greater probability than you'd get ordinarily.

POWERS: You're irradiating a cell that's on the way out?

JONES: Without the irradiation, the rate of pyknosis would be very slow.

POWERS: But the rate of death is appreciable in these cells.

FLANDERS: As I understand it you're talking about TROWELL'S data.

JONES: TROWELL'S data, yes.

FLANDERS: Now, I believe he was using conditions under which he was culturing lymph nodes in a vessel, and the conditions within the lymph node may, in fact, be rather different from those occurring within the animal.

JONES: That is true.

FLANDERS: This is shown by the fact that you need one atmosphere pressure of oxygen to get oxygen to the cells in the center of the lymph node. This is shown by the manner in which the sensitivity varies with oxygen pressure outside the node.

CASARETT: You have population cells in tissues—and certain dynamics related to the distinction between mitotic-linked death and indiscriminate death by pyknosis

of cells, that might have a bearing on these considerations. Mitotic-linked death is always limited with respect to the total number of cells entering mitosis at any particular time, whereas indiscriminate death can affect any cell or all cells and is unlimited in amount except as to dose. So that in a tissue, if one has used a dose which reduces mitotic activity, the amount of mitotic-linked death will be accordingly reduced. During recovery phases, when mitotic activity has increased greatly, the amount of mitotic-linked pyknosis or death will go up accordingly. This can occur well after 6 or 8 hours. I think one should make this distinction—especially in choosing an effect of the pyknotic reactions or the death of cells as a universal criterion of radiosensitivity.

JONES: Whatever may be happening to these cells, from the standpoint of a probability model, the risk must be applied to the majority of the cells in the system. Of course, we don't know how precise these data are, but I would say that perhaps 80 per cent or more of the cells in the system fit very nicely to this simplified concept.

KELLY: I would just like to point out that this is not a mitotically linked death.

CASARETT: It depends on what's being counted. There are medium-sized lymphocytes and large lymphocytes that are being counted—

KELLY: In TROWELL'S data, the great majority of the cells are small lymphocytes.

CASARETT: Is there any mitotic activity?

KELLY: He says no.

CASARETT: I was making a general statement. TROWELL did it both *in vivo* and *in vitro* and there's a striking similarity between the results inside the animal and in the tissue cultures. He deliberately selects an age of an animal and an anatomic area, namely the lumbosacral lymph nodes of about a 90 g. rat in which the mitotic index of the small lymphocyte is less than one in 10,000 cells. And he marks it only on the changes in the small lymphocyte and ignores completely all other cells.

BOND: With TROWELL'S data, the effects *in vivo* and *in vitro* were remarkably similar, and he was able to show that oxygen tension differences did not account for his results. I'd like to ask Dr. Casarett—On mitotic-linked death, are you referring to events that occur in cells that visibly are in mitosis?

CASARETT: Yes.

BOND: And to what degree do you feel that this contributes to death of a cell population? The mitotic index is extremely small for most tissues, and the number of cells in mitosis at any given time is an extremely small part of the population, so that on a quantitative basis can you account for an appreciable change in an organ on the basis of damage to cells that are in mitosis?

CASARETT: Yes, especially when the cells affected in this way would have been responsible for a considerable number of mature functioning differentiated forms.

BOND: Could you give an example of this? And kind of report?

CASARETT: Well, if you take a lymph node for instance, and bone marrow—let me take the bone marrow. I think most of us are familiar with the abortive attempts at regeneration of the bone marrow before ultimate successful regeneration. The first wave of mitotic activity, that is, active regeneration of the bone marrow, is aborted very often with cells dying in mitosis, and then there seems to be an elimination of mitotic lymph death here, and after, another period of lack of regeneration, then finally regeneration may come about and be successful so that this particular activity delays regeneration of bone marrow.

BOND: When one looks at the bone marrow one sees very few cells in mitosis. Are you saying that the disappearance of these cells—of the total population—is because of these?

CASARETT: I don't think there are 'very few' cells in mitosis. It is important here because each of these cells in the primitive form is responsible potentially for a larger number of daughter cells with respect to the blood—circulating blood.
HENNESSY: May we interrupt here and let Dr. Kelly speak for a moment?
KELLY: Dr. Casarett is talking about the same thing which you've been talking about, Vic. In most tissues, the deciding factor is how rapidly the cell population normally renews itself. However, you can't explain the radiation effect on bone marrow, for example (which we find to have a turnover time of about one day) on the basis of mitotic inhibition alone. If all that happened were an inhibition of mitosis for a day or two, then the cells should still be there and at the end of the mitotic delay everything would be normal again. This is not the case. The mitotic inhibition is the earliest observable effect; but then these cells die, probably very largely at the time that they are either trying to divide, or have divided and produced abnormal daughter cells. The importance of this mitotically linked death will depend on the normal rate of mitosis in the tissue.
BOND: No, I'm not sure that this is entirely the answer for the apparent differences in opinion here. What I'm asking him is, is he accounting for the death of the whole population by the irradiation of the number of cells that are in active mitosis at the time? That is my question.
CASARETT: No.
BOND: That's why I asked you. Is this visible mitosis? Or are you referring to death during mitosis of cells that have been irradiated any time in the life cycle—not only during visible mitosis? These are quite different things.
CASARETT: I suppose I should define mitotic lymph deaths in my usage. This is death that occurs when the cell attempts to divide, having been irradiated previously.
BOND: This is quite different, and I now agree completely with what you and Dr. Kelly have said.
HENNESSY: Dr. Schjeide, did you have a comment here?
SCHJEIDE: I believe it's been cleared up in the absence of comment from me.
HENNESSY: Did you have anything further on this, Dr. Jones?
JONES: I think that one way of looking at these different things is that we have a chance of some unfortunate thing happening to the cell, related to dose, and the chance associated with a given dose will be multiplied by time, as Fig. 3.1 indicates. Now, if you look at other observations, you find a fair similarity between the *in vivo* and *in vitro* effects. But in the *in vivo* comparisons, the intestinal lymphocytes are apparently different from those in other cultures or nodes by a factor of 8 or so. Something must be very different, then, with regard to these cells. Now, is it inside the cell, or is it in the environment? It might be that the environmental factors add to this effect, so that it's at least as complicated as having environmental factors perhaps multiplying another qualifying constant of some sort by a factor of 8. If a factor of 8 is involved in something like this, perhaps we ought to spend as much time looking at environmental factors as intrinsic differences between cells.
FLANDERS: I think it would be interesting at some point of this discussion to bring up the data which PUCK has collected on single cell cultures. I wonder whether Dr. Bond would be interested in commenting on that in relation to the picture he's presented.
BOND: I think Dr. Kelly is more familiar that I with Dr. PUCK's data and perhaps she would review it for us. I would only like to state that he working with cells in tissue culture from which he makes interesting deductions. It's a beautiful piece of work. But we must keep the reservation that he is dealing with a cell

that has been grown in tissue culture for many generations. It is difficult to define the status of these cells in terms of their being 'mammalian'.

HENNESSY: Would you comment, Dr. Kelly?

KELLY: Well, I think I would have to give a very short resumé of the paper, or is everyone familiar with it? Most of you probably know that PUCK's group has developed a method of plating mammalian tissue culture cells. They have managed, by treating the cells very gently, to make them grow like bacterial cultures; so that they can plate a known number of cells and observe colony formation from each of these cells. This is obviously a very nice tool for a radiobiological study of mammalian cells and they have published one paper so far. I'm sure more will be forthcoming. They describe very nicely three separate end points: if they give various doses and then plot the survival, scoring macrocolony formation survival just like a standard bacterial survival curve, they get an exponential curve which has a shoulder so that it extrapolates to a value of two and the exponential portion of the curve shows a mean lethal dose of 96 r. Cells which were plated but did not form macrocolonies now can do one of two things. They may form a microcolony in which apparently the cells have undergone four or five divisions, and then these microcolonies lyse. Or the cells may form giant cells, and, according to PUCK, if they pick the dose just right—I think around 800 r or 1000 r—they get an almost pure population of giant cells which are ten or more times the volume of the normal Hela cell. At 1000 r, the cells which are left are all giant cells, but it's only something like 20 per cent of the initial population which is left at all. This experiment illustrates that one has to specify the end point, or the criteria of damage, and the dose, before one can really discuss similarities or differences in radiosensitivity. I think most people are quite startled at first at the very low mean lethal dose but it may be just about right to account for the acute mammalian radiation syndrome.

SCHJEIDE: I would like to ask what the mode of death as expressed morphologically was with the 96 r.

KELLY: The 96 r mean lethal dose applies to macrocolony formation. This is not death in the sense that we are used to it in mammalian terms, but is the ability of a cell and its progeny to keep on dividing indefinitely.

SCHJEIDE: There is no evidence of degree after this amount of irradiation?

BOND: This is plating out on another medium—something like transplanting a tumor into another animal and then seeing if it takes or not, and it does have some complicating features. I'm sure this is not equivalent to immediate death of the cell. Quantitatively, it might be quite far from the dose that would kill a cell as we normally think of cell death.

JONES: With regard to what happened to the cell, it's very similar to what is happening to the lymphocyte in the pyknotic change. In this case, you just get cell arrest.

KELLY: To my mind, it's a very different sort of thing, as I was trying to bring out earlier. In the case of the lymphocyte, the cell is unable to function within a few hours and it lyses. Something very drastic has happened to the cell. PUCK's cells that form a microcolony undergo four or five divisions, but they have suffered mutations or chromosome damage of some sort, so that perhaps these cells eventually use up some essential substance and die after a few generations. In addition, there are giant cells which live for weeks apparently, but just don't divide. They keep on synthesizing protein and nucleic acids and become very large.

CASARETT: My question has just been answered. The giant cells do not divide. The failure in reproduction seems to be involved in the production of giant cells.

POWERS: A very common observation.

SPARROW: I want to report a paper I heard at the recent AIBS meeting that probably some of you heard, but a lot of you didn't. I believe the work was done at Johns Hopkins. They deliberately avoided using any of the standard human tissue culture cells because of the high degree of polyploidy and isolated new cultures from kidney tissue and irradiated these with doses of 25, 50, and 100 roentgens or approximately that and studied the frequency of chromosome breakage in diploid cells. And the rather startling, at least to me, the rather startling result was that in the diploid cell the spontaneous aberration frequency was approximately 1 per cent with a dose of 25 roentgen or in the polyploid cells the aberration frequency was 6 per cent. The two higher doses gave roughly the expected increase although it wasn't exact. I think this would parallel the work that you are reporting.

POWERS: They're cultured in this fashion?

SPARROW: Yes! They're cultured in this fashion, but remember these were diploid cells and probably the Helas are apt to have some polyploidy.

KELLY: I think this checks quite nicely with CONGER'S *in vivo* results with Ehrlich's tumors, where abnormal anaphases were scored.

POWERS: Is it not true that these cells are dividing more frequently perhaps than the cells in the ordinary, everyday average, private-type of cell in the body?

KELLY: No, some bone marrow, intestine, and thymus cells, for example, divide more frequently.

HENNESSY: Should we change the subject just a little?

POWERS: I think that some of us, at any rate, could be interested in the cell just by itself without necessarily wondering what role the death of *a* cell or the radiation sensitivity of *a* cell might have to the well-being of a multicellular organism after it dies. However you look at it, the radiation sensitivity of the cell is an extremely important and basic phenomenon, as exhibited by the fact that all of us are here in the first place. Because of our interest in this radiation-sensitivity, many people prefer to use radiation-sensitive cells. And this, of course, is a very, very good thing. One can perhaps recognize in the cell, some day, things which are in very, very short supply, which are limiting for the cell in some way, which have some radiation-sensitivity. Or some other person might use another kind of radiation-sensitive cell, a cell which is just as radiation-sensitive as the first cell. But both of these people, I think, would be in error if they thought for a moment that these two cells were radiation-sensitive for the same reason. There might very well be a higher degree of similarity between radiation-sensitive cell 'A' and radiation-resistant cell 'B', than there is between radiation-sensitive cell 'A' and radiation-sensitive cell 'B'. So, we have, at this point in our knowledge at any rate, to take all cells into consideration, whether they be radiation-sensitive cells or radiation-resistant cells, because the elimination of any one kind from consideration would be an unwarranted act. So we use the kind of cell that is specially suited for the purpose that we want to serve. It doesn't make any difference whether this is chondrodamonas or paramecium or the lymphocyte. We will do what we can with that cell, and try to recognize the general biological information that is derivable from it. And assuming, as we all do, that there is a uniformity among biological organisms, we hope then to apply this information generally. So, if we want to inquire into, for instance, the effect of changes of sets of chromosomes upon the radiation sensitivity, we would not consider animal cells. These systems are not the good ones. While it is true that some cells in the mammalian organism may be of different ploidy from some other cells, this, I understand, is not a predictable thing—it's difficult to recognize, define and isolate. In lower organisms however, this is not so

difficult, and perhaps Dr. Sparrow would comment very briefly on what he knows about this general subject of the relationship of change in radiation sensitivity to changes in ploidy and parent cells.

SPARROW: There has been quite a long series of papers on this topic, starting in the early 1930's, some of which deal with the incidence of mutations in the polyploid series. I think that for this group, perhaps, the data on the incidence of mutations is of less interest than the data on the general tolerance of cells in different degrees of polyploidy. Probably many of you, if not all of you, are aware of the recent work on yeast, in which there is not the expected increase in tolerance with polyploidy at all stages up to the tetraploid. To summarize the literature briefly, I would say that in the majority of studies there is an expected relationship between the degree of polyploidy, up to about 6 ploid or 6N, and increased tolerance. However, there are many exceptions in which, for instance, the hexaploid plant may be more sensitive than a tetraploid, and some cases in which a hexaploid may be of equal sensitivity to a diploid. In most cases that I am aware of, these deviations in expectancy occur in cultivated, artificially produced polyploids. I have been investigating this problem of polyploids for six to seven years and for a long while I was confused because one series of plants I was studying would show the expected relationship and the next wouldn't. Quite often we would find that a tetraploid would be of identical sensitivity to a diploid and occasionally more sensitive. I finally decided that perhaps the difficulty was that we were using cultivated plants which were under artificial environment, many of which had been recently produced by use of polyploidy, and perhaps what we should do is to try to pick a series of naturally existing polyploids. I looked through the literature and came upon a reference to chrysanthemum genus and was amazed to find that there was a series of polyploids that naturally occur in wild species which double the diploid number of 18 and goes up through triploid, tetraploid, hexaploid, octaploid, decaploid, 22 ploid and several intermediate numbers between decaploid and 22 ploid, which unfortunately are all cultivated and therefore not of use. But this was an excellent series. Furthermore, they can be propagated asexually so you would have to make one chromosome count and then assume that the plants maintain the chromosome number of their parental plant. To avoid the difficulties that you get into when you do an acute irradiation on a plant population, owing to the individual physiological differences and differences in the age of the plants, we used our gamma-radiation field at Brookhaven and put the plants into the field as small cuttings two or three inches high, and then waged weekly observations throughout the summer, to determine the length of survival of plants grown at different dose rates. We had a variety of dose rates which we had determined on previous experiments, but to make a long story short and to get to the point, picking a specific dose rate that was sufficient to kill all of the diploid plants, and still high enough to kill some of the 22 ploid plants, we found a perfect correlation between the degree of polyploidy and the radioresistance for all five levels of polyploids used—that is for the 2N, 4N, 8N, 10N and 22N, there was the expected relationship.

FLANDERS: You say the expected relationship—on what model do you derive this?

SPARROW: By expected relationship, I was referring to the relative order of power.

FLANDERS: Yes, how would you expect it?

SPARROW: Well, you would expect the higher degree polyploidy to be more resistant.

FLANDERS: This is on the basis of recessive lethal damage then?

SPARROW: Not on any theoretical grounds, but on the basis of what is previously known about polyploids. If you want to put it on a theoretical basis, there is the

genetic assumption that, as you duplicate the number of sets of chromosomes, it is progressively harder and harder to produce a lethal effect by chromosomal damage.

POWERS: The fact is that as ploidy goes up, resistance goes up, and is steady throughout the series.

SPARROW: Within the limits of the accuracy of our method, yes. Between the 10 ploid and the 22 ploid there was still a great increase in resistance. But apparently up as high as 22 ploid you're still getting increase in radio resistance. I, myself, would have expected it to level off before you reached that degree of polyploidy.

DUCOFF: Is there any evidence of increase of synthetic capabilities for the increase in ploidy? I wonder if, under your conditions with this low dose rate . . .

SPARROW: These are not low dose rates. They go up to 1400 roentgens a day.

DUCOFF: In terms of the exposure and the life of the plant these are rather long exposures.

SPARROW: Yes.

DUCOFF: And can you rule out their recovery with increase in ploidy rather than their resistance?

SPARROW: I don't know what you mean by recovery. It has appeared from chronological observations that with high polyploidy you can get cells running through conditions which may average 8 bridges and 10 fragments or something like this, and they're still able to divide. And presumably their daughter cells are still able to divide. If you want to call this recovery—yes—I don't trust to call it recovery.

BOND: How much difference did you observe in the sensitivity of the various degrees of polyploidy?

SPARROW: There are various ways of expressing this. If you use a killing dose, that is the dose required to kill all of the plants in a given time, the degree is not as great as you would expect it to be. However, if you use a somewhat different expression, namely, what is the maximum dose rate at which a diploid plant and the highest polyploid plant that I have studied can survive, then this difference becomes a factor of—I don't remember exactly—25-fold, or something like this. But the large factor we're referring to now is not in the crysanthemum series; this was another series which has a chromosome even greater—varying from diploid number of 8 to a top chromosome number of 384. And these high chromosome plants are extremely tolerant plants. These are the plants I was previously referring to which, as green plants, not as seeds, but as green plants will sometimes survive 400,000 roentgens for several months. I mean they will continue to live—and to live for several weeks—after a half-million roentgens. So it's quite clear, I think, in these cases, that the extremely high degree of high radioresistance must be related to the high chromosome number.

DOWDY: How did your doses vary with time? Are you, in effect, irradiating dead plants? When did your plant receive the critical amount of radiation? Could it continue on for days after irradiation?

JONES: Are those buds still growing?

SPARROW: This last experiment I referred to was not a chronic dose in the usual sense—it was a dose delivered in five days, and then they were observed. In this case, certainly, there was no problem of dead plants. When they are irradiated in the gamma-radiation field, our exposure period went up to 125 days. In the case of the diploids, which died very fast, the date at which they died was somewhat later than the date at which they had received the lethal dose. But this becomes less and less important as the r increases more and more, of course.

DOWDY: During the war we irradiated a large number of dogs with, I think, $\frac{1}{10}$, $\frac{5}{10}$, 1, 3, 5, and 10 r per day, six days a week. And I'm sure that we were irradiating dead dogs.

POWERS: Well, these were more resistant to at least the first part of the irradiation.

SCHJEIDE: To indicate a possibly significant difference between mechanisms, in cells of different sensitivity, I believe Lola Kelly would agree that lymphocytes from different species probably have the same order of sensitivity, irrespective of the chromosome number. I don't know if anyone ever has worked on haploid lymphocytes.

KELLY: I suppose so, but I would like to know where you're going to find these lymphocytes with different chromosome numbers.

SCHJEIDE: Well—different animals.

JONES: Salamander.

SCHJEIDE: And frogs. We have found the lymphocytes of frogs to be very sensitive to irradiation.

CASARETT: There may be a considerable difference between difference in chromosome numbers and ploidy.

SCHJEIDE: Quite true, that's why I asked if anyone had ever done an experiment on haploid lymphocytes.

POWERS: Or haploid mammals.

SPARROW: If I may continue for a moment—In this series of plants that we've been investigating, we've deliberately chosen not only a variation in chromosome number, but plants with variation in chromosome size, with the maximum degree that we could find, and while it's not always possible to get a high degree of differences in chromosome size and observe the metaphase in plants with the same chromosome number, you can get very large differences in size. By large differences, I mean, for instance, plants like trillium, which have an average chromosome length of about 10 microns, versus some succulent plants which you're likely to see growing all around here, which will have an average chromosome size of a fraction of a micron. We have deliberately picked a series of chromosome sizes in plants that are presumed not to be polyploid, on the basis of the chromosome behaviour. When we do this, all of the plants with chromosomes which we classify as large, with an average chromosome size of 5 microns or larger, or approximating that size, are extremely sensitive. By extremely sensitive I would say a sensitivity higher than that of most mammalian cells where, in the case of the lily of the valley, for example, some experiments have shown that a dose of 250 roentgens to a resting lily bulb will kill 100 per cent of the bud. They have a very high sensitivity, indeed. There are no cases in which plants with very large chromosomes do not have very high sensitivity. As chromosome size gradually decreases, the sensitivity in the average plant goes down, as would be expected on the simple target theory. However, when you get to the other end of the line where the plants have small chromosomes, even without polyploidy, there are some exceptions—there are no cases in which very small chromosome plants have a sensitivity as high as plants with the very large chromosomes, but there are some cases in which they definitely seem to be out of line with the average plant, or expected sensitivity for plants of that chromosome number and that chromosome size. I would assume that in these cases you can best explain the unexpected sensitivity, not on a target theory basis where the chromosome is the target, but on the basis that these plants must have at least one enzymatic or biochemical system of unusually high sensitivity to radiation and this is what blocks their growth, rather than genetic or chromosomal damage. We have not done DNA measurements ourselves, but there have been

enough data recorded in the literature to show that plants with very large chromosomes have amounts of DNA perhaps of the order of 40 to 60 times as great as plants with small chromosomes. Your first thought might be that the intolerance of these plants is not due to the target theory explanation, on the basis of chromosome aberration and genetic instability, but because they have to synthesize so much DNA in each resting stage that they just are severely handicapped. I think the data on the high polyploid rules this out, because these very high polyploid plants have to synthesize just as much DNA per intermitotic period as the plants with very large chromosomes. Yet they apparently can thrive without any difficulty through extremely high doses. So I think it is quite clear that the plant with the very large chromosomes must have a high sensitivity, not simply because of the high amount of DNA, but because they present a large target area.

FLANDERS: I think it is useful to bear in mind, alongside data of the kind Dr. Sparrow has been talking about, that there are rather different results for yeast. Dr. MORTIMER has found that with increasing ploidy the radioresistance at first increases and then falls again, reaching a maximum resistance at the tetraploid, while the hexaploid sensitivity is comparable to that of the diploid.

POWERS: I think that's perfectly true and probably something like that might be taking place in Sparrow's material, but there's one thing that I think that his series of observations points out and I have to ask a question before I make my comment. Is it true that the total number of aberrant figures that you see per unit dose also increases with ploidy—is that correct? The 22 ploids have more bad nuclei than the 6 ploids.

SPARROW: Yes, we have not done a detailed cytological study, but preliminary observations indicate not only a higher degree of aberration in individual cells, but a higher percentage of the cells that can be affected and still have the plants growing fairly normally. BISHOP'S work on this is perhaps the best I know of —in tetraploid cells compared to diploid he got exactly twice as many chromosome aberrations in the tetraploid as he did in the diploid.

POWERS: Now, barring the dominant lethal sort of recombination, the tetraploid obviously is in a better situation to cope with the chromosome loss, because it has all of the other chromosomes to substitute for the part which is lost. This substitution may go on, not only within a cell, but also from cell to cell in metazoal tissues. This brings out another point—that in a tissue in which cells are closely related one to the other, it could very well be that parts of the chromosomes are lost, and yet it might not be lethal to that cell so long as it is only doing its normal metabolism, because it can then derive the essentials from other cells which are adjacent and are whole and can supply the ordinary essential parts. This kind of thing has been observed in corn or something like this in SACHS' laboratory many years ago. Cells which are lethally affected if they are separate from other cells will proceed in normal development as long as they are adjacent to cells which have an integral number of chromosomes. This raises the question of the dependence of radiation sensitivity on the straight DNA content. I wonder if anyone has any comments to make on this.

JAMES: I want to ask Dr. Sparrow if he knows of any work comparable to the work that Dr. Cronkite mentioned with respect to mitosis sensitivity.

SPARROW: Yes, I have published considerable amounts of information, most of which has to do with the incidence of chromosome breakage, which will occur when you irradiate given stages of mitosis in anthers, and the difference in sensitivity—the maximum difference in sensitivity of different stages of mitosis varies by a factor of about 60-fold. The most sensitive stages are late prophase—mitosis late prophase; as you go into earlier prophase they get less sensitive, and as you

go into first mitotic division and second mitotic division, they get less sensitive —so you have a peak of sensitivity at late prophase. The minimum sensitivity so far observed is the immediate post-mitotic resting stage, the earliest stages of the interphase presumably, although you might have it later, but before any great amount of synthetic activity has begun. The difference in sensitivity here between this least sensitive stage and the most sensitive is a minimum difference of 60-fold. This difference is within a given type of cell and is simply a time difference depending on the stage. We also have observations on chromosome breakage between mitotic cells and amitotic cells in the same plant, and the data on chromosome breakage do not agree, apparently, with the animal work. The method of collecting the data was a chronic exposure which, without going into detail, I think gives better data than acute exposure. The chronic exposure shows a difference in the incidence of chromosome breakage of a factor of 10—that is the mitotic stages show ten times as many aberrations as the amitotic. Now you can try to argue this away and say—well, that's just chromosome breakage, but it doesn't really affect survival or tolerance or ability of the cell to grow. But there is parallel evidence to indicate that this really is related to the ability of the germinal lining to survive, and there are several lines of evidence to indicate this again from our chronic studies. At a sufficiently high or low dose, as you want to define the term, one of the first observations you can make on the reproductive cells is a gradual increase in the number of abortive or sterile pollen that is produced. As you increase the dose, finally you get to a dose in which you have an anther sac produced but it is full of dead pollen. In other words, it goes through the effort of trying to produce pollen but it doesn't survive. It is all lethally damaged. The next stage, when you go one step higher, is that the flower develops, but the anther, the pollen organ, is just not there. This would indicate not just a higher sensitivity in the germinal lining as such, but that the initial organ just doesn't form, so that the high tolerance of this particular line must extend from away back into the original cell.

POWERS: I wonder if there would be any interest in talking regarding the mammalian situation and discussing ploidy of DNA content as it relates to sensitivity. Is there any meat here? Does anyone have anything to say? We should try to relate what Sparrow has been saying to what Bond was saying earlier in the morning.

BOND: It would be rather logical to suppose that, if there is an increased sensitivity of cells during mitosis, it would be related to DNA synthesis. There are two pieces of work that indicate that this is probably not the case. DNA synthesis apparently is not markedly affected by radiation at doses in the lethal range. It is not the inhibition of DNA synthesis that is responsible for the mitotic inhibition that occurs. Would you like to comment further on that, Dr. Kelly?

KELLY: I think you said it very nicely. As far as I can see, there is no basis whatsoever for believing that the mitotic inhibition is due to the cell's inability to synthesize DNA. I think that there is now ample evidence that DNA is synthesized at some time in interphase—this time may vary for different cells, but occurs well before prophase. After radiation, the cells that are in very early prophase are inhibited and those are mose certainly cells which have already synthesized their DNA. We have data in the Ehrlich ascites tumor which is rather favorable for studying this effect because the Ehrlich tumor is quite radioresistant in terms of immediate death, so that one can give a dose of the order of 1000 r and have essentially all of the cells that were irradiated remain alive for around two days. What we did was to give 800 r to an animal which had a transplanted Ehrlich tumor. This inhibits mitosis for approximately two days. During

this time we repeatedly studied DNA synthesis as measured by the incorporation of P^{32} and also the total amount of DNA per cell. We found that DNA synthesis proceeded to the octaploid value but the cells did not go through mitosis. To my mind, at least, this is quite conclusive evidence that inhibition of mitosis must be due to something separate and distinct from any possible effect on DNA synthesis. If anyone has any ideas, I think this would be a fruitful thing to speculate on.

CASARETT: I don't think I can help you out, but a cell does divide partly at least as a result of the lack of oxygen when it reaches a certain size, a certain nucleoplasmic ratio and so on. I wonder whether anybody thinks there is any merit in the consideration that radiation might increase the oxygen content of the cell and delay a division by this means, and whether this might have something to do with the different lengths of time in different cells, the lengths of time during which mitosis is inhibited by the same dose.

POWERS: The more oxygen, the greater the inhibition?

CASARETT: It's an old idea that the cell grows and reaches a stage in terms of its nucleoplasmic ratio and its total size. Then division occurs and reduces the cell size. Hence the daughter cells and then the process repeats itself. In other words, the lack of oxygen may be responsible for the initiation of mitosis. Here we have a situation in which radiation is supposed to present the cell with oxygen and I wonder what you people think about this notion.

POWERS: Did you have a comment on that, Dr. James?

JAMES: Only that I've never irradiated a cell of any kind, so I don't really know, but I have worked on a related subject—the Qo_2 of cells as a function of their size and I can say that it is not completely a surface dependent phenomenon. In theoretical biology there are probably three different cell types, which can be classified with respect to their respiration. You can consider one which is absolutely volume dependent, its rate of respiration being volume dependent, another in which it is surface dependent, and the third which is the intermediate between the two. This would tend not to support the idea that oxygen was the critical limiting factor.

CASARETT: I'm under the impression also that increasing the oxygen tension in a group of dividing cells will hinder division.

POWERS: If you increase enough, you'll kill the cell.

CASARETT: Yes, of course. But if you increase radiation enough, you also kill the cell.

FLANDERS: In connection with what Dr. Casarett was saying, in an animal tissue which is respiring reasonably rapidly, the distance which oxygen must diffuse from the nearest capillary to reach cells is 20 to 50 microns. This is 5 or 10 times larger than a cell diameter. I have not heard of any histological evidence that cell division occurs most frequently in positions most remote from the capillary blood. This seems to indicate the absence of a connection between reduced oxygen tension and the frequency of cell division.

CASARETT: Another feature of this is that an adequate circulation and adequate supply of oxygen is necessary for a successful mitosis and I think that would agree with you.

POWERS: Now, I wonder if we should attempt to get back to the DNA proper.

LESSLER: Before we go there, may I make the comment that the mitosis itself is a series of slow gel changes which are thermodynamically controlled and are not oxygen dependent, and there is a wealth of work to show that this is so. A number of different people have worked on this for years. The actual division itself, the division of chromosomes from one another, the division of cytoplasm into two blobs (if you will) is a physical-chemical process.

POWERS: But the processes leading up to that point—that might be another matter.

LESSLER: Well, even the initiation.

SPARROW: I'd like to discuss the problem here of the possible relationship between DNA synthesis and observed mitotic inhibition or meiotic inhibition. In the cell I studied, meiotic inhibition is the phenomenon I observed. And it is possible by measuring the time required to go from a known stage to another stage—there is no error involved here because of the morphological distinction between stages —it is possible to determine rather accurately how much increase in time—whether it's 20 per cent or 40 per cent or 100 per cent—how much longer is required for a given stage to reach a given period, given later stage or given amount of radiation. The interesting thing is that the stages that are most susceptible to meiotic inhibition are the stages that have the highest incidence of chromosome breakage. Now to my knowledge there has not been enough cellular physiology done to indicate what the cell processes are that are going on—that might be disturbed, say, at the diploid stage, which is the most sensitive stage as far as chromosome breakage. Now when I talk about meiotic inhibition, I'm talking about inhibition between this late prophase stage and, say, first anaphase, before any chromosome material has been lost due to fragmentation. We can't explain this on the basis of the loss of genetic material. This, I think, would be an extremely worthwhile area for investigation, to try to find out what are the physiological or biochemical processes which are interrupted, say, at diploid, and cause extremely high meiotic inhibition. To the best of our knowledge, it is not DNA synthesis and it is not protein synthesis. It is probably nothing in the metabolism of the chromosome that we normally would think of here, and therefore it refers back to the comment that it must have something to do with one or the other trigger mechanisms in meiosis or mitosis.

MEAD: I want to ask a question in three parts. This is, of course, concerned with my interests. Can cell division in fact be prevented by an increase in oxygen tension? If so, what actually is prevented? Is it synthesis of DNA or synthesis in general and, not to attempt to answer that question, under these conditions of oxygen tension increase, are giant cells produced as they might be in the case of inhibition by radiation?

POWERS: To begin with, it is clear that we can inhibit cell division by a variety of agents. X-rays and nitrogen mustards apparently do nothing at all to protein synthesis and DNA synthesis. These proceed. Now what is especially effective to stop the development of the complex series of changes known as cell division—that's an entirely different matter which I didn't think we were going to be mainly concerned with today. We had a conference something like this at the Argonne about eight months ago on this question, and we have seated at the end of the table Dr. Ducoff, one of the editors. Do you have any notions on this matter, Dr. Ducoff?

DUCOFF: A number of things will affect the division *per se*, or lesion. Whether you can get similarities or not between radiation and any of these factors is a very unknown quantity. I believe that oxygen tension was one of the few things not discussed there and I have no personal information.

HOWTON: I think that, in saying that treatments such as radiation or nitrogen mustard do not affect protein or DNA synthesis, you have to remember there can be very subtle changes in the structure, even as fine as the conformation of those molecules, which can be vitally important in their further proper function. These are changes which, I venture to say, you are unable to detect in the tests, which is the basis for your saying that these synthesis are not affected.

KELLY: This is perfectly correct; we cannot tell whether our Ehrlich tumor synthesizes normal DNA or abnormal DNA. But I think there are enough other grounds for saying the same thing, the main one being the timing involved. With doses of the order of 200 to 400 r, cells do not enter prophase immediately. And this is the whole basis for KNOWLTON'S timing of the mitotic process. We know that the DNA synthesis occurs before this. HOWARD and PELC, several years ago, did a very beautiful radioautographic study in bean roots where they worked out the timing of DNA synthesis in meristematic cells. They find that DNA synthesis is complete 12 hours before initiation of mitosis. If they irradiate the cells are promptly inhibited from going through mitosis; and yet, if one would blame it on DNA synthesis, there should be a perfectly normal mitotic rate for the length of this interval between DNA synthesis and mitosis.

HOWTON: I think it is quite conceivable that the conformation of a single DNA molecule may be vital in triggering these progressive changes.

KELLY: I have no information on this, but it would have to involve a change in existing DNA.

REFERENCES

[1]PETRAKIS (*Rad. Research* **5**, 569 (1956)) carefully studied the effect of different doses of X-ray on the thymus *in vivo*. He counted the number of pyknotic nuclei, and one does get an increase in the thymus of numbers of pyknotic nuclei as a function of increasing dose. TROWEL, with a different technique, has done it *in vitro* and *in vivo* for both thymus and lymph nodes. The 'sensitivity' of the thymocytes and lymph node or spleen lymphocytes, in terms of pyknosis, is about equal. The lymphocytes in the intestinal mucosa, however, were considerably more resistant.

END OF SESSION III

SESSION IV

RADIOSENSITIVITY OF THE MODEL CELL— RECOVERY MECHANISMS

Introductory Speaker: HOWARD S. DUCOFF

THE ideas I'd like to present tonight were formulated—without benefit of template—at the Argonne National Laboratory, but they are finding phenotypic expression here under the auspices of the Department of Physiology of the University of Illinois. Needless to say, the opinions expressed are entirely my own, and do not necessarily reflect the views of the A.E.C., the University of Illinois, nor HARVEY PATT, for whom I was substituting.

The questions before us at this session concern the recovery of cells after exposure to ionizing radiation. Do cells recover? Do they completely recover? And what sort of mechanisms may be involved in recovery?

Potentially, at least, radiation affects a variety of systems. And I believe that it will be of little use to discuss recovery except in reference to particular types of injury. Furthermore, it must be recognized that even after apparent recovery from a particular type of injury, there may still be latent damage persisting in the irradiated cell. This sort of incomplete recovery is probably best exemplified in the tissue culture experiments of ILSE LASNITZKI.[1] After exposure of the cultures, cell division stopped and then resumed, and the cultures appeared perfectly normal, as if recovery were complete. But a second exposure then caused a remarkably greater deleterious effect, so that there must have persisted a considerable degree of latent injury. C. TOBIAS[2] has obtained comparable results in studies of survival of previously irradiated diploid yeast cells. Also in this class are the recent experiments of HOWARD CURTIS with aging-like effects of ionizing radiations in mice (see CURTIS and HEALEY[3]).

But if we are to discuss recovery in reference to particular types of injury, it will be preferable to limit the discussion to the three manifestations of cell damage usually encountered after irradiation:

Division block;
Cell death;
Mutation and/or other heritable change.

Perhaps these various manifestations are completely unrelated; perhaps they all follow from a single cause (e.g. chromosome injury, or DNA inactivation); or perhaps—and I would guess this to be the most likely case—these three types of damage have a more complex, and still obscure, interaction. There is an almost trivial example of such interaction, which could be more troublesome experimentally. Consider the phenomenon of mutation to auxotrophy, which may occur at a fairly high rate. Such mutants would no longer be capable of giving rise to colonies on minimal media; they would be scored as non-survivors. Nevertheless, on enriched media, such cells would give rise to colonies, so that 'survival' would be much greater. How easy it would be to get trapped into interpreting such results as indicating that nutrients cause 'recovery' of many otherwise lethally irradiated cells!

Another type of interaction may be involved in the results obtained by E. DANIELS[4] with two different species of the giant amoeba; *Pelomyxa P. carolinensis*, after X-irradiation, exhibits the 'classical' type of immediate division block, followed by eventual 'recovery'; 50 per cent lethality follows doses of about 100,000 r, and is likewise manifest almost immediately. Following lethal irradiation, most organisms of *P. illinoisensis* divide once; 50 per cent lethality is seen following only 10,000 r in this species, but death may not occur for seven to ten days. Is there a casual relationship between the delay in division block and the greatly increased mortality?

These are obviously troublesome cases. But, omitting these, is there evidence that recovery may follow radiation lesions which would otherwise lead to cessation of division, to cell death, or to 'mutation'?

The post-irradiation division block is generally described as temporary; so, as LEA and others have pointed out, recovery occurs. Division block is the effect for which there is most evidence of recovery; it is the type of damage for which there is the greatest hope of elucidating mechanisms of recovery; and it is a radiobiological phenomenon which I have personally studied, and the one I find most interesting. (I'll confess that I'm not sure whether I find it the most interesting simply because it's the one I've studied, or whether I undertook to study division block because it seemed to be most interesting.) At any rate, I'll discuss recovery from division block last, in order to have time for presentation of some original data, and some more detailed considerations of mechanism.

Many treatments will alter the percentage survival of irradiated cell populations; but when these treatments are administered prior to irradiation, factors of altered sensitivity and of degree of initial lesion, as well

as of recovery, are likely to be involved. These problems have been considered explicitly—and with far greater elegance and authority—by LATARJET and GRAY.[5] Furthermore, irradiated cells may be more sensitive to various external conditions—e.g. peroxides, temperature, inadequacy of the culture medium. In most cases, therefore, it is very difficult to decide whether a particular treatment—even if administered after irradiation—permits or enhances recovery processes, or whether it simply alleviates external influences to which an irradiated cell might otherwise later succumb. Of course, these are semantic questions, but I think they deserve more widespread recognition and discussion.

On the other hand, photoreactivation after ultra-violet irradiation is certainly a valid case of 'restoration', and, since metabolism appears to be necessary, is probably also a good example of active recovery. This is equally true of the increased survival of X-irradiated bacteria at particular temperatures following exposure (see STAPLETON, BILLEN, and HOLLAENDER[6]). Again, metabolic activity, or at least an adequate nutrient supply, appears to be necessary for what is scored as recovery.

As regards recovery from radiation-induced changes in the hereditary material, the evidence is more recent, but, I think, more satisfactorily specific. Thus, WOLFF and LUIPPOLD[7] have made the extremely important discovery of a role of energy metabolism in the restitution of certain types of chromosome breaks in *Vicia*.

A very different approach was used by KIMBALL, GAITHER and WILSON[8] for the complex situation in *Paramecium*. They have found that the first third of the division cycle in *Paramecium* is the most sensitive to radiation-induced mutation, but that the yield of such mutations could be reduced significantly by treatment during that first third with agents such as streptomycin. This work should be compared closely with that of WITKIN,[9] who found that enrichment with amino acids, but not with nucleic acids or their precursors, nor with vitamins, greatly increased the yield of mutants in ultraviolet-irradiated bacteria, provided the amino acids were administered prior to the first post-irradiation division. Furthermore, this increase of mutation brought about by amino acids could be abolished by chloramphenicol, which presumably inhibits protein synthesis. In both systems, then, suppression of specific synthetic activity during a critical phase of the cell cycle tended to enhance recovery from the 'mutagenic' lesion.

What of the other spectacular manifestation of radiation damage, the blockage of cell division?

COOK[10] and HENSHAW[11] observed the absence of recovery when irradiated

materials were maintained under conditions which kept metabolic activity at a minimum, i.e. refrigerator temperatures for *Ascaris* embryos and *Arbacia* eggs or sperm, or even storage at room temperature for *Arbacia* sperm. Absence of recovery was indicated by absence of any reduction in the radiation-induced cleavage delay, following return to room temperature in the case of *Ascaris*, or fertilization and incubation in the case of the sea urchin gametes. On the other hand, storage of irradiated *Arbacia* eggs at room temperature did lead to decreased cleavage delay upon subsequent fertilization. Some of the implications of this work were discussed by LEA.

POWERS[12] has reported much more careful experiments on *rate* of recovery of the fission process in irradiated *Paramecia*, particularly in terms of the influence of temperature. I'm sure he'll be glad to describe this work at the drop of a gavel! DANIELS' work with irradiated giant amoebae also implicates metabolic activity in recovery mechanisms. Resumption of division began much earlier when the irradiated animals were fused with relatively small amounts of unirradiated cytoplasm.

My work in this field has been done chiefly with two free-living protozoa, both of which are capable of rapid growth in completely defined media. One is a flagellate, *Chilomonas paramecium*; the other is a ciliate, *Tetrahymena pyriformis*.

Chilomonas divides mitotically, and, in the defined medium, utilizes acetate as the sole carbon source and ammonia as the sole nitrogen source, although a trace of thiamin is also required. Most of the *Tetrahymena* work was done with strain W, the object of KIDDER'S classical biochemical studies. This is a strain with macronucleus, but no micronucleus, so that its division is not mitotic.

The organisms were grown in mass cultures, and populations were usually irradiated or otherwise treated during the exponential growth phase. After irradiation, the irradiated control, and any other groups were diluted to about 5 to 10 $\times$ 10^3 organisms/cc, thus permitting 3 to 4 divisions before population growth passed the exponential phase. Samples of each group were taken every few hours, and fixed for subsequent direct counts of population density.

When we plot the logarithm of population at a given time/initial population (log N/N_0), against time in hours, as schematically shown by the solid curves in Fig. 4.1, we find the following general picture: the controls continue growing exponentially; the X-irradiated (say 10 kr, with *Chilomonas*) stop dividing, and the population remains constant for a period of time, and then growth resumes at the same rate—as far as

we can determine with these techniques—as in the controls. There is simply a delay in division, and the duration of the delay increases with increase in dose, up to about 20 or 25 kiloroentgens. With doses greater than 20 kr or so, there is not only an increase in the duration of the delay, but there is a perceptibly lower slope on resumption of division. This might indicate either the presence of organisms which have lost the capacity to divide, or a general reduction of division rate in the majority of the population. During or immediately following exposure of *Chilomonas* to doses greater than 20 kr, there is frequently a significant degree of cell death and cytolysis.

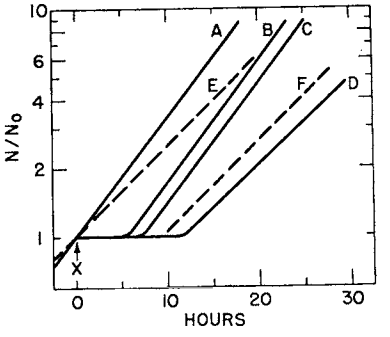

Fig. 4.1. Schematic population growth curves for X-irradiated C. paramecium. A—0 r, 25°; B—10,000 r, 25°; C—15,000 r, 25°; D—25,000 r, 25°; E—0 r, 20°; F—15,000 r, 20°. 'X' indicates start of exposure.

Let us now confine our attention to experiments in which *Chilomonas* was exposed to doses of 15 kr or less, so as to concentrate on the problem of the duration of the block, and the factors influencing it. In all of this work, of course, the duration of block is equivalent to the time for recovery of the fission process.

Using this system, it was easy to repeat the COOK or HENSHAW type of experiment, but using a wide range of physiological temperatures. At lower temperatures, there was slower growth in the controls, and a greater duration of division block, or radiation-induced lag, in the irradiated populations. This type of result is schematically shown by the dashed curves in Fig. 4.1. The remarkable feature of all this is that if we divide the duration of block by the mean interdivision—or generation—time (it is more readily measured in the control population), we get a constant for any particular dose. In simpler terminology, the normal division rate and the rate of recovery from radiation-induced division block have the same temperature coefficients.

Table IV(1)
Duration of lag in X-irradiated C. Paramecium
Influence of medium and post-irradiation temperature

Medium	Temperature (°C)	Generation time (hours)	Duration of Lag (hours)			Difference Generation time
			15 kr.	Controls	Difference	
Ammonium acetate ..	16	9.3	14.9	2.5	12.4	1.3
Ammonium acetate ..	22	6.0	8.2	1.8	6.4	1.1
Ammonium acetate ..	25	5.8	9.6	1.4	8.2	1.4
Yeast extract + Peptone..	25	4.7	7.2	1.7	5.5	1.2

We can also alter growth rate by using an enriched medium: yeast extract + peptone, for example. And again, recovery rate and normal division rate are speeded up by the same factor. Some typical data are presented in Table IV (1) (see DUCOFF[14]).

These observations are not confined to *Chilomonas*. With *Tetrahymena*, I have obtained generally similar results, although the numbers are very different; the doses employed were from 30 to 60 kr, and the generation time varied from three hours in proteose peptone at 25°C to about 12 hours in defined medium at 20°C.

In addition, I have taken the liberty of tabulating (Table IV (2)) some data published by WITKIN[13] for ultra-violet irradiated *E. coli*, and have added the calculation of radiation-induced delay/generation time; again there seems to be a reasonably constant factor.

In this connection, it is worth noting, as PATT[15] has already pointed out, that 'the duration of mitotic inhibition in various (mammalian) tissues bears a direct relationship to the duration of interphase'.

The data I've discussed so far indicate that recovery processes do occur, and that they seem to involve metabolic phenomena. Can anything be said about the sort of metabolic activity involved in the recovery from division block?

Table IV(2)
Duration of lag in UV-irradiated E. Coli
Influence of post-irradiation temperature
(*Data from Witkin*, PNAS **39**, 427)[13]

Temp. (°C) (1)	Generation time (min.) (2)	Duration of Lag (min.)			(5)/(2) (6)
		UV (3)	Control (4)	Difference (5)	
16	200	1800	640	1160	5·8
25	60	600	210	390	6·5
37	20	180	70	110	5·5

I have approached this problem with the following considerations. If a cell is lacking in some nutrient essential for growth, it may still be perfectly capable of other synthetic activities, and the performance of work. We can deplete cells of particular nutrients essential for growth, irradiate, and then restore the growth essential at various times following irradiation. If the nutrient was not required for the metabolic activities specifically involved in recovery, the longer the period between irradiation and restoration to complete medium, the greater the recovery that should have

taken place, and the smaller the difference between control and irradiated populations upon resumption of growth. Conversely, if the nutrient was required for the metabolic activity necessary for recovery, there should be little or no reduction in the lag between control and irradiated populations even when comparatively long periods intervene between irradiation and restoration to complete medium.

It proved easy to deplete *Chilomonas* of available nitrogen by washing and resuspending them in defined medium from which the ammonium salts had been omitted, and then incubating overnight to exhaust any reserve or contaminating nitrogen sources. Upon restoration to complete medium, unirradiated suspensions resumed division and exponential population growth after about seven to eight hours, at 25°C. Nitrogen-depleted suspensions exposed to 15 kr resumed population growth—at the same exponential rate—about 14 to 15 hours after restoration to complete medium, whether restored immediately after exposure, or as much as 20 hours later. These results are illustrated schematically in Fig. 4.2. So, there is no recovery of the fission process in the absence of a utilizeable nitrogen source.

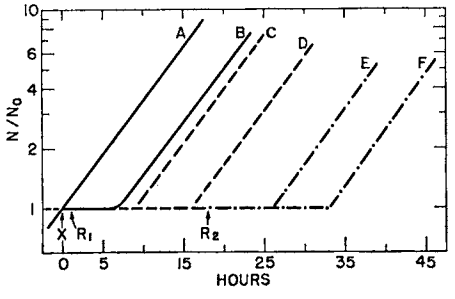

Fig. 4.2. Schematic population growth curves for exponentially growing cultures and for nitrogen-depleted cultures restored to complete medium at indicated times after exposure. A, C and E—0 r; B, D and F—15,000 r at X; A and B—growing exponentially in complete medium at time of irradiation; C and D—to complete medium at R_1; E and F—to complete medium at R_2.

Fig. 4.2 also emphasizes another noteworthy point: there is apparently no difference in radiosensitivity (as regards suppression of fission) between nitrogen-depleted *Chilomonas* and growing *Chilomonas*. At first glance, this seems somewhat at variance with the results of GIESE and his students, who have studied the effects of ultra-violet radiation on a number of ciliates; all of these ciliates proved much more sensitive when starved prior to ultra-violet exposure. Is this variance caused by differences in

the organisms employed, in the type of radiation involved, or in the nature of the nutritional deficiency?

When we did the converse experiment, and depleted *Chilomonas* of acetate carbon, instead of nitrogen, the carbon-depleted organisms were much more radiosensitive to any X-ray dose, whether we scored immediate lethality or duration of division block after restoration to complete medium. Furthermore, the sensitizing effects of acetate depletion were mimicked by treatment with the thiamin analog, neopyrithiamin, which would be expected to interfere with the utilization of acetate. Finally, KIGA and associates[16] have reported that in yeast, malonate, another inhibitor of energy metabolism, increased X-ray sensitivity. So I would suspect that starvation increases the radiosensitivity because of depletion of energy sources, rather than of nitrogenous metabolites,* prior to irradiation.

I have also done analogous experiments with *Tetrahymena* which has a long list of vitamin, purine, pyrimidine, and amino acid requirements. The only experiments which have yielded unequivocal results, so far, are those which involved depletion of an essential amino acid; phenylalanine and histidine were the two most studied. In these cases, again, there was no decrease in the duration of the radiation-induced lag on restoration to complete medium regardless of the length of time that the animals were incubated in amino acid-deficient medium following irradiation. These results would indicate—though they by no means establish—that synthesis of protein is a metabolic activity necessary for the recovery of the fission process. This might mean resynthesis of inactivated proteins, or reactivation of partially denatured molecules, or synthesis of enzymes for reactivation or replacement of other types of molecules. We do not even have reasonable grounds for speculation at present.

There is one other type of phenomenon which is sometimes interpreted as involving recovery; this is the frequently demonstrated fact that when a given dose of ionizing radiation is administered over a relatively long period of time, its biological effect is greatly diminished. My work had emphasized the role of nitrogen metabolism in recovery; it was obviously of interest to determine whether there was a dose-rate effect when *Chilomonas* was irradiated in a state of nitrogen depletion. Cobalt^{60} was used as the radiation source, and a very distinct dose rate effect was observed;

Author's note*—Since the conference was held, GIESE (*J. Bact.* **74, 271, (1957)) has reported that nitrogen deficiency is far more effective than carbohydrate deficiency in increasing ultra-violet sensitivity of yeast; and I have since found that *ultra-violet* sensitivity of *Chilomonas* also is increased by nitrogen depletion.

although the organisms had no nitrogen source available during the exposure, 10 kr administered in 10 minutes was about equivalent to 16 kr administered in 90 minutes (see DUCOFF[17]). If the dose rate effect does represent a type of recovery, it is a type of recovery which proceeds in the absence of a nitrogen source, and is quite a different phenomenon from the one we have been dealing with in our other experiments.

It seems to me that there is no doubt that cells can—and do—recover from radiation injury. But it seems probable that the nature of recovery is different for lesions leading to different manifestations of injury, and there may even be different mechanisms of recovery for any one type of injury. Study of recovery phenomena is promising—practical considerations aside—both for the light it may shed on normal cell processes, and for implications about the nature of the radiation-induced lesions. But I'm going to refrain from discussing these implications, in the hope that, given sufficient time and provocation, some of you will join me in climbing out on a limb.

DISCUSSION

POWERS: I said that I wasn't going to talk about *Paramecium*, but I'll ask a question. When you relate generation time to the length of the fission block, you're comparing the time between the cessation of cell division and the first cell division that you measure.

DUCOFF: At these doses, doses which do not result in any immediate killing, that I can detect by these methods, the usual thing is to extrapolate this population growth curve on which most of the points lie so well. Apparently, when the first one starts to divide successfully, others do follow along in prompt sequence.

POWERS: Incidentally, I have asked Howard these questions many times and he has given me these answers, but you folks haven't had the benefit of it. What about the second division after the first?

DUCOFF: They carry on for at least three, sometimes four divisions at the same rate. This implies to me that if there is any residual slower growth it is a very small thing. Now, I'll repeat that in the case of *Chilomonas* this holds for doses of about 15,000 r where we could not get detectable killing. In the case of *Tetrahymena*, similar dose effects are obtained with up to sixty or seventy thousand r; and I believe you see the same thing at 55,000 r which is, as I recall, your lowest dose without any vegetative death.

POWERS: Is it not true that in some cases the time intervals between successive divisions after treatment become shorter and shorter? In *Paramecium*, after the high doses you mention, the recovery process is observed to extend over 6 to 8 fissions.[12]

DUCOFF: I think that if this is correct, in order to get these average figures out you would have to have some cells dividing much more rapidly than the controls.

POWERS: In your case, Howard, the curve should turn up gradually at the bottom.

DUCOFF: It may turn up but this does not take place over a period of more than about two hours and the generation is about five hours, so that this is a relatively small fraction of the generation time.

POWERS: It might be different in different cells, that is, the degree to which this gradual recovery is expressed.

DUCOFF: We have sometimes argued, if this is so, whether it's the *Chilomonas* or the *Paramecium* which is the more unique organism, and I wonder if anybody has data on other types of cells of this type.

O'BRIEN: Must it be of this type?

DUCOFF: Pardon me; I meant information of this type on other types of cells.

O'BRIEN: I have been told not to do this, but I'm going to do it anyway! I've been itching to get this on the blackboard for a forty-five-minute instruction on the 'environment'! I may just very hurriedly tell you some very interesting, I might say extraordinary, events that happen in a rabbit's ear. Maybe I'll get some suggestions from this group as to whether this is a case of recovery. Compress an ear area, let us say about ten or twelve mm. in diameter, between two metal surfaces. The lower surface is beryllium so that we can get sufficient radiation through to the area in question. The upper surface is a copper cooling surface and the lower is a beryllium temperature-regulating surface. This area is completely compressed and we maintain that it is anoxic. The problem comes when you irradiate skin when it is cold. When the area is cold at the time of irradiation, the damage is less than in a warm-irradiated area. This has traditionally been interpreted in terms of decreased circulation during irradiation in the cooled area. This is a reasonable explanation. Further, if circulation is decreased, the oxygen concentration or oxygen tension is less; this fits in with the oxygen effect. If *this* area be at 40°C and *this* one at 44°C, the end result will be that the former will not exhibit a perforation after having received 38,000 r. (We are not cheap about giving r either!) But here (warm-irradiated) there will be a complete perforation and this is not surprising. This would bear out the idea that the oxygen tension being high over here, this goes to pieces. Now the question is: Will temperature *apart* from variations in oxygen tension have any effect? We presume there is no oxygen passing back and forth because the circulation is cut off. (Environment's in a bad way, in other words.) Here, where we radiate the cold anoxic area and the warm anoxic area we get approximately the same result as when we merely cool or warm the areas. Therefore, apart from variations in oxygen tension, the cold does exert what we call a protective effect. This is not the problem; I come to that immediately.

FLANDERS: Could you just clarify the results, when you do this in the cold? When you do the compressed exposures you say you get the same results. Same as what? Can you just brief us?

O'BRIEN: I didn't quite get that.

FLANDERS: Could you just repeat your observations.

DUCOFF: We micro-biologists don't understand this result. Can you simply restate it.

O'BRIEN: This area is not compressed at first *here*, just cooled, that's all. *This* is resting on a cooled surface; *this* is resting on a warmed surface. You get a large perforation *here* and in the other only some perforation, perhaps a small hole compared with the total area irradiated. Now we want to find out whether or not temperatures apart from variations in oxygen tensions have any effect. So we take *this* area and compress it, and similarly *here*, keep them quiet for about five minutes; this is done simultaneously on the same rabbit; then we irradiate an area which is smaller with the same dose in each case. However, *this* is cold and *this* is cold irradiated. And *this* is warm and *this* is warm irradiated. And we find that in the cold *here* there is no perforation at all, even though there is no question of variations in oxygen tensions, because this whole area is dememtized

with a weight of about 1,000 grams on top, pressing the two metal surfaces together. Over *here* we get complete perforation of *this* area, just as if you took it out with a cookie cutter. After about an hour and a half or two hours, in both of these areas you'll find an erythema. This erythema could be due to the radiation, could be due to the pressure, could be due to the cold, the heat, or something like that. But we have managed somehow or other to recognize what we think is the radiation erythema. We decided we would try to detect something happening in the so-called latent period. So, with this set-up, immediately after the animal was released, given his 38,000 r, we injected trypan blue into the heart. We were quite surprised to find that the trypan blue showed up only in the cold-irradiated area, very intensely and within two minutes of the injection. It might be anticipated that the trypan blue, if it showed up anywhere, would show up here (warm-irradiated area). Now, instead of injecting the trypan blue immediately after we released the animal, we laid the animal down on a table for a half an hour and then injected the trypan blue, with the result that it did not show up any place in any concentration. Of course, it begins to show in the eyes and the nose and everything ultimately, but this is a very slow process. Then we figured that perhaps we could hurry up this 'recovery' process, because where trypan blue accumulates we infer that there has been radiation injury. It doesn't concentrate in the cold non-irradiated area (control). We did several experiments to show that you never get the concentration of trypan blue except when you irradiate and cool simultaneously.

In another experiment, immediately after the irradiation of the cooled area, we changed the temperature of the chamber and brought the area up to 40°C for five minutes before releasing the animal. We did this by merely changing the flow of water into the system from cold to warm. In this case, after the five-minute warm up, there was no concentration of the dye in the cold-irradiated area. Now, is the five minute warming treatment (after irradiation) responsible for repairing the alteration and permeability of which the concentration of trypan blue appears to be a reflection? Is it not true, Dr. Dowdy, that trypan blue accumulation is taken as a measure of radiation injury?

DOWDY: I don't know.

O'BRIEN: Also, if we cool for five minutes afterwards we get no concentration of trypan blue.

MCKEE: Who would like to comment?

LESSLER: I would just clarify one point. It's very hazy in my mind. How do you get trypan blue into that area at all if you have a thousand grams of pressure on it?

O'BRIEN: We release it.

LESSLER: After the clamp is off?

O'BRIEN: That's right; we lay the animal on a table and inject the dye.

LESSLER: I see. But if the clamps are left on you had no trypan blue ever.

O'BRIEN: Correct.

FLANDERS: I have one, Dr. O'Brien. I think you ought to tell us why you use 38,000 r in this.

O'BRIEN: Somebody else asked that once before and I don't know. We did a whole series of these cold and warm areas; we started out with perhaps 5000 and found that we got the most spectacular results, the most contrasting results between cold and warm, in the region of about 38,000 r. If you go up to sixty or seventy thousand or something like that, you're just breaking some rule and everything goes. So there is a limit and we happened to find the best place is 38,000 r.

DOWDY: It's my impression that if you irradiate a substance at a low temperature the damage is probably done but does not show up until you bring the temperature of the material back to normal, after which the changes take place at a rate consistent with the changes which would have occurred had the irradiation occurred at normal temperatures.

O'BRIEN: After forty days this area is not going to have a perforation in it at all. There's a lesion, of course, but it heals and there will be no perforation.

DOWDY: That's just cooling, no pressure added to it, just cooling?

O'BRIEN: No, there's pressure added, too. If you don't compress it, but merely cool you get a saving effect of the cold but it is not as pronounced as if the tissue were anoxic and then cooled.

DOWDY: Well, you're doing two things to it.

SCHNEIDER: Do you think you're just creating a quantitative difference in the degree of local and total oxygen? Between these two factors, compression and cold, in the same area?

CRONKITE: If you could get that cold enough you might be able to do it without the compression.

O'BRIEN: I suppose so, but we don't know a way to do this conveniently except to compress it between two surfaces. In the first experiments we only had one cooling surface and the other was merely a pressure; this was satisfactory and we were told not to worry about it. However, we got some engineers to build us a chamber whereby we could effect both two-surface cooling or warming; this is much more efficient.

CRONKITE: I think what Dr. Dowdy started to comment on bears some relationship to what Dr. O'Brien is talking about and also the morning sessions. If one irradiates a hibernating mammal and keeps him hibernating, none of the cytological evidence of radiation injury develops until one warms the animal up to its normal ambient temperature, and then the entire sequence of histological events takes place at the same rate it would have normally. I do not think this type of thing bears any relationship to the recovery process, because irradiation of a hibernating animal or cooling a frog, as PATT does, does not increase the resistance, inherently, to radiation.

O'BRIEN: What I asked is what happens in the five minute period that we warm the cold irradiated area and there then follows no concentration of trypan blue? The permeability characteristics have been upset. The localization is just as precise as if you drew it with a pen; it stops at the non-irradiated area. (It begins to accumulate at the periphery.) My question was, is this recovery?

BOND: Before that, I'd like to be perfectly sure that you watch these long enough, so that there is ultimately a real difference in effect. It's not what Dr. Cronkite was getting at, but there's a delay in effect—temperature alone is producing a difference in effect.

O'BRIEN: We carried these on for about forty days and the whole area was healed.

BACHOFER: Do you find that the trypan blue correlates with the ultimate damage? The appearance of trypan blue in this immediate area?

O'BRIEN: The trypan blue concentration appears to be directly related to the dose given.

BACHOFER: Will this predict how many lesions you get later on?

O'BRIEN: With the large dose we're using we always get the same.

BACHOFER: Then this appearance of the trypan blue rather soon after irradiation does not indicate what you're going to get in terms of ultimate lesion?

O'BRIEN: Correct.

SCHJEIDE: I see the possibilities of two recovery mechanisms. One is the recovery

from over-permeability to normal permeability. Normal permeability could be reachieved upon heating in this area.

O'BRIEN: Don't you consider that you should get the trypan blue in the warm-irradiated area?

SCHJEIDE: All right, we won't call that a recovery, we'll just say that there's a change. But is it not possible that the permeability to trypan blue is an indication that the substances may enter from the environment from surrounding areas at a rapid rate which fortuitously assists in the recovery of these cells.

GLASSER: I would venture to estimate that the histological appearance of the warm area prior to perforation is probably quite fibrotic, and as such I would not imagine that trypan blue could even approach the area.

UNIDENTIFIED: Do you put the trypan blue into cardiac within minutes after the irradiation?

O'BRIEN: Within a minute.

GLASSER: How soon does your perforation appear?

O'BRIEN: Oh, the perforation in the warm-irradiated area, it would be complete and healed by forty days.

GLASSER: When does it first appear?

O'BRIEN: It shows up in about two hours in the form of an erythema.

LESSLER: In an area that is clamped off and warmed, what about the possibility of plugging? This area, due to damage within the blood vessels, particularly the small arterioles, would be more sensitive to radiation. The arterioles in the cooled area were probably initially constricted. In the constricted condition, when the clamp is removed, they will tend to dilate. Thus the cooling may protect against the plugging of these smaller arterioles.

O'BRIEN: We compressed an area here and an area there for five to six minutes before we applied the temperatures. We do this, as we say, to get rid of the residual oxygen.

LESSLER: I'm suggesting that during the irradiation, you have a different condition in the circulation. In the warmed blood vessels, although there is no circulation, you can produce plugging, whereas in the cooled area plugging does not occur. You have a rather unique condition here. You must get the blood back into the irradiated area in order to get the trypan blue into it.

O'BRIEN: When you do nothing to either one of them, nothing happens to the trypan blue; when you release them the circulation returns immediately; this you can see under the binocular microscope.

HALEY: We have two processes going on almost simultaneously. Insofar as the cooling process is concerned, it has all the earmarks of some earlier work done in frostbite. If you take an animal who has been exposed to rapid cooling, any of these dyes which will pass through the vessel wall will give you a stained area. As regards the ability of these materials to pass the walls, there isn't any evidence that I know of (and I spent about six years working on the terminal vascular bed) that indicates that plugging does occur in such a short time, so we can dismiss the idea of any plugs forming. Furthermore, after irradiation it's pretty hard to see anything plug up, even when you have petechial hemorrhage. You can see the hemorrhage beautifully, but you don't get the normal repair process in which a plug is formed. The other thing is that there is a possibility of depolymerization of the inter-cellular cement substance and that can be readily demonstrated in the intestinal tract by using the method of GIRSH. He doesn't use trypan blue, he uses Evan's blue. If you've worked really hard and have good eyesight, you'll eventually see that irradiation can produce this type of thing. Here you've got the radiation plus what almost amounts to a type

of frostbite damage. So I'm not surprised at all, under the circumstances, to see the particular area take up the trypan blue whereas the warm area doesn't. Furthermore, the present idea for the treatment of frostbite to prevent any damage to the particular area is a very rapid warming. That will explain why in a second instance the trypan blue doesn't get into the tissue.

DOWDY: I was rather intrigued; I think it's a matter of lack of adequate circulation. With 38,000 r, that's a pretty good-sized dose, it seems to me that as soon as you warm it up you get a swelling and edema of the mucosa of these small arterioles and capillaries and, therefore, the trypan blue doesn't get in.

LESSLER: I'm not suggesting that the capillaries are plugged. I'm considering it strictly on the basis of terminal arterioles which are much more liable than the capillaries to this sort of thing.

DOWDY: You mean the swelling, edema?

LESSLER: Yes, several changes like that do occur.

O'BRIEN: Dr. Lessler, *this* is the compressed area and *this* is the irradiated area; why doesn't that happen in the control? Remember, this is our control.

LESSLER: I'm suggesting that the irradiation damages the terminal arterioles, but not that it plugs the capillaries.

O'BRIEN: But then if I warm this for five minutes and there's no trypan blue accumulating here, do you suggest that there has been a recovery from this damage?

LESSLER: That I'm not suggesting. But there is a rather unique mechanism in the arterioles which shuts the arterioles off in tissue damage. You can't bleed an individual to death except through the largest arteries for this reason. If damage occurs in an area, one of the first things that happens is that these small terminal arterioles close down, completely. For example, if you lop an ear off, the animal won't bleed to death for the arterioles have the ability to constrict down. I'm suggesting that the blood flow to the area does not open up again, not because the capillaries are necessarily damaged, but because the arterioles do not open sufficiently to re-establish an adequate blood flow to the area.

O'BRIEN: But, both areas are damaged, the warm and the cold; they were both irradiated and they were both compressed; the only difference is the temperature.

BOND: It may be cheating, but did you look at the areas under the microscope at this time?

O'BRIEN: Under the binocular microscope. And we looked at them against a dental film reader, and you can see quite a bit.

BOND: You didn't make slides of the areas and take a look at the cells?

O'BRIEN: Did I make sections, you mean?

BOND: Yes.

O'BRIEN: I didn't feel it was going to teach me anything. I'm of the school that doesn't believe you can tell much about capillary permeability by looking at sections, especially of rabbit ear.

BOND: Do you think you can tell what the blood vessels looked like?

O'BRIEN: Oh, you could see the circulation return immediately.

LESSLER: Did it return in both preparations?

O'BRIEN: Yes. Dr. Haley spoke of Evan's blue which penetrates much more rapidly. In order to assure ourselves that we were having a complete block of circulation, we constructed a device, one surface of which was transparent plastic; then we injected Evan's blue in the heart and it's quite an amazing thing to see the rabbit suddenly turn blue everywhere, except in this compressed portion. And when the Evan's blue had gone from the circulatory system, we released the animal and that animal ran around the rest of his life with one white spot

in his ear and the rest of him was blue (to the surprise of the maintenance men!)
GLASSER: Would it be out of order to return to a re-examination of Dr. Ducoff's rather attractive thesis?
RUSTAD: If I may be permitted to go on with a very esoteric speculation for a moment regarding our rabbit ears. There is a phenomenon which *Amoeba* workers call pinocytosis, electron microscopists with different types of tissues call it various things, but what it amounts to is the cell pinching little globules into its interior. One of the Master's candidates at Berkeley, RINALDI, has demonstrated that, with ultra violet in massive doses, you get an independent pinocytosis which is independent of the medium, with *Amoeba*. So if we assume that very massive 38,000 r perhaps causes something of this sort on a cellular level, could this be a drinking in of whatever happens to be around as a result of irradiation? Miss DETERRA and I have obtained some data on the temperature dependence of this phenomenon. If you dump an *amoeba* into a protein solution at 2°C, it will begin to show morphological characteristics of pinocytosis in about twenty minutes as opposed to, say, thirty seconds. You pull this off and wash it several times and throw it in at 18°, and pinocytosis occurs all over the place. So, this very esoteric proposal is, simply, that there is irradiation damage here of a sufficiently high degree, so that this type of process takes place, but is inhibited by the temperature. When you remove the temperature block, the cell, for reasons of other things that happen to it, simply engulfs anything that happens to be coming along. If the dye is coming out of the blood into this area it might be taken in as a result of that.
SPARROW: This is a question for the previous speaker. I didn't understand one point. In the irradiated cold area that you heat for five minutes, is it then perforate or not?
O'BRIEN: No, no, the end result is the same no matter what you do.
MCKEE: Shall we, then, return to the considerations of Dr. Ducoff which were made initially. I think Dr. Powers alluded to the resynthesis or replacement of constituents as perhaps majoring recovery.
POWERS: I don't recall saying anything like that.
MCKEE: I knew you didn't, but I knew that wouldn't get around you.
POWERS: I know, you're being antagonistic.
MCKEE: Thanks very much.
BERNHEIM: I assume that in your *Paramecium* that grew on acetate and ammonia that the ammonia assimilation is an active process and that requires energy for assimilation; is that true?
DUCOFF: Presumably; at least this has been shown for other organisms as in the work of SYRETT on *Chlorella*. So far, we lack quantitative information on *Chilomonas*.
BERNHEIM: Does your recovery occur when ammonia starts to be assimilated? Or does the assimilation occur before you get the recovery result?
DUCOFF: When you restore the ammonia to control groups, there is about a seven-hour assimilation time after restoration of the ammonia before cell division is resumed. You can restore the ammonia, say, five hours prior to the irradiation, or you can restore it, two hours prior to the irradiation, or you can restore it at various times after the irradiation, and still get the same time relationships, so that apparently the assimilation must be just about completed before the recovery type of activity sets in.
BERNHEIM: Does that imply that your injury is in the assimilation mechanism or injury, say, to the subsequent protein?
DUCOFF: I would say that the fact that you can irradiate at almost any state of

the assimilation, and still get the same results as if you irradiate prior to the restoration must indicate that the assimilation is not impaired at all.

BERNHEIM: So the assimilation mechanism, that is the energy requiring mechanism, is relatively stable and only after the ammonia gets into the cell do you get your damage.

DUCOFF: As I visualize it, the damage consists of the inactivation or destruction of something and this can not be replaced until after the organism has built up a sufficient supply of the nitrogenous intermediates between the ammonia and, let's say, protein. It could be an enzyme. It could be just intermediates. I would think it is some sort of protein. I might say that in one case we do get a more deleterious effect, and that is if the ammonia is restored immediately prior to irradiation. I've always thought that this also fits in with the acetate depletion story. In *Chlorella*, at least, there is an increased oxygen uptake during assimilation of a restored nitrogen source. Perhaps *Chilomonas*, when just beginning to assimilate ammonia, marshalls all of its energy reserves for the assimilatory process, and thereby becomes more sensitive. If so, the 'just-beginning-to-assimilate' *Chilomonas* would be comparable to acetate—or carbon—depleted organisms.

SCHJEIDE: I think that you are pretty close to it there, Dr. Ducoff, but I wonder if you would care to get really specific (chemically) about the protective mechanism.

DUCOFF: The recovery mechanism?

SCHJEIDE: Speculate!

DUCOFF: Well, I like to speculate. But I have tried to do some experimental work on which to base the speculation. This is the reason I started doing experiments with *Tetrahymena*. Ammonia is, unfortunately, the only nitrogen source that *Chilomonas* will grow on in this simple medium. So I turned to *Tetrahymena*, and did the same sort of experiments, but here I'd withhold the essential amino acid, phenylalanine. Upon the restoration of phenylalanine to control and irradiated cultures, at various times following irradiation, the same time difference—i.e. the same duration of radiation-induced lag—was always observed. In other words, no progress towards recovery of the division process occurred in the absence of the required amino acid.

In *Tetrahymena*, as in the mammal, tyrosine will reduce the amount of phenylalanine required for a given amount of growth, but will not completely replace phenylalanine. Incubation of irradiated phenylalanine-depleted *Tetrahymena* in the presence of tyrosine still results in no progress towards recovery. On restoration of phenylalanine, there is still the same duration of lag between control and irradiated populations. So protein synthesis, I think, must somehow be involved. This may be simple protein synthesis, or it may be coupled to RNA synthesis. I tried to do similar experiments with the purine and pyrimidine requirements of *Tetrahymena*. In a few cases it appeared that prolonged post-irradiation incubation in the absence of the required purines and pyrimidines did result in a shortening of the radiation-induced lag. But closer analysis revealed that prolonged incubation of the unirradiated organisms in the absence of their required purines and pyrimidines exerted a distinct deleterious effect, manifested by a greatly increased lag, or assimilation time, on restoration to complete medium. This deleterious effect was reduced—for reasons I cannot begin to guess—by the radiation exposures employed. With suitable times for depletion, irradiation, and restoration, the irradiated populations actually do better than the controls, so we were confronted with a protective or stimulatory effect of exposure to 60,000 r.

O'BRIEN: Why did you use 60?

DUCOFF: On the basis of preliminary dose-response studies, this is the highest dose at which we don't get detectable killing.

O'BRIEN: That is comparable to our procedure.

MCKEE: This brings us into the heart of the recovery problem, I think, namely, reconstitution and resynthesis of the components of cells and I'm sure that is what Dr. Schjeide is referring to. Are there others here who have a system that they would like to talk about? I see a few hands, one is Dr. Glasser.

GLASSER: Yes; I feel somewhat like the father whose child was about to marry out of their social class, but I see some hope in hybrid-vigor in Dr. Ducoff's information that might restore a little prestige to some words I've offered at other meetings. We have had a working hypothesis in regard to protein nutrition and its relationship to recovery from injury in general and radiation injury in particular. Generally, the thesis works like this. We have felt that after irradiation the recovery of a system deprived of protein is greatly inhibited in comparison with a system in which protein is offered. This is on an isocaloric basis. To extend this further, the original work was so designed that animals were protein-depleted (protein-free diet) prior to irradiation for a period long enough to effect a reduction in liver protein concentration of about 50 per cent. This is a period of three weeks. At this time the animals are exposed to 500 r X-rays and half of the irradiated animals are put on a protein diet of 18 per cent casein; half are continued on a protein-free diet which is isocaloric and to the best of our knowledge isodynamic with the protein diet. The controls are similarly handled. A variety of organ systems are studied under these situations. The testis and its histology, certain biochemical changes in the testis, liver protein, liver carbohydrate, spleen histology, and certain enzymes in the spleen. In general, we have found that in protein dependent organ systems, the extent of injury from irradiation is much less in depleted rats than the injury in adequately fed animals. However, the rate of recovery of a given system, when the host is continued on the protein-depleted diet, is much longer than protein-depleted animals which are irradiated and recover on the casein diet. We have felt that there are a number of factors concerned here. One, that the cellular interpretation of the radiation lesion may be altered in certain specific systems which are dependent on protein for their normal physiology. The radiation lesion may be interpreted through a protein substrate enzyme mechanism. When this informational series is depleted the injury may be diminished. Once the injury is deposited in the cell, in some manner or fashion we can't define, then the energetics for recovery are dependent on the restitution of protein. Since this is not being provided, other nutrient or other bio-energetic materials must be supplied through alternative and less efficient pathways and this would account for the longer recovery period. We have some data which would support this, but before I would have to cite the data I just want to pose the verbal picture and see what kind of comment we can get on this.

MCKEE: Are there others that have any current data that would fit into this particular picture?

SCHJEIDE: I don't have data, but can I ask a question?

MCKEE: I see no other hand.

SCHJEIDE: I wonder if sulphur-containing proteins are particularly conducive towards recovery?

GLASSER: Specifically, we have not tried this although we have doubled the methionine components in the vitamin mix. But we have not been concerned with anything except the total amount of protein in the diet.

SCHJEIDE: I wonder also if it would be feasible to attempt an experiment in which one adds specific precursors for the nucleic acids.

GLASSER: It may be entirely feasible, but, again, we have not extended ourselves so far.

SCHJEIDE: The point in this reasoning is obvious. We might get to the heart of what is being replaced and what is essential for recovery.

MEAD: I would like to ask a question and perhaps make a comment, too. Perhaps I'd better make the comment first. Precursors for nucleic acids are precursors for almost anything aren't they? It would therefore be very difficult to eliminate these precursors without eliminating the energy source.

DUCOFF: That's the trouble with mammals.

MEAD: I was about to ask what carbon sources are needed in *Tetrahymena*?

DUCOFF: We use as carbon sources glucose and acetate.

MEAD: What carbon containing materials do you need for the nutrition of *Tetrahymena* besides phenylalanine or glycine?

DUCOFF: The list of essential amino acids is almost identical to those required by the mammal.

MEAD: Are these identical, I mean as far as you know?

DUCOFF: Yes; this is something that makes GEORGE KIDDER very proud of his *Tetrahymena*. On the other hand, *Tetrahymena* requires, in addition, exogenous purine and exogenous pyrimidine which makes it look a very attractive organism for this study to settle between the nucleic acid and protein. But, because of this experimental artifact or anomaly, or whatever you want to call it, the deleterious effect of the prolonged incubation in the absence of its required purines and pyrimidines, I can't get very clear cut results on this question. I would very much like to find some simpler organism that would require only one purine or pyrimidine and one amino acid and test in this way. But I don't know of any such organism.

MCKEE: I believe coffee is ready. Unless there is some other point bearing directly on this, Dr. Lessler has a system he'd like to talk about. Oh, excuse me, you have your hand up here. If it's regarding this, go right ahead.

TOTTER: I want to ask Dr. Ducoff if the rate he showed us for these recoveries was not consistent with this kind of a picture; that there's some substance destroyed with a rate, within these cells, which is linear with the dose of X-ray, and then a resynthesis commences at the same rate in the injured cell as in the unirradiated cell, until some necessary level is reached so that the cell can divide.

DUCOFF: This is at least one of the pictures, which may be consistent with what's happening . . .

TOTTER: But, isn't the kinetics inconsistent with the template construction?

DUCOFF: If it's a simple direct template construction, quite possibly. I'm not sure what you're driving at. I don't really believe that it is the destruction of template in this case.

TOTTER: It seems to me to be very unlikely that it is a destruction of template in this case.

DUCOFF: I don't know of anything that we can with any confidence call template, outside of things like DNA; and, as you know, we can increase the DNA content in *Tetrahymena* almost six-fold and still get the same duration of radiation-induced fission block. So, I would rather think that there is something else involved. Whether it's a repair of something or replacement of something, I don't know.

MCKEE: Does anybody else have any comments on this point?

JAHN: I would like to ask Dr. Ducoff one related question, and that is, did he find any evidence of synchronized division following this block?

DUCOFF: No.

JAHN: Do you believe that all of these cells are stopped at any stage in division in this average of one-and-a-half generation times and then begin division again at the same point? Is there evidence one way or the other?

DUCOFF: I don't believe they are all stopped at any one stage, although there isn't any evidence from this work. From the radioautograph studies, one would expect that this is not the case in other materials and I think we have the same sort of phenomenon. If you want to look at division as requiring a set of conditions, after irradiation, the cells may have advanced, as far as DNA content is concerned, but still some other thing has not advanced.

JAHN: I was wondering how this compared with, say, the heat treatment which blocks division, and then when the heat treatment is removed, the cell begins dividing in synchrony.

DUCOFF: This is what we do with *Tetrahymena* and you can block for the equivalent of several divisions and still get greatly increased DNA content. I don't think you can increase the total DNA content that much with radiation; the longest you can block with these doses is only slightly in excess of one division time. The most you could get, presumably, would be a doubling of DNA. We have particularly looked, not necessarily for synchrony, but for a sudden spurt of growth. We do find such a spurt after nitrogen mustard block, which would indicate a damming back of some processes. We were particularly interested in this and looked for it, and could find no evidence of any increased amount of cell division at the recovery part of the curve.

COFFEE BREAK

LESSLER: I would like to discuss the effect of X-irradiation on the erythrocytes of the frog. These are postmitotic cells whose respiration is readily damaged by 100 to 200 roentgens. The endogenous metabolism of these cells in represented as the zero line of all non-irradiated controls in Fig. 4.3. The depression in respiration following 100 r or 200 r was 10 to 15 per cent. The cells apparently recover from this respiratory depression. If you follow cell respiration for several hours, the oxygen consumption returns to normal respiration levels. These experiments have been carried out over an extended period and the hundreds of determinations of non-irradiated controls provide a strong statistical point from which to determine respiratory variation due to irradiation.

One of the things we studied in this system was where, in the respiratory chain, irradiation was causing its effect. Endogenous respiration was compared with respiration in non-irradiated cells following the addition of succinate, alpha-ketoglutarate, glucose or pyruvate. These substrates caused marked increases in frog red cell respiration. I shall discuss the two that produced the greatest increases in erythrocyte respiration, namely succinate and alphaketoglutarate. In non-irradiated cells these substrates caused a 15 to 40 per cent increase in the oxygen uptake of frog erythrocytes. They did not, however, change the general pattern of radiation damage to respiration or its recovery. One other substance we have significant data on is reduced DPN. DPN H_2 added to non-irradiated frog red cell suspensions increases the respiration up to 45 per cent.

TOTTER: Catalytic or substrate?

LESSLER: Substrate level. DPN H_2 added to 100 r irradiated suspensions of frog red cells, however, did not provide a protective effect on respiration. The general pattern of respiration remains very much the same. Our conclusions from these experiments were that the recovery from low doses of radiation of nucleated erythrocytes tends to be independent of the substrates we used. One would wonder why the substrates have so little effect on radiation inhibited respiration since they do have a very marked effect on respiration of undamaged cells.

BERNHEIM: When your irradiated cells have recovered their endogenous respiration, and you now add succinate or alphaketoglutarate, what results do you get?

LESSLER: Unfortunately, we haven't done that particular experiment. The general extrapolation that I would make is that after you come back to what would be our non-irradiated level, addition of substrate at that point would increase respiration.
BERNHEIM: Do you have any idea of what the endogenous substrate may be?
LESSLER: Whatever it is, it's in the cells, and whatever it is it is not connected with the cell division mechanism. There is no cell division and these cells respire quite well in salt solutions.
JAMES: What is the order of magnitude of the P O_2 for these cells?

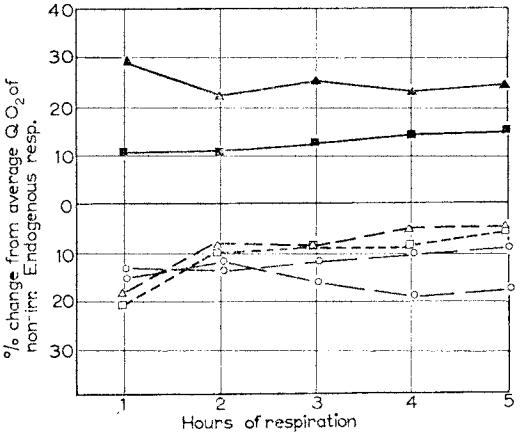

Fig. 4.3. The effect of 100 r and 200 r on the endogenous and substrate stimulated respiration of bullfrog erythrocytes.

Solid dots represent endogenous respiration following 200 r; open circles, endogenous respiration following 100 r; solid triangles, non-irradiated succinate stimulated respiration; solid squares, non-irradiated alpha-ketoglutarate stimulated respiration. The open triangles and squares represent the averages following 100 r or 200 r of succinate and alpha-ketoglutarate stimulated respiration respectively. The broken lines, which represent irradiated erythrocyte suspensions, all show depressions below the endogenous respiration level as represented by the zero line.

LESSLER: That's a tough one to answer because I have expressed numbers in percentages. You want it in cubic millimeters or dry weight of the cells?
JAMES: Yes. What is it for 10^6 cells?
LESSLER: We have expressed all our results on the basis of dry weights. These cells have a high level of respiration. Somewhere in between a sperm and a sometic cell would be a good place to put it.
KELLY: If you increase the dose, do you get a further depression of respiration?
LESSLER: Our data indicated a logarithmic rather than a linear response to dose with the frog erythrocytes.
DUCOFF: These were irradiated *in vitro*?
LESSLER: Yes, *in vitro*.
DUCOFF: If you add the substrate prior to irradiation, what happens?
LESSLER: The same thing. It doesn't matter if you add the substrate at the point of irradiation, that is after the irradiation has occurred. We ordinarily wash the cells and then add the substrate. However, we have also irradiated them in the

substrate supported medium. The amount of substrate is of the order of 0.003 molar. It's relatively small.

DUCOFF: I was just thinking of, perhaps, an energy pump priming mechanism to start using the succinate. If you then knock down your endogenous metabolism, you just might not be able to start working on succinate.

MCKEE: Were you thinking of, perhaps, altered permeability of the cell membrane?

DUCOFF: I wasn't thinking in these terms. It would be another possibility. I was thinking more of the adaptive enzyme system.

LESSLER: We have definite evidence of changes of membrane permeability following 100 r in frog erythrocytes. Isotope uptake studies, electrical impedance studies, and cytological examinations of these cells all show membrane changes.

DUCOFF: Which way? Are they more permeable or less to each particular substrate?

LESSLER: To which substrate we don't know. We haven't had the substrates labelled yet, but the substrates, in general, affect markedly the undamaged cell.

SCHJEIDE: You have irradiated these cells *in vitro*?

LESSLER: Only in our isotope uptake studies, and I can't extrapolate from these data. The reason we did not irradiate *in vitro* is that one cannot calculate at these low doses the level of irradiation to a blood cell. We can only calculate a whole body dose. When you're working at these lower levels of radiation your percentage of error becomes very great unless your dosage is very accurately controlled. In other words, I might have to give a whole animal several hundred r to get a hundred r to the blood cells.

SCHJEIDE: Another twist to this would be to irradiate a fairly large volume of blood cells, and reinject it into an animal and then take them out again, to see if any substrate at all, as provided in a normal environment, would be effective.

HENNESSEY: To turn to the question of adding the succinate before irradiation, do you mean the respiration would be depressed to normal? Would it drop from 30 to 20, or would it drop from 30 all the way down to minus 10? Would you make this clear?

LESSLER: If you add succinate before you irradiate, the level of respiration goes up to 30 per cent above normal endogenous respiration. If, at this time, you irradiate, you come right down.

HENNESSEY: You don't drop the same amount that you would from the control level down without succinate?

LESSLER: Without the succinate in, you drop a little bit less. This would be between 10 and 15 per cent.

MEAD: I would like to mention some of our experiments that I intended to mention earlier although they seem to have come up here now, and that is the absorption, if you might call it that, of various materials by ascites tumor cells following irradiation. What we are actually trying to study is the absorption of lipoproteins across the cell membrane in ascites tumor cells and the particular lipoprotein that we used first, since it is the simplest, was the unesterified fatty acid—that is albumin palmitate complex. The complex was labelled either in the albumin or the palmitic acid portion and we analyzed in two ways. We separated the entire mixture to find out where the label was and we also checked the center well of the Warburg vessel in which the incubations were carried out to find out how much activity had been respired. Of course, the activities of all fractions have to add up to the amount of material which was put in. Well, in the unirradiated ascites tumor cells, we got a certain uptake of palmitic acid by the cell and a certain amount of oxidation. It was a good steady oxidation. However, the albumin that accompanied the palmitic acid was not absorbed by the

cell nor was it oxidized in the time interval that we were studying. Irradiation with 1000 r doesn't change this relationship in the slightest. In other words, palmitic acid was absorbed and was oxidized by the cell at the same rate and albumin was not absorbed by the irradiated cell any more than it was by the normal cell. So I think that this would argue against either an increase or decrease in permeability and against any interference with the fatty acid oxidizing system by a 100 r dose.

KELLY: I have a question on the biology of these cells. Do they die off after these doses, or what happened to them?

LESSLER: They apparently recovered. See how the respiration curve tends to approach normal levels after several hours.

KELLY: They recovered respiration at this point. What happens if you keep them *in vitro* for a day or two? Do they live a long time or do they die?

LESSLER: We have studied these cells one, two, and three days post-irradiation. When they return to normal respiration levels they stay there.

KELLY: *In vitro?*

LESSLER: *In vitro.* They will even bounce a little above normal. Of course, they have been stored at 5°C and then measured at either 25° or 30°C.

KELLY: What happens if you keep them at 20° *in vitro*? Do they remain alive for any length of time?

LESSLER: Yes. Twenty-four hours studies would show the same average non-irradiated controls as the zero line shows here. They can be stored very well. The first year's work was to find solutions in which amphibian erythrocytes could be stored. Otherwise, we couldn't have done some of the later experiments. What mechanisms are we hitting here? The oxidation of those substrates we have used apparently did not materially modify the radiation effect. Dr. Mead said that he didn't find any modification with the lipid material that he used. What are the possibilities here?

LEVEDAHL: I have a question I would like to ask Dr. Mead. I am not quite sure that I got all of the evidence. It seems to me that you said that there was no change in the utilization due to irradiation, but, in saying so, you then claimed that the absorption stayed constant. Now did I miss part of the evidence? Let's assume a situation where the utilization of the fatty acid is the limiting factor. In other words, it will be utilized at some fixed low rate. You might affect permeability quite considerably, and still not be able to detect such a change in this cell, if there were a stored reserve of the acid. My notes say you claim both absorption and utilization, but I didn't hear any evidence on the utilization.

MEAD: There are two possible effects on absorption. One, an increase in absorption, and the other a decrease. Now the increase in absorption is easily measured. For instance, you can measure an increase in absorption for protein. Protein was not absorbed in the control nor was it absorbed in the irradiated cells. This is all I can say, that there was no increase in absorption of protein following irradiation. As far as the decrease in absorption goes, since all other conditions were the same and the palmitic acid disappeared from the medium and appeared in the center well as CO_2 at the same rate, we can say that there was no decrease of absorption of palmitic acid, but that's all I can say.

LEVEDAHL: This is the part that confuses me because, if there is adequate palmitic acid in the cell under both circumstances—in other words, if utilization is the rate-limiting step—then there is no reflection at all on the absorption. If it is metabolized very slowly and you need only one or two molecules, say, you could change permeability quite considerably and not see a reflection in utilization. It doesn't seem fair to say that because we have not changed permeability of protein

we have therefore not changed permeability of palmitic acid. The solubility characteristics are quite different for the two.

MEAD: I think you may be correct there. All I can say is that there was no increase in absorption of the protein, and if absorption was the limiting factor in palmitic acid utilization I couldn't measure any decrease in that.

BERNHEIM: Have you tried adding methylene blue to your cells? In other words, could you get an oxidation if you irradiated it?

LESSLER: We never have added methylene blue to the system as yet. I might mention something that just came to mind—we have also studied human red cell respiration, in such an *in vitro* system. These human erythrocytes are definitely non-mitotic, they don't even have a nucleus! The level of endogenous non-irradiated respiration drops to one order below that of nucleated erythrocytes. The radiation effects on human red cells are not manifest until you get up around 600 r. It's a rather intriguing thing that the absence of a nucleus in the cell causes such a profound drop in normal respiratory levels.

MCKEE: In this same regard, Dr. Lessler, have you tested for any of the other intermediates in the phosphate shunt?

LESSLER: We have tested, but the validity of these data depends on a thorough statistical analysis and this has not yet been done.

TOTTER: I think Dr. Bernheim asked the question I was going to ask, and that was whether or not the oxyhemoglobin content was so great that the production of some methemaglobin would not affect the endogenous respiration. Does this recovery curve, for instance, resemble the recovery of methemaglobinemia under these same conditions?

LESSLER: This is an oxygenated system and we have observed an oxygen effect. It we irradiate in 20 per cent oxygen (an ambient pressure of oxygen of about 167 mm. of mercury) as compared to 100 per cent O_2 (p O_2 660 mm. Hg) we observed an oxygen effect. I don't think in this particular system that there is much methemaglobin actually formed.

TOTTER: That is what I wondered. What is the magnitude of the exchange there? If the oxygenation is limited by oxyhemaglobin, then the formation of some methemaglobin would give you this sort of a result. If not, the order of magnitude is entirely wrong to have this, but the addition of methylene blue, as Dr. Bernheim suggested, would take care of that or, alternatively, you could use flavine phosphate, and something to reduce it, which would very rapidly reconvert the methamaglobin to oxyhemaglobin.

HOWTON: Dr. Lessler, would you please restate the evidence that permeability changes occur?

LESSLER: X-irradiation as low as 25 r causes ruffling of the cell membranes and other cytological disturbances. These amphibian erythrocytes are really quite sensitive to low doses of radiation. Cytological damage increases with dosage to about 200 r. Electrical impedance studies show changes which are interpretable as membrane changes. We have also observed changes in P^{32} and K^{42} uptake and loss in irradiated amphibian erythrocytes.

SCHJEIDE: Apparently, this permeability disturbance is visually overcome because you say that these cells survive for several days. Or did they survive at all?

LESSLER: That's what we tried to do with studies of the rate of hemolysis and cell counts. I'm not completely convinced by our own results as yet. I think that we get something of the type that ALPEN[18] describes, following irradiation of rats coupled with thermal burns. In the red cell population, those cells which are badly damaged go to pieces and the remaining erythrocytes are a relatively resistant population. Our evidence suggests that those cells that are badly damaged

tend to disintegrate during the first 24 hours, leaving a relatively undamaged surviving population.

SCHJEIDE: I would like to point out that, in irradiation of the whole frog, or tadpole, to be more specific, there is no detectable drop in red blood cells in the circulation as estimated by blood cell count during the first few days. It takes a matter of a month or two to detect a difference, implying that these cells continue to circulate.

LESSLER: Do you feel that the cell counts of tadpole blood is a reliable technique?

SCHJEIDE: Not to measure the blood volume, if that's what you're hinting at. The counts themselves are reliable enough, if you make a large enough number.

LESSLER: I see. In other words, you statistically treated the data. We've been having a lot of trouble making reliable cell counts.

SCHJEIDE: A point I wish to make is that hematopoesis is totally shut off for a matter of a month with a dose of 100 r so that this would not be a factor in what I've just said. Finally, if we can join these two experiments together (which I doubt we can really do), this would be some sort of evidence that the cells are protected somehow *in vivo* despite the irradiation of other parts of the same organism with a rather high dose.

LESSLER: Is there any storage area in the tadpole at all?

SCHJEIDE: Yes, there is the spleen and we do seem to have storage of lymphocytes before irradiation and release at the time of irradiation.

LESSLER: You might have the damaged cells taken out by the spleen and later replaced, all by the lymph.

SCHJEIDE: Quite possibly.

MCKEE: Are there other comments regarding this paper? If not, I wonder if someone has another recovery system they would like to talk about?

BACHOFER: We were intrigued by the work referred to earlier by Dr. Ducoff, namely, the work of COOK in irradiating *Ascaris* eggs. COOK found that low post-irradiation temperatures previous to incubation of the eggs gave a much increased survival. This we investigated, together with other post-irradiation treatments. Contrary to the results reported by COOK, we found that holding irradiated eggs at low temperatures (0° and 5°C) gave a lower survival when the eggs were subsequently incubated at 30°C than when the eggs were incubated immediately after irradiation without an intervening period of cold treatment. Highest survivals (completion of embryogenesis) were obtained when irradiated eggs were immediately incubated at optimal temperatures; any holding of the eggs at lower temperatures previous to incubation decreased the survival. The lower the temperature, and the longer the time the irradiated eggs were held at the low temperatures, the lower was the survival. These low-temperature post-irradiation treatments had no effect on the delay of cell cleavage when the eggs were subsequently incubated at optimal temperatures. In this respect our work agreed with COOK's work. To return to the recovery of irradiated eggs, reported by COOK, when the eggs were held at low temperatures prior to incubation: it should be noted that COOK used the eggs of *Ascaris megalocephala*, whereas we used the eggs of *Ascaris lumbricoides suum*. Lest it be assumed that the two species differ in this respect, Dr. GEORGE PAHL, who worked with me on this work, has subsequently repeated the work on *Ascaris megalcephala* and found results the same as ours with *Ascaris lumbricoides*. In short, the results of COOK could not be verified with either species. We are, therefore, forced to reject the results of COOK. I think Dr. PAHL has not yet published his results with *Ascaris megalocephala*.

Two types of post-irradiation treatment which produced genuine recovery

involved either a period of anaerobiosis or a treatment with KCN after irradiation but prior to incubation. These treatments produced recovery, both as regards the time required for cleavage, and the completion of embryogenesis. It should be pointed out that these were cases of genuine recovery, not cases of arrested damage. There are cases in the literature in which a treatment which slows up metabolic processes may superficially appear to foster recovery. When, however, metabolism is allowed to proceed normally, the damage manifests itself; so long as metabolism is checked there is no manifestation of damage or other changes. The work of WOLFF and LUIPPOLD on rejoining of chromosome breaks, it seems to me, can be reduced essentially to this type of phenomenon; under anaerobiosis or KCN treatment, rejoining does not take place; the chromosomes simply sit there since oxidative metabolism is necessary for rejoining. The recovery with *Ascaris* eggs, however, is quite different, in that the eggs do not merely sit there under anaerobiosis or KCN treatment; they actually recover during the anaerobiosis or KCN treatment. This recovery can be detected only by subsequent incubation aerobically, since this is required to manifest the damage. The damage is greater in cells not subjected to a period of post-irradiation anaerobiosis or KCN treatment; if they are incubated in oxygen immediately after irradiation they show a greater deterioration; there is, therefore, a genuine recovery involved during anaerobiosis. I do not know of any case parallel to this. At least here is definite recovery, perhaps of a different type.

DUCOFF: First of all, I think this emphasizes one thing which I said, in that when you talk about recovery of damage, you have to specify the type of the manifestation. In cases of cold you simply block recovery of the division process. Both you and COOK get this.

BACHOFER: If you block this process with cold treatment, you do not know what the effect of the cold treatment will be until you subsequently incubate the eggs, since low temperatures hold all visible activity in abeyance.

DUCOFF: Yes, and then you find that it makes no difference if you don't store them or you store them for six months. The lag between the irradiated and the controls as far as cleavage is concerned turns out to be the same.

BACHOFER: That is correct for cell cleavage but not for completion of embryogenesis.

DUCOFF: In WOLFF'S work, I believe you have the sign wrong in that, in their work, cyanide treatment between the two fractions reduced the amount of recovery; this is part of their evidence for the metabolic role or the role of energy in their restitution.

BACHOFER: As I recall, nothing happened either anaerobically or under cyanide, whereas with the *Ascaris* eggs something did happen.

DUCOFF: They figure that the oxidative metabolism is necessary.

BACHOFER: Yes, it is necessary for rejoining of chromosomes.

DUCOFF: The third point is, that there's certainly an analogous type of experiment to yours, which is probably more creditable than COOK'S results, and that is the 1953 experiments of STAPLETON, BILLEN, and HOLLAENDER. Here, by stopping some systems and permitting others to go, you can favor recovery processes. Whereas, if you let everything go or stop everything simultaneously, you don't get any improvement. In their case they have to go down to a rather critical optimal temperature. It is intermediate between the optimal growth temperature and the low temperature which stops processes. And they have to supply nutrient to get a marked recovery. In your case, you're stopping something, but probably not others by the treatment.

BACHOFER: But I should like to say, further, that we are definitely not just stopping

something, because under anaerobiosis we varied the temperature and found that at the optimal incubational temperature for *Ascaris* eggs we obtained the greatest recovery; at lower temperatures there was less recovery during the period of anaerobiosis. This indicates that there is a positive anaerobic recovery going on and not a mere holding something back. Something is being positively built up or harmful products are being destroyed.

O'BRIEN: About an hour ago I wrote a personal letter to Dr. Myers here. It asked: Do you allow any distinction between recovery and protection? He hasn't yet answered the letter! I think it important that there be some agreement between people who are working in areas like this, as to whether this is just hair-splitting to speak of recovery and protection.

BACHOFER: By no means.

O'BRIEN: The word protection hasn't been used here before today.

DUCOFF: I'm sorry but I did use it, and indicated that in some cases there is certainly a difficulty in deciding what you have. For this reason, I did not consider any of the pre-treatments, some of which give far more spectacular results.

O'BRIEN: I wasn't thinking of chemical protection in that sense. I was thinking particularly of temperature at the time of exposure.

DUCOFF: I would think that, where it's a post-irradiation temperature change, you have a recovery-type mechanism. When it's a pre-irradiation temperature which gives the result, then you just aren't sure which is concerned, and since we are supposed to be considering primarily recovery mechanisms here, I thought we should focus our attention on post-irradiation treatments only.

SPARROW: We have observed one mechanism which I think would fall in this category of recovery. I should say one phenomenon; we don't know what the mechanism is. I would like to describe it briefly and ask for comments or suggestions. This observation was made in our gamma radiation field and it occurred at a number of different times but most frequently in the irradiated snapdragon. It occurs when you put small snapdragons, maybe three or four inches high, in the gamma radiation field at a variety of dose rates starting at dose rates of a few r a day and going up to a top dose rate of, say, 500 r/day. The highest dose rates are completely growth inhibiting. That is, after a few days, there is no appreciable growth noticeable in these plants, as far as height is concerned, although, after a period of weeks, you can notice that the leaves have increased significantly in thickness. These plants just sit there, essentially as we put them in the field for two or three months and then suddenly, while they're still being irradiated, at the same dose rate, one or more buds will start to grow and at the same rate or faster than normal plants. This would seem to me to be a recovery mechanism or an adaptive enzyme mechanism which allows the plant to recover suddenly and begin to grow as if there were no radiation present. We haven't tried to investigate this ourselves. I've tried repeatedly to interest some plant physiologist in studying this phenomenon but so far without success. Is there anything comparable to this known in animal literature?

BACHOFER: Is radiation going on all the time?

SPARROW: Not all the time. Twenty hours out of every twenty-four.

BACHOFER: I mean when this growth starts up they're still being irradiated?

SPARROW: Yes. In other words, they're growing now and at a dose rate of 400 to 500 r a day.

MCKEE: I should think certainly adaptive enzymes would be considered a recovery phenomenon here.

DOWDY: I pointed out twenty to twenty-five years ago, that if you give a fractional dose to the skin of a human, and you keep right on irradiating with that same dose,

the tissue will heal while you are irradiating. I've only had the courage to try that once. One grain of sand doesn't make a beach, but as far as I could tell, this is exactly what happened. While irradiating at the same daily dose, the skin goes right through all these changes and then heals over.

CASARETT: I would like to ask Dr. Dowdy if this is the same as the work of BLOOM on the gastro-intestinal mucosa in which he gave about 80 r per day? At first the intestinal mucosa showed damage but then, subsequently, 80 r per day could be given many, many days without damage. Reconstitution went on as normal, apparently; whether it was by selection of sensitive cells, with essential net accumulation of more resistant cells, or by what mechanism was not clear.

DOWDY: Rarely does one, during clinical therapy, completely denude the entire area that's irradiated. What happens with regeneration is that it normally comes from small islands of residual tissue that survive in and around the periphery. Regardless of which it is, it certainly must be, an adaptive process because even if there are normal cells growing in the area they are being irradiated. I presume if one carried this far enough he could get a second denuding, but, as I say, I have had the courage to try this only once.

SPARROW: As far as I know, in our case there is no necrosis of tissues. We were not killing the cells. It's just a growth inhibition. They sit there presumably without cell division until suddenly they overcome the physiological disturbance that's been holding them up.

DOWDY: If you go down to a small enough dose, it produces no active necrosis. I think HARVEY did that with the finger ridges in the monkey. He gave graded dosages down to something like 25 r per day and what he got then was atrophy, but without subsequent recovery, something analogous to the sperm in dogs which disappeared when irradiated with 1.0 r per day.

SCHJEIDE: I was going to ask Dr. Dowdy or Dr. Kelly, either one, if they would care to comment on the apparent adaptation of the lymphocytes following prolonged treatment with irradiation. Of course, I realize that this may not be single cells which are adapting; the entire tissue may be adapting. I wonder if you know of any major differences between these adapted lymphocytes or lymphocytes in adapted tissues and the more sensitive kinds of lymphocytes.

DOWDY: You were probably acting on the experimental basis, I think, instead of the clinical, and the two are entirely different; so do you want to go first?

KELLY: I don't know what he's talking about.

DOWDY: Somebody asked me this question the other day, as it applied to a sarcoma or to Hodgkin's disease as a generalized disease. We'll assume each instance to be a generalized disease. You treat the patients over the peripheral node-bearing area with a tumor dose, 300–400 r and, as you know, these nodes will regress and after a period of time will return three months, six months, or a year later. You treat them again and they will regress. Finally, there comes a period of time when, regardless of how much radiation you give, there is no significant regression or change. Perhaps this is adaptation, or perhaps sensitivity has decreased by virtue of an inadequate circulation, but I'm sure that one thing happens; with each regression there is an increase in fibrosis, and finally you end up with a node, which is largely a fibrotic mass with a few dispersed cells in it. At this stage the tumor bed is not so vascular. But, on the other hand, if you have an isolated node (localized disease), I believe that one can completely irradiate cell lymphoid cells by giving 5000 r and there will be no recurrence.

BACHOFER: I wanted to ask Dr. Casarett what animal he was using in what he mentioned a minute ago.

CASARETT: I referred to the rat.

BACHOFER: And you were dealing with humans?
DOWDY: Yes.
BOND: In reference to Dr. Schjeide's question, I would like to cite the work of KAPLAN'S group on the thymocytes, shall we say, where they noted that the regenerating population histologically was different from the original population. The cell type was larger. Furthermore, they noted that if you shield a portion of the animal, a portion of the bone marrow presumably, then the regenerating population was the same morphologically as the original cell type. This is adaptation of some kind; apparently a new population takes over.
MEAD: I'd like to ask Dr. Sparrow how long he has followed these snapdragons?
SPARROW: Do you mean after they start to grow again?
MEAD: Yes.
SPARROW: Up to about six months.
MEAD: Are they normal for all intents and purposes?
SPARROW: Well, no, they grow taller than normal. The height to which they grow is quite abnormal, perhaps twice as tall as normal.
BOND: What is the total dose that you're dealing with, again?
SPARROW: This factor begins to show after eight to ten weeks' exposure to 350 to 500 r per day.
PERSON: What do the nuclei look like and what do these doses mean? Are the nuclei pretty well smashed up before the growth starts?
SPARROW: I don't know if I can answer that question. We have made a large number of sections, but I don't think that we have ever been able to say definitely that any particular bud, while it was still inhibited, would later continue to grow. Many cell abnormalities do occur of course at the high dose rates.
PERSON: Do you only see this at 500 r per day and not at lower dose rates?
SPARROW: No, I think that they taper off. There is a gradual transition from fairly normal buds up to extremely abnormal.
POWERS: In this connection quite a pertinent observation was made by PODOLSKY and HUTCHENS (1954) growing *Chilomonas* in the presence of HN_2. But since this concerns *Chilomonas* I refer the matter to Ducoff.
DUCOFF: If you treat *Chilomonas* with nitrogen mustard, and make frequent population counts, plotting log of N/N_2 against time in hours, you get curves of population growth similar to those following X-irradiation except for two features:

1. Following addition of the mustard, there is considerable population growth —perhaps 30 to 50 per cent of the cells divide—before the block becomes manifest.

2. After a period of block whose duration is a function of dose—just as with X-ray—division resumes. But at the time of resumption of division after mustard block, there is, as previously mentioned, a sudden spurt during which division is faster than the control rate for a brief period of time.

If at any time during the period of block, or during the exponential growth period following recovery, an equal dose of mustard is added, there is no further effect. For example, we might treat three groups with 0, 2×10^{-5} M; and 4×10^{-5} M nitrogen mustard respectively; and plot the three population growth curves obtained. If, following recovery, an additional 4×10^{-5} M mustard is added to each group, the original 4×10^{-5} group shows no effect; the original 2×10^{-5} group responds with a short additional period of division block so that the total block for the group is the same as if it had been treated just once with 4×10^{-5} M mustard; and the original control group gives the response expected following 4×10^{-5} M. In other words, the final portions of the three-population

growth curves are perfectly superimposable upon each other. This may represent an adaptation type of effect. But the original mustard has to be present in the external medium to get these results. If you wash the cells free of the mustard, and then treat them again, they no longer show any resistance. It disappears within a couple of hours. Now, Dr. Sparrow asked whether there were comparable radiation studies among the animal work. (When you raised your hand, Dr. Powers, I really thought you wanted to demonstrate that we microbiologists are at least aware of some of the mammalian work.) For we might cite Mrs. STROUD'S chronic tritium exposure of mouse tissue cultures. And, also to the point, BRUES' work with radioactive glass beads embedded in rat liver; eventually mitoses reappeared despite the continued irradiation. But I'm afraid I'm not as familiar with the details of their work as I am with the HUTCHENS experiments on mustard in *Chilomonas*.

POWERS: I would just like to emphasize that the cells are growing in the presence of the mustard. They are inhibited by the mustard, and then they start growing in the presence of the mustard, and it can be demonstrated that the mustard is still active. This same solution can be shown to be active against another set of cells which have never been treated.

DUCOFF: You might also say that these cells are larger and have a higher protein content during this recovery in the presence of the mustard than the normal cells do.

BERNHEIM: I'd always understood that the life of the mustard molecule is very short.

POWERS: The life of the parent compound is short.

BERNHEIM: And not the active? Or will the second compound be the active?

POWERS: All of these products of hydrolysis are active to some extent (POWERS, RAPER, and POMEROY, 1954). The parent compound is very active. The first cyclic derivative is not so active, but it is the one that most people test in solution, for it has a long life.

BERNHEIM: Long enough to count them?

DUCOFF: For this particular effect at any rate, it has a half life of at least twenty hours. We have done assays, removing the cells and then putting fresh cells into this solution and we can actually see the gradual decline.

RUSTAD: I would like to ask Dr. Sparrow about the very gross histology that you find within these repeated irradiations. Are the cells still proliferating at a certain rate throughout, and maintaining a small size, or is the mitosis essentially suppressed. What I'm driving at is, when the plant grows, is this a burst of mitosis, or is it off-seed kind of perception that would lead to the elongation of the cell?

SPARROW: As far as I can remember, the buds that have started to grow again look more or less normal. However, up until the time it starts to grow the section is fairly inhibited and very abnormal and there is a mixture of hyperplasia and cell enlargement. Sometimes you get cell enlargement without hyperplasia and sometimes you get, especially in the leaves, hyperplasia when the leaves thicken. Does that answer your question?

RUSTAD: Yes.

POWERS: There are two other investigations that should be cited in this connection. Number one, RUKIN (1954) exposed bacteria to P^{32} solutions. The phosphorus content was high enough to produce appreciable radiation effect in the growing culture. He found that the radiation effect was production of a lag in the growth culture, followed by resumption of growth in the continued presence of P^{32}, an effect quite similar to the HUTCHENS experiment just described by Dr. Ducoff. On the other hand, I learned last week that, at the Donnor

Laboratory, there is an experiment being carried on by GRAEME WECH. He is irradiating continuously a culture of yeast cells; apparently in this particular culture there is no adaptation phenomenon observed. But there is a constant depression of the growth rate beneath that of the unirradiated material. It never approaches the control growth.

MCKEE: Dr. Dowdy, do you want to expand on that? Any other comments on this particular subject right now?

LESSLER: I was just wondering if anybody had any ideas on what adapts?

MCKEE: Any comments on what adapts?

HOWTON: In this connection, I would like to ask Dr. Sparrow if, when these snapdragon seedlings resume growth under these fantastic irradiation conditions, the resumed growth ever occurs along the main stem? I believe that normally the snapdragon seedling grows straight up without branching unless the tip is pinched off. I get the impression from what you've said that resumed growth always occurs at a side bud.

SPARROW: It usually occurs from a side bud, but I don't think I can say that it never occurs from the stem apex. I think it does sometimes occur at terminal branches.

HOWTON: If not, however, then all the resumed growth occurs under these conditions, in a sense?

SPARROW: There was probably a very small bud there when the plant was put out in the gamma field. Some of the growth would have been new growth under the conditions of radiation exposure.

HOWTON: It might be interesting, once growth starts from one of these side buds, to discontinue the radiation and see if the same thing happens.

SPARROW: To see if it is dependent on radiation for its continued growth?

HOWTON: It may conceivably be the dramatic change in the environment which causes the cessation of growth.

KELLY: Are these buds highly polypoid or are they apparently the same as the original plant?

SPARROW: I should be able to answer this question, but I can't. I don't think they are from indirect evidence.

KELLY: Is this in the dose range where highly polypoid cells might be able to get along, whereas others with fewer chromosomes wouldn't? Or is this completely out of the picture?

SPARROW: The reason that I don't think they are, is that there is a characteristic morphology of flowers on the polypoid branches, and these branches don't always flower, but when they do flower they don't have the characteristics of polypoid flowers. I can't say that they're never polypoid, but I'm certain great numbers of them are not.

MCKEE: We have lots of good circumstantial evidence here for protoplasmic synthesis. Do we have any specific chemical analyses for components in recovery phases and synthesis? We haven't explored that as much as we might.

JAMES: Dr. Ducoff last night postulated something with respect to specific inhibitors, resulting from a fortuitous analog as it were, and I was wondering whether something like that may not be cited in this type of effect. Suppose you did have the fortuitous analog occur, that could block the given pathway. This would require an alternate pathway while growth goes ahead.

MCKEE: Anybody have any suggestions on analogs and competitive inhibitors?

DUCOFF: I think a word might be said about this, off the cuff. In our conversation, Dr. James was intrigued by the concepts of feedback and we came up with this sort of idea: perhaps, in order to maintain itself, or some particular function,

a cell has to have a certain minimal amount of a material X. Let's just say that it needs N plus or minus A molecules of the compound in question, and this gets used in the performance of its function. Perhaps it simply gets used up at the time of division, and in the normal course of things the cell has some feedback mechanism such that, as long as the number of molecules is less than N minus A, the cell keeps making more of the compound. N is a relatively small number. Now, suppose the effect of radiation on your chemical system is not destruction of X, but the conversion to X′. This would represent the inactivation of a prosthetic group necessary for the vital function, leaving a molecule sufficiently intact to fool the feedback receptor in the same way that a metabolic analog fools an enzyme. Under these circumstances the cell would fail to respond because, as far as the cell knew, it had its N content of X. Nevertheless, it couldn't perform its function because some of these X's, while present according to chemical test, and present according to the cell's feedback receptor system, are nevertheless non-functional molecules. This, of course, could apply to all sorts of things. I think what Dr. James is suggesting now is that under these circumstances perhaps the cell finally goes through some other pathway.

HALEY: That brings to mind all of BEADLE'S work on the *Neurospora*. He gets actual chemical lesions, on radiation of the *Neurospora*, so that in certain instances, both in the vitamin series and the amino acids, out of the same seed pod he can get one seed that will carry the process all the way to completion, but the next one, B, will take it up only through the first step. This builds up the biochemical and he has a regular factory for building this particular biochemical. The next one will take it up two steps and he builds up the intermediate at that point and has a factory for producing an additional compound. In the third case, he has a third intermediate. Now, from what I know of his work, it would appear that this is an actual block, because if he adds the vital precursor at each one of these steps, he can force the *Neurospora* to go through this complete cycle, and come out with the original end product that you would expect to get from this particular *Neurospora*.

SCHJEIDE: I would just like to say that some work is now going on in embryology which shows very clearly that the cell can adapt to certain substrates. The substrates which were used in this particular study were tryptophane and some others. The embryonic cells were placed into solutions with these substrates present, and then, upon subsequent analysis for enzymes which were responsible for acting on them, they found increases in the enzymes. I would just like to suggest that, perhaps in a more indirect way, the introduction of small amounts of irradiation might result in such adaptation of cells. Perhaps there would be fewer oxidative type enzymes present, or perhaps the cells would react by taking up or producing more antioxidants.

GLASSER: I'm a little disturbed with the pacific nature of the conversation here, which would seem to imply that all recovery is complete and all adaptations were successful in the adequate stage. I have a very distinct impression that all recovery is not necessarily complete, and that some of these adaptations, although functional, may be less than adequate, in terms of the recovery phenomena or rephased in its reciprocal of the residual injury. I think we may have to specify that these adaptations, although they present one major criterion, namely the mortality of a given cell or cell populations, may in other physiological environments be incapable of answering the requirements of response.

MCKEE: I think that's a very good point. It seems to me that we haven't exhausted the possibilities or anywhere near. Our time is growing near, and Dr. Cronkite has indicated he would get some kind of summary for us.

CRONKITE: I think I would find it rather difficult if not impossible to give any adequate summary of the session that has gone on this evening. I think that one might say that Dr. Ducoff gave a very scholarly discussion. He described some micro-organisms, the three types of cellular changes that occur after irradiation, (1) the blocking of cell divisions, (2) some aspects of cell death and mutation, and (3) some of the factors that are involved that will alter these things. Particularly in respect to the blocking of cell division he brought up some nutritional aspects that are very pertinent and interesting. From then on, I think, everyone here is really up against the stumbling block of the fact that, in part, recovery does take place for rather obscure reasons; that it is probably incomplete, and it is a subject that needs more study. Other than that I have nothing to summarize.
MCKEE: I think that's an excellent summary. Does anybody want to add to it? Dr. Schjeide, what about this movie?
SCHJEIDE: We have a motion picture sent to us from Dr. POMERAT, University of Texas. It has to do with the effect of radiation on cells and tissue culture. It would be perhaps possible to show the picture this evening. Are you tired? Should we show it sometime tomorrow? Tomorrow it will be then.

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END OF SESSION IV

SESSION V

INTERACTIONS BETWEEN CELLS AND TISSUES FOLLOWING RADIATION

Introductory Speaker: GEORGE W. CASARETT

I. RADIATION EFFECTS IN GENERAL

THE problem of interactions between cells and tissues following irradiation is essentially the problem of indirect effects of irradiation at the biological level.

Latent periods for various biological effects or endpoint criteria, varying from minutes to years or generations, are due in large part to the time taken for complex series of indirect biological effects which must be completed before the endpoints are detectable.

Indirect biological effects of irradiation constitute, at least in great part, the mechanisms of amplification of the early physicochemical events of absorption of small amounts of energy to the production of the relatively large degrees of tissue damage and clinical effect associated with morbidity and mortality.

The variety of mechanisms contributing to radiation sickness and death are related not only to damage of cells and tissues, but also to the many and complex homeostatic and repair mechanisms of the organism.

The best morphologic evidences for indirect effects are in general: (1) that radiation can cause a greater effect in some organs with total-body exposure than with local exposure, and (2) that local irradiation can result in degenerative changes in non-irradiated tissues.

For purposes of discussion the ways in which radiation has been thought to affect a cellular component of a complex organism, such as the mammal, are divided into five general categories:

(1) Relatively direct effects on the cell or its immediate fluid environment.
(2) Indirect effects on the cell due to relatively direct effects on neighbor cells.
(3) Indirect effects on the cell due to circulation of 'toxins' which are produced by direct or indirect effects on distant tissues or organs and which act in deleterious fashion on the body in general.

(4) Indirect effects on the cell resulting from functional responses of homeostatic or defense mechanisms resulting from direct or indirect effects of radiation on distant tissues and organs. Such effects may be termed 'constitutional' effects.

(5) Indirect effects on the cell caused by changes in the environment due to reduction of blood supply resulting from direct or indirect effects on blood vessels.

1. *Relatively Direct Effects of Radiation on Cells*

Some radiation cell death is linked with cell division in that cells made unfit to endure the stress of division die in the attempt. Mitotic-linked cell death is limited in amount in proportion to the total number of cells in mitosis. Cell death not so linked can be caused in any kind of cell and is unlimited, but in most cases requires much higher doses.

Death of dividing cells reduces the reproductive capacity of the cell population. However, doses less than those resulting in considerable mitotic-linked death may reduce temporarily the number of dividing cells or cell divisions in tissues.

If the dividing cells of a tissue are stem cells in an orderly series of cells, some of whose daughter cells remain as dividing stem cells while the others differentiate, function, age and die or are excreted from the body, then as long as there is failure of the stem cells to provide new cells for differentiation, there is the secondary effect of reduction in number of the differentiated forms.

In such tissues the stem cells presumably have the alternative potentials for division or differentiation. There is some evidence from studies of irradiated seminiferous epithelium and epidermis, that precocious differentiation may occur when division is inhibited. Precocious or increased rate of differentiation might then be considered as an indirect effect of irradiation.

Hypoplasia and atrophy of self-populating tissues may then be brought about by irradiation through inhibition of mitosis, with loss of existing cells through differentiation and delivery to other tissues, aging and death, or excretion. The atrophic process may be enhanced by doses sufficient to cause death of cells and more direct loss. These mechanisms of radiation effect are prominent in the proliferative radiosensitive tissues, such as hemopoietic tissues, seminiferous epithelium, epidermis, and gastrointestinal mucosae.

The persistence of abnormal cells in tissues after irradiation is undoubted. However, their influence on the functional capacities of the tissue are not adequately understood.

Some of the visible abnormalities of irradiated cells are sometimes reversible, e.g. clumping or condensation of chromatin. Some abnormalities are not reversible and may lead to cell death eventually. The process of cell division itself is one important mechanism of elimination of some of the abnormal cells. The extent to which non-dividing cells with similar abnormalities are eliminated is unknown.

The abortiveness of initial attempts at regeneration of radiation depopulated tissues, e.g. bone marrow and seminiferous epithelium, may be due to lethal mutations or other defects which cause cell death in the first flurry of mitotic activity and are, therefore, greatly reduced in number during subsequent, more successful, attempts at regeneration.

It is reasonable to presume that gene mutations may be produced by radiation in somatic cells and that there may be little elimination of such cells or their progeny from the cell populations as a result of their defects. It is possible that some of these cells are biochemical mutants and functionally inferior to normal cells or could interfere to some extent with normal metabolic processes.

The extent to which the metabolic activities of specialized somatic cells are governed by all of the genes of a cell is unknown.

2. *Indirect Effects of Radiation on Cells Due to Effects on Neighbor Cells*

Conceivably, abnormal cells may have some influence, however small, on normal cells in contact with them or close by, and vice-versa. The extent and nature of these influences are not clear. However, there is some evidence to suggest that damage and recovery may be influenced by chemical interactions between irradiated and neighboring non-irradiated regions.

3. *Indirect Effects of Radiation on Cells Due to Circulating Toxins*

There is some evidence to suggest that injury to some cells and tissues following irradiation may be brought about in part by circulating 'toxins', although no specific toxin has been demonstrated. It seems that radiation can cause a greater morphologic effect in some organs or tissues with total-body exposure than with local exposure, and local irradiation sometimes causes degenerative changes in non-irradiated tissues. However, the causes of such circumstances are often not clear, and the relation of these circumstances to circulating 'toxins' is certainly vague.

'Toxins' may be expected from tissue or cell breakdown products. Rapid destruction of a tumor by irradiation produces an 'intoxication' which has been presumed by some to be the result of cell decomposition

products and the inability of the body to metabolize them, or perhaps due to consequent metabolic changes produced by them. Toxins may also be produced by infections and bacteremia which occur in irradiated animals. The shock-like syndrome which results from high radiation dosage in a short time is thought by some to be due probably to the sudden release of decomposition products of cells into the circulating blood. The decomposition products of the cell nucleus are considered to be especially noxious.

The problem of circulating 'toxins' following irradiation has not yet been adequately investigated and is highly controversial.

4. *Constitutional Effects*

The adrenal glands are involved in some of the indirect effects of irradiation, apparently through the mechanisms of their reactions to stress. Irradiation induces changes that seem to reflect an increased demand for adrenal hormones rather than a direct effect of irradiation on the adrenal itself.

Intense localized irradiation causes generalized involution of non-irradiated lymphatic tissue in the adrenal-containing animal but not in the adrenalectomized animal. The adrenal-mediated effects are absent in irradiated hypophysectomized animals. It is not clear whether the pituitary action in this respect is a direct or indirect effect of irradiation, i.e. the mechanism by which irradiation elicits the pituitary-adrenal cortical response is not known.

Since it has been reported that some protection from lethality has been achieved by shielding of the adrenals during otherwise total-body irradiation, the possibility exists that direct effects of irradiation in the morphologically radioresistant adrenal glands decreases the capacity of the adrenals to react to the stress of irradiation.

Vascular Effects

Some of the components of the walls of small blood vessels are moderately sensitive to irradiation, especially endothelial cells. Irradiation can induce vascular changes, such as endothelial swelling, necrosis, or subsequent proliferation and also thrombosis and fissures of the wall as earlier changes, and varying degrees of fibrosis and occlusion as later changes.

Radiation affects the endothelial cells of capillaries, arterioles, and venules by inhibition of division and by more direct destructive effects. Replacement of lost cells may be inhibited for a time. Blood in contact

with damaged endothelium or with subendothelial tissue may clot and the small lumen may be rapidly narrowed or occluded thereby. Persistent swelling of damaged endothelial cells may also narrow the vessel lumen. Regeneration of endothelial cells from surviving cells may subsequently restore the endothelial lining or may overcompensate with the result that hyperplastic endothelium may obstruct blood flow.

If there is fissuring of blood vessels due to intimal and medial changes, the vessel wall may become permeable to the extent that there is leaking of blood and fluid into the extravascular tissues, with edema and inflammation. Under such circumstances repair processes in the vessel wall and surrounding regions involve the activity of fibroblasts in the connective tissue of the affected regions. This is also the case when the adventitia is damaged sufficiently to cause degenerative changes in collagenous tissue. Consequently, the more specialized cells of the vessel walls are gradually replaced by fibrous tissue, a less resilient and less resistant tissue in terms of stress or other damage. Proliferation of fibroblasts and deposition of connective tissue fibers may, however, continue for such a long time progressively after irradiation has ceased, that eventual thickening of the vessel wall at the expense of the caliber of the lumen and even obliteration of the lumen may result.

Marked changes in tissues dependent on affected vessels for their blood supply can be produced by these vascular alterations, despite the fact that the vascular changes themselves are often subtle and inconspicuous.

One reason for the inconspicuous nature of these vascular changes is that they can occur in spotty fashion and not continuously along the course of vessels, so that in any one section through a tissue, even thorough and competent examination of vessels may reveal relatively few sites of significant change. If vessels are caused to undergo progressive fibrosis however, more of the vascular course becomes involved gradually, so that at increasing periods of time after irradiation, increasing numbers of cross sections of small blood vessels per unit area of sectioned tissue reveal significant changes.

It will be immediately apparent that a focal narrowing or occlusion of a vessel supplying a capillary bed, on the arterial side distal to the last effective collateral channel of blood supply, is all that is necessary to disrupt blood supply to tissues dependent on the portion of the capillary bed in question and lead to the starvation of the tissue through ischemia.

Another reason for the inconspicuous nature of the vascular changes

is that the smallest blood vessels, capillaries, arterioles and venules are much more sensitive or affected than larger blood vessels. The endothelium of small blood vessels seems to be much more radiosensitive than that of larger vessels.

Another reason for the special importance of the small vessels is related to their small lumens, which are much easier to occlude seriously by all of the mechanisms of vascular occlusion, including fibrotic thickening of the wall at the expense of the caliber of the lumen.

The death of many radiation-treated tumor tissues has long been recognized as partly secondary to vascular lesions, among which intimal changes and thrombosis are prominent.

Vascular damage is of fundamental importance because of the consequent lowering of the resistance and reparative powers of all of the tissues of which the blood supply has been impaired.

In some regions of the body, the initial deleterious effects due to vascular injury may be followed by the establishment of collateral channels for blood supply, if the vascular damage has not been excessive and widespread. If adequate new circulatory channels are not established, recovery of the dependent tissue is impaired.

Complete or marked interference with blood supply by the more rapid mechanisms of occlusion, such as thrombosis or endothelial swelling and proliferation, tends to cause relatively rapid degeneration of dependent tissues. Less complete or slower interference with blood supply, as is the case with slow fibrotic thickening of vessel walls, produces a more gradual atrophic change in dependent tissues. The tissue changes which are secondary to vascular alterations are restricted largely to the volume of tissue supplied by affected vessels unless, or course, the vascular changes affect certain organs or homeostatic mechanisms to such an extent that indirect constitutional effects are induced.

Within the volume of tissue in which there is damage secondary to vascular change, the damage is relatively indiscriminate, i.e. it may affect all types of dependent cells regardless of their radiosensitivity or their physiologic activity.

However, tissue damage secondary to vascular changes accounts for only some of the effects of irradiation, since the doses required to affect mitosis and kill cells in radiosensitive tissues are lower than those affecting seriously the functional integrity of blood vessels during the time of greatest irradiation-induced changes in tissues.

As long as the local circulation remains intact, its action aids recovery from effects of radiation on the tissues it supplies with blood. Once the

blood circulation itself is damaged, recovery of tissues from radiation effects is impaired and the indiscriminate degenerative effects secondary to ischemia are added. The rate of recovery seems to be considerably dependent on the local blood circulation.

II. RADIATION EFFECTS IN VARIOUS TISSUES, ORGANS, AND SYSTEMS

The primary histopathologic effects of irradiation on the epidermis and gastrointestinal mucosae are probably relatively direct, i.e. the primary events are the destructive effects on the dividing epithelial cells in the gastrointestinal glands, and on the germinal cells of the epidermis. However, in the development of progressive ulceration, contributions seem to be made by secondary factors, such as mechanical trauma, infection and vascular change.

Although the role of vascular damage in the initial development of radiation ulcers may not be clear at present, it does seem probable that the damage of the vasculo-connective tissue exerts influence on the course of the lesions and accounts for their intractable nature and poor healing, on the basis that the blood supply and the damaged stroma may be inadequate to support epithelial repair.

Degenerative changes observed in the parenchyma in many of the moderately or highly radioresistant organs following substantial radiation doses seem to be due often to vascular damage. For example, disturbance of the vascular bed, rather than direct injury of the nervous elements, seems to be the cause of the damage or symptoms related to the brain or spinal cord following irradiation. Vascular effects are especially important in organs, such as the brain, where collateral blood channels or anastomoses are few.

Temporary interruption of longitudinal bone growth is due primarily to relatively direct effects of irradiation on the proliferating chondroblasts in the columns of cartilage cells in the zones of endochondral ossification, and also to direct effects on the invading bone marrow of the metaphysis. However, the more lasting effects on longitudinal bone growth are explainable, in part at least, on the basis of decrease in number and caliber of blood vessels in the metaphysis.

The primary histopathologic effects of irradiation causing sterility of the testes are relatively direct effects on the spermatogonia, consisting of prolonged inhibition of their mitotic activity and cell death, with maturation-depletion of other existing cells of the seminiferous epithelium.

In the case of the ovary, the primary histopathologic effect seems to be

relatively direct destruction of ovocytes and granulosa cells of the follicles. This effect is capable of causing indirect effects on secondary sex characteristics of the female, or artificial menopause. In contrast to the testis, the ovary reveals considerably greater damage of follicles when irradiation is total-body than when it is localized to the ovary.

The effects of low or moderate doses of radiation on lymphatic tissues are due chiefly to relatively direct effects on lymphocytes, with destruction of cells and inhibition of division. However, with higher doses the dissolution or loss of lymphocytes is to a considerable extent related to the hypersecretion of the adrenal cortex.

The effects of irradiation on the concentration of circulating peripheral blood cells are based considerably on the radiosensitivity of the precursor cells in the hemopoietic tissues, with respect to both inhibition of mitotic processes and cell death, with consequent hypoplasia or aplasia of the hemopoietic tissues. Other factors of importance in producing these effects in peripheral blood are the ability of the various precursor cells of the hemopoietic tissues to recover from damage and, in the matter of timing of effect, the survival time and rate of utilization of the mature blood cells in the circulating blood.

Furthermore, the utilization, destruction, and production of blood cells following total-body irradiation are affected indirectly by a number of general alterations in the body. For example, circulatory changes may result in a decrease of the effective blood cell mass, infection may result in an increased demand for blood cells, damaged epithelial surfaces may contribute to increased loss of blood cells, and nutritional or metabolic aberrations may lead to a decreased production of substances necessary for formation or differentiation of blood cells.

The destruction of hemopoietic tissue and the cessation of erythropoiesis may be sufficient to account for the fact that an anemia is produced by irradiation. However, in view of the long life of the radioresistant erythrocytes in the circulating blood, the rapidity and degree of the anemia produced by doses in the mid-lethal range or greater suggests that additional factors are involved.

Microscopic hemorrhages, which are common following irradiation, and gross hemorrhages when they occur, contribute to the loss of erythrocytes from the circulating blood.

Work with labeled red cells and plasma, together with hematologic and histopathologic methods, such as that done by JACOB FURTH and his associates, have elucidated many of the factors involved in radiation anemia following doses in the LD_{50} range.

During the period after irradiation in which erythropoiesis is suppressed, there is a reduction not only in red-cell mass but in plasma volume as well, which masks the degree of reduction in the red-cell mass, especially during the first week. This accounts for the fact that during this period the erythrocyte counts, hemoglobin concentrations, and hematocrit values are often within normal ranges.

The reduction in red-cell mass was found to be caused by normal aging and death of erythrocytes and by the loss of erythrocytes from the circulation resulting from increased capillary permeability and widespread minute extravasations of blood into tissues and the lymphatic system. Ultimately these red cells underwent phagocytosis and hemolysis.

The increased excretion of breakdown pigments of hemoglobin observed in the LD_{50} range is difficult to interpret in terms of a direct action on circulating erythrocytes or an increased hemolysis of circulating erythrocytes, since an unknown number of hemoglobin-containing erythrocyte precursors are destroyed in the hemopoietic tissues by irradiation.

It is still within the scope of possibility that some erythrocytes may be destroyed by toxic materials from damaged tissues or from infectious organs when they are present in the body.

The tendency to bleed after irradiation correlates strongly with decrease in numbers of circulating platelets, which in turn correlates well with the destruction and decrease in number of platelet precursors, the megakaryocytes, in the hemopoietic tissues. However, it is possible that additional factors may contribute to the bleeding tendency.

The bleeding tendency seems to be due largely to lack of platelets, perhaps not only to lack of their blood coagulation factors but also their function in maintaining continuity of the walls of blood vessels when these are damaged. Platelet transfusion has a beneficial effect on the bleeding tendency.

Bleeding cannot always be related to thrombocytopenia and the delayed clotting reaction. Damage of capillary walls by irradiation, with alteration in capillary fragility seems also to contribute to the bleeding tendency. Data on disappearance of labeled plasma, red cells, and colloidal gold from the circulation following irradiation suggest that endothelial damage is of great importance in the increased capillary permeability occurring after total-body irradiation. However, the mechanisms by which radiation alters vascular permeability or fragility are not yet clear.

J. G. ALLEN and his associates originally reported evidence for a heparin-like circulating anticoagulant in dogs after irradiation. Later,

however, he found that no such evidence could be shown unless transfusion reactions had occurred. Heparinemia could probably enhance the bleeding tendency, if and when it occurred after irradiation, but it does not seem to be the fundamental cause of it.

It is well known that total-body irradiation with substantial doses can decrease the resistance of the body to infection. Some of the possible contributing factors are depression in circulating granulocytes, inhibition of antibody formation, damage of epithelial barriers on the body surfaces, i.e. epidermis and alimentary tract, reduction in phagocytosis of bacteria, and impairment of blood supply. The precise causes and mechanisms for the bacteremia are not entirely clear however.

L. JACOBSON *et al.* have shown that the capacity of the irradiated rabbit to produce antibodies to injected antigen is largely retained if the spleen or appendix is shielded during exposure. It was earlier shown by CHROM that bacteremia could be minimized by shielding the liver and spleen during irradiation. It is not known whether the shielded lymphatic tissue initiates antibody formation or promotes the process elsewhere. This question is of considerable significance, however, in view of the beneficial effect of spleen shielding on hemopoietic recovery generally. The degree of inhibition of antibody formation following irradiation correlates well with the degree of damage of the blood-forming tissue.

Shielding of relatively small volumes of tissue seems to decrease the severity of an otherwise total-body exposure. Although shielding of the spleen is especially effective, shielding of the head, extremities, or other small regions are also effective to some extent, according to the work of L. JACOBSON and his associates.

Recovery of hemopoietic tissue is more rapid after such subtotal irradiation. Shielding of the spleen in mice and rats or the appendix in rabbits has been shown to lessen the severity of the blood changes and enhance recovery. Splenectomy prior to irradiation does not modify the hematologic response to radiation. Spleen transplants and the injection of bone marrow suspension or spleen homogenates into irradiated animals facilitates recovery. Injection of lymphocytes into irradiated rats does not seem to be beneficial in this respect.

The mechanism of the protective effects of shielding small volumes of tissue is poorly understood. JACOBSON *et al.* have suggested that the mesenchymal tissues in certain shielded areas may supply a factor which facilitates regeneration of blood-forming tissues. According to these workers, cell colonization by cells originating in the shielded tissue and repopulation of the hemopoietic tissues by proliferation of these cells, if

a factor at all, is only one aspect of the hemopoietic recovery process. The shielded tissue in some way restores the functional capacity of the fixed stem cells, the reticular cells, of the hemopoietic system to repopulate the hemopoietic tissues. The shielded tissue may likewise restore the functional capacity of the surviving free stem cells to proliferate and repopulate the hemopoietic tissues. If cells migrate from shielded tissues and lodge in hemopoietic tissue, then it is possible that they, too, contribute to recovery not only by proliferation but also by elaboration of hemopoietic recovery factor or factors.

The effectiveness of bone marrow injection in mice after irradiation seems to be due to repopulation of the hemopoietic tissues by the injected cells. To what extent the mechanisms of protection involved in tissue shielding and bone marrow injection are similar or different remains to be elucidated.

III. RECOVERY OF TISSUES FROM RADIATION EFFECTS IN GENERAL

Recovery of tissues from radiation effects in survivors is variable in type and in rate and degree of completion, depending upon dose and severity of initial damage.

Histologically, most tissues seem to recover fully and behave normally after small single doses. For example, cell division is re-established at normal or temporarily supranormal levels in radiosensitive, actively proliferating tissues, and the tissues are repopulated with the same kinds of cells that they lost. In such cases the tissue has regenerated typically.

After somewhat larger doses, regeneration of histologically typical cells and cellular arrangements may be practically complete, barring secondary complications, or incomplete to varying degrees, but the defects observed histologically, e.g. small degrees of hypoplasia or fibrosis or subtle changes in vessel walls, may not seriously affect the integrity of the tissues or constitute a serious hazard to the organism at this time. However, such tissues may be less resistant to further stresses or insults than they were previously, and may break down more easily.

Typical recovery of a tissue involves the restoration of damaged cells to their normal structure and function and the replacement of lost cells by cells of the same type through homeotypic or heterotypic production from other cells which have survived in the tissue or which may have migrated.

After still higher radiation doses, there is a tendency toward less complete and less typical recovery in many tissues. There may be increased

numbers of cells atypical with respect to size, shape and staining characteristics, and there is an increased tendency to replacement fibrosis, usually associated with relatively increased changes in the walls of small blood vessels. The replacement fibrosis is a kind of repair by 'secondary intent', as in the case of any scar tissue, and represents loss of original functional capacity and resiliency. Such tissues often may tend to degenerate progressively and break down easily under further insult such as trauma, infection and other stresses.

Throughout all of these considerations of recovery from effects of irradiation it is recognized that cytologically and probably biochemically there may be persistent radiation-induced defects in cells, as well as permanent histologic defects.

The condition of a tissue at any particular time after irradiation depends not only on the initial damage but also on the extent and type of recovery which has occurred, the extent and type of further insult or stress applied, and the nature and degree of the changes which have occurred in time as a result of the permanent, irreversible or unrepaired changes produced in the cells by radiation.

Histopathologically, and at relatively early times after irradiation, the irreversible component of radiation injury is relatively subtle in comparison with the reversible component. However, changes or injuries that are 'fixed' irreversibly in cells and tissues can be amplified by various biologic and pathologic processes and became more hazardous or damaging with time, even without further irradiation.

IV. IRREVERSIBLE RADIATION EFFECTS— GENERAL CONSIDERATIONS

There are several general categories of histopathologic or cytologic effects of radiation which can be considered, at present, parts of the irreversible component of radiation injury:

1. *Defective Cells.*

We do not know the ultimate fate of various kinds of possible mutants of somatic cells or their influence with respect to the various specialized functions of different cells and tissues.

Mutation of somatic cells have been postulated in various hypotheses to be of prime importance in the production of cancer and other diseases, and in aging and acceleration of aging by radiation. However, none of these postulations are as yet well founded on experimental data. There is a distinct possibility that mutations of somatic cells may have something

to do with such effects and should receive close attention. However, hypotheses which attribute these effects more or less simply to the accumulation of mutations of somatic cells are at odds with biologic and pathologic data which suggest that the mechanisms of these effects are probably much more complex and involve other important factors as well.

2. *Defective Cell Products or Defective Differentiated Cells*

Often, for lack of suitable functional tests or amplification systems to facilitate study, we can only speculate on the possibility that the products of a defective cell, or the fully differentiated cells or structures derived from defective precursors, might function atypically or in an inferior or deleterious fashion. In the case of at least some mutations in reproductive cells we are provided with a great system of amplification of small cell defects, namely, the development of an organism from a fertilized ovum, whereas in the case of mutations or other defects in somatic cells such a convenient system is not available or known as yet.

However, in the case of certain somatic cells the ultimate effect of some cell defects are readily seen in terms of defective cell products. For example, in the case of cataract of the optic lens, non-lethal radiation damage done to cells of the anterior lens epithelium can result in the production of defective lens fibres formed by differentiation of the damaged cells and possibly of their daughter cells. The defective fibres result in opacities of the lens.

3. *Reduction of Regenerative Capacity of Tissues*

The reduction of regenerative capacity of tissues, which has been observed after substantial radiation exposure, may be related to the reduction in the number of stem cells in some tissues, or perhaps a decrease in the reproductive capacity of stem cells in other tissues in which the numbers of such cells do not seem to be reduced. This general effect of reduction of regenerative capacity is most apparent in radiosensitive, proliferating tissues, such as hemopoietic and gametogenic tissues, epidermis and gastrointestinal mucosae. It is not clear to me to what extent the permanence of this effect is due to direct biologic effects of radiation on the stem cells themselves, and to what extent it may depend on changes in supporting tissues or in distance tissues having influence on the cells in question.

4. *Asynchronous or Atypical Repair of Tissue*

After irradiation an inferior tissue may result from a lack of the coordination and balance in recovery rates of different histologic components

of the tissue which are necessary for regeneration and recovery to the normal, typical state. This kind of change may be regarded as an irreversible defect in the tissues. For example, fibrosis of blood vessel walls and of other damaged tissues or tissue components, although in a sense a repair process of the body, is repair of secondary quality, as compared with typical regeneration. Such fibrotic changes may progress in degree and eventually become damaging or hazardous to the tissues and even the organism.

The irreversible changes which have been discussed generally are not necessarily lethal at the time of production. However, the permanent changes produced by irradiation in the earlier time periods post-exposure must be related to the delayed radiation effects and ultimately to the life-shortening effect of irradiation which occurs after an intermediate post-recovery period of relatively low mortality.

How does irreversible change ultimately express itself long after irradiation? This question brings us to consideration of radiation life-shortening and its causes, which seem to be concerned with questions and problems involving acceleration of aging and the induction or acceleration of disease, including malignant tumors.

V. RADIATION TUMORIGENESIS

A survey of the facts gathered suggests a multiplicity of mechanisms by which neoplasia might be produced by ionizing radiations. Implication of both direct and indirect effects of radiation are found, but these effects are not always precisely delineated. The immediate causes of the neoplastic changes are, as you well know, still hidden.

In the case of many of the tumors which have been caused or increased in incidence by radiation exposure, it seems that the tissues of origin have been tissues damaged by radiation. For example, osteogenic sarcomata caused by radium or strontium and skin carcinoma caused by irradiation from external sources seem to result from the actions of radiation exerted directly on the tissues in which the tumors originate.

However, some of the tumors of endocrine glands can be brought about by irradiation of other tissues. According to JACOB FURTH, interference with the pituitary-target organ relationship, with derangement of feedback mechanisms, seems to lead often to tumor development in different organs, e.g. mammary gland, thyroid, pituitary, and irradiated ovary. The development of leukemia in mice is strongly influenced by hormones, and so is the induction of leukemia in mice by radiation.

I^{131} can cause tumors of the thyroid, and also tumors of the pituitary. According to FURTH, it is possible that I^{131} thyroid tumors are caused by a combination of local effect of irradiation on thyroid and excessive secretion of thyroid-stimulating hormones by the pituitary. He indicates that tumors arising in the endocrine organs, such as pituitary tumors, represent a type in which little if any change in character of the cell has to be postulated, since multiplication of the tumor cells, like that of their normal homologues, is caused by a sustained endocrine stimulus. In metastasizing they behave as malignant growths, yet they may be checked by correction of the hormonal disturbance. Eventually the known dependent growths gradually or suddenly lose their dependency and sooner or later become autonomous.

Experimental evidence suggests strongly that cancer develops as a result of a series of events rather than from a single direct effect of the tumorigen on a single cell or cells. In radiation tumorigenesis there is a latent period between radiation exposure and the occurrence of tumors which can seldom be accounted for solely on the basis of growing time of a tumor. It seems necessary that there be a change not only in a cell or group of cells to make them potentially cancerous, but also some kind of change in surrounding cells or in the supporting vasculature or connective tissue bed, or in homeostatic mechanisms, or in the case of some cells, a change in a tissue to which the 'pre-cancerous' cells are related as a target, as in the case of endocrine relationships. Theoretically, the order in which the series of events occur to produce a tumor may be of little consequence.

If the fundamental pre-cancerous change in cells were a gene mutation, for example, mutation of a gene which might control factors limiting cell division, then conceivably a similar mutation of the other gene of the same kind in the cells might be required for development of a tumor. It is also conceivable that the pre-cancerous change in cells, or mutation in the broad sense, might be extragenic. However, mutation theories for carcinogenesis are not yet substantiated.

In view of the fact that the pituitary gland secretes so many hormones, influences directly the function of so many endocrine organs, and indirectly or directly several non-endocrine organs, and influences regeneration and growth of almost all cells in the body, it is tempting to think that subtle changes in pituitary cells may be responsible for the late degenerative and neoplastic changes and premature aging following radiation exposure. However, adequate evidence is lacking for this idea.

VI. ACCELERATION OF AGING BY IONIZING RADIATION

I'm sure that many of us have been long aware of the fact that animals whose lives are shortened by sublethal total-body doses of radiation seem to degenerate more rapidly than controls with increasing age, showing premature physiologic changes and signs superficially suggestive of senescence. They tend also to develop the diseases of their species earlier so that their clinical history seems to be compressed in time.

According to HENRY BLAIR, animals subjected to total-body chronic irradiation seem to conform to the same life-span-dose relationships, whether or not they exhibit a high incidence of specific or special diseases, such as a particular malignancy. Also, at their respective median death times, populations of animals suffering life shortening from single total-body radiation doses and populations of control animals seem to have about the same incidence of the same diseases. The data of SHIELDS WARREN on ages at death and causes of death of physicians, radiologists compared with non-radiologists, are compatible with these findings in that they suggest life shortening in radiologists related to earlier occurrence of terminal diseases generally.

Such findings suggest that life shortening is determined so largely by the more diffuse deterioration caused by irradiation that the various kinds of specific diseases which occur do not appreciably alter the outcome, i.e. they suggest that the diffuse deterioration is really the underlying cause of life shortening and promotes premature onset of diseases which were more or less likely to occur eventually.

I have been interested for many years in the degree to which, and the forms in which, residual or irreversible radiation changes might be observable in the body before they produced their ultimate effects on life span. When and how are these changes ultimately expressed in the form of actual damage or life-shortening pathology? Do these initial permanent changes remain static once laid down or do they become amplified or develop spontaneously in terms of increasing damage or physiologic hazard, without further radiation exposure?

Certainly these questions have not yet been completely or satisfactorily answered. However, some observations may be worth discussion.

The studies with which I have been concerned have been done on material from many and various kinds of radiation experiments. Much emphasis has been placed on detailed histopathologic comparisons between the basic or universal senescent changes in non-irradiated control animals and the universal degenerative changes occurring during the

lives of irradiated animals, in order to determine whether the deterioration observed superficially in irradiated animals is truly an acceleration of biological aging processes or whether it is generally secondary to disease processes.

Limitations of time permit only a general presentation of some of the findings of this work.

So far it seems that the progressive diffuse deteriorations produced by different kinds of radiations in different mammalian species are basically or qualitatively similar, and depend quantitatively on dose size and species sensitivity, although the diseases which occur eventually may vary to some extent with species.

All of the histopathologic senescent changes seen were basically identical qualitatively in both control and irradiated animals. However, in the irradiated animals they began or were initially detectable earlier, progressed at greater rate, and were therefore more pronounced at given ages.

The diffuse deterioration produced by irradiation can be identified largely and basically with acceleration of actual aging processes, as defined histopathologically, and with the subsequent effects of these processes.

I have been unable to find so far any special histopathologic mechanism or primary feature in radiation-accelerated senescence which would distinguish it fundamentally from physiologic senescence.

In general the phases of histopathologic effects of single radiation doses on the *parenchyma* of the more proliferative and more *radiosensitive tissues* are as follows:

Phase 1 is characterized generally by damage and destruction of radiosensitive cells and consequent hypoplasia and atrophy of tissues and organs. The degree of effect increases with dose.

Phase 2 is one of regeneration and replacement of lost parenchymal cells. The length of the interval between irradiation and the active beginning of this phase varies directly with dose size, and the rate and degree of completion of regeneration varies inversely with dose size.

Phase 3 is the intermediate phase of little or no change, during which there is either a normal degree of cellularity, or maximally regenerated levels which are subnormal to varying degrees depending on dose. The length of this phase varies inversely with dose.

Phase 4 is the phase of accelerated aging in the *parenchyma* of organs, and is somewhat variable with respect to time of initial detectability in different organs, individuals and species, In general, the onset of detectable increase in senescent histopathology is earlier the greater the dose, and the rate and degree of such change varies directly with dose size.

Although there is little or no detectable initial radiation damage of the parenchyma in the more radioresistant tissues or organs following doses in the mid-lethal and sub-lethal ranges, accelerated senescent changes in parenchyma occur in such tissues and organs in this last phase.

In general, the basic changes of senescent histopathology in the parenchyma of organs are progressive, and are atrophic or involutional in type, consisting of hypoplasia, atrophy, and as these conditions advance, fibrosis.

The only widespread histopathologic effect of a permanent and progressive type which could be traced continuously from the time of radiation exposure to the time of death was the effect on small blood vessels, especially arterioles and capillaries.

Subtle evidences of injury can be seen during the initial phase of radiation effect in various components of the walls of small blood vessels. They occur very irregularly and discontinuously along the length of vessels.

Doses within critical ranges may promote swelling of endothelial cells and even proliferation as a result of injury, and these reactions tend to narrow or occlude small vessels rather rapidly

Such reactions may be only transitory with single doses, depending on dose size, but may be increased or prolonged in a vascular bed when effective doses are administered repeatedly or continuously.

Whether or not occluding endothelial reactions are observed during the phase of regeneration of parenchyma or later depends on the dose size and schedule and, possibly, also on some of the later, more indirect, effects of irradiation.

When the dose is high enough to destroy endothelium in spots, some small occluding thrombi may be formed.

Injuries are seen in the initial phase in medial and adventitial elements of arterioles with sufficiently high doses also.

Subsequent to initial injuries in vessel walls there develops a subtle, slowly progressing, irregular thickening of vessel walls by deposition of connective tissue and by replacement fibrosis, ultimately at the expense of the caliber of the lumens.

This reaction to injury is heralded, according to the sites of initial

injury, by thickening of the intima with slowly increasing subendothelial connective tissue elements or by increasing fibroblastic activity in the adventitia and media of arterioles, and in relation to capillaries.

The beginning of this arteriolocapillary fibrosis can be detected with effort as early as the parenchymal regenerative phase if the dose is high, but it is more difficult to detect with lower doses until it has developed progressively during the intermediate phase. This vascular change is more easily seen, of course, in its later and more advanced development during the phase of accelerated aging of parenchyma.

All of these radiation effects in the small blood vessels are histopathologically nonspecific and are qualitatively similar to the changes involved in the generalized progressive arteriolocapillary fibrosis of aging. The difference seems to be only one of rate or degree.

Progressive arteriolocapillary fibrosis is an irreversible change which is accelerated by initial radiation injuries to blood vessel components and becomes gradually more hazardous or damaging as it increases progressively with time.

In both control and irradiated animals during the phase of manifest senescent change in parenchyma, there is a strong direct correlation between the degree and rate of arteriolocapillary fibrosis and the degree, onset and rate of development of parenchymal senescence, both generally in the body and also with respect to many individual organs.

It seems clear that the ultimate parenchymal senescence is the result at least in large part of reduced blood supply and presumably other hemodynamic effects attendant upon the vascular changes.

The parenchymal senescence in some organs may conceivably in turn cause further changes in dependent organs, and so on, until terminating disease is developed as a result of various combinations of internal and environmental factors.

For example, when these vascular changes in renal arterioles exceed a certain rate and degree as a result of irradiation, nephrosclerosis develops.

In physiologic senescence of the kidney and probably also in the accelerated senescence of kidneys receiving only relatively small doses of radiation, the tempo of the vascular changes in usually slow, so that it does not exceed the capabilities of adaptive changes, such as the growth of new vascular channels and the tubular hypertrophy of unaffected nephrons, which tend to mask the retrogressive changes and prevent or delay the development of the pathologic condition of nephrosclerosis.

In this sense the nephrosclerosis produced by irradiation may represent a very rapid acceleration of renal senescence.

Radiation nephrosclerosis may be relatively nonprogressive, after a point, or progressive to varying rates depending upon the rate, degree, and extent of renal vascular change. Under these circumstances the kidneys at various stages and rates of pathologic progression may resemble kidneys involved in a variety of clinical renal disease syndromes.

Progressive nephrosclerosis tends to promote premature death associated with evidences of hypertension, advanced generalized arteriosclerosis, and impairment of renal function.

If the hypertension is very 'benign' there may develop only mild circulatory disturbances, and life tends to end as a result of one of a large variety of common diseases, similar to the circumstances involved if there is no nephrosclerosis. If hypertension is more serious, then there is, among the common causes of death, increased incidence of deaths associated with evidence of renal failure, cardiac hypertrophy, cardiac failure, and hypertensive encephalopathy, including cerebral arteriosclerosis and cerebral hemorrhage.

The renal hypertension resulting from irradiation seems to contribute greatly to the subsequent development of generalized arteriosclerosis, and such effects in the kidneys themselves tend to advance the progression of nephrosclerosis, and so on in the manner of a vicious cycle. In a sense, nephrosclerosis is an acceleration of aging processes which accelerates aging changes in other organs.

These data support the idea that senescence of the kidney plays an unusual and important role among the aging processes in that its rate may have much influence in determining the nature of the senescence that an individual will experience and the manner of his death.

According to clinical descriptions, e.g. those by LUXTON in England, the radiation nephritis resulting from 'abdominal radiation baths' in radiotherapeutic practices, produces typical clinical syndromes very similar to acute or chronic glomerulonephritis, benign essential hypertension, or malignant hypertension. The symptoms seem to differ in degree and tempo but not in essential nature. Yet all of these clinical syndromes are apparently the result of one basic disease process, with a common etiology, produced by a single measurable agent.

These clinical observations and the present experimental observations taken together suggest that there may be a common basis for a variety of clinical types of renal disease, in the rate and extent of arteriolocapillary damage and fibrosis.

In summary, the available data indicate that sublethal irradiation of the whole-body or substantial volumes of vital tissue causes premature

death or shortening of life primarily by accelerating actual biological aging processes, as defined histopathologically, which in turn causes earlier onset of the diseases of the species associated with or promoted by aging processes.

Premature death may be due somewhat more often to certain specific diseases in strains of animals highly susceptible to special disease, or when irradiation is highly localized and intensive. However, even in the latter case, the basic degenerative changes in parenchyma and blood vessels which precede the onset of disease in the irradiated region are remarkably similar histopathologically to accelerated senescent changes. This suggests the possibility of accelerated local senescence.

Although the fundamental causes and mechanisms of arteriolocapillary fibrosis in physiologic or accelerated aging are not completely understood, the importance of this process as a mechanism of irreversible fixation of radiation injury in tissue and as a mechanism of amplification of this injury into irreparable damage and accelerated senescence of parenchymal tissues, indicates that it may also be an important underlying mechanism in the induction of many diseases, including malignancies.

DISCUSSION

GLASSER: The material offered this morning presented us, I think, with a rather broad spectrum of specific information as derived from the histopathological approach. Now, lest our colleagues of other disciplines be timid about this, I think it should be reiterated that the histological picture we see is the resultant of a galaxy of various physiological, physical, and chemical vectors which approach us from the cellular level. I think we should recall that everything Dr. Casarett reported has a basis in physiological and biochemical fact. Now, the problem we must come directly to terms with is the interaction between cells and tissues immediately following irradiation, and I would suggest to the group that perhaps the best starting point is a consideration of humoral factors and whatever thoughts we have on the humoral theory of irradiation. I invite comments from the floor.

CRONKITE: Since historical perspective is part of the intent of this conference, I'd like to commend, without deprecating any of the American endeavors in the field, that the shielding studies actually originated in 1912 by CHIARI and again by FABRICIUS NOLLER in Denmark in 1922 and, at a later date, by CHROM and then were rediscovered in the Manhatten district. I introduce this because it's a historical fact that people seem to forget. Next, humoral factors, I presume the toxins, would fall into this area. I would like to have someone clearly define first what is meant by 'irradiation toxin'. I think one can forget immediately the problems of bacterial toxins. Also, what, if any, confirmed evidence is there for the existence of a single radiation toxin that circulates?

BERNHEIM: I think that perhaps we should get an answer to this problem if one compared the more or less immediate toxic reactions that occur after a rather massive whole-body irradiation with the toxic reactions that occur when you mechanically damage cells. When you mechanically damage a cell you get the vasodilation of shock. This is presumably due to leakage of nucleotides from the

cells into the circulation. There are probably other toxic reactions, but how does the shock syndrome differ from the toxic reactions that are caused by massive irradiation? In other words, when you get a leakage from cells damaged by irradiation, you may leak the same thing from cells that are damaged by mechanical trauma. If not, and if the syndrome does vary, we must assume that there has been immediate change in some mechanical constituent in the cell that is irradiated which then leaks out and produces a different type of toxic reaction. This latter toxic reaction would then be different from that produced when cells leak due to mechanical disruption. I don't know if this is a logical way of approaching the toxin idea.

GLASSER: I think it behoves you, as Dr. Cronkite suggested, to define toxin.

BERNHEIM: I don't think you can define the toxin until you've isolated it. You can compare the toxic reactions after irradiation with the toxic reactions after the crush syndrome—are they exactly the same, or do they differ? Does anybody know?

BOND: We've run into difficulties on this for they have a very superficial resemblance, but I don't think in any sense that this means the mechanism of production is necessarily the same. They do the same thing, but this does not mean the same mechanism.

BERNHEIM: Does anybody know how they differ? I'd like to ask Dr. Bond: Are you admitting that there are such things as toxins appearing after irradiation?

BOND: No! No, most certainly I'm not. I only agree that the picture that you see following irradiation is what is referred to clinically as intoxication, a vague clinical term. This is a far cry from identifying a circulating toxin that produces the syndrome. In the same vein—I think this is pertinent—I'd like to ask the first speaker a question; I'd like you to confirm what you said or what I thought you said. Did you say that the sum of effects with total volume irradiation is greater than when the same dose is given to a localized area?

CASARETT: Yes, in some organs. However, we don't know why in some cases.

BOND: It depends on the criterion of effect. It is difficult to speak of indirect effects without bringing in shielding. Local effects in a shielded area are, of course, less than with total body radiation. If a local area is selectively irradiated, I am not sure that the extent of destruction is less than with whole-body irradiation at the same dose, although regeneration may be faster. An outstanding exception is the lymphocyte or lymphatic tissue with a well-known mechanism. I'd like to know exactly what you have in mind.

CASARETT: In giving other examples, I do not mean to imply that a specific toxin is involved.

BOND: I would like to know of some well-proved examples.

CASARETT: Irradiate the ovary of rats with 600 r and you get slight transient change; irradiate the whole body, and you destroy the ovary; if you irradiate the testes of a dog intensively this can produce an actual degeneration in the lung. I don't know what the mechanism of this is.

BOND: Can you tell us who did this, and when?

CASARETT: PAUL HENSHAW.

LEVEDAHL: I think the second one is reported in a book by SPEAR, where 24,000 r were administered to rabbit testes and lung degeneration resulted.

CASARETT: In an attempt to be provocative, I indicated, for purposes of discussion, that there is this possibility. Greater effects seem to be found in whole-body irradiation when compared with local irradiation in some organs. In the case of lymphocytes, we do know something about the mechanism of this kind of indirect effect. In the case of other organs, we don't know the precise mechanisms. And the mechanisms may have nothing to do with toxins. But these

are some of the examples which one could cite for purposes of argument or discussion on the possibility of toxins, specific or nonspecific.

BOND: I'm not convinced of any abscopal effect except that concerned with lymphatic tissue.

SCHJEIDE: I'd like to cite another example, this time from the discipline of embryology. This was reported by STRANGEWAY and FELL. If a chicken embryo older than 3–6 days of incubation is given whole-body irradiation, and if, 24 hours later, tissue from liver, muscle or lung is explanted, the cells are less able to divide, indicating damage. However, if the embryo is irradiated and the tissues are removed immediately, the cells all divide according to what would appear to be a normal pattern. If makes no difference whether the embryo is in the 2, 4, or 15 day stage. This implies that during 24 hours, *in situ*, some indirect mechanism is operating on the cells. Apparently the indirect mechanism operates only after 3–6 days of incubation since cells taken from 3-day-old embryos 24 hours following irradiation divide normally, whereas cells taken from older embryos do not.

CRONKITE: I don't want to be unpleasantly persistent about this matter of definitions, but, since no one wishes specifically to define something, I will try to state my particular position and possibly define it. All of us know that adrenaline, and many other physiological substances, can be lethal. They kill and, if you wish to, you may possibly call these toxins. Now it has been said off and on for many years, that there are substances uniquely produced by irradiation in the integrated organism, let's say mammals, which mimic the direct effects of radiation. When everyone talks about indirect effects, are they talking about the liberation of histamine? I'm sure histamine is liberated for a short period of time and can give toxic effects, but it is not a radiation toxin, *per se*. Are they talking about something that is mimicking the direct effects of radiation that are known, or are they talking about some physiological substance like corticosteroids or histamine that is being released? I think it is a tremendously important thing to bear in mind all the time. Now, for my own part I can not believe that there is anything like an irradiation toxin and I am certain that 90, plus, per cent of the effects in mammals can be explained by the direct effects of the irradiation on the cells, with a little bit of superimposition. I prefer to say abscopal effects, using a term that MOLE in England coined, to get away from the chemists' indirect effects to explain it. This is something that is superimposed upon the direct effects of irradiation on tissue. This is a positive statement of my own personal feelings, and I would welcome anybody who can really demonstrate confirmed evidence for the existence of a radiotoxin that mimics the effects of radiation.

SCHNEIDER: The work which we've done in the past using antibiotic vaccine to which we imbedded insulation tubes involved putting two rats, littermates, into union post irradiation by the technique of CRONKITE and BECKERT. Now, the question of an indirect effect is what marks my remarks because in the course of the operation, it is necessary to shave or clip the hair on the opposing surfaces of the two animals and—without explanation on our part, we found that the regrowth of hair in the unirradiation partner was slower to occur and scantier at its maximum than in the irradiation animal. Neither of the rats showed normal regrowth of hair in the shaved or clipped portions. The only reasonable explanation is that there is some indirect effect mediated eventually by the vascular osculation between the irradiated and the non-irradiated partners. I cite this as possible evidence of an indirect effect in a different animal.

BOND: I can't dispute that as evidence of an abscopal effect, nor can I explain

it, but I'd like to come back to my original point which fits in with what Dr. Cronkite is getting at, I think. The examples which you cited, Dr. Schjeide, have been noted also with tumors. It is well known that irradiation of the tumor bed affects the tumor, perhaps through damage to the vascular system. So perhaps we should distinguish between a 'local' abscopal effect and a 'distant' abscopal effect. I should still like to be sure of well-documented evidence, other than in lymphatic tissues, where the same dose of irradiation, total body irradiation, will produce a greater change in the actual area that has received localized irradiation. Under these circumstances, are there clear-cut examples where there are abscopal effects? Is there a distant effect where the whole body irradiation makes the area worse than localized irradiation?

CASARETT: Shielding lessens the effect with respect to lethality and with respect to hematopoietic recovery. Did you have any other criteria in mind, Dr. Bond?

BOND: All gross criteria . . .

CASARETT: If you say that shielding lessens the effect do you mean that it lessens the toxic effect? You see, I don't have sufficient evidence for proving or believing that there is a specific toxin, but I did cite evidence of indirect histopathologic effects.

BOND: I'm trying to get at specific examples of proved abscopal effects. Can you show a specific example where, for the same dose of irradiation in distant regions as in whole-body irradiation, there is an increased effect of a dose to a localized area of tissue as measured by a criterion in that tissue irradiated?

CASARETT: I gave that example of the ovary, among others.

BOND: I think that when this occurs it may be secondary to all sorts of things.

CASARETT: That's correct.

BOND: It is late in the game, and you'll probably find there is a nutritional basis or some such thing.

CASARETT: Perhaps you want a change that can't be explained presently on any basis. I'm not promoting the idea of toxins, because I don't know whether there are toxins produced by irradiation or not. I want to make it clear that the unsolved problem of toxins was brought up by the speaker as a matter for discussion. On the other hand, there are these local changes which are greater in individual organs when the total body is irradiated than when the organ itself is irradiated alone, and there are changes in shielded tissues of some kinds when the rest of the body is irradiated. I don't know but that this is the part that we perhaps ought to get at.

CRONKITE: May I ask for a definition of the word 'abscopal'?

BOND: It is an effect in the tissues distant from the area actually receiving irradiation.

GLASSER: I'll spell it: a-b-s-c-o-p-a-l.

TOTTER: I think that the point that Gene wants to make here is a very good one. We could perhaps lay this ghost and be sure we're talking about the same things. I am sure the early radiobiologists worried about toxins, but the more recent worry about it, if you go through recent deliberations from the Manhattan district people, I think could be summarized in this way. When chemists and biochemists were brought into the radiobiological work, the first question we were asked was, what is the stoichiometry of this stuff? When it was calculated out for them by the physicists, they naturally turned to see what this would be in chemical terms, that is the stoichiometric relationships of the chemical reactions. Then they say that this would be like a powerful toxin. We only know one or two. I think this came up purely for a comparison and not really with the idea that any such thing existed. But it gave you a notion of what you would have to look for.

GLASSER: I think that we ought to do some book-keeping right now. It is fairly apparent that there are people working in radiation biology who feel that immediately after irradiation there is liberated in the organism a substance or substances which are not common or usual but are rather singular or specific with regard to the normal biochemistry. These are perhaps *de novo* substances. Basically I feel that this is not the case, but I would like to ask specifically: Is there anyone in this room at the present time who feels that there is a specific toxin liberated after irradiation?

O'BRIEN: I believe that it would help our cause if Dr. DOWDY were here or one of his co-workers, Dr. LAWRENCE or Dr. VALENTINE, because, as many of you may know, they have written a short historical review of exactly this problem of the existence of an indirect effect.

CRONKITE: The absence of it?

O'BRIEN: They include that term.

CRONKITE: They did work on the absence of an indirect effect. That was the title.

O'BRIEN: No. The title was 'Is there an indirect effect?'

CRONKITE: That is correct.

O'BRIEN: Attached to the historical introduction there are also a few paragraphs on definitions. They want to make it clear that they're dealing with specific indirect effects and, as you say, not effects due to the disintegration of tissues and so forth. They review the cases on the plus side and the negative side and they come to the conclusion that there is as much to be said on one side as there is on the other and not very much to be said on either. Their own work, to which the review and the definitions are attached leads to the conclusion that their work does not support the idea of an indirect effect. Dr. DOWDY told me yesterday that he doesn't think there is any doubt about the existence of an indirect effect, a specific indirect effect. Now, this will be hard to detect. But all I want to contribute here is the fact that in our laboratory, when we became interested in this business, it came as something of a surprise that nobody had attempted to irradiate, solely, the circulating blood without radiation insult to any other tissue. We proceeded to rig ourselves up a little technique whereby this could be accomplished. Now, of course, red and white cells are being irradiated, but I don't consider this particularly important. I don't know how to talk much about dosimetry and so forth because we've been using extremely high doses. Some things preliminarily have happened, but we realize we could give such a high dosage to wreck completely the chemical system of the plasma and we hope to refine this business. However, ROBERTS RUGH from FAILLA's laboratory (I thought we were original) told me how he had done this ten years ago; he took the blood out of the body (dog) and then cross-fired it. RUGH figured out how many r each red blood cell had received. This last point didn't particularly interest us, but he said that nothing happened and so he abandoned the work. I wrote a little letter to Dr. LAWRENCE, and he wrote back that he thought that our approach was an important one and that he, too, had attempted to do this, but gave it up because of technical difficulties. I won't go into the preliminary results that we have, but we think the work has promise.

GLASSER: We have two things here. One is whether or not there is a humoral, and then, if you will forgive me, an indirect effect. But the thing I think is relatively important to the subject right now is: Is there a toxin? Or a toxic substance?

O'BRIEN: Anything outside of the normal constitution of the liquid portion of the blood, would you call that a toxin? If that's what you say, I say yes.

SCHJEIDE: I believe that Dr. Glasser could be the spokesman for a large group

at Rochester who are investigating this particular problem, and I wish he would make a few comments regarding their results.

GLASSER: I've been trying to avoid this by keeping the conversation out in front of the chairman's table. No, I don't feel there is a specific radiation toxin. Yes, I do believe that the effects produced in whole-body radiation may be the result of irradiation response in a specific tissue. Such tissues are capable of augmenting their usual biochemical product. This biochemical product is then carried into the circulation and its percentage or proportion in the circulation is altered. This in itself is an insult or a broad variation in the logical environment. In adjustment to this altered biological environment the organism *in toto* gives response by a variety of biochemical and physiochemical means. Some of these changes then can be elucidated under the general terminology of radiation syndrome.

SCHJEIDE: Would you be more specific in telling the exact details of this one experiment?

GLASSER: I think what Dr. Schjeide is trying to get at is the elucidation of the EDELMAN report, that immediately after irradiation there is liberated into the plasma of the irradiated animals a substance which, on cross-transfusion or on placement in recipient animals, will kill the recipient animal. Dr. EDELMAN reported this some time ago and, because of the peculiar and novel nature of this, it received immediate attention. I think it would be fair to say that at the outset that only Dr. EDELMAN at the present time, has been successful in repeating Dr. EDELMAN'S experiment. But I think that the effort put into data has been worthwhile. We got into it because of an unfortunate, successful first experiment. Would it be that the first experiment was not successful I wouldn't be discussing it today. What we did was this. We have some very exquisite survival curves on irradiated chick embryos and these curves are rather distinct; they have very short limits of variation and they've been done at various seasons so we know that they're fairly finite. In the chick embryo, the 12-day embryo dies in a rather distinct fashion measured by an acute vascular distress followed by a secondary slope descriptive of other types of death. This is very strongly characterized. What we did was to take a dog and expose it to 600 r X-rays; withdraw from that dog, within a few hours after irradiation, some of its blood, and prepare plasma. This was put into recipient 12-day chick embryos. Similarly, plasma from a non-irradiated (or I should say a sham-irradiated) dog was prepared and put into another group of embryos. It was rather spectacular that the survival curve of chick embryos which received the plasma from the dogs exposed to 600 r could be superimposed directly on the curve for chick embryos directly exposed to 600 r X-rays. The survival curve for the animals that received the control plasma was what we would expect for a control population. We have never been able to repeat these results again. And there we stand. However, in the face of the marked paucity of comprehensive data on humoral factors in post-irradiation physiology and the high level of competence in EDELMAN'S laboratory, I do not feel that we can dismiss this subject in a cavalier fashion. If only from an academic viewpoint the question of a discrete radiation toxin provides a type of introspection too often absent in this area of radiation biology.

CASARETT: How many times have you tried to repeat this?

GLASSER: Six. The total contribution by the hen population of Northern New York State came to something like 2400 embryos for these experiments. One more thing, we have since tried a rather homologous experiment in which a series of four dogs was cross-transfused to test for hematologic compatability, then one dog was irradiated with 1000 r and cross-transfusion continued. We haven't touched the dog at all.

BOND: We, too, are rather intrigued by Dr. EDELMAN'S results and we've spent about a year's time on this. We've irradiated normal and adrenalectomized rats —as near as we can tell in the precise manner that EDELMAN has done. We've taken the serum and plasma from these animals at various time intervals following irradiation, put it into recipient adrenalectomized rats and into mice that have received previous doses of radiation that are sub-lethal. We've done a number of these experiments, and in none of them have we been able to find any indication that the irradiated plasma increased the effects at all. In one other series of experiments we have used a rather sensitive indicator of radiation damage which is the decrease in lymphoid tissue weight. This effect occurs at very low dosage. We transfused the serum plasma from irradiated rats into normal mice and we've been unable to detect any decrease in the weight of the lymphoid tissue. Now, there is the possibility that there may be toxin and that we can not detect it in this fashion. A dilution factor occurs and I believe this was what Dr. Totter was probably getting at, that one perhaps wouldn't expect to find toxin by these approaches. Probably the serum is not the place to look for a toxin. But, nevertheless, none of these approaches that we resorted to yielded positive results.

HALEY: This is an old bromide and I agree with Gene Cronkite. One thing of which we can all be certain is that whole-body irradiation does release normal body constituents of the organism. We spent about a year on this histamine situation to prove the fact that it had nothing whatsoever to do with irradiation syndrome. If you'll look into the release of seratonin, you'll find that it's released. If you'll look into the adrenal, you'll find that you have a release of adrenal cortical substance and each place that you look you'll find that you have normal body constituents released. But, every time you take a real good look you can't find anything that could definitely be determined to be a toxin. Now, we have to take into consideration that maybe under stressing circumstances some of the materials that are normally present, that are normally secreted in the organism, can have deleterious effects on it. But they need not be classified as toxins, because other conditions can also produce an over production of some of these materials and in such cases they are not considered to be toxins. We just say that in such instances an animal is hyper-cortical as far as secretion from the adrenal gland is concerned, but we don't say that it's a toxin. I don't believe it exists.

MEAD: I believe that Dr. Bond has hinted at this, and I'm not sure if anyone else has said it, but the fact of the matter might be just the opposite of the release of the substance. I wonder how many of these abscopal effects are due first to the primary insult to the tissue, and, second, to the under-nutrition that this tissue must be existing in. The animal, for two or three days following irradiation, is existing in a severe under-nutrition state.

SCHNEIDER: That is not true, of course, for local irradiation, however.

MEAD: No.

SCHNEIDER: This brings up other indirect effects. I hesitate to use the word toxin because it's in such bad repute. Nevertheless some circulating material must account for the fact that, in pathological tissue, it is a common observation that local irradiation to one group, let's say of lymphosarcomatous lymph nodes, may be accompanied, and not infrequently is accompanied, by reduction in gross size of distant lymphosarcomatous lymphoid nodes in the same patient during the period of observation. One other question that arises that is perhaps not timely, but that I think ought to be brought to the attention of all of us, is the question of dosimetry. When we discuss the administration of, say, 600 r

locally and 600 r total-body irradiation, we are discussing what is produced physically by an apparatus, ignoring the vast differences in energy exchanged within the tissues irradiated, and this is far more significant than statement of the physical law. We are not dealing, therefore, with the constant factor in that dosage, but with a variable.

BOND: Would you care to elaborate on that last statement? I'm not too clear what you mean. Do you mean that the total energy absorbed in the one condition is different than the other one?

SCHNEIDER: Yes.

BOND: In other words, the integral dose?

SCHNEIDER: The integral dose.

BOND: I certainly agree with you, but I'm not sure that anyone has been able to attach any quantitative numbers on what this means though in relationship of integral dose to observed effect. You spoke of lymphoid tissue. This is, I think, a very special case, as Dr. Haley and I have mentioned. I think this has been shown in an early experiment by LEBLOND and SEGAL to be linked with the release of adrenal hormones. PATT and SWIFT showed fairly clearly that in this instance you do get the so-called abscopal effect through the pituitary-adrenal mechanism. Furthermore, it is not a direct effect of irradiation on the adrenal or the pituitary. We have irradiated these organs at very high doses and you do not get a decrease in weight of lymphoid tissues. If one gives different integral doses or the same integral dose to different parts of the body, you obtain this abscopal or indirect effect only when this energy is deposited in a region such that the animal becomes obviously ill. That is, where the region is sufficiently sensitive so that the animal grossly loses weight and looks ill. One can deposit this same amount of radiation energy in, shall we say, a non-specific tissue like muscle, and not see this effect. In other words, it seems to be secondary to the stress syndrome.

SCHNEIDER: May I ask you in respect to that: Do you mean that the animal shows clinical signs of the so-called radiation syndrome, radiation sickness?

BOND: Yes, this gets to the general problem of what is meant by 'sick'. When a mammal receives so much damage he lookes sick. He is ill. How do you define this? Does this mean a toxin is circulating? Changes in the adrenal-lymphatic system are seen following damage other than from radiation that makes an animal look 'sick'. I think this is quite different from what we are attempting to get at here; whether there is a specific radiation toxin or not is quite a different thing.

KELLY: I'm going to stick my neck out, but I don't believe there is a specific radiation toxin. That is, a substance which is specifically produced as a result of irradiation. On the other hand, I do believe that there is a good deal of evidence that if you irradiate a human in such a way that you produce very rapid losses of the cells, which is the sort of thing you were speaking of, that you do get toxic phenomena which are not necessarily associated with the adrenal axis. Now, we've been playing with this for some time and apparently tissue breakdown products are very toxic in the animal. This was shown in an inbred strain of mice given tumor tissue. To be specific, if you take a tumor and grind it up to where the cells are essentially all dead and then inject this subcutaneously, the animal gets very, very sick. In particular, you get very severe liver infection which you can see histologically and which you can also show with a function test. Admittedly we put a quarter to a half a gram of tissue, dead tissue, subcutaneously in order to measure this easily, but I don't see why such an effect shouldn't happen in the intact animal.

BOND: Certainly there are examples like this. For instance, following a burn to a large area of the skin one may get severe damage to the stomach. But, in all of the experiments that I know of, where tissue has been damaged by irradiation, this sort of thing has not been demonstrated. I quote a specific example; CONARD and QUASTLER have for a long time been taking the damaged epithelium from irradiated gut and injecting this into other animals. They expected the irradiated epithelium to be more toxic than normal epithelium; however, to date, this apparently has not been so. You cite a very interesting thing, but I'm not sure that you generally are getting to a specific radiation toxin.

KELLY: No. I think your point is well taken. This same thing happens in crush syndrome. I'm trying to say that this is not specific with radiation.

BOND: This just gets back to the original thing that Dr. Cronkite brought up. We know that these things happen in a variety of situations, but has anyone narrowed it down to something specific due to irradiation?

KELLY: I started out by saying that I do not believe in this specific nonsense.

RUSTAD: I have some evidence, regrettedly only for u.v. on a purely cellular system, against a specific toxin. I am forced to rely on our old friend *Tetrahymena* that has been discussed recently. It is possible to give *Tetrahymena* a dose which would be twice the lamp cytolysis dose for u.v. This dose would be perhaps ten times the average sterilization dose and is greatly in excess of the cell division delayed doses with the amoeba. You end up with a very unhappy bag of protoplasm which was once the *Tetrahymena* and which you know the amoeba will eat. It is possible to saturation feed these irradiated organisms so that perhaps a third of the volume of the amoeba is taken up within an hour. There would be a complete turn-over within a period of a day. There is absolutely no evidence of change in the division rate. No detectable effect at all on the amoebic formation, rather massive. Again this was u.v.

O'BRIEN: There seems to be a logical difficulty here. I think I could clarify my own position as to what you people think if I were to simplify it and employ Dr. Schjeide's model cell. If I may imagine this cell somewhere in an animal and surrounded only by plasma—I know that this will be difficult, but let me propose for the moment that surrounding this cell is only plasma, and, in some ingenious way, I am able to irradiate *only the plasma*. Upon examining the cell, subsequent to the irradiation, I find abnormalities in the cell. I want to ask, would you consider this an indirect effect? This is what I'm talking about as an indirect effect, perhaps even as a specific indirect effect, that with a given dose to this plasma, part of which ultimately will move into this cell, I would suddenly find on chromosome number four a little twist. I'm not trying to be facetious, but this is a 'repeatable thing'. If this were true, would this classify as an example of an indirect effect of radiation? I don't think this question of indirect effect can be answered by indiscriminately irradiating both the living and the nonliving portion. According to Dr. LAWRENCE's group, you have to consider specific indirect effects and in this you can envisage touching the protoplasm, if I understand them correctly. Now, would you answer my question, Dr. Glasser? Would you consider this an indirect effect or an abscopal effect?

GLASSER: In my mind what you're suggesting is a little alchemy. In my rather impoverished concept of the organism there is, as implied by the subject of this Session, an action called 'interaction between cells and tissues'. I choose, peculiarly, to feel that, to a great extent, among the chief integrative biochemical factors here are the endocrines. I can envisage that irradiation of a specific tissue brings into physiological play these integrative factors—I might use the words feed-back phenomena. Any insult to the environment will involve

hormonally modified and regulated interactions between one part of the organism and the other. Therefore, I just can't picture your conditions. But I think that we've gone far astray, if you'll forgive me, and perhaps someone could bring us back on the broad generalization that there may be some humoral phenomenon, not necessarily toxic, which integrates these things.

JONES: I would not like to bring us back on the subject we've been on, but suggest we go on to another subject.

SCHJEIDE: I would like to suggest a possible radiation specific toxin which would, in the present context, be quite abnormal because the animal would die long before it would become manifest. We have irradiated isolated lipoproteins rather than the whole blood with very high doses ranging up to several 100,000 r and, of course, have been able to oxidize the unsaturated fatty acids which produce organic peroxides and, although we have not actually taken these lipoprotein peroxides and injected them into animals, I submit that this would be quite deleterious and would have been quite radiation specific in its induction. I think Dr. Mead, who has been working with peroxides, the feeding of them and injection of them, might comment further. In other tissues such peroxides may be produced with a much smaller dose of irradiation.

JONES: Let's leave this subject of the acute effects of irradiation we are discussing now, and perhaps take one step towards the long-term effects of irradiation. I would like to select growth phenomena and perhaps give an example we could use as a stimulant to go on with the discussion. If we look at growth response after the irradiation or the suppression of growth, I think that there is good evidence that the effect is quite proportional to radiation exposure. Take RUSSELL'S data on irradiation of mice or rats *in vitro*. The decrease of the embryo is about three-tenths per cent per r exposure and it doesn't make too much difference what the exposure was during nutrient development, although he has only the earlier segment of it exposed. Over quite a dose range you get this constant per r. Also, if we look at the direct effects we can measure in humans, the follow-up of the Marshallese children, the follow-up of the children of Hiroshima and Nagasaki, and put this in terms of the loss of expected growth potential. This is from the time and age that they were irradiated for the time that they were followed, one also gets about the same constant as the remaining growth potential is decreased between one-tenth per cent and three-tenths per cent per roentgen of exposure. Also, within the limits of testing for proportionality of radiation effects, this seems to be a proportional thing, i.e. you have a linear equation as far as you can test, for this effect over the exposure range. Here is an example of growth being a very complex interrelationship between cells and tissues.

We certainly know from the animals studied over the past 40 years that irradiation exposure during the growth and development of the rat certainly takes a bite out of the growth potential. The animal never reaches the full size. The shape of the growth curve is as though the animal was made older in time. All of these fit together with about the same constant. Yet we do know that, if radiation exposure is given to a single limb, that limb will show the greatest growth relativistic decrease in growth potential, and there may not be an effect from the partial-body irradiation from the whole body. Shall I leave it there for general discussion?

GLASSER: Have we provoked anybody thus far? If not, I'll let Dr. Jones continue. You haven't offended anybody; will you continue until you do?

JONES: I believe myself, that this effect in growth potential has something to do with the killing of cells. I don't know what kind of cells, though, that are

responsible for the decrease in growth potential, but if we look at this constant that you get which is approximately three-tenths per cent per r, you find that this is the same number that you can get in mice, rats, guinea pigs, and humans for the effects of irradiation on the marrow, for the effects of irradiation on the white blood cells, perhaps for the effects of irradiation on the testicular mass. And I would interpret all this as perhaps the chance of killing itself per r exposure.

GLASSER: Can I have a point of information? When is the measureable arrest of growth inhibition picked up in any one specific system?

JONES: If you irradiate a relatively adult animal, you know you get a decrease in body weight and subsequently usually come back to, almost at least, the same size as that started with. But in the growing animals you'll have to wait a period of time to see the change and gross effect. In the embryo I would guess the result may be that of killing a certain random pattern of cells, on the basis of probability, of about three cells per 1000 cells per r. Losing this number of cells, you just decrease the whole population of cells, so you can't provide the same size embryo as that you would have had before this destruction of cells.

GLASSER: You just offended me. I agree basically but I want to draw out the discussion we started yesterday, that I challenged Dr. Schjeide with, namely, species differences. Why may there not be a differential sensitivity between the same tissue in different species through irradiation? The point I'm going to make here is drawn from the study of sub-lethal irradiation of chick embryos. In this experiment we have given 600 and 800 roentgens to 6-day embryos so that we could get two weeks of a growth phase. Daily weights of these animals would indicate that from the sixth through the eighteenth day there is no difference in the weight curve or in the growth curves, if you will, of these embryos as compared with controls. However, at the eighteenth day we get the beginning of a very sharp break in the growth curve so that by the nineteenth day you certainly get a significant difference between the embryo which was exposed to 800 r, versus the control, and by the twentieth day you get a distinct difference in both 600 and 800 r. This latency over such a long period of a relatively short 'gestation' is something that perplexed us. It is this resiliency that I'm interested in because this may have a reference in the adult of the chicken and other species. Now Dr. Schjeide is the head chicken man here: Do you have anything that may define this problem?

SCHJEIDE: For once I'm at an almost complete loss of words. But, I will ask what this has to do with our general subject today which is the interaction between tissues? I believe you might clarify that.

GLASSER: I agree with you.

SCHJEIDE: And that pertains to Dr. Jones also. Perhaps he would like to state how he believes that this involves interaction between tissues. Are you not talking about the interaction between populations of cells rather than on a chemical basis?

JONES: Absolutely, but this is a long-range interaction in respect to irradiation rather than an interaction during the acute phase.

CRONKITE: I would just like to ask Dr. Jones how he defines growth potential. What does he mean?

JONES: We have some idea of the height or weight of people in certain populations according to age. We use these as controls, and then if you gain not quite the full amount, the fraction I would use would be this amount divided by the expected growth for that age.

BERNHEIM: I assume that in these animal experiments they don't react to injection of growth hormones. Is that correct?

JONES: I don't know if they would react to it or not. As far as I know this hasn't been tried.

BERNHEIM: There are two possibilities that I think are very important to your argument. You are saying that the potential of the whole body being dealt with is less, maybe simply because of a decreasing amount of growth hormones from the pituitary. The growth hormone is now injected into this animal, and you get them to be normal or super-normal and, in that event, you've eliminated your explanation. If, on the other hand, it kills the plasma, you'd be putting a stress on cells which would be unable to grow, and this would substantiate your argument.

JONES: That is one thing that could be done. I would be surprised, however, if irradiated animals, even severely irradiated animals, would not respond to growth hormones, because the normal animals respond and they are only somewhat reduced from normality.

HALEY: That has been shown by SELYE about five years ago. The irradiated animal responds very nicely to growth hormone. In fact, he used it as a procedure for recovering the generalized weight loss that is seen after whole-body irradiation. However, I haven't seen the work confirmed.

GLASSER: I would like to ask Dr. Jones if he would care to extend this proposal of aberration in the matrix of the organism to potential considerations of carcinogenesis? Can these manifestations be adaptations which may be carcinogenic?

JONES: I don't know what carcinogenic changes are, and I would be inclined to agree with Dr. Casarett's proposal that we consider the integral of all changes that take place with age as being part of the carcinogenic mechanism. Certainly, I think that if we investigate changes of this sort, both in age and carcinogenic potentiation, we look for changes in numbers of cells and tissues, and the interplay of balances that result from relativistic shifts in numbers of cells, as well as changes in qualities of cells, if we are talking about growth, on a cellular basis. We certainly have a good deal of ammunition to believe that we have changes in the qualities of cells, both with time in the life-span, and after irradiation exposure. However, we should not leave the problem at this point; we should also discuss the multiplicity of physiologic events such as arterial sclerosis, vascular changes —all of these things, as increment steps toward total summation of deterioration, probably interact and reinforce the chances that further deterioration will take place.

BOND: I would like to bring up again, in the light of what Dr. Jones just said, the work of KAPLAN, and I wonder if he'd care to comment on it. That is, in lymphoma induction. With whole-body irradiation one gets lymphoma induction in mice. If the thymic region of the animal is shielded, the incidence of lymphoma is reduced. KAPLAN also gets changes in RNA depending upon whether he shields or not. The important thing is, in the animals that are shielded, the regenerating population in the thymus, presumably the site of origin of the lymphoma, is a different type of lymphocyte, in that it's histologically different. But if he shields the animal, the population remains the same as it originally was; there is no difference in the morphology of the regenerating population. It is only in the population where there has been a morphological change, that he gets the high incidence of lymphomas.

CRONKITE: That's correct; but you can make it even more complicated by doing thymectomy, irradiating the animal, putting the normal thymus back, and still get the cancer, from the cells that were non-irradiated. We've done the same thing with carcinogenesis of the breast. It is completely unnecessary to irradiate the breast or the ovaries. You don't even need to irradiate the pituitary. You

just irradiate most of the animal and end up one way or another with some ovaries that are functioning and you get a very rapid onset of diverse tumors of the breast, without having to irradiate the breast.*

BERNHEIM: How do you explain this without a toxin in the breast? Or an indirect effect?

CRONKITE: Estrogen in large amounts, or changing the estrus cycle *per se*, will induce tumors. One thing I think that the whole-body irradiation does, to put it crudely, is just to klobber the entire metabolism, and upset the inter-relations between organ systems, and in a rat this upset of the endocrines, I presume endocrines, results in neoplasis whether the breast has been irradiated or not.

ENTEMAN: As I understand it, you are putting the thymus or the breast back into the same animal from which you took it.

CRONKITE: KAPLAN did the thymus work. He removed the thymus from genetically uniform animals, irradiated the whole animal, put in thymuses, after irradiation, from other non-irradiated homozygous animals, and the incidence of thymomas was the same.

ENTEMAN: Is there a limit of time after irradiation when you can get this effect?

CRONKITE: I don't recall the data sufficiently well. I think I'd have to refer you to KAPLAN'S papers on this. He has a rather unique method of inducing irradiation of 144 r per week for three successive weeks and then thymomas commence three months after this treatment, and I think it was after . . .

KELLY: After the last irradiation?

CRONKITE: Right after the last radiation, he puts in a normal thymus, genetically identical, and gets the same incidence. In respect to the breast, it is purely a matter of reflecting the milk line from one end of the animal to the other, and shielding it with lead. One got an equal number of tumors in the shielded area as in the other shielded area, but irradiation only in the breast tissue did not induce any tumors with the same tissue dose. . . . (*See earlier footnote.*)

LESSLER: I think it very evident from such data that there are circulating carcinogens in the organism and that brought me back to Dr. Schjeide's original model cell. We had something in there we haven't discussed yet, namely a thing called virus.

SCHJEIDE: I had viruses.

LESSLER: That's what I say. In your cell you definitely had virus. Now that is another possibility in this toxin or abnormal substance type of thing, the presence of viruses in search of disease. I think that there is a series of papers on that at the New York Academy of Science. There is this possibility that there are dormant viral elements that come into the picture. I don't know. I just throw this out.

RUSTAD: I recall some of Dr. Jones's comments. I would like to direct a question to Dr. Casarett and, possibly, to Dr. Kelly. In some of these systems that depend on a stem cell, is there any evidence, say over a long period of time, to get away from our analogy of bacterial microcolonies, that there is an extensive killing of the stem cells, and, if there is killing, is there any evidence of a further proliferation of the stem cell itself?

CASARETT: If I have your question correctly, you say over a long period of time?

RUSTAD: I mean that you get these bursts of mitosis, for a couple of divisions. But I'm getting away from that, going to 10 to 20 cell divisions. Is there any evidence for death of the stem cell?

CASARETT: With sufficient dose, there is killing of many free stem cells in some

*Work since the Conference is not quite so definite about not needing to irradiate the breast. It is clear that irradiation of the ovaries is not necessary but ovaries must be present and functioning to some extent.

tissues, and even fixed stem cells in other tissues. However, surviving stem cells may subsequently proliferate and continue to do so without new evidence of extensive death, long after irradiation.

HENNESSEY: I want to mention some work which is very closely related to what Dr. Cronkite mentioned. It was reported at the Strangeways Laboratory in London by Dr. GLUCKSMAN, I believe. The skin in rats was irradiated with a dose that caused complete sluffing of the area. All the cells that were irradiated, they were quite sure, sluffed from the area, and carcinoma resulted later within that area. He was quite certain that no cells which had been irradiated were there because the area of the slough was much larger than the area of radiation.

FLANDERS: Wasn't his point that he made an ulcer in the skin of a rat?

HENNESSEY: Yes. It was also larger than the area of irradiation.

FLANDERS: This eventually became malignant from cells growing in from the edge, following a process of attempting to heal the ulcer and breaking down the edge.

HENNESSEY: Yes, that is correct. There were no cells irradiated which eventually produced the cancer.

FLANDERS: It wasn't so clear that it was certain that they hadn't been irradiated.

HENNESSEY: He was certain that it had not been irradiated.

GLASSER: Can we cease being so specific in quoting our numbers and very specific things, and try to milk all this information? There must be an underlying philosophy behind this.

BOND: One more specific remark in regard to what Dr. Hennessey was saying. In the case he cited there was chronic ulceration, chronic irritation. In the cases that Dr. Cronkite indicated, there is no obvious indication of a chronic irritating process going on.

FLANDERS: I would really like to ask Dr. Cronkite for his general philosophy of this, because it seems to me that this discussion in the main has been looking for exceptions in the evidence of the toxin, and we've also heard from Dr. Glasser of these little end points as seen by the pathologists. Would Dr. Cronkite be prepared to put forward the philosophy of mode of action of radiation on at least some radiation syndromes?

CRONKITE: That is a very hazardous thing to ask me, because I've been known to talk for prolonged periods of time on things like this. I think I won't say anything in respect to acute radiation syndrome because, so far as I'm personally concerned, I think that all the phenomena observed in the range, let us say, from 200 r up through a couple of thousand r can largely be explained on the basis of the disturbance in the cellular systems in the body, that are in a continual state of renewal. If the dose is sufficiently large to eradicate the proliferation of cells in the gastro-intestinal tract, within three to four days, the mucosa is denuded. There are bare villi sticking out into the intestinal stream. There is a massive loss of fluids, manifested by a bloody diarrhoea in the animal, a rapidly increasing haemo concentration, and death ensues rather promptly. QUASTLER has for a long time considered this as a stable intestinal death of three to six days with a mean survival of about 4.6 days. If one does a simple experiment in principle, extremely difficult in practice, of maintaining dogs alive by a tremendous infusion of fluids so that they do not collapse from the vascular standpoint, and time is permitted for the few cells in intestinal epithelium (that still have a capacity to regenerate) to do so, the animals do not die at the stable time but live, and die from the next clinical syndrome, which one might call the hematopoietic syndrome, which is the result of the disturbance by irradiation of hemopoiesis. I don't think that there is any direct evidence that the granulocytes and platelets *per se* produce something, which are necessary for metabolism in the animal.

They are defensive agents and after depletion one is going to get into trouble. This is just common clinical knowledge of any blood disorder where one is not making cells. There comes a day when one just bleeds to death and one gets an overwhelming infection that can not be controlled. This is lethal. Now, I am sure this is an over-simplification of so-called gastro-intestinal death and the hemopoietic death that are the common causes of death in the mammals unless one goes to extremely high doses of irradiation and evokes changes in the nervous system. That is a system that is not in a continuous state of cellular turn-over. I would be pleased to elaborate even more on this phase if desired, but I think this is my philosophy from a general standpoint. I think one further thing must be recognized, and this is based on one of the largest volumes of human experience with radiation injury. Many Japanese at Hiroshima and Nagasaki died with a regenerative hemopoietic system. This is a statement of fact. The biochemists have invoked this as indicating that there is some other obscure biochemical defect, metabolic defect, that is also lethal. What it is, is still undisclosed, if it exists. I mention it here because this is one of the things that has mystified people, when one tries to explain it on the failure of the renewal systems to undergo regeneration, though, it is the latter that I am personally inclined to think is most important.

One statement in respect to carcinogenous. I can feel free to speak on the subject because we only accidentally got into doing work on it. I have no experience and very little knowledge when you come right down to it. I think it is self-evident, from a vast amount of clinical experience and animal experience, that radiation directly by some action on cells can induce local carcinogenous. I think it is also true that, by some obscure disturbance in the overall metabolism, the inter-relations between tissues induced by radiation, that neoplasia can develop in tissues. It would look as if the tissues in which this occurred are those that are dependent for their normal growth and maturation and function upon humoral substances, some of which are well defined and others not so well defined. Certainly in respect to the endocrines, the inter-relation between the endocrines is quite well defined. These tissues go through cyclic renewal, depending upon menstruation, pregnancy, lactation, and so on. The same thing happens, I think, for hemopoiesis; there are definite humoral controlling factors that are not yet adequately defined, but do exist, which control the orderly proliferation of the hemopoietic tissues. I think every case for one brings in an indirect or abscopal —I don't know what word to use now, but let's say radiation-induced—neoplasia, without direct irradiation of the tissue, in a tissue that is in a continual state of renewal, and in which there is good direct evidence for a humoral control under normal circumstances.

SCHJEIDE: I wonder if Dr. Cronkite would weave something into his statement regarding arteriosclerosis and the existence or non-existence of a toxin.

CRONKITE: What's your question again, please?

SCHJEIDE: I wonder if anyone could read into this statement something regarding arteriosclerosis as a manifestation of radiation injury. Can we not consider 'abnormal' lipoproteins as toxins even though they may operate in a relatively slow manner?

JONES: In the experimental animals there are vascular lesions in the heart and the vessels, too, on the sclerotic side, after irradiation exposure; at least these kinds of changes come along earlier than they would do otherwise, compared to the total animal.

SCHJEIDE: Would someone be willing to state that this is an indirect or a humoral mechanism?

CRONKITE: Well, it's almost a UCLA invention, as it were, and there must be someone here who would comment on it.

SCHJEIDE: It is a Berkeley invention.

GLASSER: It is my general feeling that arteriosclerosis needs no special consideration here any more than cancer *per se* does. These are metabolic diseases, if you will, which are manifestations, dysfunction in the total integrated system physiology. Depending on this compendium of random effects, it may be arteriosclerotic, it may be renal, it may be carcinogenic, and it may be that Dr. Kelly has something worthwhile to say.

KELLY: I don't know whether it's worthwhile but, since Dr. Cronkite so nicely summarized acute effects, I think that one should at least make a stab at ageing effects. I would like again to draw your attention to PUCK'S quantitative data on the number of cells affected by 500 r. I think that there is no question at all that he has shown, that with 500 r, only one per cent of the mammalian cells which he has irradiated remain unaffected, or at least don't show a very gross abnormality. Now, quite obviously, to irradiate a mouse with 500 r, all the tissues don't die off, and the acute effect, which Dr. Cronkite has discussed, whether or not the animal survives through these two weeks, to my mind, depends on whether this one per cent of cells left in the bone marrow and in the intestine or whichever one you want to talk about, whether these cells are able to regenerate the tissue in time, so that the animal survives the acute period. I am not trying to be very specific about the one per cent; this is an abnormality. This doesn't get away from the fact; let's take a tissue like the liver; presumably each cell in that liver is no longer a normal cell. It has some sort of a chromosome break or gene mutation or something is the matter with it because it was also in the radiation field. The same goes for the kidney, and the same goes for the pituitary and every other tissue which we consider radio-resistant, because it is not normally dependent on the rapid cell renewal. For the long-term effect these cells must function properly and the same goes for the vascular system. As Dr. Casarett said this morning, if you put an additional stress on the cells, for example, if you do something to the animal so that part of the liver is damaged and the liver has to regenerate, then presumably many of these damaged cells would not regenerate or would not regenerate as efficiently. The same goes for the kidney and any other tissue where you so depend on occasional regeneration. Even if this stress is not applied, however, something like arteriosclerosis may very well be due to the fact that these liver cells—I happen to like the liver, but maybe something else—are no longer as efficient as they were and I think that one of the things, perhaps long-range things, that we will learn from radiation is how much chromosomal material or how much organization is essential to the functioning of each of these differentiated cells.

ENTENMAN: We all apparently are looking at radiation effects as being something which is depressing or destroying a function. In the regenerating liver it would seem that you might get a better functioning cell, actually, as regenerated. Certainly this is true in the regenerating liver in some respects, after partial hepatectomy. You get a better Kupffer cell activity apparently, as measured by chromic phosphate uptake, at least, and on bile production. This is shown by BRAUER in the isolated perfused liver. We've talked a lot about toxins this morning. I wonder if, again along this line, we couldn't speak for a moment on the factors which restore, and which might be brought into complying and restoring, the irradiated animal, rather than the materials which are toxic in whatever sense you want to take that term.

MCKEE: In this regard, I think I'm talking to the subject and also verging back

to some previous statements which I guess were by Dr. Bond and Dr. Cronkite, regarding the long-term effect and particularly recovery status in Hiroshima people. I've often wondered what the completeness of the nutrition of these people was in terms of the possibility of recovery. It is well known, of course, that to get a good vitamin deficiency, for example, you have to have metabolism going on in the animal, and the specific deficiency of this one component, in order to get this situation. I'm wondering if the dietary of the Japanese or in the animal experiments would lead to any speculation or conclusions regarding this thought.

GLASSER: I want to point out that we have officially reached the close of this session, but if the group is willing we can continue for a few moments more to finish this single point. Are there any specific related comments?

BOND: I think it is fairly obvious that the Japanese were nutritionally deficient at the time of the bombing. In another example of irradiation of human beings however, in the Marshallese that were exposed to fairly high doses of radiation a different situation existed. Certainly, following exposure, they were exposed to food like they never had been before. They were fed by the Navy and their 'nutrition' was something to behold. Nonetheless, there was quite a delayed recovery in these individuals manifested in the recovery of the hematopoietic system. As a matter of fact, as late as three years after exposure there is still evident that the hematopoietic systems, as evidenced by peropheral counts, has not returned to normal. Also, as Dr. Jones stated, there is some indication that the growth rate does not return to normal in the young irradiated individual.

ENTENMAN: Well, I don't want to belittle the Navy crews. I would venture to say that the nutrition offered by the diets that the Navy puts out really might not be the best kind of food to eat for the best growth.

GLASSER: I don't think we can be so vain as to think we can offer a summary for the session. But I would venture to say that, basic to everything we have discussed here, I think there must be a more generalized mechanism in regard to the total integration and inter-dependency of the organ system. I think perhaps in the free thinking session we can release ourselves from further inhibition and go on.

END OF SESSION V

SESSION VI

SUGGESTIONS FOR FUTURE STUDY

Introductory Speaker: JOHN R. TOTTER

I HAVE been asked to attempt to point out some of the developments which may be expected to occur in radiobiology. However, one cannot predict what will be accomplished in the near future, nor will I even try to predict what aspects of radiobiological research will be encouraged by the A.E.C. Most leadership in this field must come from scientists directly concerned with the development of research in radiobiology. Occasionally, urgent needs for special investigations make themselves felt in Washington as a result of problems arising in the areas of responsibility of the Division of Biology and Medicine. One can confidently predict that these needs will continue to arise in the future and be met successfully, as in the past, by cooperative efforts of the A.E.C. laboratories and independent researchers.

If there is a common denominator for the group of specialists in diverse disciplines who are at this conference, it must be a desire to obtain a real description of what happens to an irradiated cell at the molecular level. Only by obtaining such a description will we be in a position to deal rationally with radiation problems. By this I do not mean that they are not dealt with adequately and rationally at a physiological level. Dr. Casarett's talk this morning gave ample evidence that the pathology of radiation sequelae has been examined and described very thoroughly. But Dr. Cronkite brought us back to our real problem with his insistence that we must get experimental subjects past the acute phase of radiation effects or we will not be in a very good position to make use of our knowledge of the physiology and developing pathology of radiation damage.

I would like to present for your consideration and for your critical examination a biochemist's views, not on what developments I expect to see come to pass, but upon those areas of effort which I think should be most rewarding in a search for a more complete molecular-level description of the effects of ionizing radiation on living cells.

For convenience, we might arbitrarily classify cells into a few groups according to their behavior subsequent to high-energy irradiation. The first group would include those which manifestly undergo nuclear damage

at low levels of irradiation. Dr. Casarett has spoken earlier of some of these as undergoing 'mitotically linked death'; others may be those which survive mitosis but which suffer a detectable mutation or visible chromosome alterations. It is the loss of this type of cell in the animal following whole-body radiation in the mid-lethal range which may result in death, because the tissue involved is vital, and must ordinarily be rapidly replaced by continuing cell division. Plant cells with the same kind of sensitivity are common enough but the manifestations of the effects are, of course, different.

The biochemical approach to the problem presented by this group of cells is extremely difficult. In the first place, the stoichiometry of the irradiation is such that we know that there are few or no biochemical techniques that would enable us to observe the initial chemical changes more or less directly. We either must make inferences on the basis of very indirect observations or develop methods with the requisite degree of sensitivity.

Dr. Sparrow spoke of the relationships that have been observed between degree of ploidy and between chromosome size and radiation sensitivity. I will recall to you that he mentioned some regularities and some examples which were considerable exceptions. With this we might consider another type of cells—those which Dr. Ducoff and Dr. Powers have brought to our attention here. These one-celled organisms (certain paramecia, amoebae, etc.) differ sharply from those I have been considering, in that they do not appear to undergo nuclear damage sufficient to interfere with cellular division and multiplication at low doses of ionizing radiation. There may be several ways by which they could escape damage. If one has any faith in the essential biochemical unity of living organisms, however, it seems most reasonable to imagine that the nucleoprotein of these cells undergoes just as much damage as that of other cells when it is in a comparable physical state. If one admits this—and keeping in mind Dr. Sparrow's exceptions—it is almost necessary to invoke a repair mechanism to account for the observed differences (conversely, a mechanism for rapid continuing damage subsequent to irradiation could account for apparent greater sensitivity).

It is in studies of this 'nuclear repair mechanism' that I think important advances may be made. I am thinking here especially of the studies of WOLFF, CALDECOTT, STEFFENSEN, KONZAK, and their coworkers who have combined cytological methods with biochemical and physical techniques to establish the possibility of modifying effects in nuclei subsequent to irradiation.

In contrast to quantitative difficulties with the magnitude of changes which can be measured biochemically, there are cytogenic methods with which one can easily score the results of a few roentgens of irradiation and the numbers one gets are reasonably related to the damage done. The difficulty with these methods is that it has not been possible to extract much chemical information from the numbers.

There has been sadly lacking a suitable chemistry for molecules of a size between the limits of resolution of an electron microscope and above a thousand or so of molecular weight. Such a chemistry will have to develop if full benefit from new techniques in bacterial and viral genetics is to be realized. I am speaking of new developments in transformation, transduction and phage and virus multiplication studies. These methods promise to revolutionize our knowledge of genetic material and are already providing basic information about the nature of the gene. It is most improbable that advances in radiobiology will proceed rapidly without a large contribution from this field. (The biochemist is really squeezed between the kinetic approach exemplified by work on target theory and the biological assays employed by the virus and phage workers.)

To provide an introduction to a consideration of work on tissues, I would like to bring in a third category of cells. (The fourth category has all the ones that can't be placed into the other three.) This third category consists of those cells which do not undergo mitotically linked death following irradiation, but cease to function in part as a result of the insult. For purposes of this discussion I will dismiss those like the lymphocyte, which die or are destroyed quickly following the injury, with only the statement that they look to be very interesting subjects for investigation.

It seems to be established that some cells, like the functional cells of the liver, can survive a good-sized radiation dose indefinitely without being removed or replaced. That there is a latent injury is indicated by the fact that a much later stimulus to mitosis results in a sharply elevated proportion of visible chromosome aberrations. Presumably many of these irradiated cells would have genetic changes which eventually could be manifested as an inability or altered ability to metabolize substrates. Ordinarily there is little stimulus to mitosis—therefore, these cells may persist for long periods of time.

Dr. Haley has mentioned a repeated observation that irradiated but apparently recovered animals cannot handle drugs in a normal manner. This morning he brought up the subject of biochemical genetics and the contributions it may make to an understanding of radiation injury. If I may borrow the comparison he made, and state it again as a question:

Could it be that an organ like the liver with a low rate of cell removal is, after irradiation, similar to a heterocaryon in *Neurospora*, which carries two mutant nuclei, neither of which is capable of sustaining vegetative growth but which together can do so because their capacities are complementary? (In this case the total capacity would be equal to that of one unmutated nucleus.)

If such a supposition has any validity, it might offer a mechanism for a generalized loss of vitality, such as envisioned by Dr. Jones. I hope Dr. Jones will comment on the possibility, especially since I think he has some quantitative estimates that bear on the problem. Thank you.

DISCUSSION

McKEE: I wonder, Dr. Totter, if, in your statement about all nuclei being affected in a similar fashion, you were speaking specifically of bond breakage and were excluding the influence of protective factors, physical, chemical, etc., that might be in the cells. It seems to be at variance with some statements that have been made.

TOTTER: I said in a similar physical state.

McKEE: Yes, and chemical?

TOTTER: There must be a considerable variation in what damage could be sustained due to things around the chromosomes and nuclei. I don't particularly want to make a point of it. The difference between 500 roentgens and a couple of hundred thousand roentgens ought to take care of most of that.

KELLY: Are you of the opinion that chromosome breakage and gene mutation are both effects on nucleo-protein? It seems to me that chromosome breakage would be due to many other things.

TOTTER: Let us diagram some pertinent observations on the board. On the X-axis is time, and on the Y-axis is some criterion of damage which we will say is chromosome breakage. If one could look at the chromosome very quickly after irradiation, one probably would find a very sharp drop in chromosome breaks in T followed by a slower drop. What we finally measure at the end point is remaining unhealed breaks. This is a possible explanation of the results of WOLFF, STEFFENSEN, and KONZAK and various other people. I think that STEFFENSEN can show that the time constant for the rapid rejoining probably is of the order of a minute. WOLFF can demonstrate that some chromosome breaks remain open for half an hour or more and he can, by various manipulations, affect the rate at which the chromosomes reconstitute or translocate.

KELLY: Are you speaking of nucleo-proteins?

TOTTER: A stronger linkage than the ionic one seems to be involved in the slow rejoining process since energy is required for restitution. There may be covalent bonds involved. I find it very difficult to visualize what really causes the chromosomes to break. It's a large event for a single ionization. So in this area I'm very vague.

KELLY: There are a number of chemists here. I haven't heard them venture any guesses on this!

MEAD: I think there are two chemists sitting here who are primed for just such a question and I don't understand why they aren't on their feet already.

SCHOLES: May I first ask how many ionizations are assumed necessary to cause a chromosome break?

KELLY: One.

SPARROW: I believe LEA has data which suggests about 20 ionizations.

FLANDERS: If you consider the results for bacteria, the electron track was the only effective part of the irradiation and that amounts to roughly 20 ionizations. It may be that a similar figure applies to mammalian tissue. Of course, there may be a great difference between mammals and the other end of the biological scale, bacteria and viruses.

SCHOLES: One can see, of course, that the several nucleic acid or protein strands can be broken by so many ionizations.

FLANDERS: You have brought to light a very real lack in our present knowledge as to just how the chromosome in the higher organism is built up. Electron-microscopy, thus far, seems to be rather disappointing in filling in this gap. Here is a place where we badly need new ways of looking at cellular substance.

SPARROW: It disturbs me very much to hear chemists and radiobiologists trying to extrapolate Watson-Crick models of the DNA molecule up to the microscopic level of the visual chromosome break. I think the smallest you could consider the chromosome in the cross-section diameter to be is about a tenth of a micron. For a diameter of that size there would have to be thousands if not hundreds of thousands of strands of the diameter of the Watson-Crick DNA molecule and, therefore, I don't understand why people are concerned about trying to translate a breakage in the single Watson-Crick type molecule into chromosome breaks. If you accept the figure of 20 ion pairs as being required to produce a chromosome break, you must imagine some sort of a chain reaction or physiological process required to break the strands so that the chromosome falls apart. If it isn't this sort of mechanism, it must be a sol-gel situation in which the whole structure fails due to some molecular disturbance.

SCHOLES: Certainly you can relate the Watson-Crick model to some things we have learned about the radiation chemistry of nucleic acids in solution. However, we know practically nothing about the macromolecular structure of the nucleus and herein lies the difficulty. But with regard to Dr. Sparrow's remarks, I would agree that there must be processes other than a pure radiation-chemical one to cause the entire chromosome to break, because it seems rather unlikely that all these particular strands would be broken during the ionization. There must be some other process involved, perhaps some physiological process. I may point out, in this respect, that it has been found that irradiated nucleic acid is more susceptible to the action of phosphomonoesterase than the unirradiated material.

LEVEDAHL: At the risk of antagonizing both of the previous speakers, I don't think it is necessary, if given 20 ionizations, to involve either the nucleic acid's part or to worry about a pure chemical reaction. I think that KAUZMANN and the people at Princeton, who have been working on protein denaturation, have shown that protein denaturation kinetics are of the order of 15th- or 16th- or 17th-order reactions. Perhaps the chromosome break is not at all related to the nucleic acid part, but rather to the denaturation of the protein in the nucleic acid-protein combination. Fractures in the protein section by, say, a 15th-order reaction would rupture the entire chain combination.

FLANDERS: There's one point we should bear in mind. In the phenomena of chromosome breakage, you don't see a breakage immediately following irradiation. For example, in bacteria the earliest time at which you can see a breakage is just under two hours. Presumably, in that time, the chromosome condenses into what you finally see, so there may be a very large difference between the thing which is broken by irradiation and the final broken structure which is in the order of 0.1 microns in diameter.

SCHJEIDE: Whether or not the protein or the nucleic acid is the first affected, could we emphasize the role of the remainder of the nucleus in this effect by asking Dr. Sparrow a question? If the irradiation is given during the active mitotic cycle, when the cell returns to the interphase, do the chromosomes show the same amount of breakage as they would had irradiation been given during interphase? They do not show chromosome breakage with as low a dose when they're in the condensed form, do they?
SPARROW: You're asking if 50 r delivered at metaphase produce the same amount of breakage as 50 r delivered at interphase?
SCHJEIDE: That's right.
SPARROW: No, they produce a very different amount.
SCHJEIDE: Can we read into this an effect of something outside the chromosome itself as being conducive to the breaking?
SPARROW: I don't see why.
KELLY: Dr. Sparrow, if you irradiate a cell which is just part prophase, do you not see a break immediately in anaphase? Before, you said you do not.
SPARROW: If you irradiate at late meiotic prophase in *Trillium* and look at anaphase, you will see a small number of breaks. If you irradiate a metaphase and look at the first anaphase to follow, you do not see any breaks in that anaphase, but irradiation at either of these stages will show a large number of breaks when they reach the next division. As far as we can tell, it's a type of breakage that doesn't develop far enough to express itself. They often are called 'potential breaks'. What this means on a chemical level, I don't know. There is no lesion which can be detected in the light microscope.
LESSLER: We are shifting from the molecular through the electron microscope and visible as if they were not dependent upon one another in any way. When you speak of a visible break, you are speaking of something visible in the light microscope which has a resolution of 0.2 micron. Until a break is 0.2 of a micron in size, you cannot see it. As far as I know, nobody has as yet studied chromosome breaks by electron microscopy. There may be forthcoming some evidence that there are chemical changes long before the observed break. I might say, in this connection, that at the International Biological Congress at St. Andrews, extensive studies on the electron microscopy of chromosomes were reported. When this material reaches publication, a new concept of the structure of the chromosome may be evident.
RUSTAD: Perhaps Dr. Sparrow could tell us the quantitative differences between metaphase and interphase. What is the difference in sensitivity in chromosomes?
SPARROW: In *Trillium*, interphase itself has a range of sensitivity of perhaps 20–30 fold, but if you contrast metaphase to interphase, the difference is about 60 fold. I wanted to get back to Dr. Levedahl's comment. You indicated that you might be antagonizing me by suggesting that protein was a necessary part of chromosome breakage. On the contrary, I've argued for this, too, in my publications and I think it's extremely probable that the protein molecules may be more essential than the DNA molecule in maintaining structural continuity. Part of my reasoning for suggesting this is that if you can digest out all the DNA from a chromosome, leaving only the protein there, you still have an intact chromosome.
BERNHEIM: Is depolymerization of nucleic acid envisioned as the first thing that happens? Or is it the last thing to happen? Or doesn't it happen at all?
SCHOLES: Well, I should say that depolymerization occurs immediately in solutions of pure nucleic acid. What we sadly lack in this particular field is some decent radiation studies on the nucleo-protein of cells. The details of the literature are somewhat scanty and very contradictory in this respect.

LEVEDAHL: I would like to come back to Dr. Sparrow's comment. I think that there is only perhaps a fortuitous series of circumstances here, but it might be worth airing for a moment. Apparently chromosomal breakage can occur with relatively small doses of radiation and apparently sulfhydryl groupings and enzymes are affected by very low dosages of radiation. The denaturation kinetics on proteins, at least those that have been well elaborated, all involve sulfhydryl. In a study of the depolymerization of DNA (DNA in solution) (using rotary dispersion as a technique for following the depolymerization) we found very little or no detectable effect on the DNA molecule at low radiation levels. In other words, we must go to the order of magnitude of 6000 or 7000 r instead of the matter of 15 or 20 or 50 r. I think that these results imply that there is little value in pursuing the depolymerization of DNA in solution, as a means of recognizing this concept of chromosomal breakage, or trying to explain the chromosomal breakage. The DNA molecule, at least by the measurements with which we are familiar, is simply not sufficiently sensitive in solution to account for any chromosomal breakage involving nucleic acid change. One is driven almost compellingly toward the idea that the protein is going to have to serve as the intermediary in the formation of the break which, granted, you will see at a later time.

CRONKITE: I want to ask one question in respect to what Dr. LESSLER said. Is there any *a priori* or experimental reason why a chromosome should bear any relationship to the Watson-Crick model so far as its own shape is concerned?

FLANDERS: Would anyone like to answer that?

DUCOFF: I'm sure Dr. Sparrow could tell us a little more detail than I remember about TAYLOR'S concept of the chromosomal structure.

SPARROW: I have heard him explain it, but I don't think I remember it well enough to repeat it here.

KELLY: May I introduce a scratch-pad calculation? One mammalian chromosome contains something like 10^4 molecules of DNA. The Watson-Crick structure, as I understand it, refers to one of those molecules of DNA. I fail to see why there should be any relationship whatsoever.

LEVEDAHL: To what?

KELLY: The structure of one DNA molecule as Watson-Crick postulate it and the structure of a chromosome. There are 10^4 of these things in a chromosome.

LESSLER: Let me answer in part, Dr. Cronkite. There is a theory of chromosome structure which is generally accepted. The strands of the chromosome are shown to be spiral in nature. This actually is the only known relationship of chromosome structure to the Watson-Crick model, which has two spirals. These spirals would have to have secondary, tertiary, and quaternary spirals and protein as well to make up a chromosome. Unfortunately, you can't unravel a chromosome in this way by present investigative techniques.

TOTTER: I'd like to call you back again to what Dr. Sparrow mentioned. That is that chromosomes go through a cycle before breaks become evident. This is a very important observation. It has been known for quite a while. However, the concept that breaks can be reconstituted has not previously been introduced into our discussion. There must have been a metabolic event which made the breaks become evident after the injury. Now it seems there must also be a metabolic event which restores them.

SPARROW: The idea that there may be a physiological reaction involved in producing the break is not generally realized so far as I am aware. I know this has been proposed but nobody has paid a great deal of attention to the concept. That is the reason for our present difficulties!

TOTTER: There is another comment that I want to make, to get back to Dr. Cronkite's question to me: The reason I said earlier that I didn't consider it important to try to relate the chemical break in the single Watson-Crick model to the chemistry of chromosome breakage directly was that there are 10^4 molecules to break and one of these doesn't necessarily have anything at all to do with the chromosome break. There must be a chain reaction or something very complex, or a physiological process that goes on after this, that results in the breakage.
HOWTON: I don't think we should lose sight of the fact that there is nucleo-protein in the chromosome, nor of the very good possibility that at least in indirect action some change in a nucleo-protein molecule may be involved. With reference to some of our discussions on the role of protein, it seems rather unlikely (and I think Dr. Scholes will back me up on this) that an active radical of the sort that would be involved in indirect action would be able to penetrate the protein shell to get at the DNA itself. Hence, we might expect that the primary break in this structure might occur in the protein portion of it.
SCHOLES: This, of course, assumes only an indirect action of the radiation.
HOWTON: Yes.
MEAD: I wonder if there is any reason, as Dr. Levedahl says, to suppose that there is a break in any carbon-carbon bond in chromosome breakage. Is there any reason to believe that the chromosome is held together strictly by covalent bonds? Denaturation of the protein at a special spot might cause the whole structure to fall apart because of its inability to fit together. Perhaps it is held by numerous hydrogen bonds and bonds of lesser strength than covalent bonds. Has anyone any evidence on this? Is it necessary for the chromosome to be held together by strong bonds of the covalent variety?
DANIELS: Is there not some evidence that DNA extracted from chromosomes after irradiation is degraded, relatively, to that extracted from unirradiated controls? From this it is reasonable to infer that DNA degradation is associated with, or a result of, chromosome breakage. But does it necessarily mean that radiation-induced fracture of DNA chains 'causes' or initiates chromosome breakage on the molecule level? This seems to be the basic point at issue.
FLANDERS: I want to make a comment on our dilemma, and this relates again to the kind of analysis that LEA made on chromosome breaks. To obtain a consistent picture, he was forced to suppose that of the hundred breaks induced by radiation, over 90 of these would never be seen and, in fact, would simply rejoin. We have, thus, a structure showing a fantastic tendency to rejoin if it possibly can.
KELLY: May I pose another question to the chemists? According to a number of people (I happen to remember MAZIA's paper), the nucleo-protein particles, which presumably are the genes, are joined together partly by calcium or possibly magnesium. Would it be reasonable to assume that one particle going through in the right place could break that sort of an ionic link?
MEAD: That is just the point I was making. This is a very weak bond. The choromosome may be held together by secondary weak bonds of this sort and a mere change in shape would cause it to fall apart.
SCHOLES: Two possibilities come to mind. We know that polar groups can be liberated by irradiation, for example phosphate groups and amino groups, and if these are removed in this way, this would probably seriously weaken any electrostatic bond between the nucleo-protein particles. This may result in a concrete break. The second possibility, and we know very little about this, is the transfer of energy along the chains. The energy absorbed in one part of the molecule may not necessarily do damage to that portion. It can be transferred by migrating changes throughout the chain to other parts. It may very

well mean that this is the cause of splitting of a very sensitive part of the chromosome. I don't think one, at this stage, can differentiate between these two possibilities.

FLANDERS: Would anybody like to comment on the site of chromosome breakage from the point of view of what experiments might possibly be done or attempted?

JAMES: This isn't an experiment. The point was made that you couldn't resolve the thickness of a Watson-Crick type of model with that of the chromosome. However, if you assume a molecular weight of about 6 million and if the nuclear-proteins were arranged linearly, the lengths would certainly be resolvable in the light microscope. They probably would be in the neighborhood of 3 microns long, even in a relatively coiled state. This is the question I would like to pose: Does anyone have any information concerning the relative orientation of these chains in, presumably, the chromosome as such? Are they oriented parallel to the axis or in what way are they oriented?

CASSEN: Here is a speculative suggestion. Perhaps a chromosome break is not a break at all and could be visualized in terms of stretching. Suppose you had a long coiled spring, the adjoining parts of the helix are tied together by some weak kind of bond. The ionizing event could break a lot of these bonds in a specific section of the spring and it would uncoil locally. It would still be a linear helical array and the rejoining of the apparent parts of the chromosome would be the gradual rehealing process of pulling the loose spring back and tightening up on itself. This is suggested to me by the fact that there seems to be an attraction of broken ends of chromosomes over great distances, enormous compared with any known chemical forces or valences.

TOTTER: This could account for a large number. But there are always a few which are really broken because they can translocate.

BERNHEIM: There was some mention of chain reactions here, and I would like to ask: Does the chain reaction occur in the DNA itself or does it occur in some other constituent of the nucleus which then acts on the DNA? Is there any evidence?

SCHOLES: There doesn't appear to be any chain reaction in DNA.

BERNHEIM: In DNA. Therefore, you must assume that there is a chain reaction of some other constituent of the nucleus which then builds up enough energy to split the chromosome.

SCHOLES: A chain reaction? Not a chemical chain induced by the ionizing radiation. Some sort of physiological process, perhaps?

BERNHEIM: It actually attacks the DNA sufficiently to break it. Is that it?

SCHOLES: Yes; this is Dr. Sparrow's idea, really.

BERNHEIM: What sort of substance could this be?

RUSTAD: Perhaps Dr. Sparrow can comment on this better that I can, but there has been quite a bit of recent work, actually over a period of time, in the laboratory of Dr. KAUFMAN. These people treated various plant cells *in vivo* with ribonuclease. In one of their papers they summarized a large part of the experimental portion by stating that the breaks produced by ribonuclease were of the same nature as those observed after irradiation. This work has been interpreted in a variety of ways, some of which are controversial. One of the interpretations is based on a disturbance of chelating properties. An earlier one was a release of desoxyribonuclease. Possibly we are dealing with an indirect event, rather than one on the chromosome. Or, sensitization of the chromosome to some normal reaction may be produced.

SCHJEIDE: Denaturation of protein could lead to action by proteolytic enzymes.

SPARROW: I think we should consider the possibility that an initial small lesion

produced in nuclear protein (not specifying which part of it) might damage the molecule enough so that other nuclear enzymes could take over and finish the job, so to speak. I think that there is some evidence for this in the literature which suggests that depolymerase will attack only so-called depolymerized or partly depolymerized DNA molecules.

ENTENMAN: A similar mechanism prevails in the case of nucleo-protein. Irradiation of the nucleo-protein makes it susceptible to action by trypsin, whereas previously, prior to irradiation, it is not. I would like to ask a question. How can you postulate a chain reaction when often the effect (chromosome break) isn't revealed until several cell divisions have occurred? My concept is that a chain reaction is much faster.

SPARROW: The reason for suggesting a chain reaction was to surmount the almost unexplainable fact that the chromosome break is produced by such a small number of ion pairs. When a volume the size of a chromonena or chromatid is broken, it does not seem reasonable that such a small number of ion pairs could lead to a breakage. It seems that you have two possibilities: (1) a small lesion is susceptible to enzymatic attack and, the other, (2) that this small lesion magnified itself somehow. I've talked to some chemists about this and they seemed to think it is reasonable that a chain reaction could occur, but I don't recall the details of this chain reaction or how they were proposing its occurrence.

FLANDERS: Perhaps we should terminate the discussion on chromosome breaks. Dr. Levedahl has had his hand up for some time. Could this be the last comment?

LEVEDAHL: I would like to make one statement before we close this subject. The introduction of such a small number of ion pairs really presents no particular difficulty thermodynamically. You can achieve this result by a model, such as Dr. Mead mentioned a moment ago. A small number of ion pairs could institute a change which would give rise to a sufficiently high configurational entropy energy, so that you could account for the rest of the energy needed and not have to resort to a chain reaction. The changes in configuration alone could give rise to sufficient energy to activate, if you will, or continue on with the same reaction. There is no need to insist on a chain reaction to account for the energy necessary.

FLANDERS: Perhaps we should leave the subject of chromosome breaks. I wonder if it would be sensible to go first to the smaller, simpler organisms, later getting back to tissues and whole-body irradiation and its studies. Would you, Dr. Mead, like to start off?

MEAD: I can start off on something different, a really small organism, a molecule.

DUCOFF: I was going to suggest that while we discussed and apparently agreed that we don't understand the mechanism of the chromosome breaks, wouldn't it be logical to have some sort of indication of the role of chromosome breakage in the other manifestations of radiation injury? What effect do chromosome breaks exert on the cell's future? Wouldn't the subject be appropriate at this time?

FLANDERS: Yes, I think it would. Would you like to comment on it?

DUCOFF: No, I wouldn't. I'd like to learn something.

TOTTER: There are really two problems, are there not? Plant material behaves quite differently from animal material, with which we are more familiar. Almost invariably the animal cells die if they undergo an asymetric division. This is not necessarily true for plant cells.

KELLY: In theory, at least, the one daughter cell, which is missing a big chunk of the chromosome, dies. The other one with just the extra piece might get along all right.

DUCOFF: Don't the ascites tumor cells get along with peculiar sorts of chromosomal distributions?

KELLY: Yes, that's why I say it is not necessary that both of them die.
SPARROW: To reply to your question about the plant cells, I think it's very hard to generalize. It's quite clear from data other than radiation experiments that some plant-cells deficient in a considerable number of diploid chromosomes will live. On the other hand, there are plants which cannot get along if there is even a small fraction of one chromosome missing. I don't think you should generalize and put all plants in a category that makes them different from animals in this respect.
FLANDERS: At least one study in the literature has attempted to express anticipated cell death as a function of complete chromosome set. Cell death was studied in the bean root and there were a number of assumptions. A reasonable measure of agreement was obtained between the expected lethality and the lethality which occurred. Are there any further comments on the relationship between chromosome breakage and cell death?
RUSTAD: Some time ago HENSHAW found a very definite increase in formation of multipolar spindles in sea urchin eggs as a result of irradiation with comparatively low doses, such as those which might induce division delay. A tripolar spindle is going to be fatal to most organisms with a normal diploid number because the mechanisms for chromosomes getting into any definite cell no longer exist. I wonder if anyone knows of anything comparable in tissues that have been irradiated.
CASARETT: Multipolar spindles do occur in mammalian tissue cells and, as far as can be seen, usually lead inevitably to death of the cells.
SPARROW: Let us return to the question of chromosome breakage leading to lethality. A study of chromosome numbers in somatic cells of the Chinese hamster has been made by GERGANIAN and associates. I think the diploid number in the Chinese hamster is 22 and the chromosomes are very large and quite distinctive. This group believes they can identify every chromosome in every cell in which they make an observation. They feel very strongly that the so-called normal diploid cells are not always normal diploid cells, but that there is considerable deviation from this. Instead of always having two chromosomes of a given pair, sometimes there are three, sometimes four, sometimes one, and sometimes there are none. If this can happen in a hamster with a diploid number of 22, where presumably each chromosome must carry more genetic material than it does in man or mice or mammals of higher chromosome number, one would expect that it could happen in mammals with higher chromosome number.
KELLY: I thought, however, that these cells contained similar amounts of DNA. Is it not possible that some of these chromosomes were split, or that sometimes two of the chromosomes were joined together and, therefore, yield a false count?
LESSLER: We recently have concluded a study of bovine sperm—bull sperm—on five different species of dairy cattle of known inbred strains. The chromosome numbers were plus or minus four from the average diploid number of 60 and the DNA content showed similar variation. Both chromosome counts and cytospectrophotometric-DNA analyses were carried out, the latter verifying the counts and thus indicating that there was not a constant number of chromosomes per bull.
SPARROW: What were you counting?
LESSLER: We were counting the cells of testes squash preparations including spermatogonia, primary spermatocites, and spermatids.
SPARROW: Was this a variation around a mean?
LESSLER: Yes.
SPARROW: Did an individual have a different chromosome number?

LESSLER: There must have been incidental replication of an occasional chromosome. I think that LEUCHTENBERGER has suggested this also in a report dealing with studies of dwarf cattle. We found it to occur also in normal bulls.

SPARROW: Was the count always above the expected diploid number?

LESSLER: No. It was plus or minus 4. Odd numbers also were seen.

FLANDERS: Perhaps we should turn our attention to some other forms of life. I wonder whether anyone here would like to comment on ideas they have in regard to viruses or bacteria? Perhaps discussion of these will give us an indication of relationships between molecular damage and cell death.

RUSTAD: Certainly techniques that are being developed with respect to the synchronization of cell division in some of the microorganisms should be a profitable approach for radiobiologists. We are all concerned with the correlations in sensitivity of the cell with its morphological and metabolic features. Dr. Sparrow's work follows this line very definitely, and I think Dr. Ducoff has done some work of this sort.

DUCOFF: Synchronization may be a valuable tool. There should be differences in sensitivity in the various phases of the life cycle. But, in our study of *Tetrahymena*, the effective dose is 50,000 r and the required exposure time is too great a portion of the life cycle.

JAMES: Still, this has been done in bacteria. In our own laboratory we have been working with protozoa using synchronization procedures and there are also some naturally occurring synchronizations. The ocean algae show natural synchrony of a period generation time of about 24 hours during which all the divisions are precisely on time. Cellular physiologists are very much concerned with the change in the various major constituents of the cell during the course of its generation period of division. One can't make any hard and fast rule with respect to the rates of synthesis of different constituents because they seem to vary with different organisms. But I should think that simple organisms, fairly well defined metabolically, could be found for use by radiobiologists in relative sensitivity studies.

PERSON: Recent unpublished, probably unfinished, studies have been carried out by some French workers at Oak Ridge (KARR and SICARD, *et al.*). In their studies they used thymine-deficient mutants of *E. coli*. RNA and protein synthesis proceed in the absence of thymine, whereas DNA synthesis does not. Radiation sensitivity has been investigated under conditions where thymine was used to synchronize synthesis of DNA. There does not appear to be any obvious correlation between the peak of DNA content per cell, protein content per cell, RNA content per cell, and the maximum and minimum radiation sensitivity per cell.

DUCOFF: I don't think the microorganisms are the best types of material for this type of study. I would prefer to synchronize and study cells which are more suitable for cytological work. There is a method of doing this which apparently is quite effective. Most amphibia are good cytological material. The trick simply consists of starving them for a while and then refeeding. Similarly, by means of temperature change applied to tissue cultures of warm-blooded animals one can induce a synchronous division which is preceded by a synchronous DNA synthesis. This was reported by H. FIRKET. I wonder if it might not be possible to induce synchromy by this means, in tissue cultures of some mammalian tissue which is suitable for cytological study?

Perhaps the Chinese hamster material would be good. One could attempt to establish correlations between synthesis and chromosome breaks, the decline in colony forming ability, giant cell formation, etc. I have used microorganisms in

my work simply because they seem more suited for certain physiological studies. For studies of a different nature, one must settle on cells which are best for the techniques available.

POWERS: May I change the topic of conversation? We have been asked to try to conceive experiments that should be done (preferably by someone else). I know of several things which should be done in bacterial systems. We have been engaged recently in measuring the changes in radiation sensitivity in a dry bacterial system induced by changes in the temperature at the time of irradiation. These studies have demonstrated that there is a steady decline in radiation sensitivity from approximately 60°C down to about 70°K (the temperature of liquid nitrogen). At the temperature of liquid helium (6°K as measured) the sensitivity appears to be about the same as that at liquid nitrogen temperatures. Sensitivity thus decreases to 77°K, then plateaus from 77°K to 6°K. The magnitude of the change, although highly reproducible and significant, is not very large. Bachofer assayed temperature effects on dry T–1 bacteria phage with a result of the same magnitude as mentioned here. My question is, what is there in a physical system that acts like this? What changes can take place in molecules, as we decrease the temperature, which makes them less radiosensitive down to the temperature of liquid nitrogen, and then cease to take place from that point down?

DANIELS: I have been studying temperature effects of irradiation on solids, very simple solids, and have come to some general conclusions. Below 77°K down to 6°K little effect generally occurs and the radiation energy is stored unused. This has a well-known physical basis. As the temperature is elevated to 77°K, disassociation of excitons takes place. The energy can then be released so that electrons are 'floating around' in the solid.

POWERS: Could you explain what is meant by 'excitons'?

DANIELS: An exciton is a relatively stable state of an electron bound at a positive hole or vacancy. The next temperature range is up to about 100°K, where effects of radicals produced as a result of electron capture are seen, although they diffuse slowly.

POWERS: My results do not surprise you then?

DANIELS: No.

POWERS: You think that the radiation sensitivity or the initial interaction through irradiation of the bacteria is the production of these excitons?

DANIELS: The exciton is stable only below 77°K. It does not exist after the temperature is increased.

KELLY: Dr. Daniels, I am confused. Is this an ionized atom or molecule with an electron fairly close by?

DANIELS: Yes, but this is just a general theory of solids in any case. There are positive and negative poles in all solids. In the case of an inactive ionization, these things are trapped at the negative or positive poles.

KELLY: The electron started out on an atom and you knock it loose but it doesn't go very far?

DANIELS: That's right.

KELLY: As a plain ordinary chemist would say, this is a case of ionization where the electron stays with the atom?

DANIELS: Not necessarily with the atom you ionized, but fairly close—any one of the positive holes.

POWERS: Could we back up? We irradiate bacteria or molecules at low temperatures; then warm them up. The time of measurement of effect is perhaps 19 hours after they have been warmed up. Does it make a difference that we warm them up? What happens in the solid? Must one measure effects in the

cold or can one warm them up and then measure the existence of excitons or measure their existence?

DANIELS: For simplicity of interpretation, most of these effects are or should be measured at the temperature of irradiation. Results otherwise can depend on the rate of warming because of diffusion effects.

MEAD: The phenomenon that you mention might be very similar to the one that HANNAN reported for butter fat, except that this occurred at a different temperature. He measured peroxide formation and he got maximum formation by irradiation at −30°C. If he went either down to −70°C or up to −20°C he got less peroxide formation. A partial explanation may be, however, that the hydroxy radicals were immobilized at the lowest temperature.

SCHOLES: Or the organic radicals?

MEAD: Or the organic radicals. Yes, in other words, they did not travel as far at so low a temperature.

FLANDERS: I would like to put forward one or two suggestions. It seems to me that the central role of DNA as the genetic material in all forms of life has now been thoroughly established. It is possible that research directed at observing the properties of irradiation damage of either genes or chromosomes, where you can most readily get at them, might be profitable. These may range from studies of transforming principle, which is a hard material to handle, to DNA in viruses. Certain viruses can be dismantled, studied without their protein, or without their nucleic acid. The nucleic acid is infectious even without the protein. I would like to put forward the comment that studying radiation effects on systems of this kind might lead to an understanding of the chemical relation between radiation action and gene function at its simplest level.

SCHOLES: Perhaps you may indicate what exactly you mean by radiation action from a chemical point of view.

FLANDERS: Presumably, we would like a chemical and physical picture of the nature of radiation injury. We would like to say: this type of energy goes in; it breaks these bonds of these molecules because of this mechanism; the following results eventually occurred.

O'BRIEN: It does worry a chemist when he doesn't know what he's starting out with, doesn't it?

FLANDERS: That is the reason for our interest in transforming principle. One does know what one is starting with because this is over 99 per cent DNA and less than 1 per cent protein.

O'BRIEN: You would suggest that investigations on a test tube of non-living material would be the ideal approach?

FLANDERS: It would be useful. I don't know that any approach is ideal.

O'BRIEN: Is there not something to be said for what JACKEL has stated? We must admit we have not the wherewithal to attack this type of problem directly and, therefore, must proceed by indirection.

KELLY: I'd like to suggest, Dr. Flanders, that since you introduced the subject, you summarize for us the radiation inactivation of transforming principles, lack of oxygen effects thereon, and how the results fit in with whatever we've been talking about.

FLANDERS: LATARJET and EUPHRUSSI TAYLOR found the molecular weight for their transforming principle preparations to be of the order of 700,000. They assumed 30 eV per ion. One ion would inactivate the transforming principle. It may be that one should use 100 electron volts for an inactivation. This would give a higher molecular weight. Experiments on the oxygen effect, on transforming principle, have not definitely proved the absence of an oxygen effect. I think this is a question which should be re-examined.

KELLY: I would like to ask Dr. Sparrow whether irradiation in nitrogen shows an appropriate reduction in chromosome breakage.
SPARROW: I haven't done any experiments which bear on this subject, but I know that chromosome breakage is affected by the presence of oxygen.
KELLY: Would you go along with the view held by LATERJET that the oxygen effect does not apply to point mutations but does apply to chromosome breakage?
SPARROW: I think there are data which show that the oxygen effect does hold for mutation, but I'm not sure.
SCHJEIDE: I think it does.
FLANDERS: I think there are mutations of both kinds, those that do show and those that don't show an oxygen effect. An absence of an oxygen effect in non-dominant lethals in mice was reported by RUSSELL. On the other hand, certain mutations in *coli* studied by ANDERSON four or five years ago at Oak Ridge (back mutations to streptomycine resistance) showed an oxygen effect. In the same organism another did not. A rather nice demonstration.
TOTTER: You are providing a lot of grist for Dr. Mead and Dr. Bernheim to mill when you talk about an absence of oxygen effect on something that has no lipid in it.
KELLY: What about chromosome breaks? Are you proposing lipid in the chromosome?
TOTTER: I think there probably are metabolic events involved both in the breakage and in the restoration of chromosomes and this leaves a lot of territory.
MEAD: Here is a slight change in subject. Perhaps it will furnish some food for thought to be voiced after the coffee break that is coming soon. I want to ask whether there is any evidence that nutritional supplementation, other than that which would normally be of the most benefit, or be optional to the organism or animal, could be specific for optimal recovery from radiation. For example, is there any evidence that certain vitamins may aid especially in radiation recovery, or that any radiation recovery is aided by any supplementation other than that which would normally be optimal?
TOTTER: What are you speaking of—animals, bacteria?
MEAD: Anything in which nutritional studies can be carried out.
TOTTER: Much of my scientific career has been devoted to nutrition. From my experience, I think it most unlikely that you could not find a tricky condition under which you could make it show, but I don't think it very important when you must devise a tricky method to show that a special vitamin or something has an effect on radiation recovery.
KELLY: We have been encouraged to speculate, Dr. Totter. Could we assume that some of the things which have been shown in bacteria might also be valid in mammals?
TOTTER: I find it difficult to believe that mammalian cells are not always under positive nutrition. By that I mean that external starvation must be severe before metabolic starvation is obtained. One can alter the conditions around bacteria much more at will so that tricky conditions of stress are easier to obtain. One should not, however, belittle what one might learn from these simpler organisms.
KELLY: I was thinking specifically that if you supply bacteria with purines, pyrimidines, certain amino acids, etc., you get a higher survival than you would otherwise. It is certainly possible that this would make a difference in the mammal also. If, for example, one supplied cells for repopulation of the bone marrow, and at the same time supplied purines, pyrimidines, and whatever other cell constituents the cell can possibly use preformed, might the cells not have a somewhat better chance at repopulation or at division? I think that at the

present time we have no clear concept of what fraction of the nucleic acid precursors is normally synthesized *de novo* by each cell and what is supplied by the rest of the organism. The bacteria will utilize whatever preformed purines are given to them and only when there aren't any such available will they synthesize their own. It's possible that mammalian cells act in the same way.

TOTTER: Yes, I think this is quite correct. I think what you're saying is that there is a *rationale* for a nursing profession.

KELLY: Yes.

ENTENMAN: I don't quite understand this continued return to the synthesis of DNA when it was more or less decided a couple of sessions ago that DNA synthesis wasn't important, particularly in recovery.

KELLY: If one attempts to repopulate the bone marrow, the cells must divide at an extremely rapid rate, once every few hours, and before they divide they must synthesize a great many things. I think it's possible that if we supply them with some of the things that they need so that they don't have to synthesize *de novo*, they can do a little better.

SCHJEIDE: In answer to Dr. Entenman, it could be pointed out that the term 'cellular repair' has been used in two senses in our discussions. We have spoken of cellular repair in the intracellular and single cell level and we have talked about repopulation, which is repair of a tissue by multiplication of cells. One could conceive of intracellular repair occurring without synthesis of DNA, but repopulation involving cellular division certainly would require synthesis of DNA.

SPARROW: In the spirit of the title of this session, 'Suggestions for Future Study', I make this proposal: The fact that in plant material there exists a difference in radiosensitivity of a factor of 60 fold between cells which are a few hours or a few days apart in development, indicates that this provides the opportunity for the biochemist or the cellular physiologist to determine which factors make the cells suddenly go through dramatic changes in radiosensitivity. To the best of my knowledge, no one is pursuing this on plants at the moment, either on the biochemical or physiological level. Likewise, there exist closely related plants which have major differences in radiosensitivity.

FLANDERS: To some extent this refers back to the fact that we don't know enough about chromosome mechanism, structure and general behavior. It may be that some of these observations will be much more explicable when there is established a more profound knowledge of what happens in the chromosome during irradiation.

SPARROW: This is true, but there are some perfectly obvious things that have not been done in plant material. For instance, no one has tried to determine the relative activity of enzymes such as catalase, which you might expect to be important.

BERNHEIM: Returning to the role of lipids during irradiation, there is something in plants of a polysaccharide nature which is a very good anti-oxidant. If chloroplasts are isolated and exposed to white light or ultraviolet light, lipid peroxides are formed from the unsaturated fat which apparently is part of the chloroplast structure. If the antioxidant is added to the chloroplast, the formation of the peroxides is retarded. The radiosensitivity of different plants thus may be a function of the amount of this particular compound present in the plant at the particular time.

DUCOFF: Is this a result of the photosynthetic activity of the plant?

BERNHEIM: I do not know. The antioxidant can, however, be isolated. It is thermostable, unlike the antioxidants of mammals which are thermolabile. It has a large polysaccharide component. We hope to assay for its presence in

various plants, quantitatively, and then compare the amount present with the radiosensitivity.

ENTENMAN: It has been said a number of times during this session that depletion of materials will influence the ultimate death of a tissue. It expires because the cells stop dividing. What is the evidence that there is an actual depletion of an essential substance that inhibits or stops cell division?

DUCOFF: Isn't this just postulated as an explanation for the fact that a cell can go through a certain number of divisions before it stops dividing and all the cells die out?

POWERS: Studies of paramecium provide some evidence. KIMBLE irradiated well-fed paramecium and measured the intermitotic time after irradiation. He found that the intermitotic time lengthened only after several cell divisions, after the cells begin to recover from depression of cell division rate. In our laboratory, we used starved paramecium. When starved paramecium are irradiated, the cells show maximum intermitotic time immediately and the intermitotic time then shortens as cell divisions proceed from that time. The recovery curve was almost the same as that observed by KIMBLE, particularly in the portion of the curve from the maximum depression up to control rates. The simplest interpretation is that the well-fed cell, while damaged, is still capable of dividing because the damage is at some point in the biochemical supply line which is some distance removed from the cell division process. When, however, fresh metabolites are required by the cell, it turns out that irradiation has blocked the production or utilization of these. This is indirect evidence, but I believe it follows the interpretation we have given previously.

BREAK

FLANDERS: Perhaps we have been dwelling too much on chromosome breaks and on the subject of DNA. Let us go to the other extreme. Dr. Jones, will you please make some remarks about man?

JONES: The remarks I have to make are concerned with the estimation of effects of radiation in man and with an appraisal of some of the difficulties in making these estimates. As you know, only a few years back we had a concept of radiation effects which would allow a tolerance dose of a tenth of a roentgen per day. For professional people working with radiation devices, this would mean a tenth of a roentgen each working day, integral doses of 30 r per year, and lifetime doses of the order of a thousand r. This was based on the work and experience of some of the early radiologists. Earlier data dealt largely with acute radiation effects. Acute radiation effects have associated concepts of threshold effects and recovery effects, which were gelled and concentrated in the rather conservative view that for a small amount of radiation given in any short-time period, recovery processes might keep abreast of any acute change. None of the acute manifestations of radiation effect would be in evidence. Within the last 15 years views have changed rather rapidly, but the thinking in professional fields has lagged behind the generation of evidence of radiation effect, in terms of the long-term events. Consider the discovery of the genetic effects of radiation by MÜLLER. MÜLLER'S concept was largely set in a background which is quite acceptable today in terms of irreversible changes associated with irradiation—not only genetic effects—but effects on the somatic cells and systems which would seem less simple. I would recognize the effect on aging. Aging occurs in all organisms but is speeded with radiation exposure. This effect is to a very large extent proportional to the total radiation exposure. The effects are such that if a person survived an estimated average lethal dose (which according to Dr. Bond would be about 350 r) his

chances for living a normal life are pretty good. At the most, his life span would be reduced (about 7 per cent on the average) by things which would generate disease, such as vascular disease, any kind of degenerative disease, including cancers or other bizarre unsuspected events which may not be presently recognized as resulting from radiation effects. One would estimate that about 5 per cent of his total life span might be reduced because of 350 r radiation exposure. This estimate may be too conservative. Instead of five years' difference in life span, it may be ten years or more. On the other hand, it might be less than the smaller estimate. In any event, it probably is some number in such a general range. Approaching this problem at the biochemical level is extremely difficult. How can we observe a difference, biochemically, between an individual who is 30 years of age and one who is, say, 35? Or one who is 35 and one who is 40? I've always made a habit of looking closely at people and sometimes can come very close to judging their age by looking at wrinkles and hairs, skin sags, and the like, but these are hardly biochemical events. If I were to attempt to approach the aging process in general on a biochemical basis, I could not choose any test which would be strictly definable as a biochemical test which would even enable me to tell the difference between a 60-year-old and a 30-year-old person, or an 80-year-old and a 50-year old person. Some of the functional capacity tests are acceptable at the physiological level, but even with functional capacity tests one finds much individual variation. There may be as much variation between individuals of the same age as there is between individuals of widely different ages. If for an average lethal dose of 350 r we can expect a difference in physiologic age to have been generated in the order of only five years, could we, from the standpoint of today's knowledge of biochemistry, expect to cope with this on a biochemical basis? The future is hopeful, but I think the future is only as hopeful for radiation effects as it is for studying the aging problem in general.

Let us now proceed in a slightly different direction. We have some evidence that certain diseases tend to be specific for radiation effect. Leukemia is much more liable to occur in human beings who are exposed to radiation than in those who are not exposed to radiation. This enhancement, according to comparisons that have been made between radiologists and physicians at large, amounts to a factor of about 8-fold for the difference in exposure of these two groups. The life-shortening effects are on the average, perhaps two years in this instance. This means that all causes of death are only different by about 2 per cent, whereas with respect to leukemia you have an 8-fold increase, so that the relative magnitude of increase in leukemia compared with the increase in degenerative disease for this group is a difference between two-tenths and eight, approximately a 40-fold difference. This relative difference with regard to human beings is apparent in the data from Japan. It is the one fact which we can accept without reservation in the follow-up study of atom bomb survivors in Japan. The incidence of leukemia in those who were severely exposed runs as much as 10 to 100 times that of the lightly exposed or unexposed population. The leukemia problem is also a problem of the proportionate effects of radiation. Ten years ago we would have had no trouble defending the general concept of threshold effects with regard to radiation exposure. You remember that the evidence for threshold effects is largely in the events that have to do with acute effects of radiation. We are just now exploring the long-term and life-shortening effects of radiation, and we find ourselves without sufficient data to test whether correlations truly exist. Exactly ten years ago a large hassle took place with regard to genetic effects. There was considerable debate as to whether the points obtained for radiation-induced mutations could be extrapolated to zero origin. The evidence for a

threshold with respect to acute effects was so great that many argued that the curve might possibly take a unique dip at some dose less than 100 r. This possibility inspired CURT STERN and others to fill in the missing gaps. As a result of work with fruit flies, two extra points were inserted. One of these was at 50 r and the other at 25 r. Both of these points satisfactorily fit the linear extrapolation of the earlier data. With the accumulation of more data during the last ten years, I think that most minds are reasonably agreed that proportionality is associated with genetic effects, going back to relatively small amounts of radiation dose. Even so, as stated earlier, one really doesn't know whether genetic effects can be extrapolated back to a single ionization. This is a moot question at present, whether we can extrapolate back to single ionization or whether we can extrapolate back to ionizations that may be of the order of 10, 100, or 200 per cell. Perhaps one ionization may not do the whole job and another ionization may come along a little bit later and nibble away at one of these parts, and thereby events gradually accumulate to a point where molecular separation takes place. At the moment, the effects of radiation as far as induction of human leukemia is concerned rest almost entirely on an appraisal of the data from Japan. Analysis has been made by a number of people. The first crystalline evidence to come from the data was that of COURT BROWN and DOLL in the British report on Hazards to Man of Nuclear and Allied Radiations. Subsequently, it was reviewed by LEWIS. I made estimations from it about a year ago. The actual doses taken by individual Japanese have not been determined. One has available only fuzzy groupings of people with regard to exposure. Since the way you put this information together determines the results, I have made some attempts to purify the information. There are many bizarre pieces of data, such as having relatively large numbers of the survivors listed as individuals suffering severe acute effects, although their positions with respect to the epicenter of the detonation were such that, if they had been in the open at this distance, they would have received less than 10 r. Considering large discrepancies of this sort, it is difficult to determine whether proportionality does exist within the data from Japan. But, I think proportionality is nevertheless the simplest concept to apply to it and it does fit to a very great extent. I also was satisfied with the numbers that LEWIS presented with regard to leukemia induction in man until I worked with a refinement of the data from Japan. LEWIS states that there are 10^{-6} chances of leukemia induction per roentgen per year of observation after exposure. From my treatment of the same data (I limited my data to cases where there were acute symptoms indicating radiation exposure of a moderate or severe amount), it appears as though this number might be five times 10^{-6}. Obviously, this would make a considerable difference in the entire problem.

Now, why may this be relatively important to us? You may be surprised to know that the story of an 8-fold difference between radiologists and physicians, at large, rests on seventeen cases of leukemia in radiologists. Although statistically significant, it represents a relatively small number of cases collected over a long period of time from a fairly sizeable group. The reason for this is that leukemia is a rather small risk compared with all the things we are faced with. However, with respect to the American population at the present time, you can find from the vital statistics of the United States that the leukemia incidence for the whole United States is roughly 0.7 per cent of all deaths. This is a number which amounts to 10,000 deaths per year. The leukemia problem seems even more acute when it is recognized that the death rate for leukemia in the United States has increased approximately 6-fold from 1900 to the present time. Argument has raged as to whether this increase is due to improved accuracy of

diagnosis. This argument, in part, can be met by determining the age of onset of leukemia for population groups in this country. When this is done, it is found that there is very little change in incidence of leukemia from 1920 onward in individuals, either male or female, who are 20 to 50 years of age. The increased incidence of leukemia in this country is largely in the group of individuals over 50 years of age. This fact can be used as a relative base line to determine the reality of this increase in leukemia. Thus, the facts that I have presented would argue that this increase is a real one. Also, if we compare the present time, say, 1955, with the information available around 1940, it appears that the leukemia rate in individuals over a 15-year period has almost doubled over this period of time. Many other things have happened during this interval in addition to an increase of radiation exposure in the general population. But if the annual chance for leukemia induction, from the Japanese data, is 5×10^{-6}, and if we are truly justified in extrapolating back to minimum doses, then this 5×10^{-6} chance of leukemia explains the natural incidence of leukemia in Japan. And it also explains the natural incidence of leukemia as we saw it in the United States about 1920. It would also explain the total amount of leukemia seen at the present time, if we attribute the increased incidence of leukemia to the general use of X-rays and to radiological events that have exposed the general public. These are all big 'ifs', but, if we extrapolate in terms of what we are now seeing, knowing the events that are happening to older individuals, and knowing also that there are latent periods involved, it is quite possible on the basis of today's trend that such deaths, which are now 10,000 for the whole country, may grow in the next ten or fifteen years to something in the order of 20 or 30 thousand per year. This may become, then, an appreciable cause of death in the United States; so that, whether it is due to radiation exposure or not, it is something that certainly cannot be overlooked as a general public health problem. These are some of the things that must be examined, and one can see that they are problems that have to be examined at some level beyond the physiological level and certainly beyond the biochemical level (from the standpoint of today's techniques). The only techniques available are the statistical approaches to epidemiology, public health, and vital records. In some cases, this will be rather tedious, because of risk rates that run only 10^{-6} per roentgen per year in the population. Populations of 100,000 are perhaps minimal for assessing these effects. Such numbers, I think, are not unreasonable if compared with the established experiments in genetic effects of radiation. In order to establish the lowest point he was able to study for a genetic effect, CURT STERN examined the individual genetic characters of 50,000,000 flies. These effects are of about the same probability as those we are looking for in the human population. Statistical methods require very large numbers in order to give significance at the one per cent level when the probability is as small as this.

With the sum of information which we have at the present time, occupational exposure recommendations based upon genetic considerations and general effects upon health have been moved downwards so that individuals will no longer get doses of 1000 r or more from the hazards of their occupations in a lifetime. A limit of about 50 r in a person's occupational lifetime has recently been recommended. (The average individual exposure should be very much less than this.) Fifty r would perhaps mean an average life span reduction of almost a year. This reduction can be compared directly to other factors which are known to affect life span. It is on a par with that for the use of an automobile by the average person in this country, and is slightly less than the average person's risk when using an automobile in going back and forth to work. The irradiation effects are

spread out and are greater in some individuals. They may be drastic effects for the person who pays the price, just as the individual who actually experiences an automobile accident pays the real price in terms of average risk for all those who drive. Thus, we can see that, as far as an individual's risk is concerned, it is relatively small.

These facts raise problems which are beyond the scope of any of us here in this room (for our disciplines are not geared to them) regarding the legal and ethical structures of society. They involve such considerations as partial disabilities, which are a statistical concept and only approximately covered by insurance. We can, however, feel fairly confident about having controlled the radiation effects on the individual's life and health if we follow the recommendations for current occupational exposure. The risk is small compared with other risks which we accept.

One thing that is not known at this time is how much radiation the human race can tolerate over a period of many generations. There is the obvious interrelationship between the cumulative genetic deterioration and the degree of good health to be reckoned with. This gap can be bridged in many different ways, by looking at the fly, at the mouse, at man. There is definite evidence that genetically poorly-constituted individuals have health problems, suffer from reduction of life span, or are physiologically less efficient. RUSSELL'S data show that genetic factors affecting the life span of an individual can be passed on to the next generation. This concept is based on relatively limited observations of cases where the male parent was exposed to irradiation before conception of offspring. Even so, life span shortening in the offspring was found to be of the order of ten to twenty days per roentgen of exposure to the testicular tissue of the parent. It appears as though the effects of deterioration, as expressed in somatic tissue effects, or in genetic transmission from one generation to the next are statistically equal in amount. The question is how much of this can be tolerated?

POWERS: Were the data you just gave (on 1 r being equal to ten or twenty days) for man or for mice?

JONES: Extrapolated to man, it would be that one roentgen is equal to minus ten to twenty days for the offspring. The ratio which I have independently calculated for human beings, estimated from all possible sources, would be that one roentgen is equal to minus five to ten days for the individual irradiated. There is a very great uncertainty, however, when life span is calculated on the basis of a single set of observations. I would imagine that the error associated with RUSSELL'S tentative estimate may be almost as great as the number he obtained, perhaps even greater. It could, however, be statistically determined that there actually was a life-shortening effect in his experiment. That effect was certain. Doubt exists only as to whether the numbers obtained for the transmitted trait are equivalent to those to be expected in their own lifetime from irradiation of adult animals. It has been said that 50 r would at least double genetic changes. These views are, perhaps, from those who would like to minimize the genetic effects of irradiation. More recent evidence indicates that the radiation dose for doubling of mutation in humans may be 5 r. If it is, 50 r may be much more than one should allow in terms of genetic effect. Some time in the near future, we must decide what the average genetic effect is in human beings in terms of exposure, and how much is liable to be accumulated throughout several generations. I think we must also decide what effects of radiation are concerned with the deterioration of body function and the occurrence of leukemia.

A final point. Do we really know that radiation effects simulate aging? Are they actually the same as aging, or are they something entirely different? The

expression, 'simulation of aging', certainly is acceptable at the present time; but I think that if we examine the poor evidence for deterioration and aging, the end classifications are only more or less fixed. There are also certain functional breakdowns which occur regardless of what the contributing factors are. It is possible that the events associated with radiation damage may be unique. Yet the final event may manifest itself through common pathways which are recognized as purely natural. As an example of this, individuals who are hit over the head experience increases in degenerative disease of all kinds. These are all natural degenerative diseases. Who can distinguish the degenerative diseases resulting from the blow from those evolving from natural causes? Another example involves the incidence of TB. At the present time, much less than a third of the whole population in America is positive to tuberculin tests. Very few of these die of TB; yet if a person in that group doesn't die from tuberculosis, his chances of death from any of the other natural causes is greatly augmented. Again, we wouldn't argue that TB is an average contributing cause of most degenerative disease. We can make a similar argument, though, in the opposite direction, that the decline of childhood diseases experienced over the past century has contributed to reduction of incidence of degenerative diseases in adult life. Some cancers, even those that are arrested, are associated with very high death rate from other causes. Individuals in this group die sooner, of other natural causes, even though they do not die of recurrence of the cancer. Again, one wouldn't say that cancer is a common factor contributing to all degenerative disease. So, this is what we have to agree upon. It is possible that, as we become more sophisticated with regard to radiation exposure, we may appreciate effects of radiation which represent very subtle deterioration and partial disabilities. At the present time, I know of no reason to speculate unduly in this regard, because we have seen no monstrous changes in individuals associated even with relatively large doses of radiation. The trend in the past has definitely been toward appreciating effects of smaller and smaller doses; and I don't think we are near the end of this trend, even though the minimal permissible dosage has been reduced in the past two years. What we must do is test some of these notions by providing an entirely new experimental basis.

DUCOFF: Would you, Dr. Jones, comment whether or not you think it's worthwhile to apply suitable loading tests to irradiated animals? The purpose, of course, would be to determine whether we can detect physiological changes quite early without waiting for the life span to show them up.

JONES: Yes, but I really don't know of any one thing to look for. What I thought might be very worthwhile doing is to devise large batteries of tests. We've had some experience working with batteries of tests that may run up to at least a hundred different items; and this, I know, is of particular use in approaching a sharply defined problem. I feel fairly confident that this technique might show something that would have a bearing on radiation effect.

CRONKITE: From our standpoint, this is a very critical problem. All of you who are involved in problems affecting irradiation to the cell suffer the same difficulty, the inability to supply yourself with sufficient space and cages and help and so forth to handle the number of animals that would give you statistically valid data.

JONES: Yes, you have to be set up to handle, at the minimum 10,000 animals and, to get down to fine points, 100,000 or a million animals.

BOND: Dr. Jones, you indicate from the Japanese data that, even though it may be proportional, the absolute dose is subject to a great deal of error. In the COURT BROWN data there are two things I'd like to ask about. One is that individuals were selected by virtue of having a specific disease—were thought

to have a predisposition toward this disease. Therefore, the possibility exists that they may have a predisposition toward other diseases. Also, regarding the dosimetry, the proportionality was found to exist if the doses were expressed in one way and was exponential if expressed in another way. We do not know which parameter is the determining one in terms of producing leukemia. Apparently leukemia was produced by partial body irradiation under these circumstances. Yet, referring to KAPLAN's data, shielding a part of the body during irradiation prevented leukemia. So again, while you indicate the uncertainties in the numbers you have proposed, I wonder whether they aren't even more uncertain that you have indicated?

JONES: Yes, the entire mechanism is greatly in doubt.

BOND: We really don't have any data at all for extrapolating to low doses. I think that the lowest dose that COURT BROWN used in his calculations was approximately 300 r.

CRONKITE: In spite of the poor economics of some of these experiments, such as proportionality at low doses, involving over a million animals, political necessity may force these experiments, regardless of how they look scientifically. I trust that this will not happen, but it will only fail to happen if every effort is made to make the public aware that there are scientists around who can be trusted when they say it's not economical and of no use to run such experiments.

DUCOFF: I might mention a negative result obtained in an experiment designed to determine whether radiation could show an aging-like effect on specific systems. This was done with mice, not protozoa. As mice age, they become more resistant to induction of tumors by methyl-cholanthrene (this compound induces tumors), although conversely, radiation is generally considered to aid carcinogenesis. Dr. HERMANN LISCO and I thought that radiation, in this case, might inhibit carcinogenesis. Mice were given 750 r in two doses and compared with unirradiated animals of the same age and with a group of mice four or five months younger and a group of mice four or five months older. The methylcholanthrene was administered five months after the irradiation. There was not very much difference between tumor response of the irradiated and of the unirradiated mice in the middle-age group and the irradiated mice certainly did not respond like the old mice. This emphasizes that aging and the long-term radiation effects may not be unconditionally equated. Other physiological tests, however, such as those carried out by HOWARD CURTIS, seem to indicate a very close comparison between aging and late irradiation effects.

FLANDERS: Well, we have overrun our time by five minutes. However, I think we should have a vote as to whether we should go on with this interesting discussion. Is it the general wish that we go on now or would it be better to close and ask the chairman of tomorrow morning's session whether this topic could be raised again?

LEVEDAHL: Let's continue the discussion over a beer!

SCHJEIDE: We hope you will take time to view a movie at this point. This film was sent to us from Dr. POMERAT of the University of Texas and concerns the effect of 500 r of X-irradiation on HeLa cells in tissue culture.

RUSTAD: I am sorry that Dr. POMERAT was not here to comment on his film on the behavior of irradiated tissue culture cells. This film has suggested to me something that might be very important in the survival of an animal following irradiation.

The waving filaments of the irradiated cells are associated with pinocytosis or cell drinking, which is a mechanism for getting materials, especially macromolecules, into the cell by engulfment of fluid. It is somewhat like phagocytosis.

We also observed filaments connecting the cells. In Dr. Crocker's laboratory, Dr. GOLDSTEIN has been able to enucleate tissue culture cells. The isolated enucleate cell has a very short life, even though it may pinocytose freely. However, if it is in intimate association with a nucleate cell as judged by the existence of transient cross-connections, the enucleate cell has a greatly extended life. This experiment suggests that there may be a transfer of essential materials directly from the nucleate to the enucleate cell.

If the survival of a cell with no nucleus is prolonged by such a mechanism, a cell which has lost only one or a few of its synthetic pathways might survive for an indefinite period of time. It would not be unreasonable to suppose that such cells could maintain at least minimally their activities which are essential to the survival of the whole animal. Of course, such a mechanism might also lead to the transfer of toxic products from one cell to another.

My hypothesis could certainly be tested experimentally if someone with a microbeam has the patience to perform statistically significant studies on individual tissue culture cells associated with and dissociated from other cells.

Speculating even further, one might anticipate that some of the long-term effects of radiation could arise from the strain on cells maintaining each other in this manner or even a gradual failure of this system of maintenance.

END OF SESSION VI

SESSION VII

SUMMATION AND FREE DISCUSSION

MEAD: I want to mention an experience which is very similar to one that Dr. Ducoff mentioned. We were trying to determine whether whole-body irradiation had anything to do with fat stability, which is measured by spreading a very small amount of fat on a piece of filter paper, subjecting it to a temperature of about 100° in a Warburg flask, and watching the oxygen uptake. Now, what we can tell by this is, first of all, the amount of antioxidant in the fat, which is reflected in the lag period before the oxygen uptake begins, and we can tell roughly something about the composition of the fat by the slope of the curve after oxygen uptake does begin. We found in our first experiments that the lag period was inversely proportional to the amount of radiation that had been received by the rats from which the fat was obtained. When we went on to repeat these experiments and make them much more statistically significant, we found a rather disturbing fact, that the lag period was also directly proportionate to the age of the rat. Now, how significant this is I just don't know at the moment, but the variation is tremendous. In other words, a weanling rat has practically no lag period before oxygen uptake, while a rat of about a year and half to two years' age might have a six- to seven-hour lag period. These look very much like your own experiments, in that the effects of radiation and of age are acting in opposition—or is this what you implied?

DUCOFF: We were unable to detect any effect of radiation. Surprisingly, although we bracketed the irradiated animals with others, both older and younger, there was essentially no shift caused by irradiation. The peaks of tumor induction rate were the same.

MEAD: At any rate, this confused our results so that it was very difficult to tell anything about the radiation *per se*.

CASARETT: Before we leave aging, I would like to mention some work on measuring (presumably) the irreversible component of radiation injury in terms of the agent that produced it, that is, radiation. I don't know how well this method will work out or whether our data will be confirmed. Dr. JOHN B. HURSH and I have been doing experiments using second or test doses on animals receiving prior initial radiation doses. The *rationale* behind this involves Blair's hypothesis that the injury of aging is equivalent to radiation injury and therefore the acute radiation LD_{50} is proportional to the median life expectancy of animals. We realize, of course, that there is a certain amount of selection in the animal population when one gives a killing dose and then tests the surviving animals with the second dose. We did two experiments which are interesting to compare. In one experiment rats were given 600 r, which produced little or no mortality within 60 days. We then tested them 60 days later when, presumably, most of the reparable injury would have been repaired, and learned that there was a reduction in LD_{50}, at that time, of about 20 per cent. We also gave 600 r to rats of about the same age to determine by actual observation the degree of life shortening, and that turned out to be about 20 per cent. In addition, we have done experiments with rats of different ages, which indicate that the LD_{50} decreases with

age. Taken together, these facts, if confirmed, plus the observation that initial irreversible tissue changes may become amplified in time, in terms of damage, constitute a possible explanation of how the irreparable component of injury produced by sublethal irradiation ultimately meets in time what might be called a falling lethal threshold, so that it can produce premature death. I would like to propose this subject for discussion.

BOND: We have done similar experiments in which a second challenge was given to mice at four weeks, six weeks, and eight weeks after the initial exposure. The initial dose was in the low lethal range—about an LD_{10}—of either gamma or heavy-particle irradiation from the $B^{10}(n,\alpha)Li^7$ reaction. We were unable to pick up, at these times, a reduction in LD_{50} with a second challenge with either gamma or heavy-particle irradiation.

DUCOFF: Didn't HOWARD PIERCE do some experiments with late repeated challenge in which he got a marked decrease in the LD_{50} amongst survivors of X-irradiation, but not amongst survivors of nitrogen-mustard? This seems to raise another question which I had in mind to ask Dr. Casarett yesterday. You say there were certain histopathological changes which you can recognize in either the old animals or the long-ago-irradiated animals and that you can pick these up after a time. Do you visualize these changes effected by irradiation as having a latent period or as developing continuously but slowly so that you just can't detect them until a certain time has passed?

CASARETT: Some of the changes have a so-called 'latent period' only because of difficulty of early detection and others have a real latent period because they are secondary to prior effects. Some changes do not have a latent period. For example, certain vascular changes do not, for all practical purposes, have one. In the case of these vascular changes one can follow in the fine vessels from early times after irradiation the progression of change. There may be rapid acceleration of the rate of change later. However, I don't think that the initial vascular radiation injury remains completely static until a later time; for example, it accelerates aging-type changes in vessels. Other changes, such as late parenchymal atrophy of various organs often occur as a result of vascular change. Certainly, the disease processes, when they come in, create a torrent of deleterious tissue changes, which are secondary and occur late. These dynamics of tissue change dictate to some extent what must be done to test irreparable injury. While an animal has considerable reserve capacities, it is insufficient to use many of the ordinary physiological tests to measure the degree of irreparable injury. One must usually stress the animal to test this effect. According to observed histopathological processes, the irradiated animal is presumably losing reserves at the rate his 'aging processes' are progressing or are accelerated by irradiation. In time, when there is little reserve capacity left to resist stress or disease, the additional tissue changes caused by disease present a picture of rapid decline.

DUCOFF: I ask, of course, out of concern over the possibility that our negative results are simply due to the fact that we didn't wait long enough post-irradiation.

CASARETT: That is a possibility, certainly. It is difficult to detect and measure certain aging effects until long after irradiation.

DUCOFF: With reference to reserve effects, it has been proposed that many of the aging effects seen in animals might be attributed to a decrease in the number of cells rather than to a lowered capacity of each cell. Might this proposal not also be applicable to radiation damage?

CASARETT: In the case of irradiation, as compared with 'normal' aging, I think there may be more of a problem with functionally defective cells, unless one can show that with normal aging there are also increasing numbers of such

defective cells. Cytologically and histopathologically, it seems to me that in normal aging the loss of reserve capacity is associated largely with a decrease in fine vasculature and in numbers of parenchymal cells. One gets the same thing with accelerated aging following irradiation, but there is the additional probability that there are many radiation-produced, persisting, defective cells. In the case of 'normal' aging, this kind of mechanism may be relatively negligible as compared with the decrease in numbers of cells. Yet there are endogenous mutagens or conditions in the normal-aging body which might produce many functionally defective cells. If this were the case, then we might find we had the same situation in normal as in radiation-accelerated aging, at least qualitatively. There may be a difference here between radiation-accelerated aging and 'normal' aging, but at present it is difficult to demonstrate.

KELLY: This perhaps illustrates once again the fact that most of our biochemical studies, until now, have been done during the acute phase of radiation damage; it may now be necessary for some of the expert biochemists to study animals which are past the acute state, even several months past, to try to see if the liver, kidney, and other organs or tissues show any obvious biochemical defects.

ENTENMAN: It would be nice if you had a test that you could use, but I think that Dr. Jones, last night, indicated that about one hundred tests might be necessary, and that he doesn't know of any one test. I certainly don't know of any test that's sensitive enough, or reliable enough, to recommend.

CASARETT: I'd like to emphasize again that, instead of normal degrees of function, reserve capacity for function should be tested, so that most of the tests that would be successful would be based on a challenge to the animal to bring out the degree of reserve that the animal has.

GLASSER: Although I should possibly be more hesitant in reporting to this group data which may be somewhat premature at the present time, I feel moved to mention that we are in the process now, and have completed an initial pilot study, on a challenge experiment of a slightly different nature. Only a single irradiation is involved, and the challenge is a physiological one. An animal population is selected and characterized primarily by nitrogen balance studies during consecutive courses of protein depletion and protein repletion. After the animals have been effectively repleted for a month, they are then exposed to 600 r. Some eight months later, survivors of the irradiation subject to normal animal-room disease patterns are then rechallenged, and protein depletion and protein repletion are again characterized. It's been extremely gratifying to find that, at this later date, the ability of the irradiated animal to withstand his second protein depletion is markedly different from that of his pair-fed control; perhaps even more interesting is the fact that the repletion pattern itself is different. Plotting absorbed nitrogen against nitrogen balance index yields a line whose slope is related to the rate of filling of protein stores, and which indicates that the ability of the animal, some nine months after irradiation, to refill or replete his protein stores is impaired. However, although the data are very clean-cut, there are, because of normal mortality patterns, only six animals in each secondary challenge group. At the present time we are running a fourteen-month challenge group. I think this is very heartening, at least to us in Rochester, because the concept of physiological challenge presents a functional, relatively short-term mode of evaluating residual injury.

ANON: I think it would be disheartening.

BOND: I think there's another problem here that might explain differences in results, having to do with the choice of a criterion of damage. If I understand the speaker correctly, he believes that there are many types of radiation death,

or of mechanisms which lead to death, and that these are quite different. A second, acute LD_{50} may therefore not be the right criterion to use to detect latent damage, and the fact that there is or is not a decrease in LD_{50} at, say, six weeks or so does not necessarily indicate that there is not latent damage that could manifest itself if the animal were given irradiation in a different pattern, or if the correct criterion of effect were employed.

SPARROW: We have been doing work for several years on radiation-induced tumors in plants. One of the conclusions from this work is that if you wait long enough in the life-history of the plant to irradiate it, the radiation apparently has very little effect on increasing the incidence of tumors. I wonder if a similar observation is known for mammals? Would Dr. Jones or anyone else who has pertinent information care to comment on this?

JONES: Although I don't know the answer relative to age, I think the story is quite consistent for humans, for the increase in tumors of the lymphatic tissues, such as leukemia—perhaps lymphosarcoma and Hodgkin's disease should be included here—as compared to all other tumors. When these are increased by a factor of ten, other types of tumor may be increased by only a factor of two.

POWERS: Nobody knows, though, whether a dose of 100 r has the same tumor-inducing capacity in a young animal, or in an embryo or foetus, as it does in an older animal.

JONES: I am unaware of the existence of any information of this sort, and such information ought to be obtained.

DUCOFF: The young animal is much more susceptible to radiation-induced leukemia.

POWERS: That's a special thing.

DUCOFF: Yes, that's a very temporary thing.

CASARETT: There are two time factors involved here. In a control population of normal life span, certain types of tumors have at certain times a peak incidence, after which these types of tumors don't appear in the population very much. In this way some tumors 'have their day' in the population of animals, whether the population is irradiated or not. The other factor is that the older the animal gets, the less likely he is to have enough life left for the latent period for the induction of some types of tumors; something else may kill him before he can develop a tumor. One of the impressions I get from irradiated populations is that there is a lesser induction of tumors with irradiation of older animals than with younger animals, probably partly because of these two factors.

SCHNEIDER: The latent period in carcinogenesis of rats may be dose-dependent too. Using an LD_{100} dosage and saving the rats from death by parabiosis with non-irradiated partners, a high incidence of tumors of varying histological types occurred, and in many animals multiple tumors were found. These animals were about six weeks of age when irradiated, and the first tumor didn't appear until 200 days of life; most of them appeared between the 200th and 300th day of life. Here again, with a single, large dosage of radiation, there is a rather long latent period. We have not tried extrapolations to humans because of the questionable significance of such calculations. Now, it's been said this morning that we have been studying, or talking about, single irradiation dosages and studies conducted following them. However, chronic occupational exposure to radiation is a form of fractionated low (and irregular) dosage carried out over a good number of years in the human animal. About six years ago I attempted, under rather difficult conditions, to run a brief and rather crude survey of a group of radiologists attending a regional meeting. We managed to line up 57 volunteers who let their fingers be stuck for blood counts. This was the only parameter which could be investigated at the time. From the standpoint of the time it

takes to induce any changes, there were at least a couple of tempting suggestive findings. In contrast to a group of controls taken from our own campus, of approximately the same age range as the experimental group, there was a statistically significant incidence of leukocytosis in the exposed radiologists. Morever, the platelet count, which we anticipated might show great differences, showed *no* significant statistical change. The order of probability was not high enough to be significant, although this does not rule out the possibility that the results might be meaningful. But with respect to the time situation, no radiologist who had had less than five years of exposure showed any aberration in total white count, in granulocyte and lymphocyte count, or in platelet count, as noted on a scattergraph of these findings plotted against the total time of exposure. Of course, it's difficult to quantitate the exposure that various people get from the time of their training period onward; and I am sure, too (as Dr. Jones and others may agree), that from the standpoint of the incidence of leukemia connected with occupational exposure to radiation, a decrease might actually be expected, since methods of radiation protection are at least cutting down the exposure which the newer generations of radiologists are getting as opposed to the old.

CRONKITE: In respect to what Dr. Schneider had to say on the incidence of the on-set of tumors of diverse type in rats protected from otherwise lethal doses of radiation by parabiosis, I certainly concur. We've had the same results. However, in animals irradiated at approximately the same age (43 days) with sublethal doses of radiation, the on-set of mammary tumors (following 400 r for example) is very rapid, occurring within three months after the time of irradiation, and increasing for up to almost eleven months; the incidence of tumors induced in this way is dose-dependent. It would appear that irradiation, by bringing about disturbance of endocrine relationships, may ultimately affect the development of tumors in tissues which are not necessarily themselves irradiated. In the cases where supra-lethal doses of irradiation were used, there were practically no mammary tumors. It would look as if functioning ovaries were necessary for the development of the mammary tumors, although it was not necessary to irradiate the ovaries. It is also significant that in our relatively small series of supra-lethally exposed, parabiotically protected animals, there were no leukemias, presumably because the irradiated animal had normal cells seeded from its non-irradiated mate, permitting hematopoiesis to proceed normally from non-irradiated cells. This has not been proved, but is serving as a working hypothesis.

SCHNEIDER: Do you mean, by a supra-lethal dose, more than an LD_{100}?

CRONKITE: Yes, up to 1000 r in rats protected by parabiosis.

SCHNEIDER: The point is that our dose, which was of the order of 700 r, did yield a number of mammary tumors; this was, however, a considerably lower dosage than those you have been talking about. But another interesting aspect of this subject is that rats that are placed in parabiosis without radiation (in connection with other studies) were also found to develop tumors, although at a much lower incidence than in our group in which one partner was irradiated. Many of the tumors that had developed in parabiotic partners without irradiation were lymphoid, whereas we got no lymphoid tumors at all. This again suggests a destroyed lymphoid population rather than a depression or an injury from which the animals could recover and mutate with the appearance of neoplasia. I believe you had a parallel experience in that respect, Dr. Cronkite?

CRONKITE: I think it was identical.

KELLY: May I ask if there is any evidence for a milk factor, or some other virus in this rat strain?

CRONKITE: I know of none in rats.

BOND: May I add that in these same studies, as far as dose-dependency is concerned, the incidence increases with doses of up to 400 r and, from limited evidence obtained in two separate experiments, the incidence does not continue to rise above 400 r, and may even fall off; tumor incidence may either flatten or go through a maximum at about 400 r.

CRONKITE: These are mammary tumors?

BOND: Yes, mammary tumors only; we have essentially no other type of tumor.

ENTENMAN: Along this line I might mention some of the work that COWELL and NOWELL did with a strain of mice that developed a high incidence of ovarian tumors spontaneously. They protected these mice after irradiation by injection of bone marrow, then let them grow old—the incidence of ovarian tumors in the bone-marrow-protected animals was less than that in the non-irradiated animals.

GLASSER: Although these observations are all very interesting, I wonder if anybody has any new ideas or rephrasing of old ideas by which they can relate primary radiation injury to subsequent carcinogenesis.

JONES: I'd like to make a summary remark on this. The situation we face at the moment involves having a perfectly convincing and acceptable story of humoral change associated with irradiation and induction of tumors; and overwhelming evidence for changes in quality of cells. The data indicate proportional effects as far as tumor induction is concerned. I think it very possible that both humoral changes and direct action on cells occur. When we get a 360-degree view of the whole problem, then we'll know whether we've lined it up so that we can see the one thing, or have arranged the facts and parameters so that we can see the other. In the meantime, the two parametrically different viewpoints tend to lead to confused arguing back and forth, as though we were conversing in different languages.

FLANDERS: Dr. Jones suggested last night that there is a very considerable human problem in the rising frequency of leukemia. I wonder whether he would like to comment on what might be done to minimize the future incidence of leukemia.

JONES: I should think the most obvious approach would involve searching for ways to minimize exposure to radiation. I am repeatedly shocked on learning of new sources of radiation exposure that are not recognized or properly respected. Thus I learned here at this meeting about extraordinary exposure in the rural population in connection with the therapeutic use of X-rays for cattle and stock. There is also the 'Inspectoscope' device used not only in all the prisons in California, but also in many of the larger industries for detecting contraband. I have calculated that the use of this device in San Quentin alone involves an average exposure increase for the population of the State of California greater than the average additional exposure due to fall-out at the present time. Various official estimates of the increase in radiation exposure of the total population of the United States have been made. A year ago, this was estimated as being perhaps equal to as much as one-tenth of the natural exposure. At the present time, the best estimates indicate a hundred per cent increase, over natural exposure, associated with all radiation-emitting devices: things like therapeutic and diagnostic X-ray and the radioactive dials of watches and alarm clocks all add appreciably to our radiation exposure.

FLANDERS: Are these estimates not related to radiation received before the age of 30?

JONES: That's correct. If we accept the 30-year-old estimate as 100 per cent increase, the leukemia problem situation would involve a 200 per cent increase.

Moreover, these numbers are probably conservative, because I doubt that we've really properly assessed all sources of radiation exposure. Although we are quite uncertain as to whether the problem is as bad as it is directly pictured, something of this order is afoot, and we ought therefore to restrict radiation exposure as much as we possibly can. I don't yet know how much restriction of radiation exposure will ultimately be necessary, but certainly the permissible dose will be a lesser amount than it is now.

CRONKITE: In part, at least, because there is a newspaper reporter here, I would like to make this statement: We're talking about radiation hazard. There's no question that radiation can be leukemogenic. But in terms of numbers, carcinoma of the lung, which nobody wants to blame onto radiation at the present time, is a much more serious medical problem. There are also many things in our diet and in drugs administered in the practice of medicine for which there's a definite, known, calculated risk of leukemogenesis. There is no question but that radiation is a hazard. But let us not, in our thinking, forget about all the other things in our environment that are of equal or perhaps greater importance, so far as the health of the nation and of the world at large is concerned.

JONES: By way of reinforcement of these comments, I should like to point out that the smoking problem, on the basis of the data of HAMMOND and HORN, can be estimated to be between ten or twenty times more important to health than radiation exposure.

DOWDY: In Japan, an increase in leukemia in children has been noted, if I'm not mistaken. I think Dr. Jones last night said that the incidence of leukemia has increased in Americans 40 years of age or older. It should perhaps be pointed out that during the period of time we've been accumulating irradiation, the longevity of the average individual has also increased an average of 20 years, so we can't be doing too much damage.

BOND: I would also like to say I don't think the situation as far as exposure is concerned is as bad, perhaps, as it may seem. Certainly the degree of exposure is not increasing appreciably, if at all. Various medical and veterinary groups involved in this problem are aware of it, have been aware of it, and are making real efforts on their own to reduce radiation exposure. And the A.E.C. has certainly been aware of this and is investigating the problem.

JAMES: I want to direct a question to Dr. Jones. I wonder, after hearing suggestions of the possibility that TV sets are putting out a certain amount of ionizing radiation, how long I can let my little boy sit in front of my TV set? Are there any data on this?

HALEY: We have made such measurements and found that no appreciable amount of radiation penetrates the rather thick glass face of the tube. You're lucky to get your picture!

SCHNEIDER: I, too, would like to comment on this matter of the relative importance of radiation in carcinogenesis and the overwhelming importance, which is granted, of insecticides, food additives, and other things to the general health of the population, including, possibly, the incidence of leukemia. None of these agents, not even tobacco smoke (!), has the implications of genetic change that radiation-induced changes have. And this is something that nobody is yet qualified to judge, for we have not yet accumulated enough generations or centuries for these newer conditions of radiation exposure to be accurately evaluated.

MEAD: I hate to change from such practical considerations to one that may be fantastic, but would it be possible, or even desirable, to raise animals under completely radiation-free conditions analogous to those employed in raising germ-free animals? I can see, I think, how this might be done, but the problems

would seem to be tremendous.

JONES: This has been seriously talked about during the last year. HUBERT YOCKEY was the first to bring up this point and I think it would be a very worthwhile thing to do. However, in order to be convincingly effective with respect to relatively longer-lived animals such as mammals (rather than, say, the fruit fly) this would involve problems of rather extraordinary magnitude. How would a suitable radiation-free environment be produced?

KELLY: As I understand it, the natural background is about half due to cosmic radiation, which could presumably be shielded out, but the rest is due principally to radioactive potassium^{40}. How do you get rid of this?

JONES: That is the critical problem. Obviously isotopic separations would have to be carried out to get rid of natural radioactive isotopes.

MEAD: Dr. WARREN said that a few years ago the Casino here on Catalina was offered to the U.C.L.A. Medical School. Perhaps the Casino's diving bell could be sunk in the ocean and used as a laboratory in which to carry out the necessary inorganic separations.

FLANDERS: Don't forget that there's quite a lot of radium in sea water.

LEVEDAHL: The sea water would presumably be on the outside of the bell!

JONES: The problem of making a great reduction in radiation exposure is not particularly formidable; but the problem of producing an absolute absence of radiation makes the absolute vacuum problem seem like a piker in comparison.

MCKEE: I think Dr. Sparrow would not be reluctant to tell about some of his induction tumor work and that my asking might get him to do so.

SPARROW: We have worked mainly with two different plants, one of which is an interspecific tobacco hybrid (*Nicotiana glauca x langsdorfii*). This plant is rather unique in that when it reaches the flowering stage, or the seed-producing stage, it spontaneously produces a fair number of tumors. In a mature plant, these tumors may become quite large; by large, I mean half the size of your first—large enough to cause the plant to droop way over. In young pre-flowering plants, the incidence of tumors is exceedingly low. You may have to look at 100 plants before you find a single tumor. Now when these plants are irradiated, as young seedlings or as small plants, the situation is, as far as I can see, essentially parallel to the situation in mammals in that the onset of tumors occurs much earlier and the incidence of tumors is very much greater, at least up to the stage where the spontaneous ones begin to appear in significant numbers. If you let the plants grow to maturity, this difference is much harder to detect. But to take a typical example, plants grown at a sublethal but growth-inhibiting dose range such that their normal growth is seriously hampered, will produce, in terms of amount of tissue, roughly five hundred times as much tumor tissue as an un-irradiated plant.[1] Now there are some advantages, I think, to pursuing this sort of work in plants. One of the obvious things in favor of plants is that there is no metastasis; and if you have two separate tumors you can be quite sure these represent two separate events (unless they are extremely close together). I might say that these tumors can be induced by whole-plant irradiation, either acute or chronic, by partial plant irradiation, or by internally absorbed P^{32}. We have also been investigating radiation-induced tumors in the leaves of a succulent plant, *Graptopetulum paraquayense*. This plant produces a variable number of small, self-limiting tumors in leaves several weeks after irradiation. Because they are self-limiting, small, and all about the same size, these tumors are well-suited to quantitative work. They are visibly detectable when young because they are a lighter green in color than the surrounding leaf tissue. When somewhat older, they are visibly detectable by external observation of the leaf by virtue of the fact that there is

a red pigmentation deposited in or around them. This last system (*Graptopetulum*) was only discovered about a year ago and we do not have very much data on the relative potentialities of different treatments and so on, but, again, I think this system has a lot of possibilities for experimental work.

May I take one more minute to make another comment by way of contributing to the free-discussion period? The model cell previously discussed did not have a chloroplast in it. This may have been either deliberate or accidental. The reason I am bringing it up is that there are two very important radiobiological reactions that involve chloroplasts. The first is the well-known chlorophyl mutation system. I don't need to comment further on that here. The other, less well-known and but recently discovered at Brookhaven, is a reversible reaction which affects the chloroplast system in such a manner that small areas of chronically irradiated Tradescantia plants lose all of their visible chlorophyl. If you examine these areas externally, they look completely albino. Under two conditions these albino areas will return to normal. One is when the plants which have such areas are removed from the radiation field and allowed to grow in their normal environment. The other one, which is a little more difficult to explain, is when they recover spontaneously even while kept at the same level of radiation under which they developed the condition. Why they lose their chlorophyl, and why in certain cases (but not all) recovery occurs even when irradiation is continuous, I have no idea. But both of these I think are rather interesting systems and I hope you will agree that chloroplasts should be added to the model cell.

BERNHEIM: Is there any evidence that there is an alteration of the gibberellin or auxin content of these plants that have developed tumors? Could there be a hormonal misfunction here in the same sense that there might be in the animal?

SPARROW: There is no direct evidence on this point. There is some indirect evidence, however; first, that irradiated plants generally go through a depressed auxin stage; and second, that certain plant tumors are known to be associated with a disturbed auxin metabolism.

SCHJEIDE: Was whole-plant irradiation involved in this last instance?

SPARROW: The tumors develop under either whole- or partial-plant irradiation. Moreover, the leaves can be removed and tumor formation observed in the isolated leaves. This is quite a useful system because the leaves will stay alive for at least six weeks or so after being excised from the plant (*Graptopetulum paraquayense*).

SCHJEIDE: This reminds me of the case of the snapdragon. It may be a different manifestation of a basically similar process.

BOND: I'd like to ask Dr. Sparrow a question. In view of the fact, as you have pointed out, that plant tumors are not invasive, how do you distinguish between benign and malignant tumors in plants and, if so, what are the criteria?

SPARROW: This question is frequently asked. It's a little difficult to give a satisfactory answer to a mammalian investigator because he uses rather definite criteria to characterize a tumor, some of which are not applicable to plants. But the best I can say, I think, is that Dr. QUASTLER, who is very familiar with tumors in general, feels that, aside from the invasion phenomenon exhibited by the usual type of malignancy, these plant tumors pretty much parallel mammalian tumors.

BOND: They're parallel to the mammalian malignant tumor, rather than the benign tumor?

SPARROW: Well, no; I assume the self-limiting tumors in the leaves would be considered benign. They would not correspond, in my opinion, to malignant tumors in mammals. But those in the tobacco hybrid I would consider to be

comparable to malignant tumors in mammals. The plant anatomy is such that they are able to break through the epidermis of the plant and grow externally. They use up a lot of energy, of course, but they are practically never lethal to the plant as a whole. However, plants which bear a large amount of radiation-induced tumor rarely reach sexual maturity.

KELLY: Dr. Sparrow, do these tumors arise by the resumption of mitosis of a cell which normally would not divide again or do they arise from meristem cells?

SPARROW: It's quite clear that some of them arise in meristematic areas. It's also quite clear that many of them arise in areas where the rate of cell division is exceedingly low, but I do not think we can say that there are no cell divisions. Many mature plant tissues have the capacity to initiate cell division under certain conditions, such as injury. It is always possible, of course, that cell death is produced by the radiation and that this acts, as does mechanical injury, to stimulate cell division. But as to why it should become uncontrolled or tumorous, of course, we have very little evidence and lots of speculation.

MCKEE: Did you say, Dr. Sparrow, that there are no tumors formed outside the boundaries of the irradiated area?

SPARROW: We have done several experiments to try and get at this. However, the results are not wholly conclusive. I am quite sure that about 90 per cent of the tumors that form, when you treat a plant with localized radiation, are either in the irradiated area or at the margin. But there are a small number that are outside. And we just haven't done enough work to be able to say whether the number outside could be the expected number from spontaneous incidence or whether there is really some humoral factor involved.

HENNESSEY: This is probably a little out of line but I'd like to return to a statement of Dr. Schneider's a few minutes ago, concerning the genetic implication, because radiation has been studied extensively. It seems possible that genetic effects of Milltown, smog, and so forth may not be known, simply because they haven't been studied as extensively as radiation. I'd like to hear comments along that line.

POWERS: Caffeine is being recognized as a substance that can damage protozoans, and this, then, makes coffee suspect.

BOND: At what concentrations?

POWERS: The effect is linear with dose.

HOWTON: Are there any further comments on the chemical induction of tumors?

SCHJEIDE: It would appear to be quite precarious to be alive!

HALEY: HUEPER, of the National Cancer Institute, is taking quite a long and jaundiced look at a number of materials that are in widespread use in the United States. And I know he doesn't go along with the smoking proposition that's been put forth, having to do with increase in bronchogenic cancer, because he states that we don't have a good unassailable correlation. In other words you can correlate tumor incidence with the number of miles of asphalt road or with the amount of rubber that's ground off automobile tires. You can go right down the list and pick up any number of what we love to call non-noxious materials and correlate them with an increased tumor rate. And for the benefit of the ladies, he has taken a rather jaundiced view of the use of PVP hair sprays, based on the results of some intraperitoneal implantations that he made. A year later, the sites developed rather nice tumors. So, I think that, while it is hazardous to be alive, we shouldn't get too worked up about it. We should be cognizant of the situation and not be prone to blame all our troubles on radiation. There's an awful lot of other things that could be responsible.

DOWDY: The U.S. Public Health Service has published a book of the various

types of drugs and chemicals that are being used by various investigators to induce tumors in mice, dogs, rats, etc. As I recall, the last issue had something like 2200 drugs and various other chemicals, 25 per cent of which have been known to produce tumors in animals, mostly mice. So carcinogens are apparently around us all the time. But I still like my liquor!

KELLY: Sometime during this 'free discussion' period, I think we should give the chemists a chance. They've been rather overwhelmed by the biological discussion and quite unhappy that we're asking questions that are entirely beyond the scope of present-day chemistry. Dr. Daniels will start us off in the proper direction.

DANIELS: I have been rather provoked into doing this by uncalled-for inferences of laziness on the part of the radiation chemist. This perhaps arises from the biologist asking questions which chemists, attacking from the other (molecular) end of the phenomena, do not aim to answer. For example, much of the difficulty in the interpretation of the effects of radiation on chromosomes and on their behavior stems apparently from the absence of basic knowledge concerning chromosome make-up. The link-up between the microscopic and molecular levels of investigation is missing. It is hardly surprising that, under these circumstances, radiation chemists find it difficult to answer biologists' questions. What we can do and are doing, however, is to find out what is happening on the molecule level as a result of irradiation, and, as an example of this, I have a few facts here, some fairly recent findings, as yet unpublished, which I think may be of interest to you. I take it that most of the published work on DNA in aqueous solution is known to you. One of the main problems has been to get a measure of the breakage of the sugar-phosphate backbone of DNA by radical attack. Previous work—viscosity work—has indicated that there were two effects: a direct effect measurable immediately after radiation, and a slow after-effect. But the interpretation of these phenomena in terms of molecular weight change or in terms of the breakdown of the chains is very difficult because of the lack of chemical knowledge of the behavior of polyelectrolytes. A method has been used this past year in Newcastle, which we feel provides a measure of this chain breakage. When the chain breaks at either of the two sites indicated in the diagram below, terminal monophosphate esters will result. The amount of liberated terminal phosphate has been estimated using phosphomonoesterase.

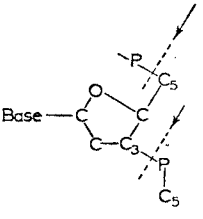

Fig. 7.1.

We have always thought this chemically very interesting, however remotely connected it may be with chromosome breakage *per se*; for example, it must be considered if a complete accounting of the radiation-produced radicals is to be attempted. It now appears that changes in the bases of DNA may have considerable genetic implication—but again, that's not really the chemist's business.

HOWTON: What precisely do you mean by *attack* on 3 or 5?

DANIELS: Radical attack. An 'OH attacking or abstracting hydrogens from these carbon atoms will lead eventually to the breakage of a C–O bond and to production of a terminal phosphate group. These are estimated using the phosphomonoesterase after irradiation. Radiation employed was either 200 kV. X-rays or Co^{60} gamma rays, in the presence of air, and with DNA concentrations in the range of 0.1 per cent to 10 per cent—somewhere near biologically interesting concentrations. The yield of terminal phosphate (in terms of G values) was 0.4, independent of concentration. How this correlates with the after-effect observed in viscosity measurements is not quite clear at the moment; further work on it is needed. It depends really on the time required for the estimation of the monophosphate and the rate at which the presumed after-effect occurs. Other work is concerned with the other end of the molecules, where splitting is possibly related to chromosome breakage and to the structure of DNA and has genetic implications; but this is not really any sort of chemist's business. Early work, concerned with the effects of radiation on the bases of DNA, involved rather high doses which resulted in very complex secondary effects, extensive degradation, formation of acidic products, etc. However, by observing changes in the optical density at 260 mμ at different pH's and by appropriate interpretation, I think we now have a measure of two sorts of change taking place in the base linkages. The overall light absorption at 260 mμ is an algebraic sum of the absorption of the chromophores in the bases themselves, upon which effects due to hydrogen bonding are superimposed. The irradiations were carried out at pH 8 to avoid as much denaturation as possible. The extinction values of the irradiated solutions were then measured at pH 8 and also pH 3 to give two different sorts of curves. At pH 8 a maximum is observed in the plot of ϵ_{260} against dose (see Fig. 7.2a). Thus, depending on the dose of radiation, either an increase in the

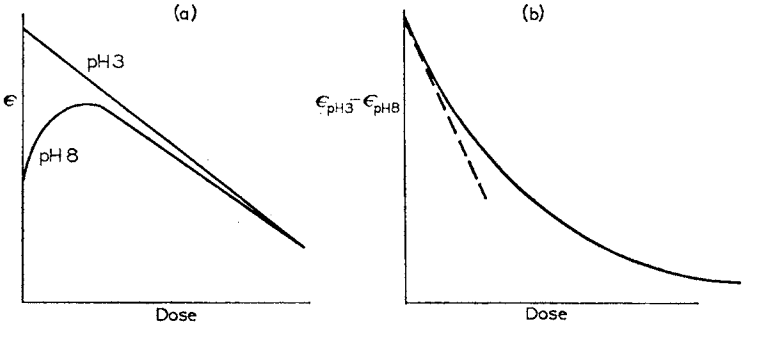

Fig. 7.2 (a) and (b)
Schematic representation of changes in optical density at 260mμ of DNA as a result of irradiation (not to scale)

extinction or a decrease may result. This phenomenon is of interest in connection with some of the apparently conflicting results which appear in the literature. The acidified solutions yield a much simpler curve, in the interpretation of which we presume that (at pH 3) all hydrogen bonds are broken; under these conditions the observed effects are those due solely to changes in the chromophoric groups of the bases themselves. Moreover, the difference between the two curves is related directly to alterations of hydrogen bonding affected by irradiation. In Fig. 7.2(b), this difference is plotted against dose. From such treatment of data

it has been shown that G for the breaking of hydrogen bonds, based on the initial slope, is markedly dependent on the concentration of DNA. In very dilute solution (0.006 per cent) the G value is 3.2. In 0.1 per cent solution it is 13—a considerable increase as far as this sort of chemistry is concerned. At the same time, attack on the bases, leading to the disappearance of absorption as the concentration is lowered from 0.006 to 0.1 per cent, decreases from 1.9 to 1.2, which is the magnitude of yields normally expected for radical effects. This is certainly not the case for the hydrogen-bond-breakage reaction. How the hydrogen bonds are broken is the subject of speculation. In this connection, there are two suggestions about which I'd like to hear your opinion: firstly, that transfer of energy from the interstitial water within the strands of DNA, excited by radiation, to the bases, breaks hydrogen bonds. This suggestion, of course, requires that there be water molecules within the strands of nucleic acid. The other supposition is that there may be, at the higher concentration, a direct energy absorption by DNA, and, in fact, by the resonating rings that are present in the bases, such as to lead to a breakage of hydrogen bonds.

HOWTON: How does your first suggested mechanism fit with atomic dimensions? Is there room to accommodate water molecules within the spiral?

DANIELS: I think so, yes. But this is simply a possibility, and whether or not there's any experimental evidence to support it I don't know.

LEVEDAHL: I have a question about the procedure. Have you measured only at pH 8 and at pH 3? At pH 3 it would appear that all of the hydrogen bonds are broken, but it would also appear that at pH's between 3 and 6, say, this method might be used to determine what bases are involved in the hydrogen bonding, knowing the dissociation constants of the individual bases. Have you followed this approach to any extent?

DANIELS: No.

KELLY: How is the DNA prepared? I notice a very large difference in the absorption of your control. Is the material ever heated in acid?

DANIELS: No, the solutions are simply acidified at room temperature just prior to determining their extinction coefficients.

FLANDERS: Is anything known about the change in viscosity and in molecular weight on acidification?

DANIELS: Yes. Viscosity decreases both on acidifying and on making such solutions alkaline. These effects are irreversible, involving the breaking of hydrogen bonds and the separation of the nucleic acid strands.

TOTTER: I'm not sure I quite understand your first possible explanation of this. Is what you proposed equivalent to what KLATZMAN talks about in the denaturation of protein, where the sudden appearance of a change results in reorientation of water molecules? I think it's been estimated that this involves energy sufficient to break 15 to 30 hydrogen bonds.

DANIELS: It's something very similar to that.

LESSLER: One thing that disturbs me about this type of data is that it always revolves around breakage of bonds, while there's very little evidence on the rate of reformation of these bonds. It seems that as you measure things like this you're measuring only an average. In addition to hydrogen bonds being broken, hydrogen bonds are also being formed. This is an easy bond to make, relatively speaking. It doesn't break irrevocably. With water molecules all about, other hydrogen bonds can form rather easily. Similar remarks might also apply to phosphate bond breakage. The observed changes may not be due to breakage alone, but to the difference between breakage and reformation in the particular chemical environment which is being studied.

DANIELS: With regard to the last point, the phosphate bond, once broken, is not likely to be reformed. That is a different matter altogether. An ester bond is quite difficult to form in an aqueous environment.

LEVEDAHL: I have some remarks that I think might be pertinent at this point. Dr. James and I have for some time been using optical rotation at various wave lengths as a measure of breakage of hydrogen bonds in DNA, RNA, and several other compounds. From the viscosity data and from the normal optical density data, after denaturation has occurred (if that term can be used for DNA) there is a partial recovery which would imply reformation of bonds. Using rotary dispersion to study this reaction, we can show rather clearly that if bonds are formed they are not the same ones that were broken. The configuration of a molecule, as measured by optical rotation, once changed, remains changed, even though the viscosity and the optical density may partially recover, so that, while it's true that hydrogen bonds may be reformed, they are apparently different from the bonds originally present in the molecule. At least there is a difference in the way they allow the molecule to reform.

HOWTON: I think it ought to be emphasized that we're talking here about hydrogen bonds that retain the 'natured' configuration of the DNA molecule. These cross the center of the spiral and are of the N–H–N and N–H–O types. Once these are broken the liberated nitrogen atoms, for example, are going to be solvated; in other words, they will form hydrogen bonds with water molecules. It should also be pointed out that a system of very high order is being destroyed, making reversal very unlikely. Moreover, some N–H–N types of hydrogen bonds are being converted to the N–H–O type, which is more stable; this provides driving force for the reaction, and tends to prevent restoration of the original arrangement.

LESSLER: Just to throw in the biological equivalent, which I for one have been trying to work back toward, we know that chromosomes break. We also know that most of these breaks are very, very rapidly repaired. Now this involves a very high order of repair of hydrogen bonds and of phosphate ester bonds in many, many thousands of strands of nucleic acid. All I ask is that the chemistry fits the biological observation.

HOWTON: What is the evidence that this process involves the reformation of phosphate ester bonds?

LESSLER: I should think it would be difficult to escape this conclusion, since chromosome breaks are known to be repaired. This must mean either that new strands are formed, which would include new phosphate ester bonds, or that there is some peculiar arrangement of the two ends.

HOWTON: I think the important point is that we don't know that a phosphate ester linkage is broken when the chromosome is broken.

FLANDERS: I'd like to make two points here—the first is in relation to Dr. Lessler's remarks. He says the chromosome thread is broken and is later reformed. I wonder if this is indeed the case. It may be that a few molecules are broken and that some of these rejoin so that after a time lapse an intact chromosome is observed. But I doubt whether the whole structure, including thousands of molecules, is ever broken and then reformed. Nor is this seen to happen, for breaks are usually observed an hour or more after irradiation. The other point I want to make is in relation to Dr. Howton's remarks on nitrogen—solvation energy. You may be familiar with the rather beautiful experiments of Dr. RICH, made possible by the availability of the enzyme, discovered by OCHOA, which induces polymerization of nucleotides to form ribonucleic-acid-like molecules. Dr. RICH has made polyadenylic and polyuridylic acids in this way. These two will

combine together. The polyadenylic acid wraps around the polyuridylic acid *in vitro* with remarkable speed, forming a twin spiral. In this case the hydrogen bonds between the bases reform with astonishing facility.

JAMES: But here, with polyadenylic and polyuridylic, one is using two consistent polymers (i.e., homogenous from end to end). The hydrogen bonding could presumably occur in this case without any specificity limitations in the linear array. A base at position x would not have to combine with its precisely opposite kind at position x on the other molecule, so that the reformation of the hydrogen bonds of two completely complementary chains would be expected. The re-association of two complementary linear patterns of polynucleotides would require more luck.

MEAD: Dr. Levedahl actually deals with these high order reactions. Isn't it true that, in a fairly rigid structure, the destruction or breaking of one hydrogen bond may have nothing at all to do with any change in the structure? A certain number of contiguous hydrogen bonds have to be broken simultaneously before you get any change, because a hydrogen bond can be broken and reformed without any real damage. Thus something of the order of 13 or so hydrogen bonds must be broken simultaneously (or practically simultaneously) before a break in the structure occurs. Is this correct?

LEVEDAHL: Yes. I think that as a matter of fact SCHACTMAN and his colleagues have made some calculations of the exact number of bonds that must be broken. I'm not sure what the number is, but it's fairly high.

TOTTER: It has changed from time to time.

LEVEDAHL: It has never decreased in size, however.

SCHOLES: There's a possibility that if a large number of hydrogen bonds are broken in a localized region, particularly at the end of the helix, then the whole molecule might unfold as it is invaded by water molecules.

LEVEDAHL: I believe the group at Berkeley has made a number of calculations concerning this unzipping of the molecule, but I don't feel that the kinetics are those to be expected for such an unzipping act.

KELLY: Would unfolding of DNA or breakage of hydrogen bonds or something of this sort, in terms of the cell, produce a point mutation perhaps? We're surely not talking about chromosome breakage, are we? Is this something that everyone is willing to agree on? That if this has a biological significance it would involve a point mutation, a gene mutation?

LEVEDAHL: I don't think we can agree, simply because we don't have enough information. The important feature, I believe, of a localized rupture of bonds is that this could be magnified by the energy made available from changes in configuration, so that ultimately some much larger structure might be ruptured. Once the modification has occurred, energy is available to continue the destructive process. But I know of no evidence that would either prove or disprove this point.

SCHOLES: I'm not sure I understand the source of this destruction-propagating energy.

LEVEDAHL: If you have a relatively rigid structure and break a large series of hydrogen bonds so that you get increased movement, you then have a large entropy increase in the molecule, and this in turn would permit the rupture of a much larger structure, or such disruption would be reflected in a larger structure. I know of no information that allows us to take even the first step, which would have to involve correlating the helically coiled DNA molecule with the chromosome.

PERSON: But there is a considerable disparity between physical studies and

biological phenomena. By the time the backbone chains are broken, biological function has long since been lost. For example, with reference to radiation effect on RNA, I think LAFNER points out that you need something like six times the 37 per cent survival dose before you start getting something like a 50 per cent reduction in viscosity.

DUCOFF: There has been some discussion of differences in sensitivity of different cell types. I wonder if the chemists can say whether there is any difference in sensitivity of DNA from different cell types? Or is all work done on the same type?

SCHOLES: Although most work is done on DNA from a single source, I believe I recall a paper having been presented at the 'Peaceful Uses of Atomic Energy' Conference held in the U.S.S.R. which dealt with this point. I believe it was reported that sensitivity of nucleoprotein *does* differ depending on the source of the material.

SCHJEIDE: I am thinking of another point which should be considered with respect to a focal point of attack in the cell. Since it has been indicated that nucleoproteins are more sensitive than nucleic acid *per se* and this is the form in which nucleic acid exists in the cell, the chemists should extend their remarks to nucleoproteins.

LESSLER: I wonder if Dr. Levedahl would comment on the consequences, say, of a few dozen hydrogen bond breaks in a nucleoprotein molecule as compared with those of a similar alteration of the double helix molecule. You've just presented what I think is a rather intriguing notion of increasing entropy which might explain a great deal of action or disintegration on the part of such compounds.

LEVEDAHL: Any comments that I have to make will be pure speculation, because I know of no one who has a good preparation of nucleoprotein with which to work. The point that I tried to make last night was that it seems to me that there is a tremendous amount of evidence slowly accumulating that would lead one to suspect, at least, that the important changes are those which occur in the protein part of the molecule. By way of reiteration—recent calculations have indicated that something of the order of magnitude of 15 to 20 ion pairs are required for nucleoprotein inactivation. Protein denaturation has an order of reaction of about the same magnitude—15th order. Sulfhydryl groups are very sensitive to radiation. Histones, I understand, contain sulfhydryl groups. Enzyme denaturation is connected very intimately with breakage of disulfide and formation of sulfhydryl groups. All of these bits of evidence seem to add up to the conclusion that a very profitable venture, if some biologist will prepare a satisfactory sample of nucleoprotein, would be to look at the denaturation phenomena of the protein part instead of the nucleic acid part of the molecule.

PERSON: But there is a very big difference in molecular weight involved here. A chromosome is a very complex thing. You're talking about protein having a molecular weight of 10,000 compared with chromosomes that are microns long. True, you have to get 20 ionizations to see an effect. But isn't it possible that lots of ionizations occur which are not detected because the breaks are repaired? With this very stable structure, there may be, say, 19 repairs for every 20 ionization breaks.

LEVEDAHL: I think that this may well be a problem of semantics. Actually, as I understand Dr. Howard-Flanders' statement, interpreting LEA'S results, there were required essentially 20 events in a relatively small area, occurring approximately simultaneously. This would then be comparable to the order of reaction that you observe for the denaturation.

KELLY: May I point out to the chemists that several years ago MIRSKY and RIS developed a method for isolating something which is, at least cytologically, very

close to a chromosome. Perhaps irradiation studies of that sort of material would be worthwhile.

SPARROW: As there are a number of chemists and physicists here, I would like to point out that spontaneous chromosome breakages occur which are apparently not due to natural radiation in the environment, but to thermal instability of the molecules. The well-known phenomenon of crossing-over, which occurs in meiotic prophase of most plants and animals, is thought to be the result of controlled chromatid breakage and reunion. The only reason I'm bringing this up is that if this can occur in the so-called natural environment, presumably the amount of energy required for this must be rather small compared to the 20 ion pairs assumed to be required for radiation-induced breakage. There seems to be a gap here between the spontaneous biological type of chromosome breakage and that which is radiation-induced. What the explanation is, I don't know. Would anybody care to comment on this?

RUSTAD: I think we've made a major breakthrough in getting a little bit off the pure DNA to nucleoprotein. Perhaps for the benefit of some people who may not be aware of this, it should be pointed out that there are certainly many other constituents of what we identify as, say, the metaphase chromosome. RNA has been demonstrated to be present in what would superficially seem to be fairly great amounts. Certainly we associate RNA with the interphase chromosome as well, as least in nucleoli; and if we assume the old DNA-makes-RNA-makes-protein type of thing, there must be a period of intimate association during such synthetic processes. I believe there is also evidence for some lipoproteins and a few things of this kind. Maybe Dr. Lessler could supply some additional information along these lines.

LESSLER: I would like to comment here, for the sake of our chemistry-minded friends, that chromosomes do indeed have a lot more in them than DNA and protein. There is a significant amount of lipid in the structure. And there is evidence of lipoprotein being present. Carbohydrate has also been isolated from chromosomes.

KELLY: I think that, since we have just half an hour left, we should perhaps see whether anyone feels that there is a vast area of radiobiology that we have not touched on in this conference which perhaps might be a subject for a future conference. We've spent a lot of time on DNA and chromosomes. Any takers on this?

RUSTAD: I think that, with the exception of Dr. Ducoff's paper, cell division delay has only been peripherally mentioned. I think we consider this to be of great importance even to the mammalian system in the overall radiation syndrome because it seems clear that a cell that can't divide for a long time may be headed for trouble; and there are certainly many theoretical implications which would indicate that this might be important. The extraordinary sensitivity of the cell-division process should in itself give us some sort of jumping-off place. Why should something that we've imagined involving a whole series of very large-scale chemical reactions be so sensitive to irradiation?

LESSLER: Before we go ahead with this, may I add one thing for the record? RNA also occurs in the chromosome in considerable amounts.

SCHOLES: As a nucleoprotein?

LESSLER: RNA is demonstrated by RNAase action on the chromosome.

DANIELS: What sort of enzymes are present in the chromosomes? Can you tell us that?

LESSLER: Oh! No!

KELLY: Any other areas of research?

SCHJEIDE: I'm not clear on just what you are asking. Are you asking for suggestions without discussion?

KELLY: I'm sure that there are rather large segments that no one has discussed, and I just thought they should at least be listed for the record, perhaps, so that people realize that we are aware of them.

SPARROW: There's one type of reaction that was implied, perhaps, in the model cell that I do not think has really come out into the open, perhaps because it's more readily detected in the plant than in the animal cell. This is the complete inhibition of spindle function at very low doses. In some plant cells, e.g. *Trillium*, doses as low as 50 r will inhibit a significant number of spindles, while doses of 400 r will inhibit roughly 90 per cent of the spindles; this is manifest in the production of polyploidy. While I think it is common to find multipolar spindles in animal cells I don't think the complete inhibition or destruction of the spindle has been reported for these low doses in animal material.

SCHJEIDE: Another area which has not been discussed, and may perhaps be fruitful, is the possible coexistence of enzymes and inhibitors and also of viruses and inhibitors. One action of irradiation may be to release such inhibition. This might prove lethal for some cells. In experiments carried out in our laboratory we have sometimes observed increasing enzymatic activity as a function of increased radiation (in the range of 500 to 5000 r). This would seem to be the result of very rapid synthesis (the cells are broken up half an hour after radiation) or, more likely, release from an inhibiting substance.

MEAD: I would like to mention a few things with the hope of provoking Dr. Bernheim and perhaps some others. In the past we have obtained evidence that irradiation of the polyunsaturated fatty acids *in vitro* forms peroxides via a chain reaction. It is undoubtedly true that these peroxides are extremely toxic to the animal both by injection and orally. It is also true that we have as yet been unable to find out why they are toxic or how they exert their effect; we have the pathologists working on this. Now the question that one asks is: Can such peroxides be formed inside the animal by irradiation? And I think we have to answer this in an equivocal manner. There is very good evidence from several groups that the peroxides can be formed. First of all, the question is, can they be formed in the depots where most of the lipids are found? I think there is good evidence that they can. Then, the question is, do they do any damage in the depots? I would think, just offhand, that the damage that might be done by this formation of peroxide *in vivo* in the depots is relatively minor. It might result in the destruction of vitamins or some other susceptible molecules, but that isn't what we're looking for. The second point is: Can they be formed in the circulation? Dr. Schjeide mentioned some results he has that show that lipoproteins are susceptible to such damage, that they do form fatty acid peroxides, and that this results in the destruction of certain other constituents of the lipoproteins. Well, is this damaging? Frankly, I doubt that this, too, is the type of damage that we are looking for, although it might be a contributing factor. This leads me to the question that I'm really interested in finding out, and that so far hasn't been very fruitful, although I think it may in the future: What will happen if such peroxides are formed intracellularly? There are two damages we want to look for. One is the formation of peroxide *per se*. The other is the destruction of antioxidants, facilitating formation of peroxide in the cell. If such a reaction took place in the mitochondria, where it might very readily, because certainly the mitochondrial enzymes are composed partly of lipoproteins containing unsaturated fatty acids, this might result in an increase in the oxidative processes, which is something that we are presently investigating.

If it took place in the microsomes it would probably result in a decrease in synthetic processes. And this we are also looking at. If it took place in the nuclei, and I think it has been very revealing to me to discover that the chromosomes do have large amounts of lipid—is this not the case?

LESSLER: Reasonable amounts—something like five to ten per cent.

MEAD: This might then give us a rationale behind the multiplication process for damage to the chromosomes or to the chromosomal protein or nucleic acid, in that unsaturated fatty acid autoxidation is a chain reaction. If you have a number of these molecules side by side you're going to get a multiplication of your original ionization effect with the resulting formation of radicals that can react further, are much more stable, but are equally damaging.

BERNHEIM: I might mention here a crude experiment with crude DNA. Using viscosity change as evidence of effect, you find that when you add unsaturated fatty esters to crude DNA, there's no change in viscosity. But if you irradiate the fatty acid ester, forming peroxides, then add it to the DNA solution, the viscosity drops tremendously. Now this might mean that if you irradiated the nucleus, producing peroxides in very close conjunction with DNA molecules, you might be able to break hydrogen bonds involved in retention of the ordered DNA structure.

MEAD: What happens if you irradiate together?

BERNHEIM: Well, then you get equivocal results, presumably because you're irradiating the DNA as well as the fat. We have therefore routinely irradiated the fat before adding it to the DNA.

KELLY: I'd like to bring up a subject which I think we have essentially completely ignored, and which may or may not be of considerable importance in radiotherapy. Dr. Dowdy, I think, might comment on this. In the literature it has been suggested that radiation inhibits cell division, but perhaps stimulates cell differentiation. It is not clear to me whether there is any good evidence for this, or if most of these observations are due to changes in cell population. I think that this might be a very fruitful area for research, particularly if the biochemists would give us some methods of studying differentiation.

DOWDY: GLUCKSMAN, of course, feels that radiation helps the cell to maturate; and, following radiation, particularly in an epidermoid carcinoma, in addition to other things that happen, he postulates that the cell may be rendered nonviable. By this he doesn't mean that the cell is dead, but that it has lost its ability to divide and reproduce other cells. If such cells continue to live out their expected life span and then die and disappear, the tumor thus eventually disappears through lack of reproduction. He has observed that the cell becomes keratinized and produces pearls and has used this observation to determine whether radiation will produce a good clinical result or not. He feels that he can tell this within perhaps a couple of weeks or so after the radiation is applied, or between the first and second radium treatment. I must admit he showed me an awful lot of material and that he seems to have something. I think if we knew ways in which we could make these viable tumor cells non-viable that we might have a more rational approach to the radiation treatment of cancer. I think this should prove to be a very fruitful field.

JONES: I think there's no question but that this does occur. But the question is, how *frequently* does it occur? According to SPEARS' discussions, it seems to be relatively rare that it occurs to the majority of tumor cells in the field.

DOWDY: I got the impression, from the material I looked at, that it was rather common for a carcinoma. Now he didn't show me anything of different types of carcinoma and I suspect that in other types it is quite clear, but believe that in epidermal it possibly is not.

POWERS: The differentiation which is being alluded to is not necessarily the same differentiation that the embryologist talks about, such as the transformation of a neuroblast into a neuron. It is likely to be highly radiation sensitive. It is also likely that radiation will not speed this process up but will inhibit it at dosages which are considerably below those doses which bring about other effects, like blocking cell division. Take, for example, the irradiation of a grasshopper embryo in such a way as not to affect cell division at all, and note that there may be no embryonic differentiation in this embryo whatsoever. A large mass of cells is formed but there is no formation of organs or organ systems.

SCHNEIDER: A parallel study to that of GLUCKSMAN, which Dr. Dowdy mentioned, might also be an interesting avenue of approach, provided that some testing method could be evolved. Such a study would be concerned with the fact that in a single field of radiation one can frequently produce a clinical cure (a destruction of a malignancy) and yet also obtain repair of the previously normal but now heavily-irradiated tissue which formed the tumor bed. Not only tissues of different germ-layer origin are involved, but also the same ones, as in the repair of epithelium, although the squamus cell carcinoma may be destroyed. Hence a different sequence occurs in these two cells from the same germinal tissue, in their ability to repair. I don't know whether this can be quantitated, or would lend itself to cellular studies by the radiation chemists.

JONES: I wonder if this sort of an effect might be closely related to the sort of thing that PUCK has described in which cells go into a giant stage without dividing. It might even explain some of the total effects that we are on the fringe of understanding about human growth and animal growth.

SCHJEIDE: There are possibly basically different mechanisms involved in the processes which we have talked about in the tumor beds and in the case of embryonic differentiation. It seems that embryonic cells which are in the process of differentiation are very similar to lymphocytes, and to the developing ova and sperm, in that they seem to be abnormally sensitive. Let me reemphasize this. When these embryonic cells are actively differentiating, they are more sensitive than they are when building up toward division. Radiation kills the differentiating cell, but the non-differentiating cell has the capacity to adapt. They will develop enzyme systems which differ from the normal enzyme system and in this case may continue to divide, forming a mass of undifferentiated cells. In the case of the tumor, you might be killing off cells which are sensitive because of lack of capacity to deal with oxidizing radicals and leaving a population which, *from the very beginning*, had better-developed oxidative enzyme systems and antioxidant properties and less tendency toward completely anaerobic mechanisms.

DOWDY: From the material I saw, I don't think that to be correct. Of course all of you who are familiar with or have seen biopsies realize that you can get almost any type of cells, depending on what part of the tumor you take them from; but as best one can tell, there is very little keratinization at all that one can observe within the areas. Moreover, by taking repeated biopsies during the course of treatment, these areas of keratinizated cells are seen to increase very, very markedly.

POWERS: But this differentiation is a destructive kind of differentiation.

DOWDY: It's what goes on normally in your skin.

POWERS: That's right, but that's destructive also. These cells are dying when the comparison is made.

DOWDY: I think they're dead.

POWERS: Right. Now, this other kind of differentiation that we're discussing is what you might call *constructive* differentiation, in which the cell transforms into another kind of living cell which then makes up a particular kind of tissue.

KELLY: I brought the point up because it seems to me that a major difference between a tumor, for instance lymphoma, and a normal thymus is the fact that in the normal thymus, you have one daughter cell, on the average, differentiating and being useful while the other one remains behind to divide again. On the other hand, in the tumor, both daughter cells divide again and exponential growth results. This is why I feel that perhaps a small change in the proportion of cells which are differentiating is something which we ought to investigate.

CASARETT: I have a point to make on this particular question from the study of normal adult mammalian tissues. It seems that the cells which can be induced to differentiate prematurely are largely those which already have the alternative potential for division and differentiation. For example, in the germinal epithelium of the epidermis and of the seminiferous tubules of the testes, and to some extent in the bone marrow, our observations have suggested that there is some precocious maturation of cells before the normal number of divisions occurs, if there is inhibition of division without killing the cells. The result is, in the germinal epithelium of the epidermis, that with repeated or chronic irradiation there may be an increase in the number of maturing epidermal cells and a decrease in the number of germinal cells. In the seminiferous epithelium, measurements of spermatogenic cells in various stages of spermatogenesis have suggested the occurrences of precocious differentiation of cells, when cell division was inhibited by irradiation. There is some evidence of this also in the bone marrow. It seems to me that the precocious maturation is brought about by inhibition of division, with the cell essentially taking the alternative potential that it already has for maturation.

KELLY: Thank you.

I'm afraid our time is up.

I'm sure I speak for all of us when I say that this has been a most stimulating and enjoyable conference and that we thank our hosts for arranging it.

REFERENCE

[1]See SPARROW, A. H., GUNCKEL, J. E., SCHAIRER, L. A., and HAGEN, G. L. *Amer. J. Botany*, **43**, 377–388 (1956).

END OF SESSION VII

ADDITIONAL PAPER

STEADY STATE CELL PROLIFERATION: ITS CONTROL AND RELATION TO RADIATION INJURY OF THE MAMMAL*†

By Eugene P. Cronkite

One of the characteristic features of radiation injury is the disturbance of the steady state of cell renewal systems. The interference of radiation with cell division has long been known. The law of Bergonié and Tribondeau was an outgrowth of this realization and has been discussed in detail by various authors and summarized by Bond at this conference. As in all natural phenomena it is necessary to visualize the normal process before one can really appreciate perturbation in the normal process. For this purpose a model for steady state cell proliferation will be formulated as I see it. No claim is made for originality. Various facets of the problem have been reviewed or considered by LeBlond and Walker, Osgood, Quastler, Patt, Weicker, Cronkite and Rigas. No attempt will be made to review previous publications or discuss previous work. The present model is purely an attempt to put down on paper many of the potential features that may be of importance. It will take experimental data and its analysis to determine the ultimate importance of the various facets of this model.

Actually the premise of a simple steady state is a gross oversimplification. Most tissues are in a state of flux; their population size varies with the balance between production and removal. For example, note the production and disappearance of callouses of the skin. However, with constant environment, there is a surprising constancy of the size of the circulating red cell mass, platelet and leukocyte concentrations. The fact that a steady state can be maintained, where cell production equals cell loss (removal by senescence and random loss), implies a delicate control mechanism response to small changes in some signal. Also the very fact that large loss can be rapidly compensated for, e.g. after burns,

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† This paper resulted from the discussion at the meeting and was, therefore, submitted by Dr. Cronkite for publication in the Proceedings.

trauma, infection, and hemorrhage, by a very rapid increase to the previous steady state level suggests the existence of another feed-back system that is sensitive to some function of rather gross changes in concentration, volume, pressure, or physiologic change induced by these losses.

Nature has established an orderly and cytologically identifiable flow sheet for cell production in the proliferative tissues. The compartmentation varies with the tissue. For purposes of discussion the flow pattern will be described for hematopoiesis. Six compartments are evident that flow from one to the other in an orderly fashion. These compartments are:

I. Primitive Progenitor Pool (PPP)
II. Multiplication and Maturation (MM)
III. Maturation and Storage (MS)
IV. Peripheral Blood (PB)
V. Extravascular Life (EVL)
VI. Removal (R)
    Senescence → RES
    Function
    External Loss

In the first compartment (PPP), cell division is proceeding. In order to maintain a constant size, one daughter cell must remain behind to divide again, thus perpetuating a primitive pool of cells. COWDRY and OSGOOD have emphasized this. Mitosis is an imperfect mechanism and it can be assumed that there will be some loss. The number of cells produced per unit time will be $N_{PPP}/t_G = K$, the flow rate from I → II, where N_{PPP} equals the number of arithmetically dividing cells in I and t_G equals the generation time. This flow rate can be increased by decreasing t_G or increasing the size of N_{PPP}. The latter would necessitate doubling divisions of a fraction of this pool. It will be assumed that the cells entering compartment II have started down an irreversible path towards a specific cell type.

In the second compartment, maturation progresses in an orderly fashion and is well established in all blood cell series. Simultaneously, mitosis goes on in the red cell series, three to four times, and in the myeloid series, about two to three times. These are reasonably well-established steady-state facts for guinea pigs (YOFFEY) and man (FLIEDNER and CRONKITE). The time consumed in this compartment is not yet clear. The repetitive divisions in the red cell series are faster than in the myeloid series (FLIEDNER and CRONKITE).

Upon completion of the last cell division, maturation is yet to be completed and cells are stored for variable periods before passing into the fourth compartment, the peripheral blood. In the case of the red cell, the life span is known to be about 120 days and is spent in the peripheral blood. There is no extra-vascular life. In the case of the leukocytes the time spent in the peripheral blood is unclear and the duration of the extravascular life is not clearly established. In fact, its existence may be questioned on histologic grounds (BRECHER). The problems of recycling of leukocytes, from the peripheral blood, to storage, to tissues, has been proposed but not established (WHITE, OSGOOD and PATT). The last compartment, the removal system, seems to have clearly three components—phagocytosis of senescent or injured cells by the RES, loss in function (leukocytes), and external loss.

The existence of the preceding flow sheet is based on facts. Under normal conditions a steady state exists and its very existence necessitates a controlling mechanism. Nature has not made the problem of control simple. There are continual unpredictable losses from minor traumata, hemorrhage and infections. There are occasional extreme demands from severe hemorrhage or infection. Radiation, drugs and disease impose severe stresses upon all steady cell renewal systems and within rather wide limits, the systems compensate for the losses induced within the various compartments enumerated. The usual stresses of life result in losses from compartments IV and V, and many spontaneous or induced human diseases result in increased loss by overactivity in compartment VI (autoimmune disease), whereas hypoplastic states may result from impairing production in compartments I or II. It appears to me that radiation particularly affects compartment II and, to a lesser extent, compartment I in the lethal dose range.

Perhaps further insight into the normal problems can be attained by a more detailed discussion of one segment of hematopoiesis, namely erythropoiesis. Here many facts have been established. Oxygen occupies a critical role. Erythropoietin, a humoral substance, can be detected in plasma under certain conditions. This substance will stimulate erythropoiesis in the absence of hypoxia. It has been identified as a mucoprotein (BORSOOK). The nature and physiological role of this agent is being extensively studied (ERSLEY, BORSOOK, STOHLMAN, JACOBSEN, MIRAND, and PRENTICE and others). It is quite easy for me to visualize the sequence of oxygen deficit to liberation of erythropoietin (perhaps none is circulating normally), to an increased production of red cells in the case of severe hemorrhage, or reduced oxygen tension. It is not at all easy to

visualize the mechanism by which the red cell mass is kept constant when one removes a tiny volume, day after day, that does not influence blood pressure or oxygen tension of the blood or tissues. I can't help but think of 'erythropoietin' as a 'panic system' which pushes production to a maximum, that is analogous to the water injection emergency power system in aircraft engines. The strong positive stimulus does not appeal to me as being the sensitive governor of the normal steady-state level. More appealing is the negative inhibiting influence from mature cells as postulated by OSGOOD and others. If, in the case of red cells, the slow removal of senescent red cells from the circulation, by the reticuloendothelial system, does feed back a physiologic brake for a spontaneously dividing system, then small losses of red cells to the outside would mean less inhibitor, hence more production, and the steady-state level could be maintained with fine constancy without induction of hypoxia or other severe disturbances in the physiologic state. In this case, one does not need to invoke the existence of an erythropoietic factor to maintain the steady-state level by lesser or greater secretion. In fact STOHLMAN has integrated all evidence on control of red cell production, and has produced a very strong argument for a dual control mechanism—one fine, the other coarse.

One may very logically wonder what all of this has to do with whole-body radiation injury, or sectional irradiation of the bone marrow. Certainly irradiations result in a random deposition of energy in all cells, so why focus upon only the dividing cells? By so focusing, one does not really lose perspective, but recognizes the dose dependence and variation in radiosensitivity of different tissues. One only says that the hemopoietic and epithelial tissues with mitotic potentials are more sensitive, and that life of the mammal is dependent upon the continuous renewal of the mature functioning cellular elements by the mitotic cells. Accordingly, cessation of renewal of the GI tract is compatible with life for only a few days—three to six—the stable survival time of the gut syndrome. The gut syndrome, and its relation to the mature and dividing cells of the GI tract, have been analyzed in detail (QUASTLER). Cessation of hemopoiesis is compatible with life for only one to three weeks, the survival range of the hemopoietic syndrome. The key to survival after doses in the lethal range is regeneration of these renewal systems. In fact this is borne out by a large volume of experimental evidence (BOND, CRONKITE, JACOBSEN, COLE, QUASTLER, LORENZ, CONGDON). Transplantation of marrow or spleen containing the PPP cells reestablishes hematopoiesis and increases the survival rate strikingly. Furthermore,

the sublethally-irradiated mammal develops a moderate anemia from impairment in cell proliferation. If the sublethally-irradiated dog or rat is promptly bled (one-third blood volume), or submitted to severe hypoxia, hematopoiesis is strikingly enhanced. In the case of bleeding, the regeneration will progress as rapidly as in non-irradiated bled animals. (VALENTINE, STOHLMAN, BRECHER and CRONKITE). Hence the bled, or hypoxic, sublethally-irradiated animal can liberate increased amounts of erythropoietin, and the erythropoietic tissues can respond to the 'panic stimulus'. However, after larger doses of irradiation in the lethal range, there is no acceleration of regeneration of erythropoiesis to bleeding or hypoxia (STOHLMAN, BRECHER and CRONKITE). Is this due to an inability to produce erythropoietin or an inability of the appropriate cells to respond? The lethally-irradiated, hypoxic dog is the best source of erythropoietin (STOHLMAN). Therefore, the failure to respond is either due to ablation of the responsive cells in compartment II, if this is where erythropoietin acts, or the prevention of response by cells in compartment I. From the work of ALPEN it appears that the latter is the case. From these observations, it appears reasonably certain that death or survival in the lethal range is intimately associated with regeneration. The latter is intimately connected with reestablishment of the normal flow of cells. It therefore appears appropriate to establish, for each radiosensitive cell system, a flow model for cell production subdividing into compartments, so that one can look at the probability of profits and losses at each stage. From these models, one can then speculate about control mechanisms, and design experiments on the normal and the irradiated animals. It is inappropriate, at the present stage of development, to attempt to equate all the factors that control production rates. In fact, this becomes a most complex problem in population statistics, and is being formulated by Professor VON FOERSTER at the University of Illinois. Another mathematical analysis has been recently published by RIGAS.

Since the generative cycle is the essential ingredient of cell proliferation, it is appropriate to look briefly at this phenomenon common to all radiosensitive systems. The generative cycle can be dissected into certain clearly defined time periods, as shown in Fig. 8.1. These time periods are subject to experimental attack in the living mammal and have been studied extensively by LAJTHA in tissue culture of human bone marrow and are under study by ourselves in human beings after *in vivo* labeling with tritiated thymidine.

In the scheme shown in Fig. 8.1, exponential doubling of the population would occur if there were no control mechanisms. Growth from

conception to adult life only involves a relatively few doubling divisions. The actual factors that determine which of the daughter cells will divide, and which will mature, simply are not known. It is assumed that once a cell doubles its DNA content, it is destined to divide. With a *constant* number of cells capable of division, the production rate can be increased only by shortening the generative cycle. If the mitotic time is short in reference to interphase time, as is generally believed to be the case, significant increases in production can only occur by shortening the

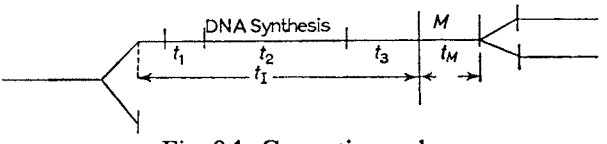

Fig. 8.1. Generative cycle.
t_1 = Rest period after mitosis is complete
t_2 = DNA synthesis (doubling)
t_3 = Rest period after completion of DNA doubling
t_M = Mitotic time
t_I = Interphase = $t_1 + t_2 + t_3$
t_G = Generation time = $t + t_M$
M = Mitosis

interphase. The increase in production will then be proportionate to the decrease in total generation time as long as arithmetic division continues. Certainly DNA synthesis is a complex and intricate process, involving the replication of the genetic code. To my mind it would appear unlikely that nature would select this time interval to shorten and speed up cell division and probably increase the chances of error of replication of the genetic code. However, nature often does the illogical. If the length of this process can be defined, and if this process can not be shortened, then the maximum increase in production rate is limited by the duration of DNA synthesis. If DNA synthesis occupies a large fraction of the generative cycle, the effective increase in productive rates will be small. Of course, it may be that all phases of generation can be shortened. If, in addition, one can superimpose doubling divisions of a fraction of the PPP cells in compartment I, or additional doubling divisions in compartment II, within the *same period of time*, the productive rates can be increased quickly and by a large factor. In fact, in some compensated hemolytic anemias, red cell production may be ten times the usual rate. All of this discussion is leading up to a consideration of the things that are measureable by experimental means and are essential to know in order to study normal cell proliferation (reviewed by LEBLOND) and perturbation by radiation of normal measureable parameters such as:

Turnover time (T) is the time that it takes a tissue to renew itself. It represents the mean life span of the adult cells of the tissue. The individual life spans may be, and probably are, for all tissues, distributed around a mean value.

Turnover constant (I/T) is the reciprocal of turnover time and represents the fraction of cells being renewed per unit time.

Turnover rate is the number of cells produced per unit time in any compartment. For compartment I it equals N_{PPP}/t_G. Determination of this number necessitates knowledge about the size of the total population. In most cases, this is almost impossible to achieve in the living animal.

Mitotic index (M) equals the fraction of cells of a series (preferably of the generative compartment) that is in mitosis.

The mitotic index is determined by counting mitotic figures. There are certain inherent difficulties. First, recognition of mitotic figures poses a problem. Mitotic counts with various stains tend to vary. Next, fixation of tissues must be prompt, so that one does not lose the later stages of mitosis by completion between killing the animal, biopsy and fixation.

Turnover time (T) equals the ratio of mitotic time to mitotic index.

$$T = t_M/M$$

This relationship is straight-forward in principle. However, if one accepts a mitotic time for mammalian cells of between 30 and 90 minutes (which seems to be a fairly certain range) then turnover time (T) is dependent simply upon the accuracy with which one can determine the mitotic index.

Mitotic rate is often studied by use of colchicine which stops mitosis in metaphase for a variable period. In this technique, mitoses are allowed to accumulate for a specified time. DUSTIN has analyzed the method in detail. All of the necessary assumptions about action of colchicine are discussed by DUSTIN. To reiterate, if colchicine does not influence interphase, and if no cells escape from the effect of colchicine, then M_n^s, the mitotic index n hours after colchicine, is inversely proportional to the turnover time (T), and directly proportionate to duration of action of colchicine; hence:

$$M_n^s = t_n/T = Mt_n/t_M,$$
$$\text{and} \quad t_M = Mt_n/M_n^s.$$

In addition to the classic histologic studies and the mitotic arrest by colchicine, various methods of DNA labeling are available. For this purpose, tritium-labeled thymidine, developed at Brookhaven by HUGHES, is ideal. This pyrimidine deoxyriboside is a specific precursor of DNA. It is either catabolyzed to tritiated water, and/or non-volatile tritium

compounds, or incorporated into DNA. The tritium beta is ideal for radioautography, thus making a visible label in specific cell types. Thus, in addition to the direct enumeration of mitotic and non-mitotic cells, one can determine the proportion of cells which are synthesizing DNA, because availability of the label is short in respect to the time of DNA synthesis. In fact this has been done in mice (QUASTLER, BRECHER and CRONKITE) and in human bone marrow (CRONKITE, BOND, FLIEDNER and RUBINI). Thus one can measure a labeling index L.

$$L = L_e/L_A$$

where L_e = number of labeled cells;

L_A = total cells in generative compartment;

$$L = t_2/t_G \quad \text{or} \quad L_e = L_A \,.\, t_2/t_G$$

However, in the situations where the label is available for longer periods and where one must consider problems of transfer of label and influence of length of time that the label is available, a more complicated situation arises.

$$L_e = L_A \,.\, t_2 \,.\, a\, t_a/t_G$$

a = a transfer function of label to DNA.

t_a = time label is available.

As t_a increases, $L_e \rightarrow L_A$ 100 per cent, at which time all cells will be labeled, and $L_e/L_A = 1$.

$$t_2 \,.\, a\, t_a/t_G = 1, \text{ or}$$

$$t_a^{100\%} = t_G/a\, t_2$$

In actual determination of the labeling index L, one does not have to assume anything about the shape of the DNA synthesis curve. One only assumes that sufficient label is incorporated to be detectable by radioautography. Admittedly, a sigmoid DNA doubling curve, with slow rates of synthesis at both ends, and a short term of availability of label, might seriously underestimate the index. However, as discussed previously, an instantaneous labeling index can only be estimated, if availability time is short, as compared to DNA synthesis time.

A more powerful technique is the measurement of the appearance of labeled mitoses. This is experimentally difficult and necessitates either serial sacrifice or serial biopsy at very frequent intervals. From observing the flow of labeled cells into mitosis for any *one cell type*, one can estimate pre- and post-DNA synthesis rest periods, mitotic time, DNA synthesis time, the ratio of DNA synthesis time to generation time, and

calculate generation time. The period between labeling and the appearance of the first labeled mitosis estimates the minimum duration of the post-DNA synthesis period. The time for build-up of labeled mitoses to 100 per cent is a function of the mitotic time and the distribution of the post-DNA synthesis rest period. The duration of the plateau at 100 per cent labeling is a function, primarily of the duration of DNA synthesis, with some perturbation by the distribution of pre- and post-DNA synthetic rest periods. The decline after this plateau is a function primarily of the duration of the pre-DNA synthesis rest period. Ultimately, the per cent of labeled mitoses will settle down to represent the ratio of DNA synthesis time to generation time. The slope of decay in the intensity of the labeled mitoses will be a function of the generation time. Therefore, it is feasible, by study of the serial changes in per cent of labeled mitoses, to establish all of the time elements for the generative cycle of specific cell types. If these values differ with cell types, then mixed populations, such as in the bone marrow, will be more difficult to analyze but are subject to experiment and ultimate analysis, when the appropriate distributions are established by experiment for a single cell type. Studies of this type have been completed in mice (QUASTLER, BRECHER, SHERMAN and CRONKITE) and are underway in patients (CRONKITE, FLIEDNER, BOND and RUBINI).

From the preceding considerations and techniques it is quite evident that the normal steady state and its perturbations can be studied and quantitated. Of particular value are the DNA labeling techniques. It has already been shown by QUASTLER and SHERMAN, BOND, FLIEDNER and CRONKITE, using irradiated thymidine, that radiation significantly interferes with both DNA synthesis and mitosis. The interference with each is dose dependent. It appears that mitosis is the process more sensitive to radiation. The relative importance of these phenomena, after various doses of radiation, can easily be evaluated experimentally by simultaneous study of labeling of DNA, and enumerating the subsequent appearance of the label in mitotic figures, as a function of time after irradiation. By a combination of the classical techniques, with labeling of DNA by tritiated thymidine, much more will be learned about the nature of mammalian radiation injury at a cellular level. These data will be more interpretable, when viewed in reference to the appropriate histologic models, for normal steady-state cell proliferation, and upon the establishment of the appropriate mathematical models, on proliferating cell population statistics, as are being formulated independently by RIGAS and VON FOERSTER.

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