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BIOCHEMICAL ASPECTS OF BASIC MECHANISMS IN RADIOBIOLOGY

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Editor

BASIC MECHANISMS IN RADIOBIOLOGY

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Conference Committee

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PREFACE

Like its predecessor on "Physical and Chemical Aspects of Basic Mechanisms in Radiobiology" (NAS-NRC pub. no. 305, 1953), this volume was prepared from the transcript of an informal conference held in Highland Park, Illinois on May 13-15, 1954. The conference was dedicated to biochemical phenomena in radiobiological actions, a topic which is obviously of paramount importance, since biochemical events must bridge the gap between the physical processes of energy transfer and their ultimate biological expression. It is our hope that this volume will at least convey the perspective of the participants on current problems in this area.

Each chapter consists of a discussion prefaced by a more or less formal review of a central theme by a principal essayist. The discussions are interspersed and followed by comments from all participants. There is considerable interplay between the chapters, a reflection of the intimate relationship between the various topics and their ramifications to basic radiobiological mechanisms.

The work of the participants, as well as that of other investigators, was quoted freely, whether available in published form or not. It is, therefore, important to emphasize that the data presented in this report should not be referred to without permission of the authors quoted. In some instances, references were provided by the participants. These appear at the end of each chapter and may introduce the reader to selected topics.

The transcript, as originally prepared, required considerable editing. An effort was made to achieve continuity and intelligibility and to tighten the discussion without seriously compromising the informal and speculative flavor of the meeting. The editor was assisted by the participants and, particularly, by the principal essayists and by Mrs. Antreen Pfau in this effort.

Harvey M. Patt

CONTENTS

PARTICIPANTS

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THE DIRECT EFFECT OF RADIATION ON PROTEINS, VIRUSES AND OTHER LARGE MOLECULES

Ernest C. Pollard

Well, I richly deserve what I am getting today. In reading through the very fine summary of last year's conference, I realize that I was operating with a needle most of the time and it is very fair and just recompense for having done this. Here I am in a position of putting up or shutting up and so I had better talk about it.

The work we have done at Yale had as its original interest the use of radiation to study structure. This follows directly along the pattern set by Lea, and we consider ourselves, if you like, descendants of Lea in our philosophy and outlook in the way in which we seek to use radiation. The use we have made of it has somewhat surprised us; some of the methods of using ionizing radiation to study viruses have been summarized in my book. (1)

Somewhat to my surprise and pleasure, one structure that we sort of postulated for Newcastle disease looks very much like the electron micrographs that are now being turned up in New York by Morgan et al (2). So there is evidently satisfactory validity to this method of using radiation to study structure. I am acutely sure it has limitations, and it would be very foolish to use it without knowing these limitations. One of the reasons for this discussion today is to send me away with a clearer idea of what the limitations are.

In this work there is one basic aim; i.e., to preserve the space relations of ionizing radiation. You use these space relations to tell you something about the nature of the system you are studying. There is a second feature to this: the ionizing events that occur must make some change in whatever you are looking at. This change is, at the present, rather too inclusive. It involves, as a rule, the removal of activity or some very vital change like that. It would be preferable if the change were more moderate and could be studied in some detail after it had occurred, because then we would get more information.

Because space relationships have to be preserved, we have operated almost entirely in the dry state. Everyone will ask, "How dry?", and the answer that I can give to that question is in the following terms:

First, the specimens are exposed in high vacuum, usually inside the vacuum chamber of a cyclotron, so that the total vapor pressure goes down to 10^{-5} mm. Hg.

Second, the thermal inactivation constants for these materials are completely different from those that are obtained in the wet state. If you heat-inactivate, under the condition that we usually use for work on a virus or an enzyme, you will find that the inactivation is characterized by a low entropy of activation, zero or negative. This seems to be characteristic of inactivation in the dry state, and you can show a very striking contrast between how the material behaves as far as heat is concerned. This we look on as an auxiliary sort of evidence. Whether any biological material can be considered as absolutely dry is doubtful; but that radiation can migrate via the medium of water under these conditions I would strenuously deny. I don't see how it can.

Now I should like to say a little about what I call a theoretical approach to radiobiology. Looking back, the strongest needle I put into the conference last year was in the form of a somewhat impassioned plea for recording of all the effects that radiation produces. I felt that we were relieved when we learned that radiation can produce an effect on water and that this in turn can produce an effect on the cell, and I tried to indicate that that relief was perhaps a little excessive, that there had to be a consideration of all the effects that radiation can bring about, and that among these effects, the direct action on the biological components of the system was most important.

I should like now to suggest that it is altogether possible to formulate a theoretical approach to radiobiology in the following terms. We can say what the parts of the cell are and list them, not just talk about them in absolute generalities but list them. Then we can inquire as to what the radiation action is on these parts separately. Having done that, we can inquire as to what function these parts have in the work of the cell and then we can synthesize a probable explanation for radiation action.

I am amazed, to tell the truth, that this is being done so little. We have tried it in what I thought was an amateurish way, and in the progress reports of our work we have, each year, written a sort of statement as to what we think this sort of theoretical radiobiology should be like. Among the things that we have discussed has been the relative proportion of direct and indirect action based solely on this theoretical approach.

The fact is that when you get into this theoretical approach you become acutely aware that a lot of data that you need are not only unknown, but are not even being sought. In the first place, it is urgently necessary to know the lifetimes of all the products of radiation action everywhere: not only lifetimes in water, but lifetimes in the solid state, and lifetimes of things such as HO_2 and, in fact, any agent that can be thought of as being involved in the response to radiation. The study of these lifetimes is at least not clearly visible in the literature.

In this connection, Dr. Smith, working at Yale during a leave of absence from the Department of Radiotherapeutics at Cambridge, conducted, I thought, a very nice experiment along the line of the one first used by Dr. Mazia. He made a measurement of the lifetime of radicals in water (3), and came out in two cases with a magnitude of about 3 microseconds. Just this one value alone completely modifies Lea's own speculations as to the relation between direct and indirect action. Lea adopted, without measurement, a figure of 0.3 microsecond. Clearly, when you have a lifetime as low as 0.3 microsecond, a radical formed far away from the important biological molecule would not be effective. It will hit something unimportant before it gets there, and the unimportant thing may even be the thing that causes it to recombine as measured in these experiments. A figure 10 times greater modifies this.

The conclusion we come to from the sort of broad theoretical approach in which we consider the effects of radiation on proteins, nucleic acid, and the microscopic cytoplasmic components is that direct and indirect actions are split about 50:50 but that they are both variable. You can, on occasion, get a cell in which only 25 percent of the action is direct, or the other way around. One must not consider either of these to be the functions of radiation that are constant. They depend on the condition of the cells and on the molecules that are in them, and we have to recognize that both of these can be variable. There is no question whatever about the variability of indirect action with respect to the nature of the cytoplasm.

I should like to suggest that this can also be said of direct action, and this is a somewhat bolder statement than I think anybody has made in the past.

One tremendous and absolutely vital datum is sitting, waiting to be found by somebody. No one knows the ionic yield for indirect action on large nucleic acid molecules. Large nucleic acid molecules obviously play a most dramatic role in cellular function, particularly in any function that takes time and requires the cell to develop. Yet I do not know of any work in which the ionic yield for indirect action on nucleic acid molecules is given. By a nucleic acid molecule, I mean one that has a biological function. I discount measurements in which the nucleic acid is either polymerized or depolymerized without a guarantee that at the same time biological function goes along with it.

CARTER: Do you mean transforming principle?

POLLARD: Transforming principle, for example, would be beautiful, but at the moment I don't think it is pure enough to measure.

CARTER: I think transforming principle is pure enough to measure and I am surprised that Dr. Chargaff does not have the data.

CHARGAFF: It is very difficult to say really what proportion of the transforming principle you have in any nucleic acid preparation. That is one of the great difficulties. We are now fractionating preparations. Maybe we will know soon a little more about that.

POLLARD: We know the yield for direct action. This has already been measured in three places and the agreement is good. But we do not know the efficiency for indirect action. It is not known, for example, whether it takes 10 ion pairs to inactivate one transforming principle molecule or, say, 1,000. Empirically, we would expect a figure of 1,000 but it might be as low as 1. If, indeed, it is 1, it would be well worthwhile to concentrate on that one subject as the probable single basic radiobiological action. But until we know that, the basis for this theoretical radiobiology is missing. It does not seem to be an impossible experiment.

CARTER: Actually the obstacle is that Dr. Chargaff has not purified the transforming principle.

CHARGAFF: You are faced with an almost philosophical dilemma. You may have between 1,000 and 10,000 different species of nucleic acid per nucleus, and if you equate the nucleic acids with the genes obviously you really don't know what you are measuring. If you are measuring one transformation feature, e.g., Hotchkiss' sulfonamide factor, you don't know what proportion of the total nucleic acid really corresponds to this particular activity. I think it will be a long time before we will be able to answer that question.

POLLARD: Well, you can answer it for direct action.

It is interesting that Fluke, Drew and I (4) measured the radiation sensitivity of the transformation of just rough to smooth. Fluke and Marmur (5) have measured the streptomycin and, I believe, one other transformation from Hotchkiss' laboratory with substantial agreement, although with a rather interesting extension that there may be two classes of molecules present.

I had a letter from Dr. Latarjet to the effect that he has found somewhat similar, though rather less, sensitivity. One can say that the apparent radiation sensitive volume for the direct action of radiation on nucleic acid corresponds to a molecule in the order of between 3 and 7 million molecular weight -- very sensitive. So that the temptation for me, as a direct action man, is to see this enormous figure and to want to say, "Well, that is the key to radiobiology." It is a great temptation because it is a huge figure. Nevertheless, it will be most unwise to take this attitude until we have the other data as to whether, by any chance, 1 ion pair can also inactivate such nucleic acid molecules by the medium of water. If that is true, than we have just as good, in fact, a better line of approach in terms of the action of water. So these are important data. Moreover, I think it is so important that it would be quite all right if we knew it only within a factor of 20. So if one had a purified transforming principle with only 40 percent inactivity it still would be worth working on.

BENNETT: I don't know if this is the type of thing you are thinking about, but Dr. Stent at the University of California is incorporating essentially carrier-free P^{32} phosphate into phage and determining how many disintegrations are required to inactivate them. It is a different type of phenomenon, I think.

POLLARD: There are two things there. In addition to the ionization, there is actually a change of atomic species plus a violent recoil and actual motion of a heavy atom, which is a very drastic thing indeed, in the case of a big molecule. Dr. Kamen knows about this because he did pioneer work on the subject. (6) The effect of incorporating P^{32} is some 30 times as great as the effect of radiation from the outside. So that there is clearly something else taking place that is not normally present in ordinary radiobiology. Radiobiology is not concerned with making nucleic acid radioactive. If that were so, the cross-section would be very small, I believe.

KAMEN: I wonder if you could tell us how the half-life was measured for radicals in water.

POLLARD: I cannot describe it in detail because it involves a lot of plane diffusion constants. The procedure was essentially that of Mazia and Blumenthal. Two separate monolayers, one of catalase and one of bovine serum albumin, were deposited on a chromium-plated glass slide which was placed in a clean water solution, with precautions for no oxygen, and then X-irradiated. Loss of function was measured by the ellipsometer technique in one case. In the case of the catalase, it was described in terms of the amount of hydrogen peroxide converted. In the case of the bovine serum albumin, it was measured for the amount of specific antibodies that would hook on to the surface of the albumin. Both gave quite similar figures. But the significant thing about the experiment is that in both cases, exposures of the order of 200,000 r were needed to produce any effect. This is quite different from an enzyme that is distributed throughout the material; for example, for catalase in bulk dissolved in water. Catalase would undoubtedly be inactivated by a

fraction of this exposure, perhaps 5000 r or something of the sort.

The point is that because there is only one place where the catalase is, namely, on the slide, the time for diffusion is important, and if the time for diffusion is longer than the time for recombination, then the radical is not effective. By calculating for plane surfaces one would come out with a theoretical figure.

I don't really want to spend time on this part since I want to get to the details of direct action studies. What we have done has been to pick up where Lea, Smith, Holmes and Markham (7) left off. They investigated the effect of X-rays on dry myosin and dry ribonuclease and measured what is called their inactivation volume. This is the volume within which one ionization, randomly distributed, will cause inactivation or loss of function. It is to be thought of as a parameter and it is only by chance or, by what I hope to bring out, by some process that we would like to be able to describe, that this parameter agrees in any way with anything known about the molecule at all.

Lea and his associates found that in both of these molecules something like the molecular volume was involved in the figure for the inactivation volume, and that if, in particular, one allowed for the way in which ionization comes in clusters, the calculated molecular weights based on this method of inactivation agreed tolerably with the figures that were accepted at the time. Lea did not follow this up in the years before he died, and when we began our work on irradiation of viruses, it was suggested by Dr. Forro that we should study the effect of radiation on enzymes as well.

The experiments are threefold in character and quite elaborate. First of all, whatever you study has to be brought into a condition whereby it can be dried and handled stably. For most enzymes and antigens, this is easy. In fact, most of these things seem to have a higher stability in the dry than in the wet state. For example, catalase can be heated to 100°C when dry but is quite temperamental when wet. These are then irradiated with fast deutrons, slow deutrons, alpha particles, fast electrons of over 500,000 volts, and also with electrons of limited penetration, of energies below 4000 volts.

We have brought every piece of physical equipment that we could to bear on this major type of study. That is one advantage of being a physicist of some reputation. You can get hold of apparatus that otherwise is a little hard to get your hands on. We have not hesitated to go right after it and we have studied what is a surprisingly large array of things.

We have found, first of all, that you can consider a molecule as having an inactivation volume and an inactivation cross-section, depending upon whether you deal with ionization that is random in volume or with ionization that is confined to dense swaths and so can be considered as a sort of linear problem. The two usually go together, although not exactly. It is not a perfect fit unless you start to introduce other factors.

But the first thing to say is that in no case when we calculate the molecular weight do we come out with something wildly wrong. As a matter of fact, we have had some remarkable successes. For instance, we insisted that the molecular weight of urease would be somewhere in the neighborhood of 100,000 and we held to that in the face of opinion that it was 480,000. Well, later determinations are giving 100,000. We hit some things rather accurately. For pepsin we find a figure of 39,000 as against the accepted value of 36,000. For others we haven't done so well. We come out with a figure of 31,000 for trypsin,

whereas the present accepted figure is 17,000. We have a number of figures for radiation molecular weights which have not been checked in any other way. As they are gradually being checked, we find that our figures are quite often in the right league.

A very interesting one is that of DNA. If DNA is assayed as the pneumococcus transforming principle, then we come out with a molecular weight of 6,000,000 and we also require that the molecule be long and thin. The figure given is 45 Å wide and 3800 Å long. When we assay DNA, however, by something quite different -- the capacity to act as a substrate for its enzyme -- we come out with a figure of something like 2100 molecular weight and a cross-section of about 500 square Å. The conclusion is that perhaps 8 nucleotides are sufficient to be specific for digestion by DNA ase.

CARTER: What are the criteria for the enzymatic activity that you used?

POLLARD: This was done by Dr. Smith. I think he measured the amount of substrate converted in a fixed time, being certain that the amount of substrate was not the limiting factor.

CARTER: What was the endpoint?

POLLARD: No endpoint was measured.

CARTER: Was this loss of viscosity?

POLLARD: No.

CARTER: It would have to be done like everything we do in a Beckmann apparatus, and this would be measured by the amount of specific changes in absorption, probably at two wavelengths.

CHARGAFF: I am not sure that this is a very good criterion.

POLLARD: I am rather interested that you grabbed on to that as a method of measurement. That was not our point. Our whole point is that there is a completely different response to irradiation.

CARTER: The other point is that if you inactivated the desoxyribose nucleic acid by three different methods you might come out with three different answers.

POLLARD: We bombarded the nucleic acid to see whether it could still be used as a substrate. The enzyme was not bombarded in these experiments.

CARTER: That irradiation can do so many different things to the molecule is the point that we want to establish.

POLLARD: I would confidently expect that if one actually studied these separately there would be significant differences between them.

CARTER: There may be significant areas of agreement.

POLLARD: Yes, quite possibly.

CHARGAFF: The transforming molecular weight was about 6,000,000,

if I understand correctly.

POLLARD: That is correct.

CHARGAFF: That is not to say that if you break it in half it is no longer transforming or is that the minimum? I really don't know what you are measuring when you say 6,000,000. Is it the molecular weight?

POLLARD: What is done is what I call a mental transformation and one has to undergo this before he can understand it. You take a preparation of the transforming principle, dry it and then take part of the dried specimen out as a control. You irradiate it with fast electrons and fast and slow deutrons and you measure, after irradiation, the amount of activity which is left. In the case of the early experiments, this was a very difficult thing to determine. One had to determine the concentration of the irradiated material on which a fixed number of transformations would take place. It was rather nasty, and the observations were not very precise. In the modern experiments, it is much easier. You can simply measure the number of antibiotic-resistant forms that are transformed. These colonies can be measured as a definite number, and you can get some estimate of the activity of the transforming principle that is left at the end. This loss of activity follows approximately a logarithmic function.

I must say with regard to the transforming principle, in view of the crudeness of the assay, that we did take the logarithmic inactivation on faith. If you believe then that the logarithmic inactivation requires that there be a constant, which is volume in one case and area in another, the volume corresponds to the volume of the sensitive unit. This can be re-expressed as a molecular weight. Bombardments that measure the volume give a value of six million for the equivalent molecular weight. Bombardments measuring area do not agree with this unless the substance is very long and thin. Agreement between the volume and the area can be obtained by saying that it is 45 Å units across and 3800 Å units long. That is all we can say about this.

CHARGAFF: That is roughly a ratio of about 100?

POLLARD: Roughly, 100.

I have spent too long on this since I am not too sure that our work is at its best in these two cases because the assays are somewhat an open question. But the substances that we have studied show remarkable radiosensitivity in the dry state, sensitivity that is apparently confined to a region that is approximately that of the molecule.

I should like to summarize the facts as we know them. I think I have nine. Incidentally, I must stress that I have a very fine group that is doing all this work, and I am on top of a pinnacle that they support.

1. All the inactivation volumes are within a factor of 4 of the molecular volume, on the basis that a single ionization will inactivate the molecule. Just a single ionization, not primary, but any ionization.

2. The cross-section of a molecule measured with densely ionizing radiation, such as alpha particles or deuterons, is a varying function of what we call the ionization density. Dr. Zirkle and Dr. Tobias call it linear energy transfer. Either is all right. Generally, this shows a trend to a maximum value and that value corresponds ordinarily to the diameter and area of the molecule.

3. The curve for this cross-section versus ion density can usually be fitted by a theoretical relation, and the theoretical relation rests on the random production of a definite minimum number of ion pairs in a molecule of definite thickness. These measurements enable you to get an independent measure of thickness.

4. Both these quantities, cross-section and volume, vary with temperature during irradiation. If the material is cooled to dry ice or liquid air temperature, it is likely, although not guaranteed, that the sensitive volume will be smaller. If you want to get a most dramatic variation you can get it every time by irradiating just below the temperature where you would inactivate thermally. If you hold the material about 20°C below that for thermal inactivation and irradiate at the same time, the volume and cross-section will both be of the order of 3 to 5 times larger than normal. It is not a small effect. It is definite.

5. You can have partial damage due to ionizing radiation. This shows up in the case of hemoglobin. If you irradiate hemoglobin and then look for any change in it by any method you like, the first thing you need to do is to put it into solution. If this is attempted at an adverse pH, the irradiated material will not go into solution. However, hemoglobin is soluble at pH 4 or 5 and once in solution it will not appear to be damaged. Since there is a change in the solubility at high pH, partial damage of some kind has occurred (8).

6. Radiation action can migrate. It can migrate across an enzyme inhibitor or an enzyme substrate bond. We have measured the effect of trypsin and soybean trypsin inhibitor separately and combined and the effect of hyaluronic acid and hyaluronidase separately and combined. In both cases we conclude that energy can migrate. We are now studying this in the case of antigen antibodies.

7. On the other hand, radiation action does not readily migrate from one molecule to another in a dry solid.

   We have a rather simple experiment to show this, being done at the moment by Hutchinson. If you take electrons of finite range, e.g., 200-volt electrons, and you bombard a layer of invertase, you cannot burn off more than one monolayer no matter how long the radiation is applied. You only eliminate from this invertase preparation the top layer that corresponds to one molecule. This means that the transfer of radiation energy from the top layer to the second layer is very difficult.

   More recently, Hutchinson has shown that this is difficult even if the temperature of invertase is increased. So that the transfer from one molecule to another in dry solid is actually difficult in the case of invertase.

8. Previous treatment of a molecule, e.g., by heat, can condition its radiosensitivity.

9. Loss of solubility is an important response to radiation. It is not necessarily the most sensitive index, although on occasion, this is the case. For example, the main effect of irradiation of bovine serum albumin in bulk is the loss of solubility. If it is put on a

monolayer and irradiated, then its antigenic property is lost or its ability to combine with antibodies is lost, but it is lost after considerably more radiation than will remove its solubility.

Now, to start the discussion, I should like to suggest that we have an explanation for these events. This is largely aimed at Dr. Platzman. If we can get him started we have succeeded.

We feel that two things occur. I rather like the method of approach that is used by Augenstine in the remarkable little book on "Information Theory In Biology", that Quastler edited (University of Illinois Press, 1954). Augenstine analyzed protein denaturization in the following stages:

1. The breaking of a bond such as an S-S bond, which is a definite strong bond. This is associated with no entropy change and involves an energy change of about 20,000 calories per mole.

2. The breaking of a number of hydrogen bonds which opens the structure. They have entropy associated with them, and each has a much smaller amount of actual energy, in the neighborhood of 6000 calories per mole.

3. Another bond is joined, and, in Augenstine's approach, this is a new S-S bond, not the right one for the original configuration.

We should like to take almost exactly the same viewpoint for radiation action. Being a physicist, I know no chemistry and, therefore, I shall just draw the whole structure.

A physicist's idea of a protein backbone, with cross-linkages here and there is shown in Figure 1.

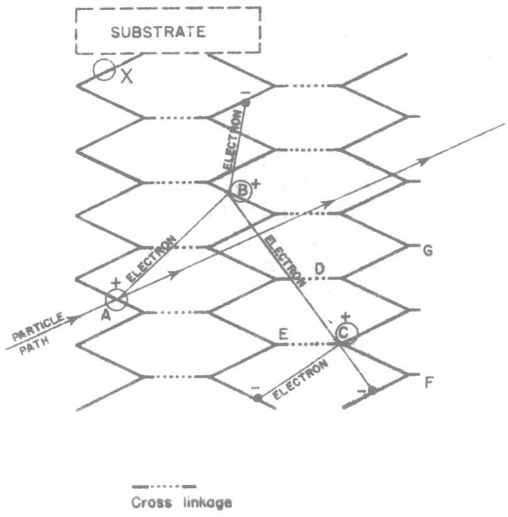

Figure 1. Schematic representation of events associated with the passage of a fast-charged particle through a protein molecule.

What is said about protein obviously can apply to nucleic acid also. Let us imagine that the particle traverses the molecule as shown in the diagram. This is the path of the fast-charged particle that does the ionizing. We will say that all it does is produce a primary ionization at A. As a result of the primary ionization, first of all, a plus is formed at A and then an electron is also released. We will say that the path of the electron is as indicated and that it ionizes again at B and then moves away. There are now two electrons produced, one of which comes to rest while the other ionizes at C before coming to rest. There are now three pluses and three minuses where electrons have been captured. Now all this must happen in the order of 10^{-13} seconds, perhaps even less because very little time is required.

What follows this and how it is related to the loss of biological function

of the molecule? Well, I feel that the things one has to think about are these: In the first place we have atoms that have lost an electron. The positive attribute, it seems to me, cannot possibly stay there, or at least there is no reason why it should. It would be quite natural for the neighboring atom to feed an electron into it, in which case, the plus is now in the next atom, even though the positive charge itself does not move physically. But from the place where it started it can go all the way along these chains and probably does so very rapidly. So we have a concept of migration up and down from one end to the other.

We have a specific functional region in the molecule, and let's say that this is attached in some way to the substrate or is hooked on to something else. It won't matter. Let us say that a bond is broken. Suppose I indicate a broken bond at X. The breaking of the bond is my conception of the removal of a valence electron by the migrating positive charge. This broken bond will mean that the structure will essentially break here, and the fragment can move off with the material of the substrate, or whatever you like, that is bound to it. In which case, the molecule no longer has its specific configuration and its biological activity is lost. This is inactivation by a single event and corresponds to the fact that the single event occurs in a place where just that one event is sufficient to cause inactivation. This might, for instance, be a prosthetic group that dropped off. The concept I want to state is that of the high energy single event. Let's call that category 1. This, in Augenstine's picture, would be the equivalent of the breaking of an S-S bond.

Now let's look at something else that can occur. If these positive positions wander around, they can move, for instance, into a place like E and that can mean that for a moment a bond will be broken. Now suppose that for some other reason, e.g., thermal agitation or another ionization, the bond at D is also broken temporarily. Then there can be a motion of the whole end of the chain outward.

Bear in mind that Figure 1 is not drawn to scale because I have drawn it linearly and, in actual fact, the ends are closer together. It is possible that this outward motion will then cause a cross-linkage between, for example, F and G, and this cross-linkage will make permanent the sort of damage that has occurred. This second method, too, is clearly dependent on the strength of the hydrogen bonding. This is something that may be dependent on temperature.

I feel that there is a lot of significance to the fact that proteins have a high coefficient of thermal expansion, and this may mean that they contain bonds that are actually capable of being weakened just by the fact that they are a little further apart when the high expansion is taking place. When this type of inactivation involving two bonds takes place, we observe a temperature effect.

In any event I should like to point out that the migration of the energy up and down these chains may take place by means of migration of the plus charge; this seems to me to be the significant thing.

I have concentrated on the plus charge, but what I have said also applies equally well to the minus, which will be stopped in the vicinity of an atom. Of course, in time these opposite charges will come close enough together so that a recombination can occur, and in a period of time of about 10^{-8} seconds recombination will be completed. It must be as small as that or we would not observe time-dose rate reciprocity in radiation action.

CURTIS: If I can get one thing clear, both of these events really occur at the outside of this molecule. That is, you have a volume here, and if I have