KFK-22

KERNFORSCHUNGSZENTRUM

KARLSRUHE

Juni 1960

KFK 22

Institut für Strahlenbiologie

# The development and prospects of quantitative radiobiology

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KERNREAKTOR
BAU- UND BETRIEBS-GESELLSCHAFT M.B.H.
KARLSRUHE

Reprinted from IMMEDIATE AND LOW LEVEL EFFECTS OF IONIZING RADIATIONS.
Conference held in Venice, June 1959.
A Special Supplement to the *International Journal of Radiation Biology.*

# The development and prospects of quantitative radiobiology

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## 1. INTRODUCTION

There does not seem to exist any generally-approved definition of the field of quantitative radiobiology. Nor do we propose to discuss this problem in detail here. We would rather limit ourselves to stating that most if not all of the results leading to the formulation of quantitative hypotheses in radiobiology have been obtained by using biological units having neither a nervous system, nor an involved physiology. Such a statement seems to hold for most of the work done during the past decades, and even today it is very difficult to use higher plants or animals for testing quantitative hypotheses. We shall, therefore, in the following discussion refer to work done on more elementary biological entities only, though we realize the desirability of obtaining a better understanding of the actions of radiations on higher organisms.

Quantitative investigation of the interactions of radiations and living matter did not start before the beginning of this century, in spite of the influence of radiations on life processes having been well recognized since antiquity. Also, the basic law governing all interactions of radiation and matter had already been established at the beginning of the last century by Grotthus and Draper, who clearly stated that actions can be brought about by absorbed radiations only, but not by transmitted or by reflected ones. Nevertheless, the first investigations which tried to correlate biological effects with the amount of radiation absorbed (usually called dose of radiation) appeared no earlier than about 50 years ago. These investigations, however, opened up immediately the exciting field of quantitative radiobiology, as they had been leading to some rather unexpected results obviously requiring quantitative treatment.

## 2. BASIC OBSERVATIONS

Actually, the early workers in the field made two observations which they found very difficult to understand, and we should add at once that in spite of the enormous amount of work done since those days we still have no generally-accepted explanation of these two observations. One is the fact that exceedingly small amounts of energy bring about very pronounced biological effects if the energy transfer is by radiation. Though there are many others I think still the best analogy to make this point clear is the following: The amount of energy contained as heat in an ordinary cup of tea would be lethal to men if absorbed as radiation from an X-ray beam instead of as thermal energy from tea. This observation, we feel, is so interesting as to justify any amount of work that may be required for its explanation, as this might well lead to a better understanding of life processes.

The second fact was more closely related to the type of experiment leading to its observation. It had been usual, probably in analogy with experiments in the field of chemical disinfection, to irradiate a population of biological units, e.g. bacteria, and to determine the ratio of individuals showing a certain effect after a certain dose. It had also been realized from the beginning that in order to make these experiments meaningful the population had to be as homogeneous as possible in all biological parameters, such as age or size of individuals. Similar experiments using chemical agents (poisons) had usually given a certain type of dose-effect curves showing practically no effect up to a 'threshold-dose', and thereafter a steep rise to 100-per-cent effect. Experiments using radiation, however, had frequently yielded dose-effect curves of entirely different shape, rising very slowly to 100-per-cent effect and often having no threshold at all. In experiments with chemical agents, the difference between threshold-dose and 100-per-cent dose had usually been explained by the unavoidable variability of biological parameters (spread of sensitivity). The results of radiation experiments seemed to require some entirely different explanation, for applying the same way of reasoning would have meant to postulate most unusual and highly improbable biological parameters. Fortunately, radiation physics was already sufficiently advanced at that time to provide the ideas necessary for formulating a useful working hypothesis which made much further progress possible.

## 3. HYPOTHESES OF DIRECT ACTION

It has become usual to assign the hypotheses of direct action and many other hypotheses in the field the rank of theories, and we feel that there is little hope for changing such widespread practice, though it should always be kept in mind that we still have no satisfactory theory capable of explaining the observations quantitatively. Nevertheless, we shall use common terminology.

The first to introduce modern physical concepts into radiobiology was Dessauer (1922, 1954) who suggested that the shape of dose-effect curves might be due to the fact that the absorption of radiation is not a continuous but a quantisized process and governed by a statistical law known as Poisson's law. Together with Blau and Altenburger (1922) he formulated the 'hit theory', according to which a certain minimum number of absorption events (called hits) within a biological entity should be necessary for the entity to show the effect under consideration. In fact, a strong similarity could be demonstrated between the dose-effect curves observed and the curves calculated for the necessary number of absorption events to occur at a given dose with a probability equalling unity. In these calculations the unavoidable variability of biological material had not been taken into consideration. Though this was neither an oversight nor a disregard of facts, but just a first approximation, it still led to a fierce discussion with some authors who did not approve of the application of purely physical methods to the problem. This discussion is no longer of interest, as the possible influence of various types of biological variability on hit curves has in the meantime been studied in detail (Zimmer 1941, 1943 a, 1956).

Much more important was a new concept introduced by Crowther (1926), who after independently developing the concepts of the hit theory, proceeded to propose the 'target theory'. He realized the possibility of calculating from the

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dose-effect curve a volume (target) within which the required number of absorption events would take place with a probability of unity during an irradiation with a given dose. Comparing the hit and the target theory, it is evident that the former is largely formalistic. It is closely related to the theory of reaction kinetics in chemistry and can well be described in such terms (Puck 1952). The target theory on the other hand requires a well-defined physical event to be chosen as a hit. A three-dimensional target, as originally envisaged by Crowther, can only be calculated if the dose is expressed in terms of ' absorption-events ' per unit mass or volume, though one can choose between a variety of physical processes, such as ionization, excitation, absorption of a photon, etc. Alternatively, a two-dimensional target (effective cross-section) can be calculated by expressing the dose in terms of ' passages of particles ' per unit area. But when using the target theory, the hit has always to be clearly defined. This necessity may facilitate the elucidation of subsequent processes (cf. Timoféeff-Ressovsky and Zimmer 1947).

The possibilities outlined above were first made use of by Holweck (1934, 1938), who suggested the use of radiation methods for determining the size of biological entities or structures. He named the method ' ultramicrométrie statistique ' and postulated the existence of biological structures or functional units (possibly of invisible smallness) corresponding to the targets as determined by irradiation. It should be emphasized here that the hypothesis of the ' ultramicrométrie statistique ' tacitly assumed that every hit occurring within a target would lead to the effect under observation (probability of action equalling unity). Unfortunately, nothing can be said *a priori* about the probability of action. But this difficulty can be overcome—at least to a certain extent—by using radiations of very different linear-energy transfer together with appropriate methods of calculation worked out by Fano (1938, unpublished) and Lea (1940, 1946) and developed to considerable accuracy (cf. Zimmer 1943 b, Pollard, Guild, Hutchinson and Setlow 1955).

It has already been mentioned above that the hit theory furnished a way of explaining the otherwise surprising shape of many dose-effect curves in radiobiology. The target theory, moreover, opened up a first possibility of explaining why radiation is so effective in causing biological actions. If it should be true that biological effects of radiations are brought about by transfer of energy to certain small targets, as distinct from the transfer of energy to the bulk of the biological material, one can readily see the difference between the actions of radiation and of thermal energy. Because of the statistical nature of the absorption of radiation, small targets can obtain comparatively large amounts of energy even at very small overall energy transfers to the bulk of the material. It was again Dessauer who coined the term ' point heat ' in order to illustrate this idea, though the local heat denaturation of proteins envisaged by him does not seem to be the most likely physico-chemical process to be considered. Many authors regarded chemical reactions started by the local-energy transfer to be more likely (Crowther 1938, Holthusen 1924, Lea 1946). Other processes have also been discussed, such as quantum jumps (Timoféeff-Ressovsky, Zimmer and Delbrück 1935), charge separation (Read 1949, 1951) and polarization effects (Platzman and Franck 1958). Lately, considerable evidence has been gained that the formation of free organic radicals cannot be neglected when studying the physico-chemical reactions which follow the absorption of radiation by living matter (Zimmer, Ehrenberg and Ehrenberg 1957, Howard-Flanders 1958, Howard-Flanders and Moore 1958, Zimmer 1959). But before discussing this promising aspect of quantitative radiobiology in detail, we have to turn first to other quite different hypotheses.

### 4. Hypotheses of indirect action

Though some of the hypotheses of indirect action in radiobiology can be dated back to the first investigations in the field, they have never been properly defined and could not, therefore, be really tested. This holds, for instance, for the idea that radiations produce cell poisons, bringing about the damage which we observe.

A more specific hypothesis has been proposed by Dale (1940) who applied to radiobiology some results he obtained by irradiating dilute aqueous solutions of biochemicals by ionizing radiation. He had been able to show that enzymes are inactivated by quite small doses of radiation if in very dilute aqueous solution. It had also been known since the extensive work of Fricke (1935) that chemical reactions in dilute aqueous solution are not brought about by direct absorption of ionizing radiation by the solute, but by some reaction product of water. As it was further known that most biological materials contain considerable amounts of water and only small concentrations of enzymes, it seemed well worth while to test such an indirect mechanism. After it had been emphasized by Weiss (1944) that water is decomposed by ionizing radiation, roughly speaking, into H-atoms and OH-radicals, the hypothesis of indirect action has been investigated very thoroughly. Two results of radiobiological research which became widely known at about the same time made the hypothesis of indirect action look very attractive: the dependence of many radiobiological effects on oxygen pressure and the discovery of so-called radioprotective substances. The possible existence of such phenomena had never been considered within the framework of direct action hypotheses and—at first glance—seemed to be difficult to understand in terms of these hypotheses, especially when taking the somewhat similar case of classical photochemistry into consideration. However, detailed studies showed that the results of photochemistry had been misleading when applied to our problem.

(i) A strong dependence on temperature was discovered for the inactivation of dry enzymes (Setlow 1952).

(ii) It could be demonstrated by biological and spectroscopic methods that, contrary to expectation, many of the radioprotective substances do not act by competition for the reaction products of irradiated water (Eldjarn and Pihl 1956, 1958, Gordy, Ard and Shields 1955, Norman and Ginoza 1958, Smaller and Avery 1959).

(iii) Radioprotective substances were found to be quite efficient in the dry state too, i.e. in cases which can clearly be described by direct-action hypotheses (Epstein and Schardl 1957, Ginoza and Norman 1957).

(iv) No satisfactory agreement could be established between the dependence on oxygen pressure of radiobiological effect and of formation of reaction products of water (Alper 1956, Alper and Howard-Flanders 1956).

Consequently, neither the occurrence of oxygen effects, nor the existence of protective substances, forms substantial evidence against direct-action hypotheses. In fact, it became quite clear from many studies that the indirect action via reaction-products of water is not the preponderant mechanism in

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radiobiology, though it certainly is of some importance. This finding led to experiments aiming at the determination of the relative importance of direct and indirect mechanisms, as well as to the formulation of combined hypotheses.

Before turning to a description of such work, it may briefly be mentioned that there are, of course, a number of other hypotheses of indirect action, e.g. via radiation-produced hydrogen peroxide or organic peroxides (Scholes, Weiss and Wheeler 1956, Latarjet 1958). In spite of considerable effort having been spent, no clear-cut evidence seems to have been reached as to the relative importance of such mechanisms.

## 5. Hypothesis of combined action

The hypothesis of combined ' direct plus indirect ' action was first formulated mathematically by Zirkle (1952) and by Zirkle and Tobias (1953) and later by Wijsman (1956). Because of the great number of parameters entering the calculation it is not easy to apply the theory to experimental results. Much quiet experimentation will still be required to make application easier. Nevertheless, considerable progress has been made in estimating the relative importance of direct and indirect effects in some special cases (Hutchinson 1955, 1957, 1958, Wood and Taylor 1957). It has also been possible to determine the average distance the radiation-produced reaction products of water travel inside a cell before becoming inactivated (Hutchinson 1957, 1958). This determination led to the rather small value of approximately $3 \times 10^{-7}$ cm, which is of the same order as the diameter of many targets calculated by applying the direct-action hypothesis. It bears out, in a quantitative and convincing way, the fact stressed much earlier by Lea (1946) and by Timoféeff-Ressovsky and Zimmer (1947) that cells do not consist of dilute aqueous solutions (cf. also Buzzati-Traverso and Cavalli 1948, Errera and Herve 1951).

At this point another fact should be mentioned which may prove to limit the usefulness of geometrical calculations in the fields of direct- as well as of indirect-action theories. It has been well known for about 20 years that intermolecular and intramolecular transfer of energy over distances of about $10^{-6}$ cm occurs in certain living systems (cf. Riehl, Timoféeff-Ressovsky and Zimmer 1941, Bücher 1950, Timoféeff-Ressovsky and Zimmer 1947). As has been pointed out before (Ehrenberg and Zimmer 1956, Zimmer 1958), the possible occurrence of energy transfer should always be kept in mind as it may well make calculations of target size and of diffusion paths meaningless in certain cases. Many types of energy transfer are, of course, closely connected to the fact that the interior of cells is usually organized in some kind of structure which makes cells even more different from dilute solutions. Consequently, some authors (Eidus 1956, Szent-Györgyi 1940, 1957) tend to attribute great importance to the consideration of energy-transfer mechanisms in explaining radiobiological effects. But this field is only at the beginning of being investigated, and mentioning the possible importance of energy transfer already leads to a discussion of what prospects there are for further developments in quantitative radiobiology.

## 6. Prospects for further development

The situation described in the preceding sections has—in recent years—led many authors to work rather from the other end of the chain of events that starts with the absorption of radiation and ends with the manifestation of some biological effect. Though experimentation of this kind has undoubtedly produced a wealth of very valuable results, we see no reason for neglecting any possibility of continuing the investigations into physico-chemical reactions immediately following the absorption of radiation by biological materials. It has already been mentioned that the results of classical photochemistry have generally been adopted without much attention being paid to the differences that obviously exist between the highly-purified gaseous systems or solutions the chemist uses and the very complex and structurally-organized material within a cell. As this over-simplification originated mainly from the lack of suitable experimental methods, it should be considered very fortunate that a new experimental technique has recently been put at our disposal: microwave spectroscopy. This new tool has already enabled very interesting new facts to be found and is certain to be a great help for further development of quantitative radiobiology.

We do not propose here to give details about the method and the experimental techniques (cf. Zimmer 1959), but we may mention that microwave spectroscopy yields information on the number and concentration of free radicals as a function of dose and dose-rate, of time after irradiation, and of external conditions such as temperature and water content of the specimen or type and pressure of the surrounding gas. Unfortunately, we cannot discuss the many interesting results that have already been obtained by applying the new tool to irradiated biochemicals as well as to irradiated living matter (for details see review by Müller and Zimmer 1959). The more important conclusions to be drawn from these investigations (Zimmer *et al.* 1957, Fairbanks 1957, Ehrenberg and Ehrenberg 1958, Norman and Ginoza 1958, Sparrman, Ehrenberg and Ehrenberg 1959, Smaller and Avery 1959, Conger and Randolph 1959) can be summarized as follows.

(i) Ionizing radiation produces free radicals in living matter.

(ii) The concentration of free radicals produced by radiation increases with increasing doses.

(iii) The concentration of detectable free radicals depends on the surrounding gas and on the water content of the specimen.

(iv) The concentration of free radicals decreases after the end of irradiation comparatively slowly. It remains easily measurable for intervals of minutes to many hours, depending on the material under investigation as well as on other parameters such as water content and surrounding gas.

(v) The hitherto generally-accepted view of absorption of radiation by living matter leading within microseconds to stable states has, consequently, to be abandoned.

(vi) A comparison of microwave spectra of various mixtures of radio-protective substances and of biochemicals with those of the components shows a strong ' molecular interaction '. The same effect could be demonstrated for unicellular organisms.

These observations certainly provide prospects for further development of quantitative radiobiology. They also lend considerable support to theoretical considerations derived by Howard-Flanders and Moore (1958) from quite different experiments, which also led to assuming the production of free radicals in living matter. The detection of long-living free radicals might well explain such observations as the so-called after-effects in irradiated biological entities. As far as the present investigations go they seem to support the direct-action hypotheses to a certain extent, but they also create new possibilities for developing the indirect-action hypotheses (cf. Howard-Flanders 1958). In fact one may hope for really important advances in the explanation of biological effects of radiation to come in the near future. It might even be possible to remodel and to develop the existing hypotheses in such a way that a satisfactory theory of quantitative radiobiology emerges.

## References

Alper, T., 1956, *Radiat. Res.*, **5**, 573.

Alper, T., and Howard-Flanders, P., 1956, *Nature, Lond.*, **178**, 978.

Blau, M., and Altenburger, K., 1922, *Z. Phys.*, **12**, 315.

Bücher, Th., 1950, *Angew. Chem.*, **62**, 256.

Buzzati-Traverso, A. A., and Cavalli, L. L., 1948, *Teoria dell urto ed unità biologiche elementari* (Milano: Longanesi & C.).

Conger, A. D., and Randolph, M. L., 1959, *Radiat. Res.*, **11**, 54.

Crowther, J. A., 1926, *Proc. roy. Soc.* B, **100**, 390; 1938, *Brit. J. Radiol.*, **11**, 132.

Dale, W. M., 1940, *Biochem. J.*, **34**, 1367.

Dessauer, F., 1922, *Z. Phys.*, **12**, 38; 1954, *Quantenbiologie* (Berlin-Göttingen-Heidelberg: Springer Verlag).

Eidus, L. Ch., 1956, *Biofizika*, **1**, 544.

Eldjarn, L., and Pihl, A., 1956, *Progress in Radiobiology*, ed. J. S. Mitchell, B. Holmes and C. L. Smith (Edinburgh and London: Oliver & Boyd); 1958, *Radiat. Res.*, **9**, 110.

Ehrenberg, L., and Ehrenberg, A., 1958, *Ark. Fys.*, **14**, 133.

Ehrenberg, L., and Zimmer, K. G., 1956, *Hereditas*, **42**, 515.

Epstein, H. T., and Schardl, D., 1957, *Nature, Lond.*, **179**, 100.

Errera, M., and Herve, A., 1951, *Mécanismes de l'action biologique des radiations* (Liège and Paris: Desoer, Masson & Cie).

Fairbanks, A. J., 1957, *Radiat. Res.*, **7**, 314.

Fricke, H., 1935, *Cold Spr. Harb. Symp. Quant. Biol.*, **3**, 55.

Ginoza, W., and Norman, A., 1957, *Nature, Lond.*, **179**, 520.

Gordy, W., Ard, W. B., and Shields, H., 1955, *Proc. nat. Acad. Sci., Wash.*, **41**, 983.

Holweck, F., 1934, *Radiophysiol. et Radiother.*, **3**, 101; 1938, *C.R. Acad. Sci.*, **207**, 380.

Holthusen, H., 1924, *Strahlentherapie*, **18**, 241.

Howard-Flanders, P., 1958, *Advanc. Med. Biol. Phys.*, **6**, 553.

Howard-Flanders, P., and Moore, D., 1958, *Radiat. Res.*, **9**, 422.

Hutchinson, F., 1957, *Advances in Radiobiology*, ed. G. de Hevesy, A. G. Forssberg and J. D. Abbatt (Edinburgh and London: Oliver & Boyd); 1958, *Radiat. Res.*, **9**, 13 and 132.

Hutchinson, F., and Preston, A., 1955, *Radiat. Res.*, **3**, 326.

Latarjet, R., 1958, *Les peroxydes organiques en radiobiologie* (Paris: Masson et Cie).

Lea, D. E., 1940, *J. Genet.*, **39**, 181; 1946, *Actions of radiations on living cells* (Cambridge: University Press).

Müller, A., and Zimmer, K. G., 1959, *Strahlentherapie*, **109**, 192.

Norman, A., and Ginoza, W., 1958, *Radiat. Res.*, **9**, 77.

Platzman, R., and Franck, J., 1958, *Symposium on Information Theory in Biology*, ed. H. P. Yockey, R. L. Platzman and H. Quastler (London: Pergamon Press).

Pollard, E. C., Guild, W. R., Hutchinson, F., and Setlow, R. B., 1955, *Progr. Biophys.*, **5**, 72.

Puck, Th. T., 1952, *Symposium on Radiobiology*, ed. J. J. Nickson (New York: J. Wiley & Sons).

Read, J., 1949, *Brit. J. Radiol.*, **22**, 366; 1951, *Ibid.*, **24**, 345.

Riehl, N., Timoféeff-Ressovsky, N. W., and Zimmer, K. G., 1941, *Naturwissenschaften* **29**, 625.

Scholes, G., Weiss, J., and Wheeler, C. M., 1956, *Nature, Lond.*, **178**, 157.
Setlow, R. B., 1952, *Proc. nat. Acad. Sci., Wash.*, **38**, 166.
Smaller, B., and Avery, E. C., 1959, *Nature, Lond.*, **183**, 539.
Sparrman, B., Ehrenberg, L., and Ehrenberg, A., 1959, *Acta chem. scand.*, **13**, 199.
Szent-Györgyi, A., 1940, *Nature, Lond.*, **148**, 157; 1957, *Bioenergetics* (New York: Academic Press).
Timoféeff-Ressovsky, N. W., and Zimmer, K. G., 1947, *Biophysik.* I. *Das Trefferprinzip in der Biologie* (Leipzig: S. Hirzel).
Timoféeff-Ressovsky, N. W., Zimmer, K. G., and Delbrück, M., 1935, *Nachr. Ges. Wiss. Göttingen*, VI, N.F. 1, 189.
Weiss, J., 1944, *Nature, Lond.*, **153**, 748.
Wijsman, R. A., 1956, *Radiat. Res.*, **4**, 257 and 270.
Wood, Th. H., and Taylor, A. L., 1957, *Radiat. Res.*, **6**, 611 and **7**, 99.
Zimmer, K. G., 1941, *Biol. Zbl.*, **61**, 208; 1943 a, *Ibid.*, **63**, 72; 1943 b, *Phys. Z.*, **44**, 233; 1956, *Acta radiol.*, **46**, 595; 1958, *Naturwissenschaften*, **45**, 325; 1959, *Radiat. Res.*, Suppl. **1**, 519.
Zimmer, K. G., Ehrenberg, L., and Ehrenberg, A., 1957, *Strahlentherapie*, **103**, 3.
Zirkle, R. E., 1952, *Symposium on Radiobiology*, ed. J. J. Nickson (New York: J. Wiley & Sons).
Zirkle, R. E., and Tobias, C., 1953, *Arch. biochem. biophys.*, **47**, 282.

## Discussion

*Bacq:*

As far as I know the method of microwaves spectrometry gives indication about the *number* but not the *nature* of free radicals. Is there any hope that in the future the method may be improved to indicate the *nature* of these free radicals, many of which may be perfectly uninteresting from the biological view-point ?

*Zimmer:*

In certain selected cases the nature of the free radicals formed by irradiation can, by analysis of hyperfine structure, already be determined in a fairly convincing way. Also, the method is being improved continuously.

*Lipetz:*

The question proposed by Dr. Bacq should be extended still further. To determine whether the free radicals detected by the new techniques are of biological importance, it will be necessary to know more than their identity. We must also know where they are—whether they are located near enough to structures of biological importance to affect those structures. For example, you pointed out that the free radicals formed in water have only a limited range of action. What is being done to determine the locations of free radicals in biological structures ?

*Zimmer:*

The importance of the problem of where the free radicals are located in biological structures is clearly seen by radiobiologists. There are already some relevant studies and more will surely appear in the near future.

*Altman:*

Are there any data pertaining to microwave spectra during the exposure to ionizing radiations ?

*Zimmer:*

I do not know of any detailed data pertaining to microwave spectra during exposure to ionizing radiations. There is just a short remark in *Biofizika* (Vol. 4, page 93), 1959 that such experiments have been done by V. V. Vojevodski leading to very interesting results.

*Magni:*

Am I right in understanding from the data you presented that, as far as the initial amount of free radicals that are produced with X-rays, a higher concentration of them is obtained in systems irradiated in the dry state as compared with wet systems, in addition to the fact that in dry systems the decay of free radicals is shown ?

If this is so, it links up well with a fact that Dr. Cavalli-Sforza and I have recently observed irradiating *E. coli* cells with soft X-rays, namely that when bacteria are irradiated in the lyophilized condition they are more sensitive to X-rays than non-lyophilized bacteria by a factor of 2 or 3.

*Zimmer:*

Nothing much can be said so far about the number of free radicals *formed* as this would require measurements *during* irradiation. Otherwise there will always be the possibility of errors by very rapid decay. However, the studies by Ehrenberg and co-workers seem to indicate that biological damage depends on the long-living radicals rather than on the short-living ones.

*Nilan:*

The curves you have presented for magnetic centre production after irradiation of *Agrostis* seeds at different moisture levels are similar to those Dr. Caldecott, Dr. Konzak and I obtained for chromosome damage in barley embryos. We find greater post-irradiation damage in the embryos at 4 per cent moisture than in those at 16 per cent.

*Zimmer:*

You are quite right. In fact, this similarity, as has been pointed out by Ehrenberg and co-workers, is quite striking.