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From Target-Theory to Molecular Radiobiology

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The Eleventh Douglas Lea Memorial Lecture†

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1. Introduction

A foreigner's attempt to lecture on radiobiology in Britain bears, of course, a strong similarity to bringing coals to Newcastle—or, should I say these days, ' to bringing atomic know-how to Harwell '. Let me, therefore, start by recalling a day in late spring 1936 when I was just about to leave my modest laboratory in a clinic in Berlin to drive out to a research institute where I used to spend my afternoons and evenings. It may interest you that for my half-days' work as the only physicist of the largest radiological hospital in Berlin I earned exactly £105 a year: a sum on which I had to live as my research-work was not connected with any remuneration whatsoever. On this day I had just got up to seize my raincoat when suddenly the door burst open and two energetic young men entered my room. I had never before seen either of the two and felt rather relieved when they introduced themselves as Harold Gray and Douglas Lea, wanting to discuss radiobiology with me. They would not know, of course, that in those days in Berlin being visited by two unknown men and without previous warning often meant trouble with the political police! In the present case I suggested driving together to the Kaiser-Wilhelm-Institut where I worked with N. W. Timoféeff-Ressovsky on the production by radiation of gene-mutations in *Drosophila*. My visitors readily agreed to join me in my recently acquired though very old motor car. From their publications I knew the ' intellectual weight ' of my passengers but I did not, at first, give due consideration to their ' physical weight ', which turned out to be comparable to the weight of my little car made mainly of plywood. This error of judgment combined with my lack of experience in driving led at a hair-pin bend in the road to dangerous instability, and we very nearly fell into one of the basins of Berlin's extended harbour. Fortunately, the accident with its probably fatal consequences was avoided, and there were no further dramatic events on this afternoon.

But the discussions were so interesting as to be resumed several times over the years. The two groups met in Britain or Germany as occasion arose, and exchanged ideas and manuscripts right to the day when the war inhibited further communication. Experimental and theoretical work, however, was actively pursued during the war by both groups and along lines largely determined by the aforementioned discussions. The outcome was summarized in two books written independently of each other during the last years of the war

† Delivered to the Hospital Physicists' Association on 11 September 1969.

and published soon after its end. The well-known book by Lea (1946) put more weight on those aspects of the problem that can conveniently be subsumed under ' target-theory in radiobiology '. The other one by Timoféeff-Ressovsky and Zimmer (1947) rather stressed the ' hit-principle ' and some related problems in general biology. Naturally, this second book, appearing in German, had a difficult start in those years. However, it was considered by the military government of the eastern part of Germany to be of such interest as to make them officially collect and pulp as many copies as they could get hold of.

Such official views tend to change rather rapidly. I suggest, therefore, to consider now some questions of more permanent importance: i.e. the conceptual roots of ' hit '- and ' target '-theories, their aims, achievements, and limitations.

2. The roots of ' hit '- and ' target '-theories

In retrospect it is quite easy to see why the basic ideas of these theories developed 40 to 50 years ago nearly simultaneously though independently in several countries. Given the well-known and still undeniable facts that (i) absorption of energy from radiation is a quantized process following statistical laws, and (ii) that living things show a structural hierarchy, it was nearly inevitable that several scientists tried to extrapolate towards biology from Einstein's famous postulate of photochemical equivalence:

$$\phi = \frac{\text{number of changed molecules}}{\text{number of absorbed photons}} = 1.$$

This postulate was meant, of course, to hold for a homogeneous system made up of small and relatively simple molecules which photochemists used in the early days for their studies. Biological entities are more complicated. Nevertheless, replacing in Einstein's postulate ' *number of changed molecules* ' by ' *number of biological entities showing the reaction under study* ' and ' *number of absorbed photons* ' by ' *number of targets ionized* ' already yields the basic idea which Lea set out to prove in his experiments as well as in his book. Here, ' target ' may mean any functionally important structure of the biological entity, e.g. a DNA-molecule, a chromosome or a cell wall, thus allowing for the structural and functional heterogeneity of biological systems. We now know that Einstein's postulate is seldom fulfilled in practice, because of the occurrence of chain-reactions, of processes of energy transfer and of rapid dissipation of absorbed energy as heat. Moreover, it was shown that bigger entities, such as the minute crystals of silver bromide used in photographic emulsions, usually require the absorption of two or more photons to become developable. Analogous or more complicated deviations from the ideal case were bound to be detected in the highly heterogeneous biological systems with targets of molecular weight of 10^6 to 10^8 in the case of DNA, and about 10^{10} in the case of the wall of a *Bacterium coli* (this wall according to recent results also constitutes a single giant-molecule).

But we also know that Einstein's postulate formed a considerable stimulus to research in photochemistry. Just the same must be said about hit- and target-concepts. Though seldom sufficient to explain a given set of results

they were of great heuristic value and will remain to stimulate research in radiation biology for a long time to come. Nevertheless, the aim of radiobiology remains to explain in detail what processes lead to reaction, and we should turn our attention to some parameters which in the way of a first approximation were deliberately neglected in the earlier treatments of hit- and target-theory. An attempt to overcome the limitations set by these first approximations requires transition to molecular radiobiology.

3. Transition to molecular radiobiology

Let me use Einstein's postulate again to demonstrate the roots of more recent developments in the field. If the 'ideal' relation $\phi = 1$ does not hold we can, obviously, be faced with the following possibilities:

$$\phi = a, \text{ where } a = \text{const} < 1$$
$$\phi = b, \text{ where } b = \text{const} > 1$$
$$\phi = c, \text{ where } c = f(D) \neq \text{const.}$$

There is no need to invoke the tiresome mathematical formalism for explaining the meaning of the three cases in terms of hit- and target-theories. The first possibility, $\phi < 1$, means that either a hit on a target acts with an efficiency less than unity or, alternatively, that a structure assumed to be the target comprises an insensitive as well as a sensitive region. Such possibilities were already provided for, in a formal way, even in the earliest papers in the field but on the ground of quite plausible arguments, considered unlikely to occur in most of the experimental work done or analysed by Lea. The case of $\phi > 1$ has now been observed quite frequently when heavy particles are used to bombard small biological entities such as phage. In the language of target-theory it means that a target is damaged or somehow influenced by a bullet passing at some distance from it. Such a possibility was not foreseen either by Lea or in any of the other early treatises. The third case $\phi = f(D)$ denotes that the efficiency of energy absorbed in the target to cause an observable reaction is not constant but depends on dose D of irradiation. Such results are often obtained when the systems under study are capable of repairing damage induced by irradiation. This case is briefly mentioned in Lea's book as well as in some other publications, but not considered in any detail as sufficient experimental evidence was lacking at the time.

The mathematical formalism of hit- and target-theories has now been developed to cover a great variety of possibilities. Among these are the cases just mentioned and others which are not likely ever to be found in an experiment. These advanced exercises in laws of probability are certainly interesting in themselves but usually difficult to apply and seldom helpful in radiobiology—quite at variance with the simple concepts the great heuristic value of which was emphasized in the previous section. Progress in elucidating mechanisms is more likely to result from biological and physico-chemical studies on problems such as: why is, in a given case, ϕ smaller than unity, how, in other cases can ϕ be larger than unity, etc. The problem of repair or recovery from damage can most conveniently be studied using materials and method

commonly employed in molecular biology and molecular genetics. This kind of work, if applied to study the biological effects of radiations, might well be named ' molecular radiobiology ', using the word ' molecular ' in the same loose and ill-defined way as many biologists and geneticists tend to use it. It is difficult to see how the physicist can apply his knowledge to these largely biological and biochemical studies though, admittedly, it is in fashion for physicists to work in biology.

There is, however, another branch of molecular radiobiology urgently requiring the attention of physicists. In analogy to Einstein's postulate, Lea postulated ' ionization of a target leads to biological effect ' and left the problem of what really happens to the target to future studies. This was a necessary and acceptable first approximation in a most valuable and serious attempt to create some kind of theoretical framework for radiobiology. Nevertheless, the problem needs further and careful investigation. Here, the physicist can make adequate use of his training and skill, as I hope to demonstrate by reporting briefly some of our work devoted to this problem.

4. Some examples of the biophysical approach to molecular radiobiology†

A physicist wants for his experiments a biological object easily obtainable in large numbers of as nearly identical entities as possible. Preferably, the material should consist of functionally important molecules only, and, finally, it should withstand high vacuum and a large range of temperatures without losing its functional activity. Remarkably enough a material meeting these requirements can be found: bacterial virus, more commonly called phage. Quite at variance with most biological materials, phage can easily be produced in amounts of 10^{14} individuals in one batch. Such a sample weighs after freeze-drying only about 100 milligrammes and withstands high vacuum and temperatures from 4° to about 350°K without much loss of infectivity. Moreover, some phages contain just one molecule of DNA representing roughly one half of the weight of the whole entity, and finally DNA can by suitable treatment be obtained in pure form again without prohibitive loss of infectivity. Even the most cautious biologists will subscribe to the statement that DNA is indeed a functionally important biomolecule. Much to the physicists' delight DNA can be prepared to withstand vacuum and an even larger range of temperatures than whole phage. Afterwards it can still be tested for its biological function, i.e. for production of new phage in an appropriate host. Thus, phage and its free DNA are well suited for investigating the processes occurring in an ionized target.

As is well known, DNA usually has the form of a long thin fibre consisting, in some of the phages, of a single, in others of two strands, and occasionally assuming circular form. Just to illustrate the extraordinary structure of this

† It is considered inconvenient to burden this section with a great number of figures, tables, and references. For these, and for more detailed description of experimental methods and results, the interested reader is referred to reports by Zimmer and colleagues (1969) and by Dertinger and Jung (1969).

type of molecule: accepting the width of the part of a DNA molecule (fig. 1) as it appears on the screen (say 1 metre) the whole length of one molecule would, on the same scale, be of the order of the distance from Teddington to Trafalgar Square (20 km). Every physicist would expect breakage of such a long thin fibre to be the most probable damage by radiation. Studies by means of an analytical ultracentrifuge show, in fact, breakage to be caused by ionizing radiation but, somewhat surprisingly, to be responsible for only 20 to 40% of the inactivation observed in most phages. Using more refined methods of investigation (and with these a physicist can be of valuable help) it is possible to calculate the average molecular weight of the pieces resulting after irradiation, and also their distribution in number and weight. Such measurements show intramolecular cross-linking to occur besides breakage. But this form of damage accounts for only a few per cent of the lethal effect observed biologically.

Before considering what other processes ionization might cause in a DNA molecule one would, of course, like to know a little more about the processes leading to breakage. This question has hitherto been attacked by biochemical

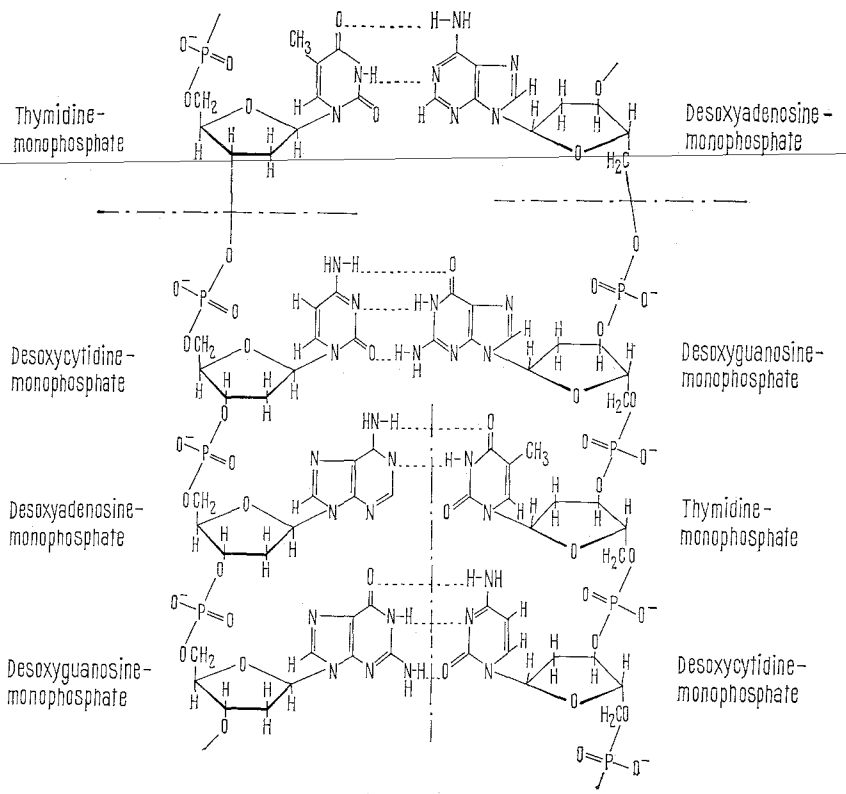

Fig. 1. Part of a two-stranded DNA-molecule showing the structure of the four more frequently occurring bases, the ways in which strand breakage often occurs, and the opening of hydrogen-bonds as observed after ionizing irradiation.

methods mainly. By chromatographic and enzymatic techniques it could be shown that breakage occurs with comparable frequency at the 3′-carbon and at the 5′-carbon of the desoxyribose part of DNA (fig. 1). In addition chromatographic studies led to finding quite another kind of damage: hydrogen-bonds holding the two strands together are opened by irradiation. The biological consequences of this type of damage are, however, not yet known.

Returning to more physical methods of investigation a careful study of the dependance of radiation damage in phage on temperature was indicated. As mentioned before, such experiments present no particular difficulty and were actually done *in vacuo* using γ-rays and 1 Mev protons at temperatures from about 4°K to well above room temperature. The results can be plotted in Arrhenius-diagrams to obtain apparent energies of activation for the reactions involved. These come out to amount to about 1 kcal/mole over a range from that of liquid nitrogen to 0°C and to a few kcal/mole at higher temperatures. These values are too small by one to two orders of magnitude to be connected with ordinary chemical reactions but suggest free-radical-reactions.

The production of free radicals by irradiation of dry organic materials is, of course, amenable to investigation by electron spin resonance (e.s.r.) in its quantitative and qualitative aspects. We concentrated on the quantitative aspect first. Unpaired spins due to free radicals were demonstrated to be caused by radiation in complete phage, in its DNA, and in all constituents of DNA such as various bases, sugar (desoxyribose), and nucleotides. The yields of detectable radicals (G-values) were found to range from 0·1 to 0·5 per 100 ev of energy absorbed from radiation. They are comparable to or rather higher than the yields of inactivation of dry phage which in our experiments amount to G-values of 0·01 to 0·3, depending on temperature and on other circumambient conditions during irradiation. Consequently, free radical reactions might well be an important link in the chain of events following ionization of a target.

Studies on e.s.r.-spectra can also furnish information on the kind of free radical induced by irradiation. Here, substantial progress could be made only after the major constituents of DNA were available in the form of monocrystals. To grow these in sufficiently large size requires much skill and patience. It is, however, feasible and permits investigation of the spectra as they depend on the relative orientation of crystal axes to the direction of the magnetic field. Fig. 2 illustrates some of the results obtained in our laboratory as well as in others. Some of the details are still controversial. Nevertheless, it is already evident that reactions involving addition or abstraction of hydrogen are important if not preponderant. They are easily caused by atomic hydrogen, a species known to be abundantly produced by irradiation of organic chemicals. We felt, therefore, quite satisfied that our e.s.r.-spectra of phage and of its DNA taken in the X-band showed, in fact, the two lines 506 oersted apart and with an apparent magnetic g-factor slightly higher than that of the free electron to be expected for trapped atomic hydrogen.

These results suggest that the formation of atomic hydrogen is one of the important consequences of ionizing a dry target, and responsible for the

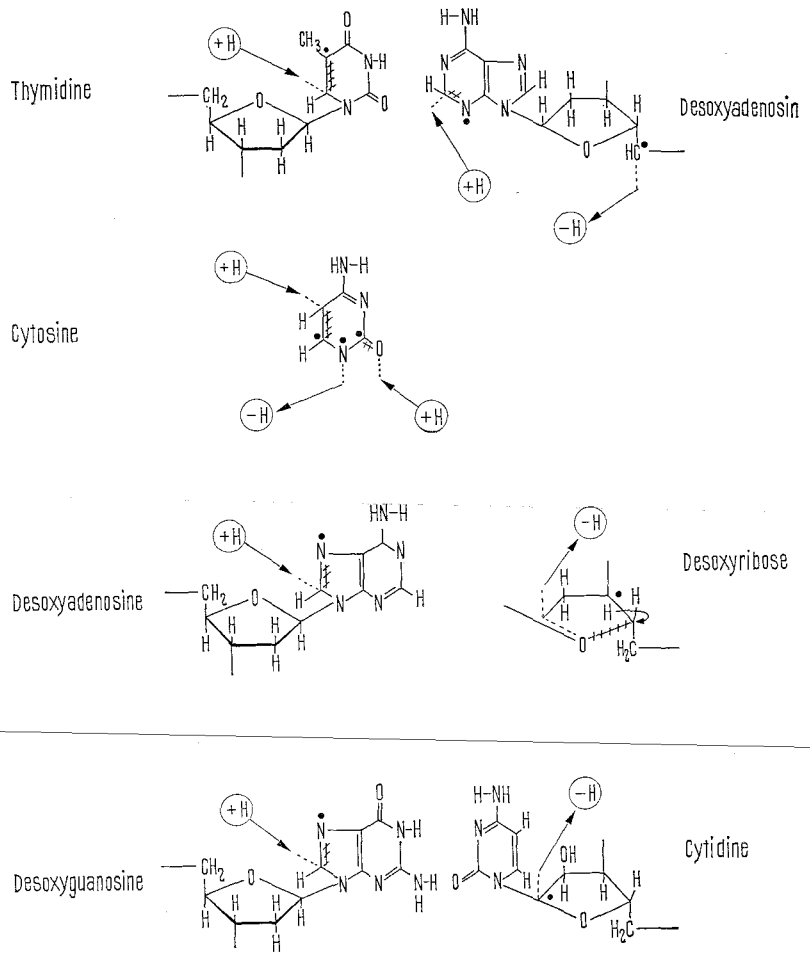

Fig. 2. Some of the free radicals proposed to account for the e.s.r.-spectra found in monocrystals of DNA-constituents after ionizing irradiation. Though some of the radical-structures given here are still open to further discussion all of them suggest addition or abstraction of hydrogen atoms as common cause.

appearance of other free radicals, which in turn may lead to biologically observable damage in DNA. In support of this view one should demonstrate, however, that atomic hydrogen generated outside of phage and DNA can cause the same biological effects in these materials as does ionizing radiation. To this end we used two different methods. In experiments with method I, dry phage or its DNA were exposed to atomic hydrogen produced in the ' classical ' way by an electric high frequency discharge in hydrogen gas. Method II simulated more closely conditions assumed to prevail during irradiation. Here a foil of organic plastic (polyethyleneterephthalate) was bombarded at a small angle by 2 Mev protons. Well shielded from these fast protons and also from any scattered electrons, biological samples arranged at some distance

were exposed to the atomic hydrogen liberated from the foil by the ionizing fast protons. The equipment used in method II somewhat resembled a parallel plate ionization chamber *in vacuo*.

As expected, inactivation of phage, of its infectious DNA, and also of the enzyme RNase was observed on exposure to atomic hydrogen, produced either directly from gaseous hydrogen by electric discharge (method I) or indirectly from a plastic foil by ionizing radiation (method II). With both methods and for all the materials tested, inactivation by atomic hydrogen followed the same kinetics (first order or single hit) as observed with ionizing radiation. Moreover, method II permitted experiments to be done at various temperatures. These yielded an apparent energy of activation of about 1 kcal/mole, in good agreement with the value found in experiments in which ionizing radiation impinged directly upon the specimens.

Such consistency of results lends strong support to the view that the production of atomic hydrogen and the radical reactions caused thereby are important consequences of ionizations in dry targets. But my aim here was to give some examples of the biophysical approach to molecular radiobiology rather than to prove or disprove a particular pathway of reaction. Other examples might be mentioned briefly, such as the use of vacuum-ultraviolet: a method still in its beginnings, though promising results were already obtained. The elastic nuclear collisions between protons in the energy range below 1 kev in biological materials are also of interest for elucidating processes of damaging targets.

Returning for a moment to the target-theory in its original geometric-mechanistic form: the importance of atomic hydrogen (made probable by the experiments described above) might weaken the hit- and target-concept to some extent. For atomic hydrogen can diffuse freely in nearly all materials and at all temperatures. However, before drawing any definite conclusion we have to know many more details of these processes. To mention just some of the problems:

(i) What is the probability of reaction of atomic hydrogen with the molecule(s) forming a target?

(ii) What does the apparent energy of activation of about 1 kcal/mole really mean? Is it the energy needed to initiate a radical reaction, to lift atomic hydrogen out of a trap, or to remove it from its parent molecule? Could it be the sum of these energies?

(iii) Additional e.s.r.-measurements are also needed particularly at very low temperatures in order to establish a more complete scheme of reactions occurring in an ionized target.

These and related problems provide ample opportunities for the physicist to make meaningful contributions to molecular radiobiology without requiring him to leave his proper field of work. Such studies will, however, not necessarily disprove the target-theory, but should rather aim at giving its somewhat formal basic concepts a meaning in terms of physics and chemistry. This task obviously requires intermediate steps too. Here, the approaches put

forward by Howard-Flanders (1958), and more recently by Butts and Katz (1967), should be mentioned. In his track-segment-method Howard-Flanders showed that many sets of experiments can adequately be explained by considering the amount of energy absorbed from the track of an ionizing particle crossing a target; whereas Butts and Katz demonstrated that for very small targets it is more convenient to regard such targets as dimensionless mathematical points and to pay closer attention to track-structure. These approaches are very valuable extensions of the earlier forms of target-theory, though they too are somewhat formalistic. It is tempting to draw an analogy here: Mendel's laws of heredity were highly formalistic and the theory of crossing-over developed later was again a rather formalistic but extremely useful extension. Nevertheless, molecular genetics cannot do without these concepts but aims at explaining the underlying processes on the molecular level, i.e. in terms of physics and chemistry. In a very similar way molecular radiobiology has to build on the foundations laid earlier, and of these Lea's contributions are surely an important part.

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