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Radiation Inactivation of Enzymes, Nucleic Acids, and Phage Particles

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INTRODUCTION

IT is worthwhile, before the description of experimental work, to say a word about the motivation of the researches to be described and also something of the people engaged in them. The purpose has been to use ionizing radiation as a powerful, localized, and penetrating agent to study cell structure in relation to cellular function. This purpose was clearly in the mind of the late D. E. Lea, and in many ways we have been continuing lines which he began. The purpose can be usefully directed only if there is some knowledge of the actual character of ionizing radiation action on the three key elements of cellular systems: proteins, nucleic acids, and polysaccharides. Such knowledge is still imperfect, and what is here described is a series of studies, which enable one to make some preliminary hypotheses as to the action of ionizing radiation. The promise of immediate future progress is excellent, and, if research effort on the right scale were forthcoming, a year or two more would see the major features of the pattern properly exposed.

The people in the Yale Biophysics Department who have contributed are Hutchinson, Setlow, Guild, Preiss, Woese, Powell, Forro, Fluke, Jagger, Wilson, Till, and Whitmore. The author writes as representing this group and the work is theirs as much as his in every respect.

In the process of studying the inactivation of these key molecules, the basic problems of radiobiology are also being studied; and since radiobiology cannot be separated from biology, the problems of biology are also involved. This will become apparent, as has been stressed already by Zirkle (p. 269).

GENERAL CHARACTER OF RADIATION INACTIVATION

The physical action of radiation is complex. All optically allowed molecular transitions presumably are capable of occurring, together with many forbidden transitions. The gradual dispersal of the intense local energy releases, and the accompanying "pre-equipartition" local high-temperature regions, plus the probable effect of local high temperatures, conspire to give the molecular physicist a choice of almost any personally favored mechanism of action. Such choices have been made, and it would be foolish to exempt this paper. In view of this great complexity, the traditional physical introduction is foregone and the experimental results on biological macromolecules are discussed directly.

The sort of experiment which is readily performed requires a set of assay tubes for some enzymes and a color agent that is dark for active enzymes. Since the reaction time is kept constant, the activity of the enzyme shows in the relative color density, and, for an enzyme heavily irradiated with γ-rays, the activity is clearly low. Such experiments were carried out first by Northrop[1] and by Hussey and Thompson.[2] Similar experiments on viruses are described later. Interestingly enough, the earliest recorded quantitative measurements on bacteriophage were made by the Sinclair Lewis hero, Martin Arrowsmith, which probably reflects the active mind of Paul de Kruif.

The dose-response curve found in these experiments generally obeys the relation

$$\ln(n/n_0)=\text{const}\times\text{dose}. \tag{1}$$

It can be explained statistically very simply, as suggested by Dessauer,[3] Crowther,[4] and Condon and Terrill[5] many years ago, by supposing that I inactivating events per unit volume are distributed randomly and that there is a critical sensitive volume V which may intercept one of these events. Since the average number of events in a volume V is IV, then by the Poisson formula the probabilities of 0, 1, 2, 3, etc., events taking place in the volume are $P(0)$, $P(1)$, $P(2)$, $P(3)$, where

$$P(0)=e^{-IV} \qquad P(m)=e^{-IV}(IV)^m/m!$$
$$P(1)=e^{-IV}IV$$
$$P(2)=\frac{e^{-IV}(IV)^2}{2!}$$

These are mutually exclusive events so that $\sum P(m)=1$, and, accordingly, one can reason thus: If the probability of escape is measured by the ratio of the number left active, n, to the number at the start, n_0, then for complete escape, $P(0)$, one has

$$n/n_0=e^{-IV}. \tag{2}$$

If one "hit" can be withstood,

$$n/n_0=e^{-IV}+e^{-IV}IV. \tag{3}$$

If two hits can be withstood,

$$n/n_0=e^{-IV}+e^{-IV}IV+\frac{e^{-IV}(IV)^2}{2!}, \tag{4}$$

and so on.

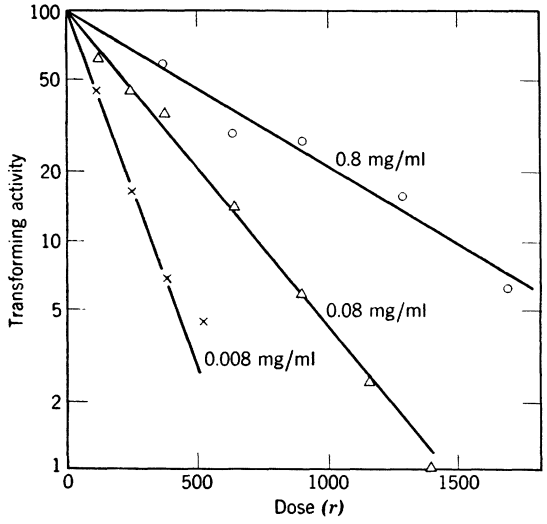

FIG. 1. The loss of transforming activity in pneumococcus DNA preparations in dilute solution. This is typical of irradiation in dilute solution where the majority of the effect is owing to the migration of radicals. That this is so can be seen from the increased inactivation in more dilute solution. Data due to DeFilippes and Guild.[6]

Usually, for enzymes and viruses, one finds the simplest, complete escape expression holding, or

$$\ln(n/n_0) = -IV. \tag{5}$$

Thus, I can be identified somehow with the dose and V with the constant in Eq. (1). The data of Figs. 1 and 2 provide examples of two inactivations which obey this relation. Figure 1, taken from work by DeFilippes and Guild,[6] shows the effect of x-rays on the transforming principle of pneumococcus, which Hotchkiss has shown to be pure DNA, and which was referred to by Rich (p. 191). The ordinate, the activity, is plotted logarithmically *vs* the dose in roentgens, and the relation for a one-hit effect holds. If the inactivation is ascribed to primary ionizations per unit volume, using a conversion factor of $5\times10''$ primary ionizations per cubic centimeter in water, an absurd value for V is found, moreover one which depends upon the concentration of transforming principle (TP). The inactivation is due clearly to "activated water," probably free radicals which can migrate and so make V many times larger than the volume of the molecule. In principle, if the radicals in activated water have infinite lifetime, and if the water and TP are pure, there always should be one molecule of TP inactivated per radical. In fact, radicals recombine; Hutchinson and Ross[7] and Smith[8] have estimated their half-life. In very pure water, it is as long as 10^{-4} sec. In yeast, it is 10^{-9} sec.

As a contrast, Fig. 2 shows some new data, taken by the author and Nancy Barrett, for inactivation of β-galactosidase. With one exception, the irradiations were in the dry state. All were done with a cobalt γ-ray source. The black dots show the effect of radiation on enzyme extracted from lactose-adapted bacteria. The relation $\ln(n/n_0) = -IV$ is obeyed. If one uses for I the number of primary ionizations, or clusters of ions, one finds $V = 4.7\times10^{-19}$cc. Since one usually is not familiar with the volumes of molecules, this figure can be converted into a molecular weight by assuming a protein density of 1.3 and multiplying the mass of one molecule by Avogadro's number to get the molecular weight. The figure found is 370 000. Estimates for the molecular weight of β-galactosidase are mostly guesses, but, if one takes the molecule to be spherical, a sedimentation constant of 18, which has been quoted, and which is approximately checked by work in our laboratory by Langridge, gives a molecular weight of 390 000. So, in this case, the dry measurements lead to a statistical

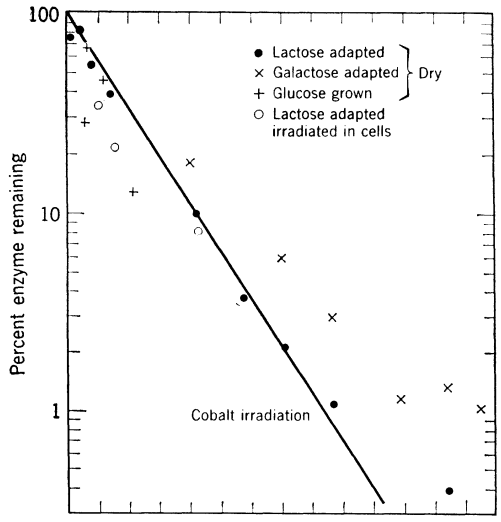

FIG. 2. The irradiation in the dry state of β-galactosidase grown under various conditions. The characteristic behavior is that an essentially uniform method of inactivation is observed dependent only on the direct hitting of some sensitive region. Irradiation in the wet state is put in for comparison. It is clear that in the wet state inside the bacterium the concentration is so high that inactivation is similar to the dry inactivation.

analysis in which the volume V may well be the volume of the molecule.

The "constitutive" enzyme, that small fraction which is present in unadapted cells, behaves about the same as does enzyme from galactose-adapted cells. The sensitivity of the enzyme in cells irradiated in C-minimal medium is not noticeably different.

Thus, one may draw radiation action on a molecule in two ways, as shown in Fig. 3. The shaded part in the center is possibly identifiable with the macromolecule itself, while the dotted lines are owing to migration of active chemical agents. For the purpose of the study of biological systems, the system can be forced very often into conditions where only the shaded part is operative. Sometimes this cannot be done, a familiar restriction to biologists.

QUANTITATIVE STUDIES ON PROTEINS

The result that the "inactivation volume" V is closely related to the volume of the molecule first was suggested in work by Lea *et al.* in 1944.[9] In our laboratory, their work has been extended greatly to include many enzymes, albumin, and hormones; contributions by others, notably Fluke,[10] also have been made. A graph summarizing these, owing to Guild, is shown as Fig. 4. A log plot of the "radiation molecular weight" *vs* the accepted molecular weight is shown. There are some clear deviations, but the relation hardly can be overlooked. It is most remarkable and unexpected, and for some time no reasonable explanation could be given. In 1955, the suggestion was put forward[11] that, in a covalently bonded structure, there would be no reason why an initially positive region produced by ionization should be confined to one atom, but rather it should migrate and settle possibly in some point of weakness. Recent work by Gordy[12] using paramagnetic-resonance hyperfine structure has shown that, in irradiated material as extensive as proteins, there can be excited two characteristic patterns, one of which is clearly identified as owing to cysteine. Figure 5 shows some of his results.

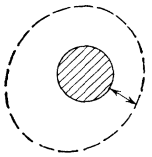

FIG. 3. Indication of the distance of migration of radicals in a cell. It has been estimated by Hutchinson that an increase in distance of 10 to 30 A corresponds to the distance of migration of a radical through a sensitive target. In dilute solution the distance of migration is much greater.

That irradiation, which hardly can have been so intense as to excite the whole set of amino acids, should consistently give such patterns, strongly suggests that migration of the positive charge indeed does occur, resulting in its statistically settling in one or two favored spots. We propose to call this the *primary lesion.*

Subsequently, the protein is exposed to water or to water and oxygen, and the primary lesion now exhibits a reactivity which is called the *chemical action.* This chemical action, by breaking an $-S-S$ bond, or by removing a side chain, gives an altered molecule. We thus feel that, as of 1958, the process is to be regarded as in Fig. 6. On this view, there should be a possibility of modifying the action at two steps, A and B, and a lesser possibility at a. It has been shown that invertase and catalase are markedly more sensitive at temperatures just below the thermal inactivation region. This is possibly action at a or A. Braams, Hutchinson, and Ray[13] have shown that ribonuclease dried in acetic acid is four times as sensitive, and in glucose, twice as sensitive as normally found. A variety of additives have no effect, among them salt and glycine, while glutathione

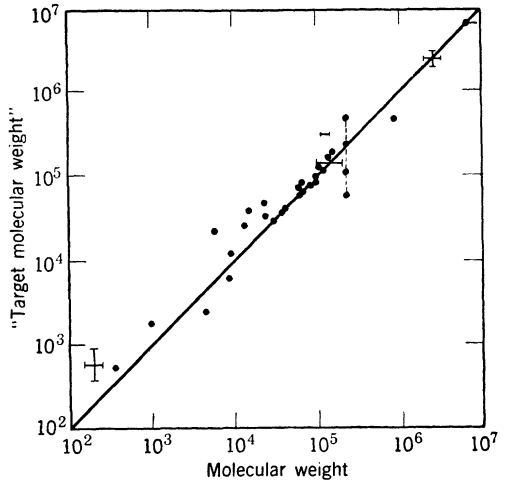

FIG. 4. A plot comparing the observed molecular weight with the target molecular weight, for a wide variety of bombarded substances. This graph has been prepared by Guild and shows the plausibility of the idea that a primary ionization anywhere within the molecule will cause the loss of biological activity. There are exceptions and quite clearly there can be means of influence which will alter the sensitivity of the target, but by and large there is a good relationship.

reduces the sensitivity by a factor of two. A sensitivity modification which is eightfold thus can be achieved. Hutchinson[14] also has confirmed the finding of Alexander[15] that dry trypsin irradiated in air is 2.3 times as sensitive as in nitrogen, for γ-radiation. For deuteron or α-particle irradiation, the effect is far less.

The fact that the relation shown in the log plot of Fig. 4 holds, means that most additives which occur in ordinary separation procedures are fairly uniform and not able to produce the effects described above.

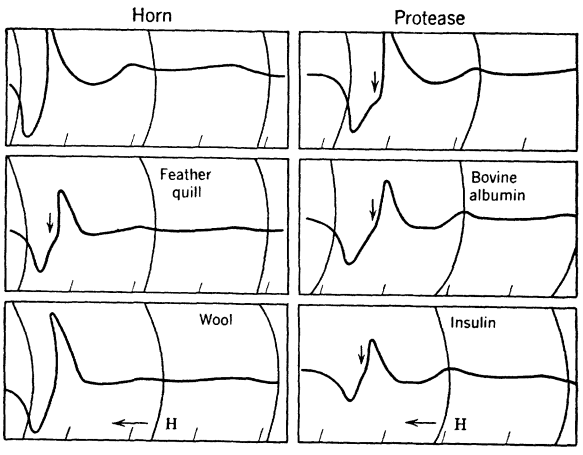

FIG. 5. Reproduction of data due to Gordy showing the paramagnetic resonance in a number of irradiated proteins. It is significant that while a variety of substances are shown quite similar resonance is observed in many of them. This means that there are probably regions in the molecule at which the formation of an unpaired electron is preferred and these can be regions of weakness for chemical action later on [from W. Gordy, W. B. Ard, and H. Shields, Proc. Natl. Acad. Sci. U. S. **41**, 983 (1955)].

Ionization $\xrightarrow{a}$ primary lesion $\xrightarrow{A}$ chemical action $\xrightarrow{B}$ altered molecule

FIG. 6.

QUANTITATIVE STUDIES ON NUCLEIC ACIDS

The transforming principle is pure DNA, and so affords a possible (though not very convenient) measure of the biological activity of DNA. Studies of radiation action on this DNA have been made by Fluke *et al.*,[16] by Marmur and Fluke,[17] by Ephrussi-Taylor and Latarjet,[18] and by Guild and DeFilippes.[19] A graph from the work of these last is shown as Fig. 7. The DNA was irradiated in the dry state and fast protons were used to bombard. The logarithmic relation is not simply obeyed, except at high doses. Two components are found, one with a very large inactivation volume which, when re-expressed as a molecular weight, is between 5 and 15 million, and a homogenous component of molecular weight equivalent of 300 000. The reason for the components is not clear. It may be that a fraction of the long DNA chains are so placed that they can become crosslinked by radiation action, and so cannot enter the pneumococcus to cause transformation. The smaller fraction cannot be crosslinked and is organized as polymers of an essential unit of 300 000 molecular weight, which must intercept an ionization to lose activity.

These studies again show the great sensitivity of DNA. More physical measurements have been made, notably early work by Hollaender and co-workers. More recently, Butler[20] has observed the change in viscosity of thymus DNA when irradiated by x-rays (Fig. 8). It is not plotted logarithmically, but the inactivation, as expressed by the fall in viscosity, is approximately logarithmic, and in the dry state the inactivation corresponds to an equivalent molecular weight of one million. Thus, both physical and biological measures show a strong sensitivity of DNA to ionizing radiation.

The sensitivity in aqueous solution is also high. Guild estimates that a unit of $1\frac{1}{2}$ million molecular weight can be inactivated by 100 ev of energy employed to make radicals in water.[6]

No clear proof of the precise nature of radiation action on DNA is available. Since it is so highly sensitive, and since it is a long thin molecule, the attractive hypothesis, which we adopt, is that it may be either broken, or crosslinked by ionizing radiation, whether dry or in aqueous solution. When this occurs, the result is biologically measurable. It is the biological sensitivity which is really responsible for the radiation sensitivities observed. If one chooses as the "measure" of DNA activity its ability to be digested by DNase, the radiation "molecular weight" is 4000, as was shown by Smith.[21] This is in contrast to the figure of 300 000 for transformation.

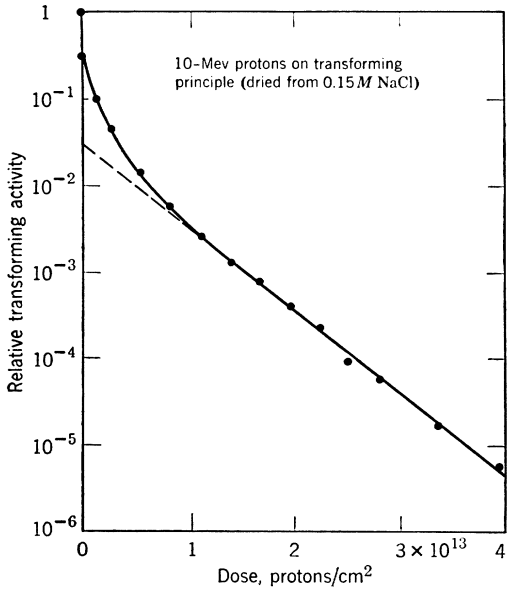

FIG. 7. Inactivation of transforming principle in the dry state as observed by Guild and DeFilippes. There is one very clear single component which corresponds to a molecular weight in the neighborhood of 300 000. For low doses, the behavior is more complex and possibly corresponds to the crosslinking of material which can then not enter the cell [from W. R. Guild and F. M. DeFilippes, Biochim. et Biophys. Acta **26**, 241 (1957)].

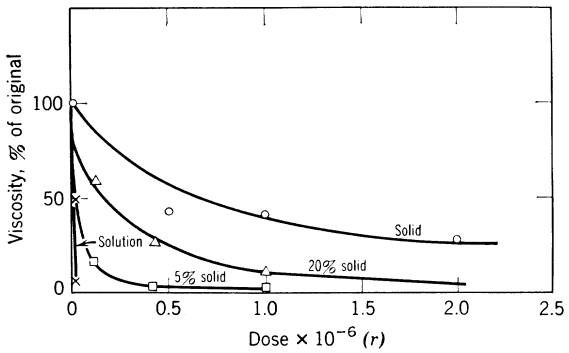

FIG. 8. A reproduction of data quoted by Butler showing the effect of 15 000 000-v electrons on solid and aqueous DNA. The sensitivity of the molecule DNA can be inferred from these data. [from J. A. V. Butler in *Ionizing Radiations and Cell Metabolism* (J. and A. Churchill, Ltd., London, 1956, and Little, Brown and Company, Boston, 1957), p. 59].

POLYSACCHARIDES

Almost no work has been done on polysaccharides. A dysentery toxin was studied by Caspar and shown to have an inactivation volume corresponding to 11 000 molecular weight. Further work is clearly needed.

EXAMPLE OF A RADIATION STUDY ON DRY PROTEIN

The statistical analysis of radiation action on an enzyme, antigen, or antibody can give more than a

simple estimate of a volume. As an example, it is shown in outline how an enzyme, not purified particularly, can be characterized. Since the purification and measurement by physiochemical means has to be done yet, this work offers some chance to test the predictions of such studies. The enzyme is β-galactosidase, and one aspect of the study was shown in Fig. 2 where cobalt irradiation was employed. Such radiation is random in volume, and the product IV can be found from the data, yielding V, the radiation sensitive volume. Using heavy charged particles, such as deuterons or α-particles, a fraction of their ionization, generally about 75%, is confined to narrow tracks. A fraction is spread more widely, as seen in Fig. 9 where a crude representation of the ionization produced by a 4-Mev deuteron is shown. If that fraction of the inactivation of the enzyme due to the on-track ionization can be found, then two quantities can be measured: the area S of the molecule exposed to radiation action, and the thickness E. Estimating the fractional inactivation due to the track is subject to some uncertainty, but can be done by using the Bohr

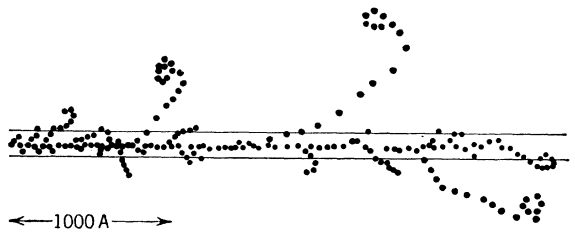

FIG. 9. Representation of the detail of a track of a deuteron. The black dots represent the ionization and the long spurs are δ-rays. In considering the part of the ionization which is along a track, allowance has to be made for the δ-rays which are off the track. The method of doing this has been worked out and permits consideration of just the part which is on the track.

theory of δ-ray production, the Bethe-Bloch energy loss expression, and some modern range measurements for slow electrons in protein.

The effect of 4-Mev deuterons on β-galactosidase is shown in Fig. 10. The data can be analyzed by a relation

$$\ln(n/n_0) = -SD, \tag{6}$$

where D is a measure of the number of deuterons per square centimeter and S is a cross section, which after correction, should bear some relation to the area of the molecule. In order to exploit the linear ionization process effectively, such cross sections can be found for different absorbers in the path of the deuteron beam. There results what is known in nuclear physics as a "Bragg curve," as seen in Fig. 11. From such a curve, the variation in cross section with the rate of energy loss of the deuteron or α-particle can be found. This is shown in Fig. 12, which also shows the result of applying the correction for the off-track ionization.

From Fig. 12, a molecular area of 8.2×10^{-13} cm^2 is deduced, and it is noted, in addition, that, for a rate of

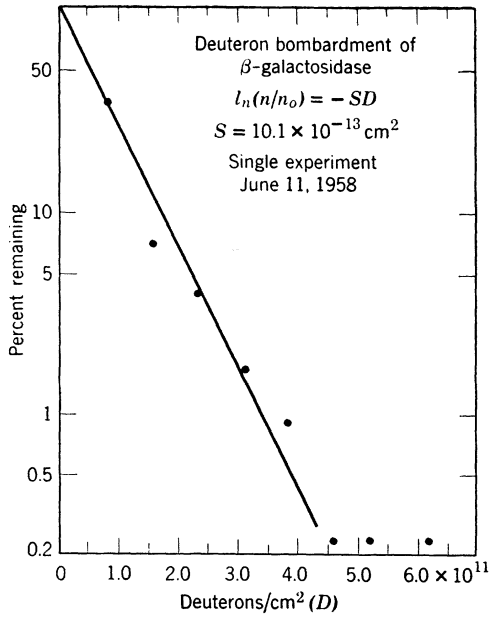

FIG. 10. The inactivation of β-galactosidase by deuteron bombardment. The relationship as indicated is obeyed and from it a target area can be inferred.

energy loss of 300 ev/100 A, the particles are 63% efficient. If this loss of efficiency is because of the fact that the ionizations are not dense enough along the track and that some "straddling" is occurring, then one should expect a relation

$$S = S_0(1-e^{-it}), \tag{7}$$

where S_0 is the maximum cross section, i is the ionization per unit track length, and t is the effective thickness. If the primary ionizations require on an average 110 ev one has, for 300 ev/100 A, $it=1$ and $i=2.7$ per 100 A, so $t=37$ A. Thus, one has the following data about

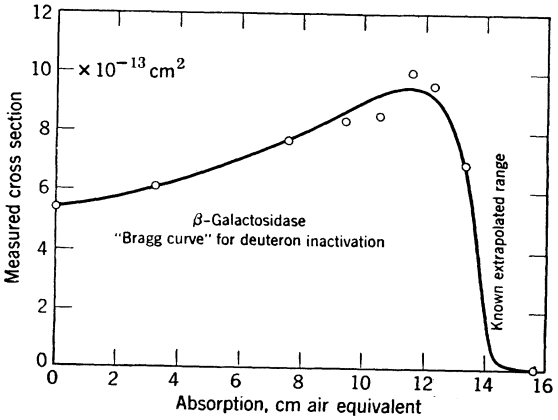

FIG. 11. A "Bragg curve" for the deuteron inactivation of β-galactosidase. The deuterons were systematically covered with foils of different thickness and their effectiveness measured. It can be seen that there is a rise as the ionization per unit path increases and then an abrupt fall as the end of the range is reached.

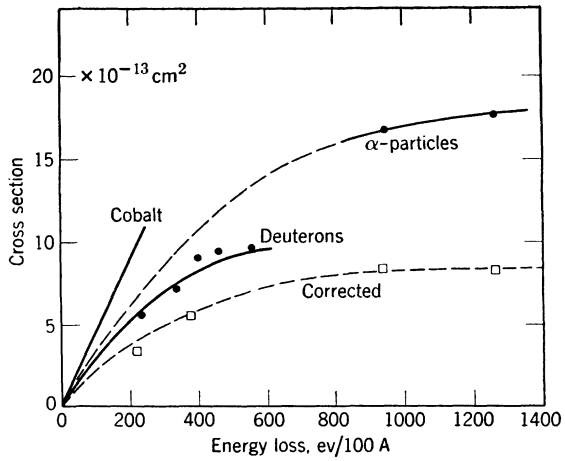

FIG. 12. The plot of the cross section *vs* rate of energy loss for deuteron and α-particle bombardment. A corrected line has been drawn in which allowance has been made for the δ-rays.

β-galactosidase:

$$\text{volume} = 4.7 \times 10^{-19} \text{ cc}$$

$$\text{area} = 8.2 \times 10^{-13} \text{ cm}^2$$

$$\text{effective thickness} = 3.7 \times 10^{-7} \text{ cm}.$$

If it is assumed that the molecule is a cylinder, radius r and length l, these can be solved for, from the volume and area, and $r = 36$ A and $l = 114$ A are obtained. The effective thickness should be $4r/\pi = 47$ A, which has to be compared with 37 A, as deduced from Fig. 12. The agreement is fairly satisfactory. Thus, the enzyme should have a molecular weight 370 000, a length of 114 A, and a diameter of 72 A, and so is roughly spherical. Such a prediction should be subject to verification.

TABLE I. Virus properties available for cross-section measurement.

Type of virus	Property	Rough results
Plant Animal Bacterial	Infectivity	Close to whole virus
Bacterial	Attachment	Very small part
Bacterial Animal	Interference	About 1/20 of virus
Bacterial	Host killing	About 1/6 of virus
Plant Animal Bacterial	Serological affinity	Very small indeed
Plant Animal Bacterial	Ability to make antibodies	Not tested
Animal Bacterial	Genetic recombination	Not tested
Some animal	Hemagglutination	Small
Bacterial Animal	Complement fixation	Not tested
Bacterial	Latent period extension	About 1/3 of virus
Some animal	Enzyme action	Small

INACTIVATION OF VIRUSES AS RELATED TO THEIR STRUCTURE

Inactivation of a whole biological system, having a structure and a number of functioning parts, presents a new problem. It is reasonably certain that radiation inactivates at least one of the molecular units of the system, but now inquiry must be made as to whether or not it has any effect on the biological functioning, or rather, any measurable effect. Viruses offer perhaps the

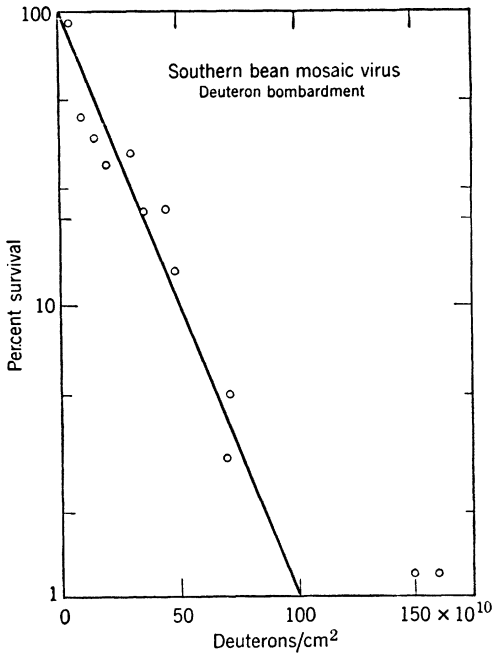

FIG. 13. Inactivation of Southern bean mosaic virus by deuterons as observed by Dimond and the author. From such data the cross section of the virus can be inferred and comes rather close to the observed cross section in electron micrographs.

the simplest system, yet a virus *is* a system and this must always be remembered. The problem can be rendered schematic as follows:

radiation → molecular inactivation →
reduction of function
↓
biological consequence.

Thus, when a virus is irradiated and its ability to multiply in the host is studied, one investigates the sum total of: (1) ability to survive in outside world, (2) ability to attach to host cell, (3) ability to invade the cell, (4) ability to multiply in the cell, and (5) ability to return to outside world. Thus, bland statements

about "virus inactivation" should be regarded with suspicion.

Before treating separate inactivation studies, brief mention is made of two empirical correlates. The first is that the virus-inactivation cross section measured with heavy particles as bombarding agents (deuterons, α-particles) is close to the electron-micrograph cross section. This correlation fails for the large viruses but works for influenza and below. The second is that the inactivation volume measured with γ-rays is close to the volume of the nucleic-acid content. This is less well established and does not work for polio or influenza, for example, but has been rather widely adopted as a

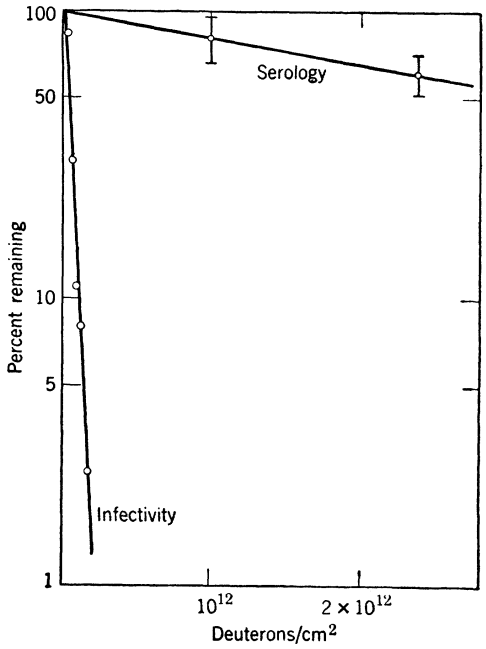

FIG. 14. Differential effect of deuterons on the infectivity and the serological combining power of tobacco mosaic virus. It can be seen that very much more radiation is needed to have any effect on the surface whereas the infectivity is very readily affected. This shows that there is a clear cut difference in the two functions of the virus and enables some estimate of the relative sizes to be made.

simple interpretation of radiation inactivation. It has some validity, but must be used with caution.

A chart is given, in Table I, of the various properties of viruses which are susceptible of study. It is clear to anyone who makes such differential radiation studies that a virus is *not* a simple molecule. In what follows, a few examples of virus studies are given, and in concluding consideration is given to what can be deduced about one virus from this kind of work. Figure 13, shows the inactivation of Southern bean mosaic virus by deuterons as measured by the ability to form local lesions on beans. The familiar relationship is found and the cross section, properly analyzed, corresponds to a

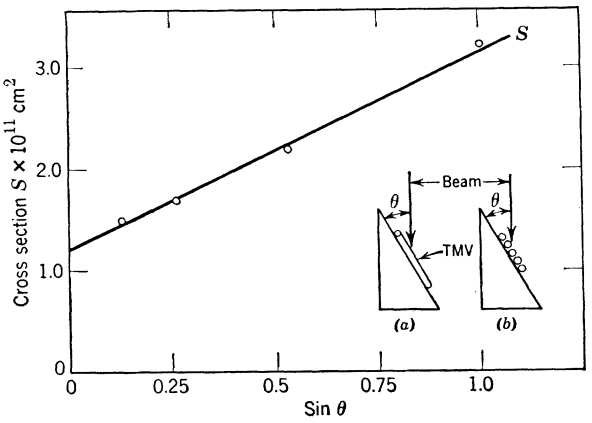

FIG. 15. Proof of the reality of irradiation target. Tobacco mosaic virus which is in long thin rods is irradiated in the manner shown in the insert of the diagram. In the first case, as the angle is varied, the target presents different aspects to the beam. This shows in the measurements as an increase in the cross section [from E. C. Pollard and G. F. Whitmore, Science **122**, 335 (1955)].

spherical particle of radius 150 A, which agrees with electron microscopy.

Figure 14 shows the effect of deuterons on dry tobacco mosaic virus (TMV) measured in terms of (a) its ability to produce local lesions, and (b) its ability to precipitate antibody to TMV. The loss of precipitating ability is

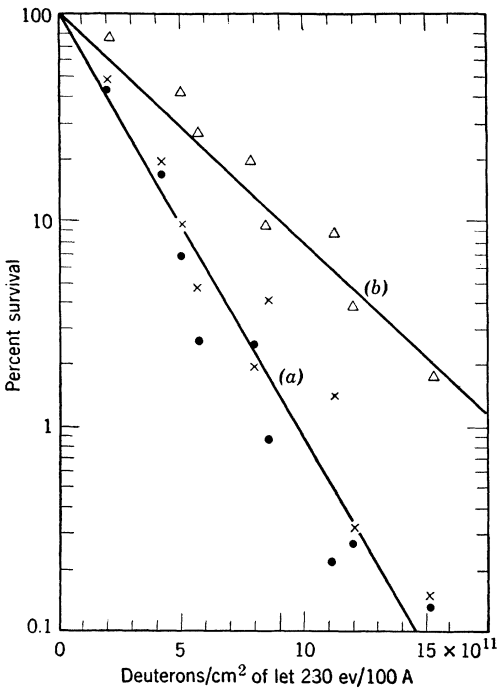

FIG. 16. Effect of deuterons on the cross reactivation of $T1$ bacterial phage as observed by Till and the author. In case (*b*), the bacterium was superinfected with undamaged virus and the fact that part of the virus material could be given from the undamaged virus to the damaged virus is shown in the lower sensitivity. The part which must be intact for cross reactivation to occur is approximately 60% of the whole virus [from J. E. Till and E. C. Pollard, Radiation Research 8, 344 (1958)].

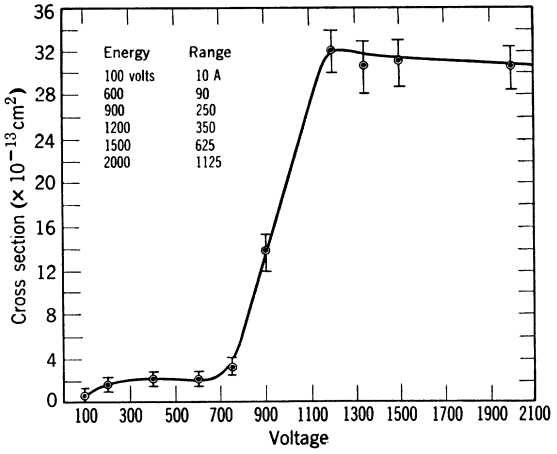

FIG. 17. The inactivation cross section of $T1$ bacteriophage with electrons of low voltage and penetration as indicated. It can be seen that, apart from a small inactivation owing presumably to some effect on the surface of the virus, the majority of the effect only occurs when the energy of the electron is sufficient to penetrate something like 250 A. This means that the sensitive part is inside the virus and also that none of it resides in the tail [from M. Davis, Arch. Biochem. Biophys. 49, 417 (1954)].

very slight. In fact, it proved that most of the observed effect was on the virus and not on the combination with antibody. If one calculates the sensitive volume for the removal of ability to precipitate with antibody, it corresponds to a molecular weight of less than 20 000. Similar experiments on Southern bean mosaic viruses indicate that there are possibly two active sites of molecular weights 30 000 and 6000, respectively.

In view of all of this statistical reasoning, one sometimes needs more real evidence to bolster one's faith in numbers and deduction. An experiment, by Whitmore and the author,[22] on oriented samples of TMV is shown in Fig. 15. For samples of TMV held pointed at the beam, with varying angles, the cross section is least when the virus particles, which are long and thin, are held pointing at the beam and greatest when perpendicular to the beam. Controls held as at (*b*) showed no orientation effect. This experiment confirms the true physical character of the target.

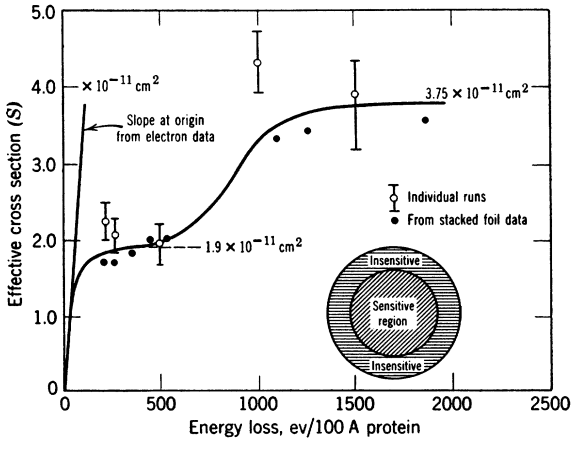

FIG. 18. Data taken by Jagger and the author on influenza virus. As the energy loss in the bombarding particle is increased, the effective cross section varies in the two-step manner. This would seem to fit with the idea that there is an inner sensitive region and an outer insensitive region and so that the virus as an internal structure [from J. Jagger and E. C. Pollard, Radiation Research 4, 1 (1956)].

BACTERIOPHAGE EXPERIMENTS

A series of phage properties has been studied. Two are selected as examples. The first is by Till and the author[23] and shows that $T1$ phage, when irradiated and allowed to enter a cell which has a second infection by a genetically different phage, can still "cross reactivate" the second phage, by which is meant that it can donate markers necessary for the progeny of both phages to function on a new host. The cross section for the loss of this ability is 60% of the single infection cross section, and it implies that 60% of the phage DNA is genetic in character while 40% is not. By quite different methods, for $T2$, Levinthal[24] has concluded somewhat similarly. The data are shown in Fig. 16.

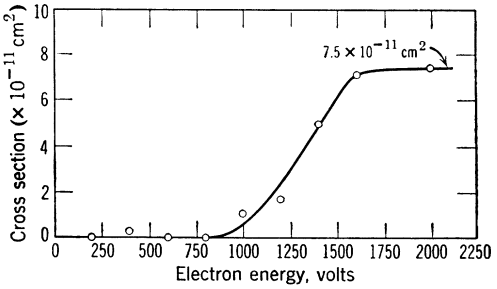

FIG. 19. Effect of low-voltage electrons on the infectivity of Newcastle disease as measured by Wilson and the author. It can be seen that there is no affect on the Newcastle disease until depth of penetration of about 200 A is observed. This means that the outer part of the virus is not concerned with the infectivity but there is a region inside which is actively concerned [from D. Wilson and E. C. Pollard, Radiation Research 8, 131 (1958)].

A technique of low-voltage irradiation, using electrons of known penetrating power, has been developed by Hutchinson *et al.*[25] Applied to $T1$ (Fig. 17), only when electrons penetrate 125 A can they produce appreciable effect on $T1$. That is, the sensitive material is imbedded in the head, and, since the tail has a thickness of only 150 A, cannot be in the tail.

NEWCASTLE DISEASE AND INFLUENZA VIRUS

Figure 18 shows an early experiment by Jagger and the author[26] on influenza virus. The infectivity in eggs was measured, and the cross section *vs* energy loss is shown. One way to analyze the data is indicated, in terms of an insensitive region surrounding one which is more sensitive. Figure 19 shows data by Wilson and the author[27] on Newcastle disease virus (NDV) using the low-voltage electron technique. Quite independent

evidence for the existence of a protective coat is apparent.

Figure 20 shows the assembly of conclusions about inactivation experiments on infectivity, hemagglutination, and hemolysin ability for Newcastle disease virus. Such a semispeculative figure is representative of what can be done on one virus. So far, no very contradictory evidence has come to the attention of the author which invalidates this model of the virus.

FURTHER APPLICATION AND OUTLOOK

The study of cellular structure in relation to its function by ionization inactivation can be carried beyond viruses. The effect of radiation on amino-acid uptake in *E. coli* can be analyzed to indicate that ribonucleoprotein particles, or ribosomes, are involved in the uptake. The low-voltage electron technique has been used by Preiss[28] to determine the location of the invertase in yeast cells. Currently, β-galactosidase in *E. coli* is under study and seems to be in the protoplast membrane. The technique is not harder than many others now in use and in the next few years should contribute appreciably to our knowledge of the cell.

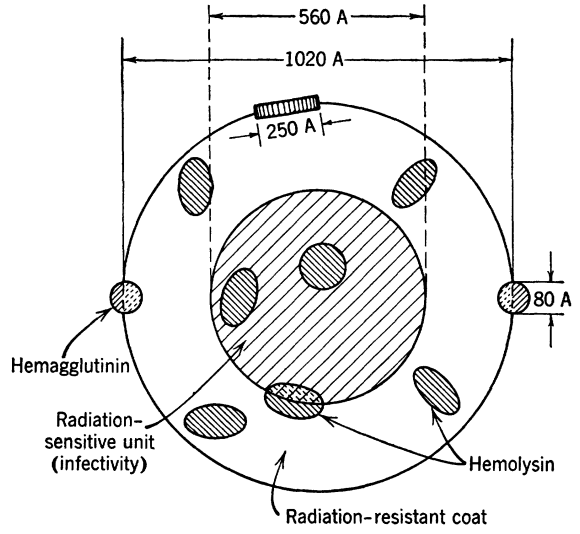

FIG. 20. Sketch of Newcastle disease virus, showing the relative size of several components. Such a drawing is schematic and serves primarily to indicate relative sizes [from D. Wilson and E. Pollard, Radiation Research 8, 131 (1958)].

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