KERNFORSCHUNGSZENTRUM

KARLSRUHE

August 1966

KFK 490

Institut für Strahlenbiologie

Some Unusual Topics in Radiation Biology

K. G. Zimmer

GESELLSCHAFT FÜR KERNFORSCHUNG M. B. H.

KARLSRUHE

Reprinted from RADIATION RESEARCH, Volume 28, No. 4, August 1966

RADIATION RESEARCH **28**, 830–843 (1966)

Some Unusual Topics in Radiation Biology[1]

K. G. ZIMMER

Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe, Karlsruhe, Germany

Mr. President, Ladies and Gentlemen:

It is my privilege today to speak to you in honor of an outstanding scientist: Dr. G. Failla. To mention here any of his most valuable and diversified contributions to radiation research seems hardly possible. We all are well aware of his great achievements; moreover, the time allotted to me would be much too short, as his activities included so many fields. Unfortunately I never had a chance to meet Dr. Failla personally. Therefore I should like to begin by telling you how I remember sitting nearly 33 years ago on the shore of a beautiful little lake near Stockholm, Sweden, reading a series of extensive papers that had just appeared in the *American Journal of Roentgenology*. Dr. Failla was one of the authors, and the papers that interested me so deeply were concerned with comparing the biological effects of 200 kev, 700-kev X-rays, and radium γ-rays. This was surely one of the pioneer studies in a field which today we call dependence of RBE on LET. These papers were of particular importance for my own work, for at this time I had just started to do similar investigations using genetic effects in *Drosophila* as a biological indicator. These experiments were done in cooperation with Timoféeff-Ressovsky, a cooperation that was to last for nearly 20 years and in various places as determined by the most inconvenient political events overshadowing the life of many of us.

Nevertheless, and in spite of all surprises that fate held in store for us, we managed to complete the aforementioned investigation—and some others as well. In fact, the outcome of our early experiments on the dose effect and RBE–LET relationships for radiation-induced mutations in *Drosophila* is closely related to the first of the "unusual topics in radiation biology" with which I plan to confront you today.

1. Do Single-Hit Effects Occur in Radiobiology?

The main results of our experiments as described and interpreted in a paper by Timoféeff-Ressovsky *et al.* (*1*), with the terminology of "hit" and "target" theories

[1] Fourth Failla Memorial Lecture delivered Tuesday, February 15, 1966, at the Fourteenth Annual Meeting of the Radiation Research Society, Coronado, California.

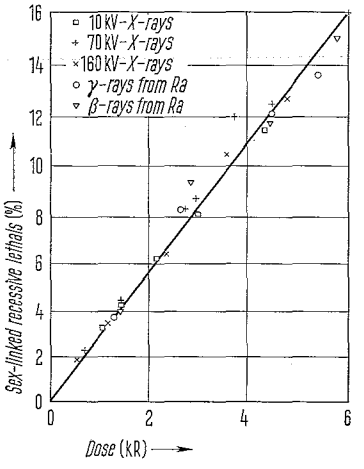

FIG. 1. Dependence of the rate of sex-linked recessive lethals in *Drosophila melanogaster* on dose of various radiations. After Timoféeff-Ressovsky *et al.* (*1*).

as given in Zimmer (*2*), can be stated briefly as follows: (1) The fraction of sex-linked lethal mutations in an irradiated population of *Drosophila* males rises with the dose, D, of radiation according to the equation $N^*/N_0 = 1 - \exp(-vD)$ indicating a one-hit process (Fig. 1). (2) The formal volume, v, of the target as calculated from the same equation is (within certain limits) independent of the spatial density of ionization (linear energy transfer) if the doses, D, are counted in numbers of ionizations per unit volume of *Drosophila* (Fig. 1). Consequently one ionization within the formal target may be considered one hit. (3) Taking into consideration additional data on temperature dependence of radiation-induced as well as spontaneous mutation, a "quantum jump" was regarded as the physical process produced by a hit in a target and leading to mutation.

There is no reason to discuss item 3 in any detail here, but results 1 and 2 seemed certainly well established in 1935. Later on, further experiments lent additional support. In fact, the deviations of the target volumes, v, found in single experiments from the weighted mean value, $\bar{v} = 1.77 \times 10^{-17}$ cm^3, were so small that the data formed one of the most carefully tested cases of a one-hit curve in radiobiology. Nevertheless, as time passed by I became worried about the approximation inherent to this reasoning: the complete neglect of a possible biological variability. At first there was no clue for its existence in the material under investigation, but theoretical studies showed how badly the hit curves can be distorted by a quite moderate variability assumed to occur in target volume, hit number, or multiplicity of targets (*3*). Some years later, and actually incited by unpublished experiments on the action of X-rays on the eggs of some water snails, I investigated graphically the possibility that approximate single-hit curves could arise through superposition of multihit curves. Thence it appeared that curves can be obtained, based on different, quite

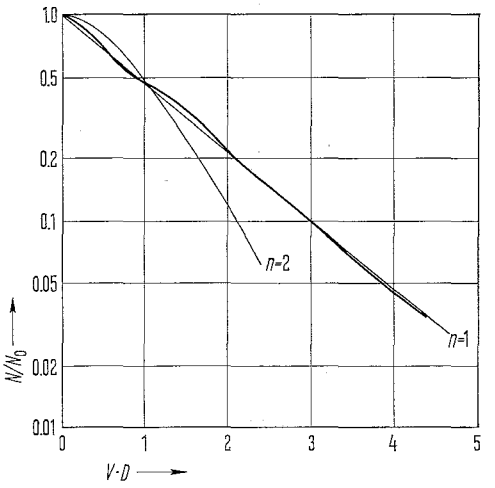

FIG. 2. Approximate agreement of a single-hit curve with a two-hit mixed curve formed by superposition of four two-hit curves with differently sized targets. After Dittrich (*4*).

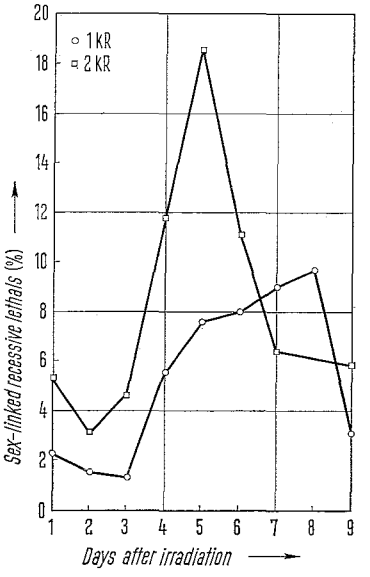

FIG. 3. Dependence of radiation-induced rate of lethals on sensitivity of stage at time of irradiation by X-rays; of 3- to 4-day-old B-males of *Drosophila melanogaster*; nine 1-day broods (stages). Spontaneous rate subtracted. After Traut (*5*).

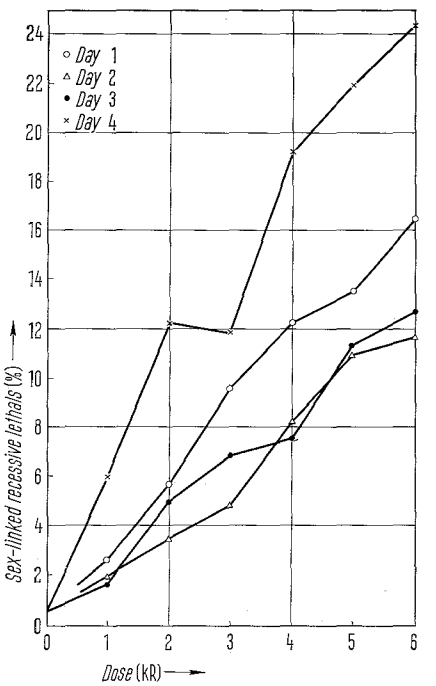

FIG. 4. Dose-effect curves for lethals induced in stages with different sensitivity; 3- to 4-day-old B-males were irradiated. After Traut (*5*).

plausible assumptions, which wrap sinuously round single-hit curves within the limits of accuracy obtainable in radiobiological experiments (Zimmer, unpublished observations, 1950). About a decade later a really comprehensive investigation of these possibilities was carried out at our suggestion (*4*). An instructive case of deception by an apparent single-hit curve is shown in Fig. 2.

In the meantime the influence of the stage of germ cells of *Drosophila* on their X-ray-induced mutability became more clearly recognized. The usual way to investigate this phenomenon had been to work out a so-called "brood pattern" giving the rate of mutation for germ cells of various stages induced by irradiation with a given dose of radiation. Comparison of such brood patterns obtained at various dosages made the single-hit curve described above appear quite unbelievable (Fig. 3). To elucidate the meaning of this apparent contradiction, the tiresome task was undertaken in my present laboratory to obtain statistically significant dose-effect curves for various stages of germ cells (*5*). An example of the results is given in Fig. 4. Here, it must be emphasized that the strange form of the curves is significant as shown by careful statistical analysis with an electronic computer. Nevertheless,

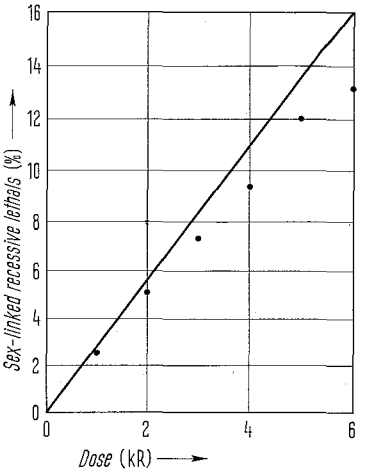

FIG. 5. The solution to the apparent contradiction: ——, dose-effect curve as in Fig. 1. This curve had been obtained by neglecting stage dependence of mutability—that is, by "integrating biologically" over the first days after irradiation, ●, mean frequency of sex-linked recessive lethals integrated arithmetically over the first 4 days after irradiation. After Traut (*5*).

summing up arithmetically all the mutations obtained in the various broods for given doses resulted in a dose-effect curve (Fig. 5) closely approximating the single-hit curve found and published 30 years earlier. Complete agreement cannot be expected, as arithmetic summation is, of course, not identical with neglecting stage dependence of mutability which may be considered as a process of "biological summation." Thus, the old single-hit curve turned out to be quite reproducible and to hold for all practical purposes (such as problems of protection from radiation damage). But it became also clear that, although it has the form of a single-hit curve, it certainly does not have the meaning.

At this moment I can almost hear biologists say: "We told you before that biology is not that simple. You physicists had better stick to inanimate systems such as molecules." I am not so sure about this point. In fact, I rather feel that being doubtful is one of the more important prerequisites for doing scientific work. Nevertheless, let us follow the advice given and consider the inactivation by radiation of a fairly simple and well-known molecule: ribonuclease. There are, of course, many papers to be found in the literature dealing with this subject, and I do not intend to discuss the various aspects of the problem in detail here. However, recent experiments done in my institute by Jung and Schuessler (*6*) brought to light a particular feature of the inactivation mechanism relevant for our present considerations. RNase A was separated by chromatography from commercially obtained RNase and purified further by various chromatographic procedures. After being tested for homogeneity,

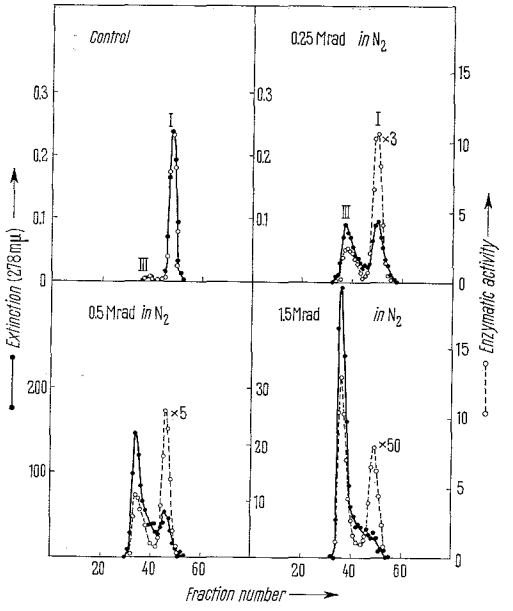

FIG. 6. Chromatographic separation of RNase A before and after irradiation by Co^{60} γ-rays in aqueous solution under nitrogen. After Jung and Schuessler (*6*).

the material was subjected to γ-irradiation under various conditions—in aqueous solution in air or nitrogen, in the presence or absence of a protective agent, and also in the dry state. Subsequent chromatography over Sephadex permitted separation into fractions of different molecular weight and determination of protein content and of enzymatic activity in these. Figure 6 shows, in the control, the presence of a small fraction (about 1.5 %) of larger molecular weight (III) besides the main fraction (I) of RNase A. Inactivation curves in air and in nitrogen are given in Fig. 7 for the enzymatic activity of both components taken together (I + III). One would hardly doubt that this is one of the "usual cases" of inactivation of a protein following a single-hit curve and showing an oxygen effect. When looking at the inactivation curves for component I alone (Fig. 8), one notices that the oxygen effect has vanished, and one might or might not attribute importance to the fact that the points at 0.25 Mrad are a little low. But one would still feel that one is dealing with a single-hit inactivation curve. Figure 9, however, reveals at once that things are "not that simple," not even with inanimate molecules. The enzymatic activity in component III rises first with increasing doses before being inactivated at the same rate as component I. Consequently, the apparent single-hit curves in Fig. 7 again cannot have the meaning of single-hit curves. What really happens on irradiation under nitrogen can clearly be seen in Fig. 6: 60 % of the monomeric RNase A–com-

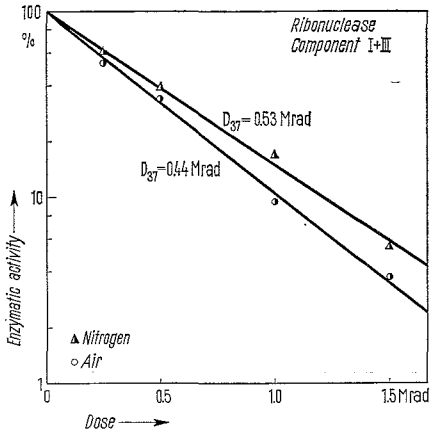

FIG. 7. Change of total enzymatic activity of RNase A (components I + III) by γ-radiation in aqueous solution under air and nitrogen. After Jung and Schuessler (*6*).

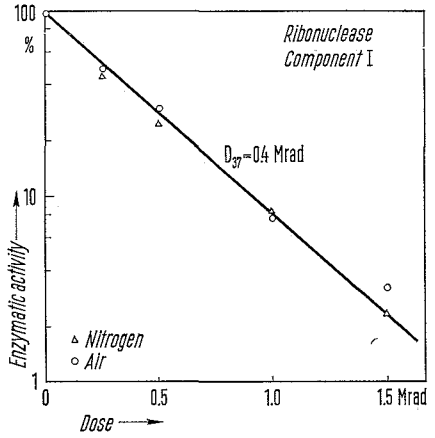

FIG. 8. Change of enzymatic activity of RNase A, (component I) by γ-radiation in aqueous solution under air and nitrogen. After Jung and Schuessler (*6*).

ponent I is dimerized or polymerized into component III prior to or in conjunction with inactivation, and hardly any inactivated monomer molecules can be detected.

Returning to the problem set out above as the first of some unusual topics in radiation biology, we should emphasize that by these experiments we have neither proved nor disproved the occurrence of single-hit effects in radiobiology. But we have demonstrated that even carefully tested cases of single-hit curves can be completely deceptive. This result, exemplified in a biological and in a molecular system, has a variety of implications. Those interested in philosophical problems will have

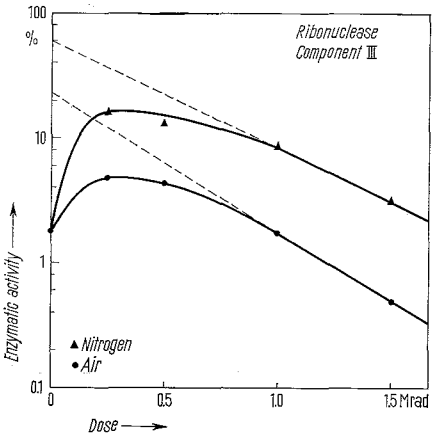

FIG. 9. Change of enzymatic activity of RNase A (component III) by γ-radiation in aqueous solution under air and nitrogen. After Jung and Schuessler (*6*).

heard about the far-reaching conclusions that were drawn from the occurrence of apparent single-hit events in radiation genetics. Many heated discussions appeared in the literature over this case which seemed to have a bearing on the general problems of determinism or indeterminism. The results given above make these discussions, valuable and interesting as they are in themselves, appear a little premature. The conclusion to be drawn for the field of radiation biology (and maybe for some branches of molecular biology as well) is clear enough: single-hit curves require most careful further testing. But so do many other concepts in radiation biology, particularly those favored for a long time. One of them comes to mind quite easily when one is speaking of single-hit curves, as these have frequently been connected with the concept of "one ionization to bring about the effect." Processes of energy transfer other than ionization have received far less attention, although some of them (for example, excitations) occur with high probability. A more unusual but highly interesting process of energy transfer to biological material is by elastic nuclear collision, a topic on which I shall speak next.

2. Energy Transfer by Elastic Nuclear Collisions as Related to Radiobiology

This is not the place to describe in detail the physics of energy transfer from radiation to matter (cf. *7*). Suffice it here to draw your attention to the fact that the fraction of the total stopping power due to electronic interactions decreases with decreasing velocity of the incident particles, whereas the fraction due to elastic nuclear collisions rises, as shown in Fig. 10 (*8*). At low velocities elastic nuclear collisions by far outweigh electronic interactions; that is, processes of energy transfer leading to ionization become of negligible importance. These facts have been well

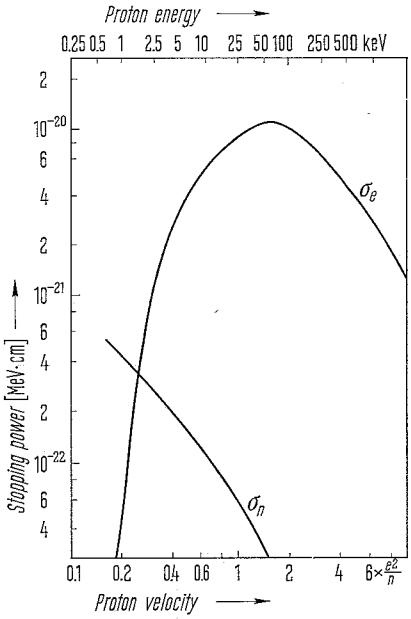

FIG. 10. Electronic stopping power (σ_e) and nuclear stopping power (σ_n) for protons in tissue. After Neufeld and Snyder (*8*).

known for about 15 years, and it has been pointed out repeatedly that experimental studies on the possible importance of energy transfer by elastic nuclear collisions are desirable for radiation chemistry and radiation biology. It is easy enough to see why such experiments should be done: (1) Particles of exactly the low velocities in question are produced abundantly by epithermal neutrons in human tissue; hence the results might be of considerable interest for fixing tolerance doses in this range of neutron energies. (2) Since the manner in which radiation energy is transferred to molecules by elastic nuclear collisions is totally different from that which it has been customary to envisage in radiation chemistry and radiation biology, the physical processes in question may well have some importance in illuminating fundamental aspects of the action of radiation on biological materials.

However, it is not easy to design a suitable experimental setup for the purpose nor to conduct studies leading to meaningful results. These difficulties provide a convincing explanation for the fact that very little, if any, work has been published relevant to this field. The main trouble is, of course, the exceedingly short range of slow particles in matter requiring a detecting system rather less than 100 A thick. This necessity, in turn, demands the possibility of working with and of accurately measuring induced changes in very small quantities of material (of the order of

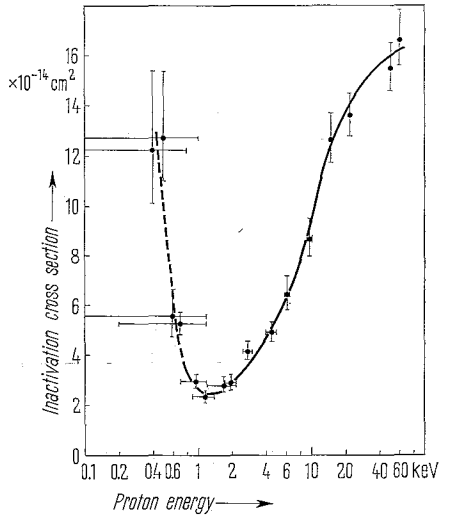

FIG. 11. Cross section for the inactivation of RNase by protons of various energies. After Jung (*9*).

1 μg). Only quite recently and after some years of patient work, suitable systems and methods were found in our laboratory in Karlsruhe by Jung (*9*).

There is no point in describing details of the experiments here. Briefly, the cross section for inactivation of dry RNase was measured for protons of various energies from 60 kev down to about 400 ev and in layers thin enough to allow not only integral measurements over the whole range of the protons but also differential determinations for part of the range. The main result of one series of such studies is depicted in Fig. 11. Starting from high energies, the cross section falls with decreasing energy of the incident protons as does the stopping power for electronic interaction. From a minimum reached at about 1 kev, however, the cross section rises again quite steeply and follows closely the rising cross section of elastic nuclear collisions for protons of still smaller energy.

An evaluation of the question to what extent this clearcut demonstration of the possible importance of elastic nuclear collisions for causing damage necessitates reconsideration of tolerance limits of epithermal neutrons should remain with the International Commission for Radiological Protection (ICRP). From the point of view of radiation biology, application of the method to fundamental problems appears most interesting. In this connection a preliminary result just obtained may be mentioned. Whereas damage to dry RNase by ionization (γ-rays or fast protons) can be protected against by a sulfur-containing compound in the usual way and to normal extent, damage to RNase by elastic nuclear collisions could not so far be influenced by a protective agent (Jung, unpublished). Such a difference

might well be of help in our attempts to decide between the various schemes proposed to explain the mechanism of protection. Elucidation of the mode by which protective substances act in the molecular level is a most intriguing field where help by new experimental approaches is badly needed, as I hope to demonstrate in discussing the third, and last, of my topics today.

3. Protective Substances: Action or Reaction?

Continuous use of a once-established terminology may develop into a habit and eventually lead to preoccupation of thought. What I want to say can conveniently be exemplified by returning briefly to the subject of the preceding section. Historically, ionization was the most easily measurable effect produced by some kinds of radiation. For convenience they were called "ionizing radiations," and this term was used to such an extent and for such a long time as to make one nearly forget about other effects which are also produced by the same radiations. A similar narrowing or restriction in thinking may, in our opinion, follow from the continuous use of the word "action" in connection with protective substances. After all, these are just ordinary chemicals, capable of undergoing ordinary chemical reactions.

Again, I do not plan to give here a summary of the many hypotheses proposed to explain the mode of action of protective substances. Even if our considerations were limited to the molecular level—that is, speaking of biochemicals and of viruses only—an exhaustive discussion would at the same time be rather exhausting. In-

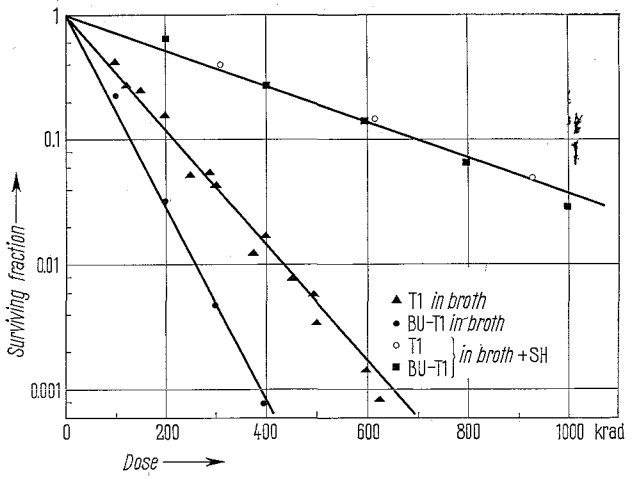

FIG. 12. Inactivation of plaque-forming ability in phage T1 and in bromouracil-substituted phage (BU-T1) by γ-radiation. All irradiations done in air on phage suspended in 4% Difco nutrient broth. Normal T1 (▲); dense fraction of CsCl–gradient centrifugation of BU-T1 lysate (●); 0.1 *M* cysteamine in the suspension of normal T1 (○) and of BU-T1 (■). After Hotz and Zimmer (*10*).

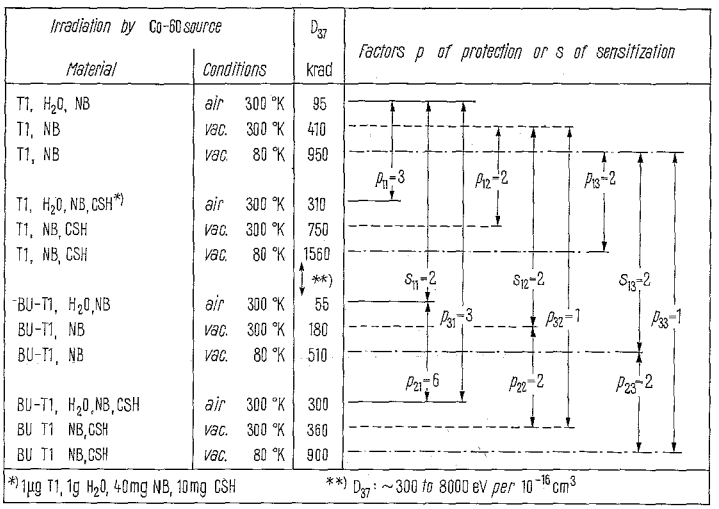

FIG. 13. Summary of several experiments on inactivation of plaque-forming ability in phage T1 by γ-radiation. D_{37} stands for mean inactivation dose, NB for nutrient broth, CSH for cysteamine, and BU-T1 for phage in the DNA of which about 80% of the thymine is replaced by 5-bromouracil. The factors p and s give ratios of slopes of the inactivation-versus-dose curves, all of which were rectilinear in semilogarithmic plot. After Hotz and Zimmer (*10*).

stead, I want to draw your attention to one special case which is particularly instructive and supports the view just mentioned.

The experiments in question were published and extensively discussed by Hotz and Zimmer (*10*). Figure 12 illustrates some of the results. When irradiated by γ-rays after suspension in broth, T1 phage is inactivated at a certain rate; bromouracil substitution for 80% of the thymine in the same phage (BU-T1) leads to a much faster rate of inactivation. If, however, normal T1 or BU-T1 are suspended in broth containing an SH compound, both are inactivated at exactly the same and much slower rate. In other words the SH compound exerts the well-known "normal" protection on normal T1. It does the same in BU-T1 but, in addition, eliminates completely the sensitization caused by substituting BU for thymine. Similar experiments were done for various circumambient conditions. All the curves found for inactivation of plaque-forming ability by γ-radiation were straight lines in a semilogarithmic plot. Consequently we can in a purely formal way, and without touching on the question of whether these curves have the meaning of single-hit curves, discuss effects of protection and of sensitization in terms of ratios of slopes.

A more complete survey of the experiments is given in Fig. 13. In passing, attention might be drawn to the footnotes in the figure stating a few facts in an unconventional way. (1) In a suspension used conventionally in such experiments there is

very little phage as compared to dry substance of nutrient broth and to protective agent. (2) The amount of energy transferred to a volume corresponding to that of a T1 phage at the mean inactivation dose (D_{37}) varies enormously with varying conditions of the experiment and is always quite noticeable. Returning to the experiments just described in connection with the foregoing graph (Fig. 12), we see the same results in more succinct form in Fig. 13: (1) Base substitution T1 → BU-T1 causes sensitization by a factor $s_{11} = 2$ if irradiation is done in broth. (2) Suspension in SH-containing broth yields an overall protection against radiation damage in T1 by a factor of $p_{11} = 3$ and in BU-T1 by a factor of $p_{21} = 6$.

This result was unexpected enough, but the point I really want to stress is the following. The outcome is totally different if one repeats the same experiments, irradiating the freeze-dried suspension *in vacuo* at room temperature or at 80°K. Here sensitization by BU is the same as before ($s_{12} = 2$); overall protection by SH amounts to $p_{12} = 2$ in normal phage but remains unchanged in BU-T1 ($p_{22} = 2$). In the first publication we mentioned that this difference of effects in aqueous suspension and in the dry state seems to indicate an ordinary chemical reaction of the SH compound which might be possible (or going on at a noticeable rate) in solution only. This simple approach does seem to have met with the interest of "molecular biologists." For some time we could not follow up this line of work because of other, more urgent problems. But recent experiments done in our laboratory show that keeping an aqueous solution of bromouracil and cysteamine for some time at slightly elevated temperature leads to conversion of bromouracil into uracil. We do not know yet what happens in bromouracil containing DNA, but one could imagine the disappearance of radiosensitization by bromouracil in the presence of the radio protecting SH compound to be caused by ordinary chemical reaction even if no radiation ever reaches the system.

Once more I have talked about a problem without offering an answer. I hope you do not mind, but to me problems are often more interesting than answers, as they tend to stimulate further work, theoretical and experimental. Judging from his publications, I expect Dr. Failla in whose memory we have come together here might have shared such a view.

RECEIVED: February 1, 1966

REFERENCES

1. N. W. TIMOFÉEFF-RESSOVSKY, K. G. ZIMMER, and M. DELBRÜCK, Über die Natur der Genmutation und der Genstruktur. *Nachr. Ges. Wiss. Göttingen* [VI] **1**, 189–245 (1935).
2. K. G. ZIMMER, *Studies on Quantitative Radiation Biology.* Oliver & Boyd, Edinburgh and London, 1961.
3. K. G. ZIMMER, Zur Berücksichtigung der biologischen Variabilität bei der Treffertheorie der biologischen Strahlenwirkung. *Biol. Zentr.* **61**, 208–220 (1941).
4. W. DITTRICH, Treffermischkurven. *Z. Naturforsch.* **15b**, 261–266 (1960).
5. H. TRAUT, Dose-dependence of the frequency of radiation-induced recessive sex-linked

lethals in Drosophila melanogaster, with special consideration of the stage sensitivity of the irradiated germ cells. In *Repair from Genetic Radiation Damage* (F. H. Sobels, ed.), pp. 359–372, Pergamon Press, London, 1963.

6. H. JUNG, and H. SCHUESSLER, Zur Strahleninaktivierung von Ribonuclease. I. Auftrennung der Bestrahlungsprodukte. *Z. Naturforsch.* **21b,** 224–231 (1966).
7. H. JUNG and K. G. ZIMMER, Some chemical and biological effects of elastic nuclear collisions. In *Current Topics in Radiation Research* (M. Ebert and A. Howard, eds.), Vol. **2,** 69–127, North-Holland Publishing Company, Amsterdam, 1966.
8. J. NEUFELD and W. S. SNYDER, Estimates of energy dissipation by heavy charged particles in tissue. In *Selected Topics in Radiation Dosimetry,* pp. 35–44, International Atomic Energy Agency, Vienna, 1961.
9. H. JUNG, Zur biologischen Wirksamkeit elastischer Kernstösse. I. Inaktivierung von Ribonuclease durch langsame Protonen. *Z. Naturforsch.* **20b,** 764–772 (1965).
10. G. Hotz, and K. G. Zimmer, Experiments in radiation chemistry of T1-phage. *Intern. J. Radiation Biol.* **7,** 75–86 (1963).