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Effects of Radioactive Elements on Biological Systems

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INTRODUCTION

Radioactive forms of elements important in biological systems have been used as tracers to demonstrate the course of a reaction or a series of reactions or to trace the fate of particular compounds or elements; they have been used also as radiation sources in order to induce effect.

Unfortunately, we discover, neither of these uses is entirely straightforward. Undesired biological radiation effects may intrude in the tracer experiments, and serious measurement and other problems may arise in the radiation experiments. While these difficulties do not preclude the use of the radioelements, they do necessitate the use of certain precautions. But we can also discover, I believe, that characteristics appearing to be disadvantageous often can be used to yield more information concerning biological systems than we would have had without them. We shall consider several examples which demonstrate some aspects of the biological use of radioisotopes.

ISOTOPE EFFECT

In any tracer experiment, the assumptions are made that the chemical behavior of the radioactive species is identical with that of the non-radioactive species and that the radio element substitutes at random for the stable forms of the element. It has been known for some time that this is only approximately true. All radioactive isotopes differ from their stable forms in mass, sometimes to a degree which appreciably alters reaction rates and equilibrium conditions. This is known as the isotope effect. In some cases the effects are large enough to inspire doubt as to the correctness of interpretation of certain quantitative measurements. Ropp (37), in a recent review on the effect of isotope substitution on organic reaction rates, gives a number of examples. Differences in specific reaction rates of organic compounds containing C–14 as compared to those containing C–12 range from several percent up to ten or more percent. In experiments in which a series of equilibria may be expected, this effect may well be an important limitation on interpretation of results.

An interesting biological example of the isotope effect involving C–14 was provided by Buchanan and colleagues (8) who showed that the alga *Scenedesmus*, during the initial phases of growth, contained only 94 percent of the C–14 expected on the basis of the amount offered to the plant. A similar effect was observed with C–13. The effect may be expected to be much larger with lighter isotopes, for example with tritium, which has a mass three times that of ordinary hydrogen. In short-term experiments, then, it is important to be certain that the isotope effect is not neglected in the overall expression of error, especially in instances in which several equilibrium states may be involved.

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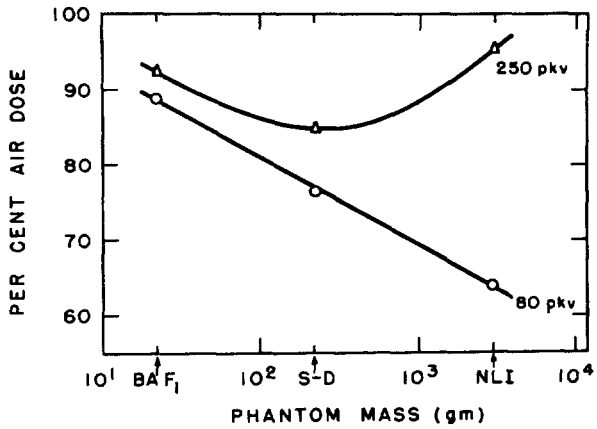

FIGURE 2. Difference in attenuation with depth within a phantom between hard X-rays (250 kvp) and soft X-rays (80 kvp). From Grahn, Sacher and Walton. (16)

effective) exhibited an LD_{50} of 805 roentgens (measured in air). The same animal receiving soft radiations (0.38 angstrom effective) showed an LD_{50} of 2,500 roentgens. The soft radiation appeared to be only about 30 percent as effective as the hard radiation in bringing about the result. The result is apparently accounted for by the relatively greater protection of internal sensitive organs by the outside tissue in the case of the soft radiation (high LD_{50}), as indicated by the results of similar experiments in smaller animals. In mice, the LD_{50} for the hard radiation is 630 compared to the 816-r LD_{50} for the soft radiations; or, the soft radiation is 70 percent as efficient as the hard radiation. Figure 2 shows the difference in absorption in phantoms between the soft radiation and the hard radiation. It is clear, therefore, that the biological response is determined not only by radiation quality (that is, the penetrating ability of the radiation), but also by body size. The distribution of the energy within the organism must be known, and should be controllable.

RADIATION EFFECTS FROM INCORPORATED ISOTOPES

The difficulties associated with dose distributions when external sources of irradiation

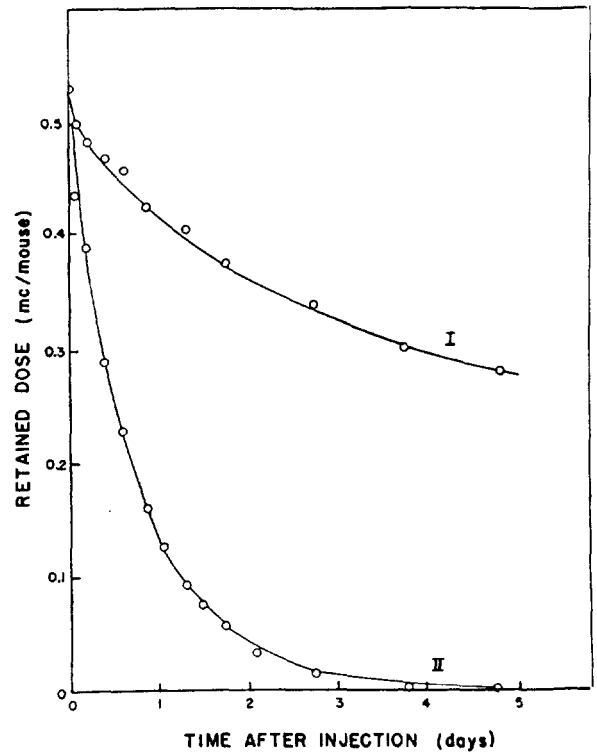

FIGURE 3. The effective dose curves for Na-24 injected in mice. Curve I measures the retention of the injected dose. Curve II is curve I decreased by the physical decay of the isotpe. From Snyder and Kisieleski. (43)

such as X-ray machines or γ-ray sources are utilized are controllable to a large extent, and errors arising from bad distributions can be minimized. The situation with respect to radioactive elements, however, is a little more complicated and is not so easily manipulated.

Consider the relatively easy case of Na-24. This element is expected to be widely distributed through all tissues and the radioactive isotope should, then, from the distribution standpoint, cause little difficulty in dosage estimation. But sodium immediately enters the physiological pool, and the physical laws concerning radioactive disintegration continue to operate. So, as shown by Snyder and Kisieleski (43) the radiation dose to the animal begins to drop immediately as the result of excretion and radioactive decay. Figure 3 demonstrates the rapid net rate of change in

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the organism. Comparison of the effectiveness of the irradiations from Na–24 with a base-line radiation such as 250 kvp X-ray, then, becomes difficult. If one is using as a biological end point a phenomenon which is dose-rate sensitive, such as acute death post-irradiation, the continuous change in rate of radiation constitutes an almost insuperable difficulty in interpretation.

With almost all other radioactive elements, differential distribution throughout the body is observed in mammals. The extreme case of I–131 is very well known. Not only do the thyroid glands themselves collect and store almost all of the iodine in the blood stream, but even metastatic thyroid tissue is known to retain its iodine-storing capacity. Actually, this extreme situation makes radiation dosimetry of iodine-treated animals very easy, because in thyroids of any appreciable size almost all of the radiant energy from I–131 is absorbed by the thyroid gland itself, with very little damage to adjacent tissues.

Other cases are intermediate; for instance, P–32 injected into a mammal will be found eventually in those organs in which appreciable nucleic acid synthesis occurs, such as the blood-forming organs and the gonads. There is an interim period before the P–32 is "fixed" in specific locations, during which the distribution is more general. This phenomenon is demonstrated also by the behavior of Ca–45 intravenously injected in rats. (29) Figure 4 demonstrates the fate of the isotope in several selected organs. It disappears rapidly from blood and more slowly from muscle, but rises slowly in bones to plateau levels. It is obvious that dosage calculation in the organism has to be done on an organ by organ basis. These dose distributions are different from those of P–32, which are different from those of most other elements, which are different from those of hard X-rays homogeneously distributing energy throughout the organism. The intercomparisons between and among these are not simple, and, at present, one can only try to relate specific effect within the organ to the amount of radioactivity deposited in that organ.

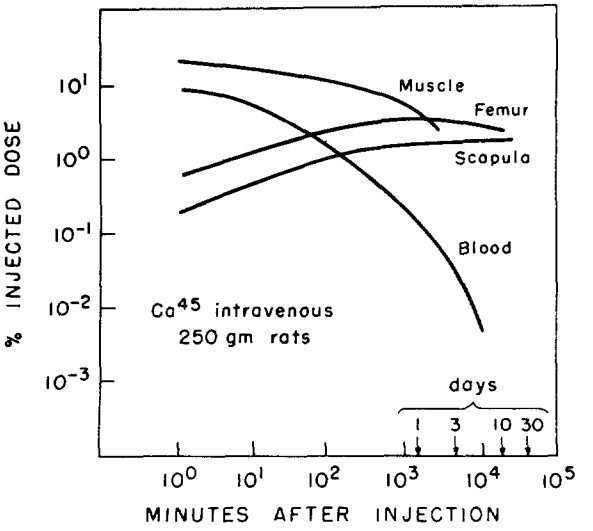

FIGURE 4. The retention of Ca–45 given intravenously to rats. Reconstructed from Norris and Kisieleski. (29)

When the path length of the particle emitted is small compared to the size of the organ and distribution is homogeneous, there is no serious dosage problem. When, however, the distribution is inhomogeneous and the path of the emitted particle is small, and certain parts of the organ receive higher dosages than other parts, a complex problem is simply moved down from the organ-system to the tissue level. For instance, how does one describe the relation between radiation dosage and the incidence of malignant tumors in bones containing radioactive elements? It has been demonstrated that these calculations must be made in reference to the microdistribution of the elements in the bones themselves. In figure 5 (48) it can be seen that Pu–239 has deposited in the bony material in areas that face on the marrow cavity. Other experiments indicate that the Pu will not move very far, if at all, from this bone material although its position relative to the marrow cavity may change. Figure 6 (24) demonstrates the distribution of Ra in a human bone—many years after the individual was exposed. The tracks mark precisely the area irradiated by the alpha particles being emitted by the Ra. It is clear from the two pictures that areas very close to the active area are

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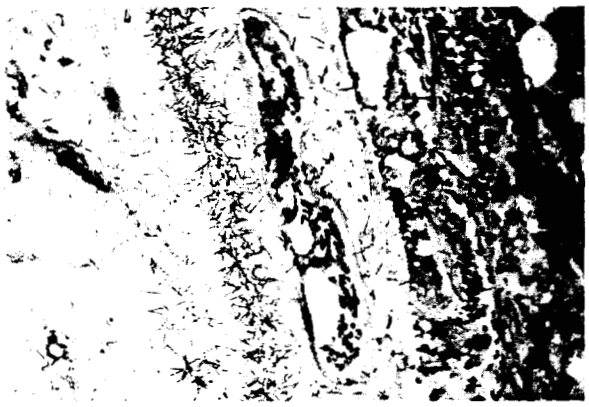

FIGURE 5. Radioautograph of a section of the undecalcified femur of an adult rabbit injected with Pu–239 15 days prior to sacrifice. The alpha tracks demonstrate the accumulation of Pu near the endosteum. From Woodruff and Norris. (48)

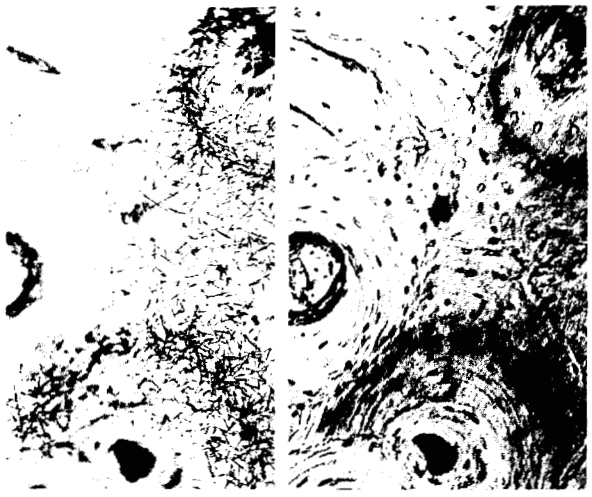

FIGURE 6. Radioautograph and photomicrograph of the same region of the cortex of the humeral shaft of a human exposed to radium chloride 22 years before death. From Looney and Woodruff. (24)

receiving hardly any irradiation, and that radiation dosage, if calculated on the basis of total radioactive content in a bone, would be incorrectly related to effect. Some areas of the bone receive no irradiation, while others receive large amounts, and dosimetry must be done on a micro scale.

Effects brought about by radioelements in microorganisms are more easily related to dose of radiant energy delivered to the biological object than they are in mammals, since, usually, the difficulties arising from heterogeneous distributions are much smaller. An example is the effect of H–3 on *Paramecium* (35). When grown in culture medium containing tritium, paramecia become equilibrated within 30 minutes. After the animals are allowed to grow for 2 days in this medium, it is found that death after autogamy (presumed to be a genetic effect) increases with radioactivity in the culture medium (fig. 7). The effect was compared

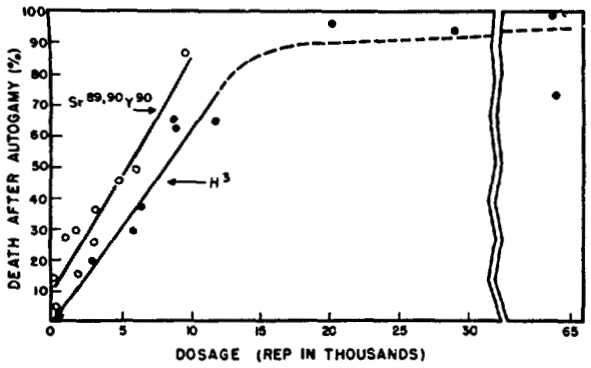

FIGURE 7. Comparison of the effect of Sr–89,90Y–90 and of H–3 on death after autogamy in *Paramecium aurelia*. From Powers and Shefner. (35)

with that brought about by a mixture of Sr–89,90Y–90 which emit very strong beta particles. In this case H–3 is homogeneously distributed throughout the cells and medium. The SrY beta particles traverse the cell because of their large energy (as shown later there may be a 2 percent decrease in dose in the center of the cell). Therefore, there should be no difference due to differences in distribution of energy. On the basis of dosage delivered to a given volume of medium, it is seen that the SrY combination is a little less efficient than H–3 in bringing about the effect measured, for the slopes differ by about 15 percent. If there is any difference, H–3, which emits a beta particle of 5.7 kev producing about 180 ion pairs per micron of track in water, is less efficient than the SrY combination, which emit beta particles of about 700 kev on the average,

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producing about 12 ion pairs per micron of track. This is just the change expected if it is due to a "point effect," and such an inference is possible only because we can compare dosages.

With the same organism and another isotope the situation is more complex. When *Paramecium aurelia* is grown in P–32 solutions (34 and unpublished observations) for a 48-hour period, during which the animals reproduce themselves seven to eight times, P–32 must be randomly distributed throughout the organism. As in the tritium experiment, death after autogamy was scored. At the levels of phosphorus used (about 0.1 mc./ml. maximum), no appreciable physiological effects, such as depression of growth or preautogamous death of cells, were observed. The radiation control consisted of solutions containing SrY mixtures which emit particles of approximately the same energy as those emitted by P–32 and thus constituted an accurate control for dosage rate and density of ionization.

Figure 8 compares the results of P–32 and SrY treatment. In each case the effect increases steadily as the dose is increased. The relative efficiency of P–32 and SrY in producing damage may be calculated from the slopes of the two lines. The ratio of the slopes is almost precisely six. In other words, it takes six times as much activity of Sr–89.90Y–90 in the medium to produce the genetic effect induced by a unit amount of activity of P–32 in the medium. This is in contrast to the result of the tritium experiment, where the effect is about the same.

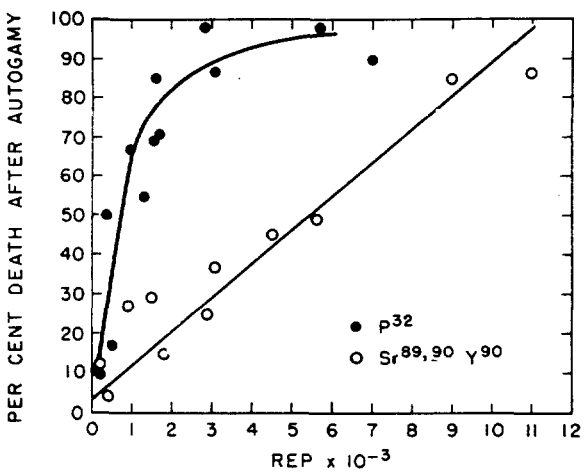

FIGURE 8. Comparison of the effect of P–32 and of Sr–89,90Y–90 on death after autogamy in *Paramecium aurelia*. From Powers (34) and unpublished observations.

One reason for the great difference in effect, between P–32 on the one hand and H–3 and SrY on the other hand, is that under the conditions of growth in these experiments the concentration of phosphorus (measured by radioactivity) within the cells may range from 150 to 240 times that in the external medium, as a result of accummulation and concentration of phosphorus by the cell.[1] Since we may have as much as 240 times as much P–32 within the cell as without, the higher effect of P–32 may be due to the concentration factor alone, and allowance has to be made for it.

This brings up the general problem of radiation dosimetry of radioactive elements in solutions and in cells, a matter which we should examine briefly at this point. The subject has been reviewed most recently by Marinelli and Taylor (27), and by Marinelli (26). The most recent pertinent measurements have been made by Clark, *et al.* (10), Loevinger (23), and Sommermeyer (44). The problems of geometry are described [2] also by Rossi and Ellis (38),

[1] Two methods of measuring the concentration of P–32 in the cells were utilized. A. A known number of cells in a known volume of suspending medium was counted with an end window GM tube. A known volume of medium centrifuged free of cells was counted. Since the volume of each cell (10^{-7} ml) is known, the difference in the counts can be used to calculate the concentration of P–32 in the cells which can be compared to that in the medium. At 7.2 μg/ml P in the medium, an average ratio (N=17) of 150 was measured by this technique. B. The other method gave consistently higher, though more erratic, ratios. It consisted of centrifuging cells into a capillary, measuring the length of the column of cells, washing them out after removal of supernatant into ashing capsules, and measuring the radioactivity of the measured volume of cells. The average value of the ratio at 7.2 μg P/ml medium was 220 with a maximum of 240. Therefore, we assume that the true value lies between 150 and about 220.

[2] After this manuscript was prepared, another pertinent paper appeared, viz., Loevinger, Robert (1956), The Dosimetry of β Sources in Tissue. The Point Source Function. *Radiology, 66*, 55–62.

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Richards and Rubin (36), and by Oddie (30). In a homogeneous distribution the energy absorbed from the beta particles whose tracks are short compared to the size of the object is obviously the total energy of the particles. In terms of dosage, it is very nearly (within 1 percent)

$$d=55\overline{E}_\beta C \text{ rep/day}, \quad (1)$$

where $\overline{E}_\beta$ is the maximum energy of the beta particle in *Mev* and C is the concentration in microcuries per gram for elements with a half-life greater than 5 days. And it is

$$d=0.023\overline{E}_\beta C \text{ rep/hr} \quad (2)$$

for half-lives greater than 5 hours. For many microorganisms these two equations apply, since the organisms are small compared to the ranges of the particles, provided that the amount of radioactivity within the microorganism is the same or less than that on the outside. When, however, concentrations of isotope occur in compartments smaller than the ranges of the particles and when the concentration is higher inside than on the outside, then the radiation dosage lost from the cell must be taken into consideration and the simple formulae do not apply.

Several approaches have been made to this problem. Based on the assumptions made by Richards and Rubin (36), the fraction of radiation lost from a cell whose dimensions are smaller than the mean path of the beta particles is given by

$$F=1-0.435(V/R)^{1/3}, \quad (3)$$

where V is the volume of the organism and R is the average range in water. For *Paramecium* with a volume of 10^{-7} ml, on the assumption that its shape is spherical and that the average range of the β^- is 3.25 mm., we calculate the fraction lost as 0.994, i. e., approximately 0.6 percent of the energy of the beta particles is retained in the organism. If the phosphorus content within the animal is 150 times that of the outside, then we expect a ratio of effectiveness between SrY and P–32 to be 1:1.9. If the concentration of phosphorus is 240 times the outside, we should expect a ratio of effectiveness of 2.44. The actual ratio is 5,[3] and there is obviously an excess to be accounted for.

By another approach derived from Oddie (30) and Marinelli and Taylor (27),

$$\frac{\text{Dose internal}}{\text{Dose external}}=1+\frac{3(C_2-C_1)(1-e^{-\mu r})}{4C_1}, \quad (4)$$

where C_1 and C_2 are concentrations of radioactivity outside and inside, r is the radius of the sphere, and μ is an absorption coefficient. This ratio for our two cases comes to 3.1 for the 150 $\times$ and 4.3 for the 240 $\times$ as compared to the observed ratio of 5. The most generous calculation leaves a difference of 70 percent to be accounted for, while, in fact, the most probable value of concentration leaves an excess of about 200 percent to be accounted for. Thus, even in microorganisms, the dosimetry of the situation enters heavily into the interpretation of results obtained with radioactive elements. What is the interpretation in this instance?

These experiments were designed deliberately to test, with a very accurate physical method, for the presence of phosphorus in the genetic material of *Paramecium*. While many believe that nucleic acids and gene material are equivalent, and that, therefore, phosphorus is contained in the gene, a direct demonstration of this is, of course, desirable. It is well known that when a beta emitter sends out its particle from the nucleus that at least one change and most probably two occur in the atom. In the first place the atom always changes to one whose chemical characteristics are those of atoms of one higher atomic number, i. e., as the beta particle leaves the nucleus, the latter accumulates one additional plus charge, and a P–32 atom then immediately becomes a S–32 atom; this phenomenon is known as transmutation. The other change that may occur with some frequency is that of position. The energetic par-

[3] The ratio of the slope of the P–32 and SrY curves is 6. If the SrY contribution is the same as that of the external P-32, then $\frac{\text{Dose from P–32 external}}{\text{Dose internal}+\text{Dose external}}=6$; therefore, dose internal = 5 dose external.

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ticle has a certain momentum as it leaves the nucleus and according to the rules of conservation of momentum, the nucleus should have a compenating momentum. Therefore, if the beta particle and one other particle(?) known as the neutrino leave at certain angles to each other, the new atom will move in position with a certain kinetic energy. This phenomenon is known as recoil. It is obvious that the energy of recoil will depend not only on the angle of exit of the beta particle with respect to the neutrino, but also upon the energy of the beta particle and the mass of the nucleus. The mass of the nucleus is constant, but the energy of beta particles varies considerably from almost zero up to the highest recorded maximum energy for the particular species. Generally, the average energy of beta particles leaving radioactive nuclei is about half of the maximum. In table 1 we see certain of the results of beta disintegration of radioisotopes. Each of the atoms

TABLE 1

PHYSICAL RESULTS OF β^- DISINTEGRATION OF BIOLOGICALLY IMPORTANT RADIOISOTOPES

Atom	Product	Maximum energy of β^- (Mev)	Approximate kinetic energy of new atom (e. v.) [1]		Bond energies (e. v.) [2]	
			Max.	Ave.		
C-14	N-14	0. 155	7. 0	3. 5	-C-C-	3. 5
					-C=C-	6. 3
					-C≡C-	8. 6
					-C-C-	4. 3
P-32	S-32	1. 70	76	38	-P-O-	~6. 9
S-35	Cl-35	0. 167	3. 0	1. 5	-S-O-	~4. 3
					-S-C-	2. 4
					-S-S-	2. 8

[1] Calculated from Libby (22).
[2] Based on Pitzer (33).

(carbon, phosphorus and sulfur) changes to an atom the chemistry of which is quite different. None of the molecules in which these new atoms would occur should be expected to be the same as the previous molecules, so the transmutation process itself should result in considerable change in the chemistry of the situation. In addition one sees that the kinetic energies of the new atoms are in the ranges of the bond energies that the old atoms possessed (22). Indeed, one expects that in most instances that the bond energies would be exceeded and that, therefore, actual cleavages of molecules would take place as a result of the recoil phenomenon. If either of these phenomena occurs in an atom which is an integral part of the gene, there should result a change in the characteristics of the gene in some proportion of the instances. If this change is, for example, suppression of duplication of the gene and if the gene concerned is sufficiently inportant to the organism, then it would be recognized as a lethal mutation.

It is not yet possible to calculate the number of disintegrations necessary for the inactivation on one gene in *Paramecium*. It would be desirable to set up the experiment in several other ways to see whether the effect changes according to expectations; for instance, a change in specific activity with total radiation dose remaining the same in a phosphorus solution should result in a particular kind of change in the transmutation effect.

There are observations in other microorganisms which also suggest that transmutation can be recognized as a cause of biological effect and from the standpoint of dosage determination some of these organisms (which are much smaller) offer even more favorable opportunities. Studies have been made on bacteria and bacteriophages. One of the early workers to call attention to this method of analysis of genic material was Rubin (39, 40), who studies the pattern of mutations induced in *E. coli* by metabolized P-32. In this cell (volume 10^{-12} ml) only about 0.01 percent of the total energy remains in the cell, according to the calculations of Richards and Rubin (36). Mutations differing in quality as well as in quantity from those produced by X-irradiation appear after growth and incubation of the bacterial cells in P-32. When the phosphorus content of the culture medium is changed, there is a corresponding change of P-32 content in the cell, but the genetic effect changes much more slowly. It was found that

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the increased P–32 could be accounted for in the RNA of the cell, but that the DNA P–32 content remained approximately constant throughout the 16-fold range of concentration. It appears that the RNA P–32 is not concerned in this effect, for the constant number of P–32 disintegrations in the DNA is sufficient to account for most of the mutations observed.

An excess of biological effect over and above that expected from ionization of beta particles has also been noted in studies with *E. coli* bacteriophages. Hershey *et al.* (18) showed some time ago that the coliphage T–4 randomly labeled with P–32 atoms cannot reproduce as a consequence of transformation from P–32 to S–32. In these experiments survival of phages with incorporated P–32 was compared with that in phages which contained no P–32 but which were suspended in a solution containing P–32; the latter constituted "beta particle ionizing radiation" controls. These authors have concluded that about one in every 10 disintegrating P–32 atoms is lethal to the phage particle and suggest that a recognizable small portion of the P–32-containing material is necessary for the self-reproducing capacity of the phage particle.

These results have been extended by Stent and Fuerst (45) who have observed the effect of P–32 disintegration in a number of coliphages at several temperatures. They report also that the efficiency of inactivation at 4° C. is approximately 10 percent for all six phages tested. In addition, they observed a change in the efficiency factor with changes in temperature—at −196° C. the efficiency is only 60 percent of its value at 4° C. for all the phages. These effects led the authors to infer that the cause of inactivation is indeed the transmutation of the P–32 atom, and that the variation in efficiency caused by temperature changes is explained by temperature effects on hydrogen bonding in helical DNA structures. At low temperatures the hydrogen bonding with the other portion of the double helix helps to hold the injured portion in place. In this case many P–32 disintegrations can occur with damage to only one strand with no obvious damage to the phage particle itself.

On the other hand, Castagnoli and Graziosi (9) have recorded inactivation of the phage against *B. megatherium* as a function of time of exposure to P–32. Because they do observe a decrease in efficiency at −79° C. as compared to 2° C., they conclude that transformation of P–32 to S–32 can contribute only slightly to the inactivation. The disintegration itself is certainly temperature-independent and, therefore, they argue, the biological effect brought about by it should be; they do not allow for temperature-dependent processes subsequent to radioactive disintegrations. Also, in the bacteriophages *Salmonella* phage P–22 and coliphage T2 (14) it has been shown also that inactivation by decay of P–32 appears to be directly proportional to the DNA content.

In *Neurospora* Hungate and Mannell (19) tested the efficiency of S–35 as a mutagenic agent. They varied the content of S–35 by varying the specific activity of the medium offered to the organism for metabolism and noted that there is an increase in the number of biochemical mutants as the specific activity increases. The increases are not proportional, but the mutation frequency may be in excess of that expected from the radiation from the beta particles of S–35. While there are still several open questions concerning the relative efficiency of S–35 as a mutagen, the study shows that it should be classed along with C–14 and P–32 as a valuable isotope for the study of biological structure and function with the transmutation technique.

These studies of radioactive decay in microorganisms seem to show that there is an excess in effect over that expected from the ionizations brought about by the beta particles from several isotopes in *Paramecium, Neurospora, E. coli* and in some bacteriophages. It is not unreasonable to think of the excess effect as resulting from beta disintegration of the atom. Even if this is accepted as a conclusion, however, there are still several open questions concerning the effects. For one thing, it is impossible to say whether the effects result mainly from the transmutation process itself (change of chemical state) or whether recoil

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has to occur as well (rupture of molecule). If one or the other is established as the more important change, a difference in interpretation may be required. Secondly, we have no assurance that this phenomenon is actually a measure of sensitive spots, for the results obviously do not show unequivocably that phosphorus or sulphur itself is in the critical position. There may be some transfer of effect within the molecule, and the temperature results reported by Stent and Fuerst (45) and by Castagnoli and Graziosi (9) might very well be telling us this. This is especially significant in view of Alexander and Charlesby's (1) results on temperature effects on irradiated organic systems, which favor the interpretation that energy transfer occurs along macromolecules and that this transfer is affected by temperature changes of the magnitude used in these studies. If this is true, we see in these studies measurements of the number of radioactive atoms that are close enough to sensitive regions to bring about the effect, but there remains the large problem of how far is "enough."

In spite of these uncertainities, however, the technique is one allowing recognition of effect brought about by an event in a single atom. This is the smallest change that can have biological effect. When particular compounds whose biochemical fates are reasonably well recognized are used in transmutation studies, considerably more precise information concerning the structure and function of the genetic material is likely to come to light.

In multicellular plants and animals, measures of transmutation effects are more difficult. In *Drosophila*, for instance, the necessity of incorporating the radioactive element into the germ line requires exposure of flies to radioactive media throughout the period of development.[4] This means that the organisms are continuously exposed to irradiation from within and from without throughout their development, and the variations in sensitivity of different tissues to radiation during development make interpretations awkard. Also, in the adult fly, the grossly inhomogeneous distribution of the isotope makes dosage calculations difficult. Nevertheless, some studies have shown promise. Bateman (5) shows that sperm from P–32-containing flies exhibit mutation rates in excess of those expected from the calculated radiation dosage to the sperm, but he is not satisfied that he has stabilized the system sufficiently for recognition of the transmutation effect. For instance, in successive sperm batches from the same fly the number of mutations observed varies in a way which cannot be fully explained at the present time. King (20) and King and Wilson (21), on the other hand, believe that they have successfully related transmutation of P–32 in *Drosophila* to at least one effect—the reduction in fertility. In these experiments he simulated the irradiation pattern from internal P–32 with an external source of gamma irradiation. F_1 females from flies exposed to a dose similar to that delivered by P–32 laid as many eggs and as many viable eggs as the unirradiated control, whereas F_1 flies whose parents had received the P–32 treatment laid only 30 percent as many eggs as the controls with 30 percent of control hatchability. Despite its difficulties, the *Drosophila* work shows promise of contributing to our knowledge of genetic determination—and the fact that it is *Drosophila* work makes it especially important to most geneticists.

PLANT STUDIES

There have been numerous studies in plants along the same general lines, and we shall consider a few examples.

There are a number of reports describing biological effect which presumably occurs as a consequence of concentration of the isotope in question (see review by Scully (42)). For instance, Blume *et al.* (7) showed that nominal tracer levels of P–32 in the nutrient solution have marked effects, presumably as a result of

[4] A review of radioisotopes effects in insects has been made by Dahm (11).

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accumulation by the plants. Figure 9, which relates microcuries of phosphorus in the nutrient solution to the length of the young leaves

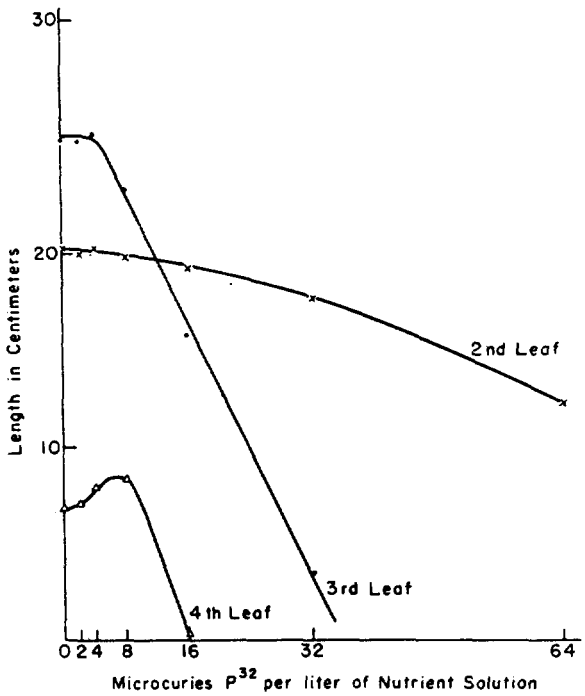

FIGURE 9. The effect of P–32 concentration in the nutrient solution on the length of barley leaves. From Blume *et al.* (7)

in barley plants, demonstrates an appreciable effect in the 10-microcurie/liter level, with the effect differing in magnitude for different leaves. Effects are visible at levels as low as 8 microcuries/liter, where they consist of increases over the controls. This recalls the results of Russell and Martin (41) who observed increased shoot weight in barley at 10-microcuries/liter levels.

Similar reports have been made by Ehrenberg and Granhall (12) for P–32 and S–35 effects on cherry and apple trees, by Thompson *et al.* (46) for P–32 on barley, and by Michaelis and Kaplan (27) in *Epilobium* by P–32 and S–35. Marked effect is reported by these investigators using a number of criteria.

Cytological effect has been scored by a number of investigators—namely, the induction of chromosomal changes by C–14, P–32 and S–35 in many kinds of plant materials (2, 3, 4, 6, 13, 15). The physical bases of these effects have not been recognized, but it seems clear that incorporation of certain kinds of radioisotopes into the chromosome can bring about numerous chromosomal rearrangements. The studies of C–14 effects are particularly interesting (42, 47) for they were observed in uniformly labeled plants and thus constitute a measure of "toxicity" made at the cellular level. All of them offer interesting possibilities for analysis on a physico-chemical basis since external radiation controls can be easily set up. In addition, they demonstrate that incorporated isotopes can bring about genetic change efficiently, and can then be used as sources of variants. It should be noted, however, that somewhat different results have been observed by Osborne and Elliott (31). In view of the promise offered by induced plant mutants, it is very desirable from the practical standpoint to compare quantity and quality of these effects with those produced by other methods.

SUMMARY

The following generalizations can be made from our brief survey of biological effects of radio-elements:

1. Energy from internally deposited radioactive elements probably brings about the same biological effects as external sources of radiation, if the physical variables such as dosage rate, linear energy transfer, and total dose are the same in the two general cases.
2. There are major difficulties in making comparisons. Rapid, continuous changes in dosage rates are the rule when many radioactive elements are utilized as internal sources of radiation. And, frequently, distribution within an organ is spotty and discontinuous, resulting in very large differences in dose delivered to different parts of the organ. Dosage determination in these cases must be done on a micro scale.

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3. The fact that certain isotopes are concentrated by living organisms has been used to advantage. Selective irradiation of organs and tissues has been possible in some instances, and conceivably may become more common. Furthermore, as a consequence of the incorporation of radioactivity into important molecules, a change in a single atom can be related to biological effect in some cells and viruses. The implications of this, for expansion of our knowledge of the structure and function of the determining elements in cells, are very great. But there is need for more work of this kind, using more compounds and different kinds of organisms, especially plants, because of the genetic and cytological advantages they offer.

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