Tritium-Induced Effects in *Paramecium Aurelia* (19116)

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Measurements of genetic effects of certain metabolized radioactive isotopes are of practical interest in the general evaluation of their toxicity and of theoretical interest in that they may lead to clues of the nature of genetic mutations. This report records observations on the genetic effects of tritium (T or H^3), one of these isotopes, in *Paramecium aurelia*.

Methods. The preparations were made by mixing 1.8 ml culture fluid(1) and 0.2 ml water containing suitable concentrations of tritium oxide. Distilled water was added to the fluid in which the controls were grown. These solutions were inoculated with a small drop of a suspension of *Aerobacter aerogenes* which was allowed to grow at room temperature about 14 hours. Then about 0.8 ml of the bacterial suspension was transferred to a deep depression slide, a single paramecium was introduced, and a cover slip was sealed with petroleum jelly over the depression to prevent evaporation of the radioactive water. The animals were allowed to grow in these preparations for about 48 hours. The final activities of each of the solutions and the incubation periods are given in Table I. In any one experiment, the animals were sister cells derived from one recently out of autogamy and, therefore, presumably homozygous(2). All animals were of Variety 4, mating type VIII, containing kappa(2). After the exposure period, the organisms were washed 5 times in inactive culture solution by serial transfers with micropipettes, diluting the original activity by a factor of about one million. From the group of washed animals a number of cells were isolated into separate depressions (Table I). The isolates grew until they had exhausted the food (3-5 days) in approximately 1 ml culture fluid, at which time the members of a clone generally entered autogamy as a unit. From each autogamous culture 36 animals were isolated and, after an incubation period of 4 days, observed for survival. While it is difficult at times to distinguish between surviving and nonsurviving clones, it was found satisfactory to classify as nonsurviving any isolate which, during the period of 4 days, did not give rise to a culture of at least 60-70 animals with few or no abnormal cells. In Table I the statement of effect (% death) is an average of the % death after autogamy induced in all the individual isolations from each treatment. Thus, the first line of Table I indicates 16 isolations from the treatment; at autogamy 36 isolations were made from each of these 16 (576 in all); and 11 of the 576 did not survive (1.9%). The activity of the stock solution of T was assayed by reducing the water to H_2 and measuring the ionization within a chamber caused by a known volume of the H_2 gas* (error not larger than 5%). The radiation dosage delivered by long-lived electron emitters homogeneously distributed in volumes that are large with respect to the range of the particles may be calculated(3)† according to rep/day $=$ 55 E C, in which E is the average energy of the β particles in electron volts, and C is the activity in curies. In the case of T, with an average energy of 5.69 kev(4), the dosage is 0.313 rep/day/μc/ml.

1. Sonneborn, T. M., *J. Exp. Zool.*, 1950, v113, 87.

2. Sonneborn, T. M., *Adv. in Genetics*, 1947, v1, 264.

* We are indebted to Dr. K. E. Wilzbach and his associates of the Chemistry Division of Argonne National Laboratory for this measurement.

3. Marinelli, L. D., Quimby, E. H., and Hine, G. J., *Am. J. Roentgenol. Radium Therapy*, 1948, v59, 260.

† The formula given is actually 61 E C; however, the constant 61 contains the number 83 as the energy in ergs dissipated in a gram of tissue corresponding to one roentgen unit gamma radiation in air. It is now generally agreed that 93 ergs/gm is more nearly accurate, and 55 E C is offered as a closer approximation of the equivalent dosage. See Evans, R. D., *Nucleonics*, 1947, 1, 2, 32.

4. Jenks, G. H., Ghormley, J. H., and Sweeton, F. H., *Phys. Rev.*, 1949, v75, 701.

TABLE I. Death After Autogamy Following Growth in Tritium-Containing Solutions.

Exp. No.	T conc., mc/ml	Exposure time, hr	Dosage, rep	No. exp. isolations	Avg % death after autogamy	
					Treated	Control
2	1.03	47	630	16	1.9	.8
1	1.03	48	645	23	1.7	.6
3	5.15	44	2955	27	19.6	1.2
3	10.30	44	5900	27	28.8	1.2
1	10.30	48	6445	15	37.3	.6
4	13.90	48	8700	23	65.2	.7
3	15.45	44	8860	23	62.5	1.2
3	20.60	44	11810	25	64.8	1.2
4	32.45	48	20300	26	96.4	.7
4	46.35	48	29000	24	93.9	.7
2	103	47	63100	18	98.1	.8
1	103	48	64450	10	73.3	.6

The dosages shown in Table I for the various treatments were calculated on this basis.

Results. In regard to number of divisions during treatment (3-4 fissions/day), vegetative death of cells (0%), and the growth behavior after treatment until autogamy, the treated animals did not differ from the control animals with the exception of the experiment reported in the last line of Table I. There was an unexplainable preautogamous depression of growth rate in the treated animals which might be related in some way to the atypically low death after autogamy observed in these animals.

With increasing activity of T in the culture solution, an increasing degree of death after autogamy was observed. The response seems to be linear up to the region of 15,000 rep (Fig. 1). This type of response has been observed for other isotopes and X rays(5,6). In view of results in Variety 1 of this species (7) which demonstrate a sigmoid type of response, the question as to the precise nature of the curve is left open. However, for purposes of comparing it with the response induced by other isotopes, a straight line function for the first 8 points was assumed, and the best straight line was calculated. The line is $y = 6.34\,x - 1.1$; y being death after autogamy in % and x being radiation dosage in thousands of rep. The standard deviation of the slope (σ_b) is 0.69.

The $Sr^{89,90}Y^{90}$ experiments with which the T response is compared are from an earlier study(5). It had been established that equivalent absorbed energies from $Sr^{89,90}Y^{90}$ and X rays(6) produce approximately equivalent results, and in order to demonstrate that the response of the animals at the time of the T experiments was similar to that observed in the Sr experiments, several X-ray control studies were made. Previous experience indicated that 10,000 r X radiation induced 76% death after autogamy, measured by our criteria of death. At the time of the T experiments, two separate exposures to 10,000 r (200 kvp X rays through 0.25-mm Cu and 3-mm Bakelite at 400 r/min) involving 53 isolated treated animals and 1900 isolations after autogamy yielded an average of 71% death after autogamy. This result is well within the error of the X-ray line, and it was concluded that a comparison of these experiments with the earlier ones is permissible.

The Sr response, also assumed to be linear,

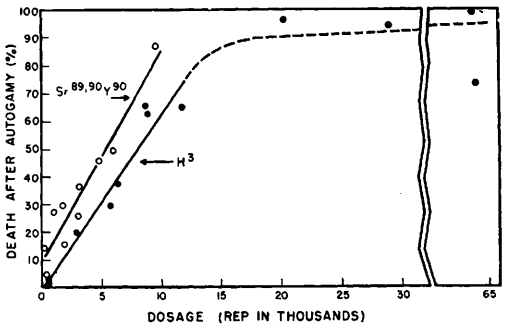

FIG. 1. Comparison of effect of $Sr^{89,90}Y^{90}$ and of H^3 on death after autogamy.

5. Powers, E. L., *Genetics*, 1948, v33, 120.

6. Powers, E. L. and Sheiner, Deborah, *Genetics*, 1948, v33, 624.

7. Kimball, R. F., *Genetics*, 1949, v34, 210.

which is shown in Fig. 1 is described by $y = 7.70\ x + 8.5$; with a σ_b of 0.86. The high value of the y-axis intercept is consistent with the higher control deaths observed at that time. Comparison of the slopes of these two responses reveals no difference between them, the t value being 1.24 with 14 degrees of freedom indicating $0.20 < P < 0.30$.

Discussion. It has been shown that the macronuclei of exautogamous cells are derived from the micronuclei of preautogamous cells under the conditions of these experiments, and that the process of autogamy ensures homozygosity and expression of the genetic constitution of the micronuclei(2), but it is not as certain that all death following autogamy subsequent to treatment by a mutagenic agent is due to disturbances in the nuclear genetic system. The nature of this death has been examined in Variety 1 of *P. aurelia* by Kimball(8) who shows that while the factors causing low vigor and death for the most part are inherited as if the micronuclei have been damaged in some way, there were certain departures from expectation that are unexplainable at the present time. These exceptions were observed in one experiment and involved a small percentage of the total number of crosses analyzed. While these exceptions must be taken into consideration and while all death after autogamy observed in experiments reported here is not necessarily due to lethal micronuclear aberrations expressing themselves in the usual manner, Kimball's analysis permits us to regard a great portion of the death after autogamy as being due to lethal changes of some kind in the micronuclei of exposed animals. It is not unreasonable to believe that two mutagenic agents of the same type induce proportional amounts of regular genetic damage even though the actual extent cannot be measured accurately.

On the assumption that the biological effect induced by T and Sr-Y is, for the most part, an expression of genetic damage, we can examine the significance of the result. There are two reasons to suspect beforehand that the effects of T may be different in degree from those of certain other radioactive isotopes, the biological system receiving the same total radiation dosage from all.

First, there is dependence of genetic effect on the kind of radio-element. It has been shown that P^{32} induces death after autogamy much more efficiently than $Sr^{89,90}Y^{90}$(5), that part of the increased efficiency is ascribable to accumulation of P^{32} in the cell and a corresponding increase in dosage to the cell, and that the accumulation phenomenon probably does not account for all the excess effect of this isotope(9). The likely interpretation of such a result is that effects are being induced as a result of the transmutation of P^{32} to S^{32} and the associated recoil occurring at the time of expulsion of the electron from the nucleus of the atom. The same kind of evidence has been reported for bacteria(10) and bacteriophage(11). This effect must be in excess of that induced by β particles, and it depends on the number of radioactive atoms present in strategic spots and the disintegration constant of the isotope. In the case of T, the relative mass difference between it and H, which it is replacing, is very great. This difference could result in much lower specific reaction rates for T and compounds containing it. In consequence there would be differential selection by enzymatic systems of non-tritiated compounds and a resulting frequency of T in H positions below the expectation indicated by the T/H ratio offered to the organism for use (this effect is known for deuterium). Since specific activities (T/H) have not been measured in these experiments, a critically proved statement cannot be made; the apparent lack of a transmutation effect is expected if it is small and there is biochemical selection against T in the organism. Therefore, it appears that if transmutation of the incorporated T contributes to the genetic damage being measured, it is so small that it is unrecognizable

8. Kimball, R. F., *Genetics,* 1949, v34, 412.

9. Powers, E. L. Unpublished.

10. Rubin, B. A., *Genetics,* 1950, v35, 133.

11. Hershey, A. D., Kamen, M. D., Kennedy, J. W., and Gest, H., *J. Gen. Physiol.,* 1951, v34, 305.

even after growth of the cell through 7 cell generations in concentrations of T up to 100 mc/ml.

The second reason for uncertainty in predicting the response of the organisms to exposure to T stems from ignorance of the role of specific ionization in the induction of the effect. An electron with an average energy of 5.69 kev (T) produces on the average 184 ion pairs per micron of track (12p.24), while one of 700 kev ($Sr^{89,90}Y^{90}$) produces about 12 ion pairs per micron of track(13). This difference obtains in these experiments. Zirkle(14) has examined the literature in some detail and agrees that with an increase in specific ionization, total radiation dosage remaining the same, the observed decrease in lethal effect of ionizing radiations on small systems such as vegetative bacteria and viruses is real. In the case of induction of lethal mutations, however, the situation is not as clear. Lea(12) states that the evidence to date warrants the conclusion that the differences in mutation rate (X chromosome recessive lethals) noted by several investigators following treatment of *Drosophila* with agents producing varying degrees of specific ionization in tissue are significant, and are indicative of an inverse dependence of mutation induction on the number of ion pairs per unit length of track. However, because of the difficulty of estimating dosages from α particles, soft radiation, and neutrons to the germinal tissue of *Drosophila,* the evidence is not as convincing as it is in the case of bacteria and viruses.

If the small difference in slope of the two response lines described here is considered real, we note that the ratio (slope T/slope Sr) of the rates is 0.82. This ratio is entirely consistent with Lea's summary (p. 153) of the relationship of mutation yield and increasing ion density of radiation. Indeed, it is very near the value of 0.78 reported by Wilhelmy *et al.*(15) for the relative effectiveness of X rays of 6.2 kv effective compared with those of shorter wavelengths. This correspondence may be significant since the ion density produced by electrons from T (average of 184 ions pairs/μ) is close to that expected from X rays of this low energy. It should be noted, however, that these authors were uncertain of their dosage measurements, and our numbers do not indicate a real difference in response. Thus, while there may be some significance in the coincidence, the similarity of the results may be fortuitous.

Because no information on the distribution of T within the cell is available, a dosimetry question which may have bearing on the interpretation must be considered. If the micronuclei contain no radioactivity they can be considered spherical "holes" in the radiation field, receiving a dose from the surrounding cytoplasm.‡ In the case of energetic electrons such as those arising from the Sr, Y isotopes, the 55 E C formula is usable for calculating the dose delivered to the "hole." But when the energy (and, therefore, the length of track) becomes small relative to the radius of the "hole," the calculation is uncertain. In the condensed interphase stage the micronuclei are spheres of 0.75 μ radius, and the length of path of a 6-kev electron (the mean energy of the tritium beta particle) in water is cited as 1 μ (12,p.24). There is, then, conceivably delivered to the center of the micronucleus, if it contains no tritium, a dose smaller than that indicated by the calculation. Until there is available a description of the absorption in water of tritium β particles, calculation of the precise dosage to the micronucleus in these circumstances is impossible. However, it is highly unlikely that the micronuclei contain no tritium, since they are duplicated 6 or 7 times during the exposure period within

12. Lea, D. E., *Actions of Radiations on Living Cells,* Cambridge, 1947.

13. Gray, L. H., *British J. Radiol.,* 1947, Suppl. 1, 5.

14. Zirkle, R. E., Unclassified Doc. MDDC-444, 1943.

15. Wilhelmy, W., Timofeeff-Ressovsky, N. W., and Zimmer, K. G., *Strahlentherapie,* 1936, v57, 521.

‡ This and similar problems of radiation dosage from radioactive solutions are discussed by Richards, P. I., and Rubin, B. A., *Nucleonics,* 1950, v6, 42 and by Rossi, H. H. and Ellis, H. Jr., *Nucleonics,* 1950, v7, 18, 19.

cytoplasm which, according to our observations on the rate of water output by the contractile vacuoles, is nearly equilibrated with the aqueous radioactive environment within 30 minutes after the beginning of exposure. Nevertheless, a definite answer to this dosage question must await solution of the difficult problems of microdistribution of T within the cell and the absorption of very soft β particles in water.

Summary and conclusions. Effects induced by metabolized radioactive hydrogen (tritium or T) and presumed to be genetic in nature have been measured in *Paramecium aurelia*. Organisms were allowed to divide 6 to 8 times during 2 days in culture fluid containing T in concentrations varying from 1 to 100 mc per ml. With increasing radioactivity of the medium, increasing death after autogamy was observed, but no preautogamous effects were noted. The response observed after exposure to T appears to be slightly lower than that induced by emitters of more energetic β particles at similar dosage levels. The conclusions are that no part of the effect can be ascribed to transmutation of T, the emitted electrons alone apparently inducing the biological changes; and that the difference in efficiency between T and $Sr^{89,90}Y^{90}$ in inducing the effects observed is consistent with the observations of others concerning the dependence of the mutation constant upon rate of energy loss by densely ionizing particles.

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Electron Micrographs of Newcastle Disease Virus Preparations from Chick Embryos Following Infection by Virus Propagated in the Short-tailed Shrew (*Blarina brevicauda*). (19117)

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The short-tailed shrew is one of the smallest of the mammals and has an extremely high rate of metabolism. Electron microscope studies were conducted to determine the morphology of Newcastle disease virus (NDV) recovered from this sensitive mammal following infection by intracerebral inoculation and intranasal instillation. In previous experiments tailed virus forms were demonstrated from purified egg-adapted virus material by Bang(1,2) and Cunha *et al.*(3). Tail-like forms were also demonstrated in hamster-adapted, mouse-adapted, and bat propagated NDV after culture in 10-day embryonated eggs by Reagan and his coworkers(4,5)

Procedure. Three-hundredth ml of allantoic fluid from 10-day embryonated White Leghorn chicken eggs infected with the 25th egg passage of California strain (No. 11,914) was inoculated into the right frontal lobe of each of three *Blarina brevicauda* short-tailed shrews. Three-hundredth ml of the same material was instilled intranasally in each of 3 additional shrews. Typical Newcastle disease symptoms developed within 24 to 30 hours in the shrews infected intracerebrally and within 5 to 6 days in the shrews infected nasally. The symptoms from both series were irritability, malaise, rhythmical contraction of muscles starting in the limbs and spreading over the entire body, and

1. Bang, F. B., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 135.

2. Bang, F. B., *J. Exp. Med.*, 1948, v88, 251.

3. Cunha, R., Weil, M. L., Beard, D., Taylor, A. R., Sharp, D. C., and Beard, J. W., *J. Immunol.*, 1947, v55, 69.

4. Reagan, R. L., Hickman, J W., Lillie, M. G., and Brueckner, A. L., *J. Infect. Dis.*, 1949, v85, 256.

5. Reagan, R. L., Smith, E. J., and Brueckner, A. L., *J. Bact.*, 1951, v61, 37.