Reproduction in Paramecium as Affected by Small Doses of X-Ray and Beta Radiation. (20450)

GEORGE E. DANIEL AND HELEN D. PARK. (Introduced by Willie W. Smith)

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Md.

During the course of work on the effect of high energy radiation on protozoa it was observed that isolation cultures of *Paramecium caudatum* in a certain group of depression slides consistently produced a larger number of individuals than similar cultures in supposedly identical slides. Chance measurements showed that the former group of slides was slightly radioactive, while the other was not. The active slides were of Czechoslovakian manufacture and had been purchased some 20 years ago. Rough measurements with a G.M. counter held over the slides indicated an activity some 20-30 counts per minute above background. The radiation was rather soft since only 0.040 in. of aluminum was required to reduce the count to background value. These slides had never been used and were still in their original tissue paper wrappers. So far as we know there had been no opportunity for accidental contamination of the slides with radioactive material and, furthermore, neither the wrappers nor a water detergent mixture used to wash the slides showed any activity. The inactive slides were new and of recent American manufacture. Both sets were approximately 75 x 25 mm with depressions 3 mm deep and 15 mm in diameter.

The following observations indicate the degree of differences observed: 1) Five single cell cultures were established in each type of slide using comparable amounts of food and media (lettuce extract). At the end of 24 hours there were 5 paramecia in the inactive slides and 10 in the active ones. 2) Ten isolation cultures were prepared in each type of slide. No transfers were made for 6 days. At the end of this time the active slides had a total population of 2459 paramecia and the inactive slides a total of 1859. 3) Again, 5 cultures in each type of slide were set up and daily examinations were made. A single individual from each slide was transferred to a clean slide of the same type each day, with new media and food. At the end of 7 days the active slides contained 85 paramecia and the inactive 61. There had been two deaths in the active slides and 7 in the inactive. 4) Nine cultures in each type of slide were established and allowed to run for 6 days. At the end of this time there were 238 and 156 paramecia in the active and inactive slides, respectively. The data from the experiments conducted in the radioactive slides are not conclusive because the differences may not be due entirely to the radioactivity of these slides. It may be that the glass liberates into the solution some substance which of itself is stimulating to growth, or such soluble materials may favorably alter the medium. We feel, however, that the difference observed in 4 experiments of 3 different patterns is more than a fortuitous coincidence. It is logical to infer from the above results that rather low concentrations of radiation may have contributed to growth and division. In order to test this hypothesis under more controlled conditions a series of experiments using radiosulfur (S 35) were undertaken.

A clone of *Paramecium caudatum* growing in lettuce extract and fed *Aerobacter aerogenes* was used as a source of experimental material. Experimental and control cultures were set up on the same day from the same stock culture using the same solutions except for the addition of the sulfur to one group. No particular attempt was made to choose stock cultures of the same age as a source of inoculum. A single paramecium was isolated into each of the depressions of a clear nonradioactive pyrex glass spot plate and the number of organisms living at the end of the experimental period was determined. At the time of starting the amount of food was adequate and roughly the same in each depression of both series. Each depression is considered as one culture. Experimental and control

TABLE I. Influence on Population of Radiation from Radioactive Sulfur, 13 Experiments.

Radiation /hr (rep)	Avg No. of paramecia/culture Exp.	Control	No. of cultures (same in exp. and control)	P (<)
3.13	158.8	96.1	9	.01
3.13	154.0	91.5	9	.01
3.13	155	96	9	.01
3.13	36.6	9	36	.01
3.13	11.2	6.9	36	.02
3.13	11.0	3	36	.01
1.55	152.8	81.4	9	.01
1.55	151.4	83	9	.02
1.55	152	78	9	.01
.77	133	110	9	.02
.77	110	62	9	.05
.38	91	105	9	.4
.38	106	119.4	9	.6

cultures were kept in the same moist chamber. Room temperature varied about 5°C during the course of any one experiment. The experiments were done at various times over a period of several months. The radioactive sulfur was secured from Oak Ridge, in the form of labeled, carrier-free sulfuric acid in 0.1 N hydrochloric acid and had, when received, an activity of 12.6 millicuries per ml. Neutralization was done with NaOH and where necessary, because of high concentration, the Na content of experimental and control solutions was equalized. The concentration of sulfate as used for experimental solutions was so low (0.0005 mg/ml) that it was not considered necessary to equalize the amount in the 2 groups of cultures. The desired amount of radiation was secured by dilution, correction being made for the decay of sulfur activity. The radiation from S 35 is pure beta having an average energy of 0.055 Mev. There may be a small amount, of the order of a few microcuries per milliliter, of contaminating P 32 in the material as received from Oak Ridge. Calculation of the energy absorbed in the medium has been made using the S 35 beta energy and the measured number of disintegrations per second per milliliter of solution as used in the cultures.

The data from the experiments with S 35 are shown in Table I. The data show that the number of organisms developing in the sulfur solutions, containing between 1.5 and 3.1 rep per hour of beta activity, was significantly greater than in control solutions* It is not safe to compare the actual numbers of paramecia produced in experiments done at different times or to consider that controls are indiscriminately applicable. This is especially well shown in experiments 4, 5, and 6 (3.13 rep/hr) in which conditions were obviously much less favorable for growth and development than in the first experiment. The data also indicate a loss of stimulation at dose rates of about 0.77 rep and below.

The sulfur experiments indicate quite clearly that relatively low doses of radiation over an extended period of time favor the growth and division of paramecium. Calculation shows that the energy liberated in the solution containing the greatest concentration of sulfur is equal to approximately 3.13 rep per hour or for the entire experimental period, 6 days, a total of 450 rep.

The stimulating effect of a relatively small total dose of beta particles given at a very low dose rate could not be reproduced by an equivalent amount of soft X-rays (48 KV, 50 ma) given in one dose requiring about 3 minutes for delivery. Four hundred fifty roentgens of X-ray were given to culture media, water used for diluting media, and to the paramecia. Several combinations of irradiated and unirradiated fluid and cells were tried, using a total of about 200 paramecia.

* In interpreting these differences it is necessary to bear in mind that small or moderate differences in rate of growth may result in very large differences in number of animals. If no animals die, and if we denote the final number of X-irradiated animals by N_r, the final number of control animals by N_c, the initial number (the same for both groups) by N_o, the number of divisions for the irradiated animals by d_r, for control animals by d_c then

$$N_r = N_o \cdot 2^{d_r}$$
$$N_c = N_o \cdot 2^{d_c}$$
$$\text{Log}_2 \frac{N_r}{N_c} = d_r - d_c$$

Thus the logarithm (to base 2) of the ratio of the final number of animals in the treated and control groups is equal to the difference in the number of divisions. Whenever N_r and N_c differ significantly so will any function of them (d_r and d_c) differ significantly. The results are essentially the same whether the computation is performed on the total or separately for each culture.

TABLE II. Effect of 4 r or 8 r/Hr on Number of Paramecia Produced in 53 Hr.*

Exp. No.	4 r/hr		Control		8 r/hr
1.	53		34		54
	52		36		48
	59		37		53
	41		7		45
Total	205	(P = <.05)	114	(P = <.05)	200
2.	66		39		59
	49		37		65
	65		38		57
	70		44		59
	250	(P = <.01)	158	(P = <.01)	240
3.	13		9		13
	13		7		12
	13		8		14
	9		8		12
	48	(P = <.01)	32	(P = <.01)	51

* Four plates of 9 depressions each (36 isolation cultures) in each group of each experiment. Figures in body of table are number of paramecia per plate of cultures. Totals are for 4 plates.

In no instance could we distinguish a difference in population between experimental and control cultures.

Since the single dose of X-rays produced no increase in growth and division we decided to try a very low dose rate for a relatively long time. A 200 KV machine operating at 0.3 ma was used. By suitably adjusting the distribution of the cultures in the radiation field it was possible to secure dose rates of 4 and 8 r per hour and to irradiate both groups simultaneously. Controls were kept in the room in which the irradiations were made but were completely shielded by a lead box. Temperature of experimental and control cultures differed randomly by one or 2 degrees.

Three experiments with low X-ray dose rate were made. Each experiment contained 36 isolation cultures set up in 4 pyrex spot plates. All experiments covered an irradiation period of 53 hours. Results of these experiments are shown in Table II. There is a highly significant difference between the number of paramecia produced in the irradiated and control cultures. Apparently conditions in one control dish of Exp. 1 were not comparable with the other in the series. When this aberrant unit is included in the calculation P = <.05. If it is left out of consideration P = <.01.

In order to test whether or not the observed increased rate of growth and division of the irradiated cell was a permanent characteristic or a transient phenomenon the following experiment was made. Immediately after determining the number of cells in control and experimental cultures of Exp. 2, Table II, 18 paramecia were chosen at random from each of the experimental and control groups and set up in isolation cultures. No further irradiation was given to any of the paramecia. The total number of paramecia in the 3 groups at the end of 53 hours was: controls, 186; 4 r/hr, 220; 8 r/hr, 226. There is no significant difference between these groups.

In view of the results the likelihood of a mutation involving either nucleus or cytoplasm or both is indeed remote. Mutation cannot be operative in this case unless one assumes a fortuitous selection of a group of animals showing a rapidly developing forward mutation and an equally rapid and very precise back mutation.

The data show clearly that under certain ill-defined but reproducible conditions cells capable of autonomous existence are stimulated to grow and divide by continuous exposure to very low dose rates of either corpuscular or electromagnetic radiation of high energy. Experiments, carried out in the radioactive slides, where only a single individual was continuously exposed to the irradiation (Exp. 3) indicate strongly that the increase in population is not a response to alterations in the environment produced by products arising from injured cells of the same species as those used as experimental material. Biological entities can show functional stimulation under conditions such that no tissue injury can contribute to the increased activity associated with the absorption of high energy radiation by the reacting system. At the present time one can only speculate concerning the modes of action and sites of origin of the stimulant. An attractive and plausible hypothesis may be based on the production of free radicals by the absorbed energy and the stimulation by such radicals of metabolic activities. Low dose rates would produce radicals or other reaction products in amounts small enough to be stimulating while

high dose rates would lead to toxic concentrations of the same or other substances.

Summary. Beta radiation administered at a level of from 0.77 to 3.13 rep per hour and X-ray radiation given at 4 or 8 r per hour stimulates reproduction in paramecium. The stimulation is not due to mutation since it disappears immediately on removal of the cell from the influence of the radiation.

Received May 21, 1953. P.S.E.B.M., 1953, v83.

Effect of Intravenous Administration of Paritol-C* on Serum Lipids of Hypercholesterolemic Rabbits.† (20451)

J. C. FORBES AND O. M. PETTERSON.

From the Department of Biochemistry, Medical College of Virginia, Richmond.

Previous work in our laboratory showed that only a relatively small percentage of the cholesterol of lyophilized normal human or rabbit serum can be extracted by cold chloroform even in 24 hours, while a large percentage of the serum cholesterol of nephrotic patients and hypercholesterolemic rabbits can be thus extracted(1). The fraction extractable in a 3-hour period, which is usually practically the same as extracted in 24 hours, has been called the "readily extractable" cholesterol.

Gofman and associates(2,3) have shown that heparin administration causes a shift in the lipoproteins of high Sf values into those of successively lower ones in the serum of hypercholesterolemic rabbits and human subjects. Nikkilä(4) obtained the same general effect. Injection of heparin also has been shown to suppress the rate of development of atherosclerosis in cholesterol fed rabbits(3). Since the anticoagulant Paritol exerts the same general effect as heparin on blood coagulation and has been shown to affect serum lipoproteins (5), it was decided to determine if its administration would favorably influence the concentration of the "readily extractable" fraction and that of total cholesterol in hypercholesterolemic rabbits.

Experimental. Male rabbits, weighing between 4 and 5 lb except when otherwise stated, were put on a 0.5% cholesterol diet prepared as follows. Fifty g of cholesterol were dissolved in 100 ml of chloroform and added to 500 g of Wesson oil. After warming on a water bath until practically all of the cholesterol was again in solution, the whole was added to 9450 g of Rockland rabbit pellets and thoroughly mixed. The diet was ready for feeding after all the chloroform had evaporated. It was fed to all the animals throughout the whole experimental period. The Paritol was injected intravenously using a 5% solution. Blood for the analytical studies was obtained by nicking an ear vein after the ear had been warmed with a heat lamp. The analytical procedures employed have been described previously(1).

In Exp. 1, after the animals were on the high cholesterol diet for about 12 weeks, the Paritol injections were begun. The total cholesterol by this time had reached an average of 1750 mg/100 ml of serum and the "readily extractable" fraction constituted 90% of the whole. The Paritol injections were given in amounts corresponding to 0.1 ml of a 5% solution/lb body weight. It will be seen from the results, which have been averaged in Table I, that the Paritol injections caused a marked drop in all the fractions studied. This was true in each individual animal as well as in the average for the group. The percentage of the total cholesterol present in the "readily extractable" form tended to decrease as the total cholesterol decreased. This was very marked in all individual cases where the total cholesterol had dropped to

* A polysulfuric acid ester of polyanhydromanuronic acid kindly supplied by Wyeth, Inc., Philadelphia.

† Presented at the annual meeting of the Virginia Academy of Science, May, 1953.