STUDIES ON X-RAY EFFECTS.

XIV. The Effect of X-Ray on the Division Rate of Paramecium.

By ROBERT T. HANCE,* Ph.D., and HARRY CLARK, Ph.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, September 14, 1925.)

Reports on the biological action of x-ray have been contradictory owing largely to the fact that, in the absence of a unit of dosage, accurate comparison of results has been impossible. Furthermore, it has generally been considered that dosage could be disregarded as it was to be expected that larger doses would have simply a more pronounced effect than small ones. As a matter of fact, this idea is erroneous for it has been shown that doses of different intensity may have diametrically opposite effects on a tissue (Murphy and coworkers).

With the development of an x-ray apparatus capable of delivering rays of a constant quality and intensity (Clark), a major difficulty has been eliminated. For the purposes of the present work, paramecium was selected as the biological material because its rapid multiplication renders possible the numerical expression of any reaction to radiation. This selection was made in spite of the fact that no protozoon or simple metazoon has thus far been demonstrated to give any certain response to x-ray exposures. But the ease with which such forms can be handled in large numbers either in mass cultures or individually and the facility with which they may be subjected to a variety of conditions have seemed to make it worth while to investigate anew their response to radiation.

Since reproduction is one of the most easily affected vital functions, we have chosen to test the action of various doses of x-ray on the division rate of these unicellular organisms.

Material.—Two races of paramecium have been utilized; *i.e.*, *Paramecium caudatum* and *Paramecium multimicronucleatum*. For brevity, the former will hereafter be called Race C and the latter Race M. In general appearance they

* Fellow in zoology of the National Research Council.

are similar although Race M is the larger. Race C has but one micronucleus whereas Race M has four; and Race C has two contractile vacuoles while Race M has from two to seven, depending on environmental conditions (Hance, 1917). Although the paramecia used in the experiments came originally from two pure lines, in many of the experiments the additional precaution was taken of starting new lines from single individuals in order to insure a uniform physiological make-up.

About 15,000 animals were observed individually and many times that number were studied in mass cultures.

Method.—A standard hay infusion was made daily, with 0.5 gm. of hay in 100 cc. of spring water. The mixture was boiled for 5 minutes. For containers, Syracuse watch-glasses boiled in water containing a trace of paraffin were used for the small mass cultures or the isolated individuals. The thin film of paraffin on the glass resulting from this treatment prevented the medium from adhering to the sides of the dishes and rendered easier a complete survey of the animals in the dish.

In order to eliminate the secondary radiation from the glass container in the preliminary experiments, the paramecia were placed in 1 or 2 inch sections of paper soda straws one end of which was plugged with paraffin. With a slender tipped pipette it was possible to place a single individual in such a tube and to recover it without difficulty after the exposure to x-ray. With one end of the straw stopped the surface tension was sufficient to hold the liquid in place during the manipulation. The radiated animals were seldom in the straws more than 1 hour and the absence of any deleterious effects arising from the sojourn therein was shown by the fact that controls allowed to remain inside for 2 days were apparently unharmed. When it became evident that no considerable differences were to be found as far as the rate of division was concerned, the tube was discarded for the simpler one of exposing the animals in the watch-glasses.

The X-Ray Outfit.—The x-ray outfit used in this work has been described (Clark). A broad focus Coolidge tube mounted in a lead-lined cabinet and kept cool by means of a fan is operated on 60 cycle current rectified by kenetrons. Ionization measurements made with apparatus previously described (Clark) have shown that the outfit may be kept in operation continuously without a variation of current or voltage exceeding 2 per cent. In the experiments the rays were produced at 30 kilovolt peak and 22 milliamperes, filtered through very thin cardboard, and used at a target distance of 25.5 cm. Under these circumstances the rays produced, according to our measurements, about 6×10^{12} pairs of ions per gm. per second in air.

EXPERIMENTS.

Experiment 1. To Test the Effect on the Division Rate of Single Exposures to X-Ray.—

(*a*) Samples of the two races of animals were placed in soda straw tubes and exposed to the standard dose used in these experiments for 1, 2, 5, 10, and 20

minutes. One-half of the controls were also kept in tubes for the duration of the exposures and the other half were kept in watch-glasses. After exposure five animals from each batch were placed in five individual watch-glasses and the division rate noted for 5 days. No difference in the division rate of the two sets of controls was observed. The samples of both races exposed for 1 and 2 minutes showed no reaction, while the division rate of those subjected to the same treatment for 5, 10, and 20 minutes was slightly lowered for 2 days, after which it slightly exceeded that of the controls. At the end of 5 days the average number of divisions per day was:

	Race C.	Race M.
Control	0.48	0.6
X-ray (all exposures)	0.46	0.6

No differences in the effects of the various lengths of exposure were noticed and the total number of divisions of both x-rayed and control lines were the same at the end of 5 days as shown above. The tests involving 10 minute exposures have been repeated three times, and the 20 minute exposures eight times always with similar results. See Charts 1 and 2, Graphs 1 and 6.

(*b*) In all of the following experiments mass cultures of the two races of paramecium were first exposed in watch-glasses. Then from each exposed culture from ten to twenty-five animals were isolated in individual watch-glasses and the division rate followed for from 5 to 15 days. The average rate of division was obtained by dividing the total number of animals found in all of the watch-glasses by the number of individuals originally isolated. In this series of experiments exposures lasting 1, 1½, 2, 3, 4, 5, and 6 hours were used. Exposures up to 3 and 4 hours long failed to produce any more marked or different effects on the division rate than those lasting less than 1 hour but after the 5 and 6 hour treatments the division rate was slightly increased over that of the controls. As the results seemed to be complicated with temperature the detailed account is omitted here to be reported when more complete data are available. With the exception of the 1½ hour period the other exposures have been repeated from two to eight times. See Charts 1 and 2, Graphs 2, 3, 4 and 7, 8, 9 for a diagrammatic representation of the results reported above.

As the protocols show, paramecia were exposed to a range of the x-ray for periods of from 1 minute to 6 hours. Even the largest dose proved insufficient to cause the death of the animals. It was observed, however, that there was a slight but constant depression in the division rate lasting from 2 to 4 days and occasionally for 5 days. After this period the x-rayed lines divide slightly faster than the controls. This

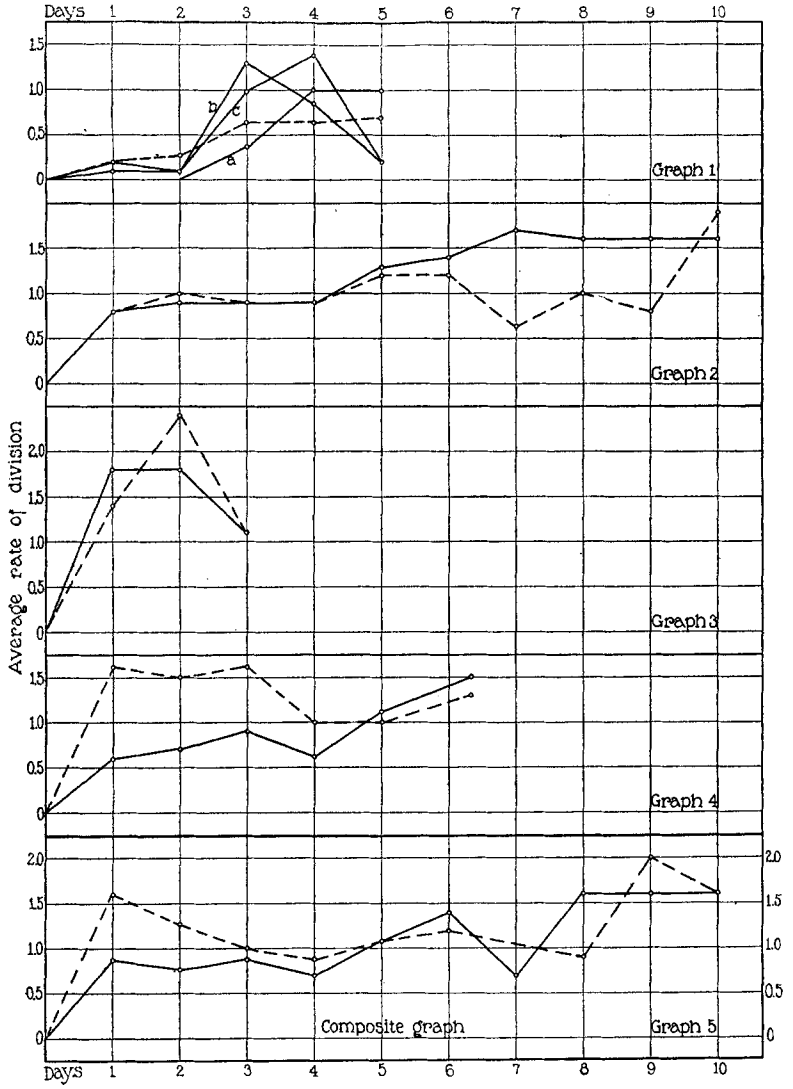

CHART 1. Graphs illustrating the effect of x-rays upon the division rate of *Paramecium caudatum*. The rates of division in tenths of a division are plotted vertically, while the number of days following exposure to x-ray are given horizontally. In all the graphs the broken lines represent the division rate of the control or non-radiated paramecium, the unbroken lines the radiated forms. Graph 1. Curve *a*, 5 minute exposure; curve *b*, 10 minute exposure; curve *c*, 20 minute exposure. Graph 2. 1 hour exposure. Graph 3. 2 hour exposure. Graph 4. 4 hour exposure. Graph 5. Composite graph made by averaging the figures upon which the first four graphs are based.

acceleration was about sufficient to offset the depression so that after awhile the x-rayed and control strains had produced about the same

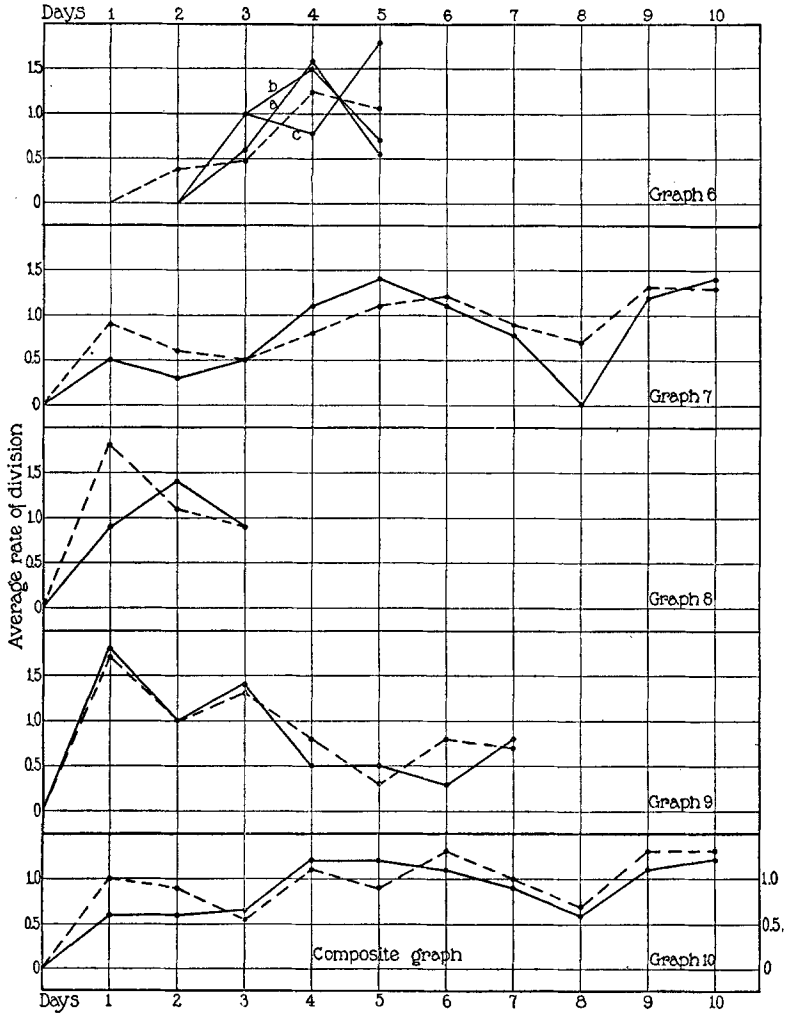

CHART 2. Similar to Chart 1 except that the reactions of *Paramecium multimicronucleatum* to x-ray are illustrated. Symbols and descriptions as in Chart 1, Graphs 6 to 10 being comparable in order to Graphs 1 to 5.

number of individuals. Both races of paramecium responded in the same manner. The period of depression was practically as marked

from the short exposures as from the longer ones although some incomplete data indicate that long exposures cause an initial stimulation rather than a depression of the division rate.

Experiment 2. To Test the Effect on the Rate of Division of Repeated Exposures to X-Rays.—

(*a*) The exposure was repeated once within 24 hours after the first dose.

Twenty individuals of both races were isolated in separate watch-glasses and for 2 weeks their normal rate of division was recorded. At the end of this period one animal from each line was isolated and allowed to multiply until there were four descendents. Two of these were held as controls and the other two were placed in paper straw tubes and x-rayed for 1 hour. The next day one of the two rayed specimens or its descendents were again exposed for 1 hour. The rate of division was noted each day for 6 days. In all cases, with but one exception, the animals exposed twice behaved as did those that had only one exposure. The rate of division was temporarily lowered but returned to the normal in about 3 days. This experiment has been repeated six times with comparable results with the single exception noted in which the forms died that were exposed twice. In this case, of fourteen paramecia x-rayed on 2 successive days, ten died on the 1st day after the second exposure without dividing, one died without dividing on the 3rd day, one divided and died the 2nd day, one divided and died the 3rd day, and one divided after 3 days and lived. These results have not been again obtained.

(*b*) Exposure repeated twice a day for 8 days.

Mass cultures of both races were exposed in watch-glasses for 10 minutes, at 4 p.m. and at 10 a.m. for 8 days. The animals were exposed fifteen times in all without any obvious reduction in their number as compared with the controls. At the end of the period all appeared slightly swollen and somewhat more sluggish than usual.

(*c*) Three exposures about 12 hours apart.

The technique was similar to that used in (*b*) except that after each exposure ten individuals were isolated from the mass culture and their division rate followed. Two lengths of exposure were used, 15 and 30 minutes. The first two exposures lowered the rate of division below that of the controls although after the second it was not as much lowered as after the first. After the third exposure there was no depression, and, in the case of the 15 minute sample, there was a slight increase in the rate of division. The average number of divisions for the first days after each of the exposures is shown below.

	Time exposed.		
	1	2	3
15 min. exposure	0	0.6	1.4
30 " "	0	0.4	1.2
Control	0.2	0.9	1.2

At the end of 4 days the average division per day for both control and radiated animals was 1.

(*d*) Exposure repeated at intervals of 3 or 4 days.

Since our early work seemed to show that the division rate returned to normal in about 3 days, mass cultures were rayed every 3 or 4 days to determine whether a more enduring effect could be produced. Mass cultures of both races were placed in watch-glasses. One culture of each was held as controls and the other cultures were rayed for 20 minutes. Four individuals from each x-rayed culture were isolated and the rate of division followed for 3 and in some cases 4 days, when the culture was again exposed to x-rays for 20 minutes. This was repeated four times in all and after each exposure four paramecia were isolated from the controls and the x-rayed samples. The C race showed the usual initial depression in the rate of division followed by a complete recovery in 4 days. Each succeeding exposure produced the same effect. The M race had its division rate lowered after the first exposure but after the succeeding exposures it was slightly raised. In all cases, however, the rate of division of the x-rayed samples and of the controls was approximately the same at the end of 4 days.

In the observations described under Experiment 2, we have attempted to extend the initial depression of division by repeating the exposure once or twice daily or at intervals of 3 or 4 days. As many as fifteen exposures have failed to cause a further definite decline in the rate of multiplication or to continue the original depression. One exception to this general statement should be recorded. A number of individual paramecia were rayed for 1 hour and half of them were given a second exposure the following day. During the period that it took the controls to divide five times, the animals receiving a single x-ray dose divided twice and those that had two doses with one exception failed to divide at all and died in from 1 to 3 days. Thus far it has not been possible to duplicate this result.

Repeated doses on mass cultures of paramecium have consistently caused the animals to round out so that they appeared slightly swollen, and to become somewhat sluggish in their movements; but no lethal effect has been noted.

The data presented in Experiment 2 (*c*) and (*d*) seem to indicate that the M race may develop what might be termed an immunity to x-ray after several doses. What actually happens may be that the animal approaches the limit of its reactive power, after which it shows either less or no reaction to this physical agent. In the table of Experiment 2(*c*) the second dose produced less effect than the first,

and the third produced no change in one case and a slight acceleration of division in the other. The same phenomenon is recorded in Experiment 2 (*d*). In this instance the exposures were 3 days apart and after the first dose the division rate was initially stimulated rather than depressed. In both cases, however, the number of animals produced at the end of a given period as in the case of the controls was the same. The length to which this progressive inactivation, if it may so be called, may be carried has not as yet been determined.

To determine whether one end of the cell was more sensitive to x-rays than the other, the anterior and posterior ends of radiated animals were isolated after fission and followed for several generations. No differences in division behavior were noted.

To discover whether exposure to x-rays would be more potent during the process of fission (mitosis) the division of the individual was timed so that it would occur during the process of radiation. The descendents of these animals were studied for several generations but the results were not different from those described above.

DISCUSSION.

The inability of earlier workers to obtain any results after exposing protozoa or small metazoa to x-rays is probably due to the fact that they were largely concerned with attempts to determine a lethal dose. Since the animals did not succumb even to very long exposures it was concluded that they are non-sensitive to this physical agent. To demonstrate the reactions of paramecium to x-rays as reported above it was necessary to observe a large number of individuals and to follow their descendents for several days.

The slight initial check on cell division followed by a rapid recovery is the most obvious of our results. The slight stimulation of the rate of division which occurred after exposures of 5 and 6 hours as well as the results (with Race M) after repeated exposures at intervals of several days suggest that the reaction of paramecium to x-radiation may be reversible. Treatments lasting for 10 minutes to 3 or 4 hours depress division, while longer or repeated exposures may under some conditions raise the reproductive rate.

While it is not possible on the basis of the present data to draw

conclusions regarding the character of the physiological reactions, certain inferences seem warranted. The actual mechanism of reproduction (mitosis) cannot be affected since animals radiated while dividing behaved no differently than the others. It seems more probable that the reaction to x-rays must be a more or less general one throughout the organism, since experiment has shown that one end is no more affected than the other, and since, furthermore, there is apparently a residuum of the primary effect remaining for several days, even after the animal has divided. The residual effect is indicated by the lack of a reaction or the lessened reaction to later exposures at various intervals. This residual effect disappears in 3 or 4 days as far as its influence upon cell division is concerned.

We are able to offer no direct evidence as to why protozoa or small metazoa prove so slightly reactive to doses of x-ray that are many times more than sufficient to kill a small mammal. It may be owing to the circumstance that paramecia very rapidly react to their maximum, after which no change in them occurs.

SUMMARY.

Two races of paramecium were submitted for varying lengths of time to x-radiation and a large number of individuals were observed to determine the effect on the rate of division. The division rate of both races suffered a slight initial depression lasting for 2 to 5 days following the exposure. This depression is followed by complete recovery.

Within rather wide limits the length of the exposure has, in these experiments, made no appreciable difference. Apparently the maximum effect of the x-rays is produced by relatively short exposures. Continued radiation produces little further change until exposures of 3 and 4 hours are used, when precisely the opposite results are obtained from those obtained with shorter exposures.

Doses repeated at various intervals have in general failed to interfere more markedly with the division rate than a single dose. Repeated radiation causes the cells to become slightly swollen without apparent interference with their viability.

BIBLIOGRAPHY.

Clark, H., *Am. J. Roentgenol. and Radium Therap.*, 1924, xi, 445.

Colwell, H. A., and Russ, S., Radium, x-rays and the living cell, London, 2nd edition, 1924.

Hance, R. T., *J. Exp. Zool.*, 1917, xxiii, 287.

Hance, R. T., *Tr. Am. Micr. Soc.*, 1916, xxxv, 135.

Murphy, Jas. B., *J. Am. Med. Assn.*, 1914, lxii, 1459. Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800. Taylor, H. D., Witherbee, W. D., and Murphy, Jas. B., *J. Exp. Med.*, 1919, xxix, 53. Thomas, M. M., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 75.