RATE OF RECOVERY FROM THE ACTION OF FLUORITE RAYS.

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This paper is a report of some experiments upon the rate of recovery of *Paramecium caudatum* from the cytolytic action of fluorite rays. The organisms were exposed to the radiation emitted through the fluorite window of the hydrogen discharge tube described in previous communications.[1,2] The intensity of the radiation was such that an exposure of 8 seconds caused cytolysis in 57 per cent of the exposed organisms. In order to study the rate of recovery from the action of the rays, the entire 8 seconds of radiation was not given in one exposure, but in two portions of 4 seconds each, with a longer or shorter interval of time intervening between the two exposures. The relation between the length of this interval of time and the percentage of organisms cytolized was observed.

The organisms used were from a pedigreed race of *Paramecium caudatum*, cultured in drops of nutrient infusion on concave microscope slides. A single organism was placed in a small drop (measured to uniform size) and exposed to fluorite radiation on a special microscope slide provided with a fluorite window. The rays passed through the fluorite window of the microscope slide from below. After the exposure the small drop containing the organism was flooded with from one to two drops of infusion and the organism was transferred to a new concave slide and placed in a damp chamber for observation. The following day, the cytolized organisms were disintegrated, while the organisms which survived were active and

[1] Bovie, W. T., The action of Schumann rays on living organisms, *Bot. Gaz.*, 1916, lxi, 1.

[2] Hughes, D. M., and Bovie, W. T., The effects of fluorite ultra-violet light on the rate of division of *Paramecium caudatum*, *J. Med. Research*, 1918, xxxix, 233.

had usually undergone fission. From previous experiments[2] on the effects of these rays on *Paramecium caudatum* we have learned that if the organisms are actively swimming 24 hours after the radiation, they will continue to live and will multiply at the same rate as the non-radiated controls. The experimental results are given in Table I.

It will be seen from Table I that as the interval of time between the two exposures increases, the per cent of cytolized organisms decreases. During the time between the two exposures the organism recovers from the effects of the first 4 second exposure so that when the second 4 second exposure is added the total effect is less than that of a single 8 second exposure. The amount of this recovery in-

TABLE I.

Rate of Recovery from the Action of Fluorite Rays.

Interval of time between exposures.	Total No. of organisms exposed.	No. of organisms cytolized.	Cytolysis.		Calculated No. of organisms cytolized.
				Corrected.	
min.			*per cent*	*per cent*	
0	72	41	57.0	50.0	41.0
7.5	47	20	42.7	35.7	19.75
15	54	17	31.5	24.5	17.0
30	49	9	18.3	11.3	9.35
60	50	3	6.0		4.9

After one exposure of 4 seconds the per cent of cytolysis was 7.

creases as the interval of time between the two exposures increases. For example, when the interval of time between the two exposures was 1 hour, the organism had so completely recovered from the effect of the first exposure that the combined effect of the two exposures was no greater than that of a single 4 second exposure.

7 per cent of all the organisms receiving a single 4 second exposure cytolized. If we subtract 7 per cent from each of the percentages given in Column 4 of Table I, we obtain the values given in Column 5. These values are plotted in Fig. 1 as ordinates against the intervals of time as abscissæ. The points fall upon a smooth curve. The shape of the curve suggested that the process of recovery might be adequately represented by the equations which govern homogeneous

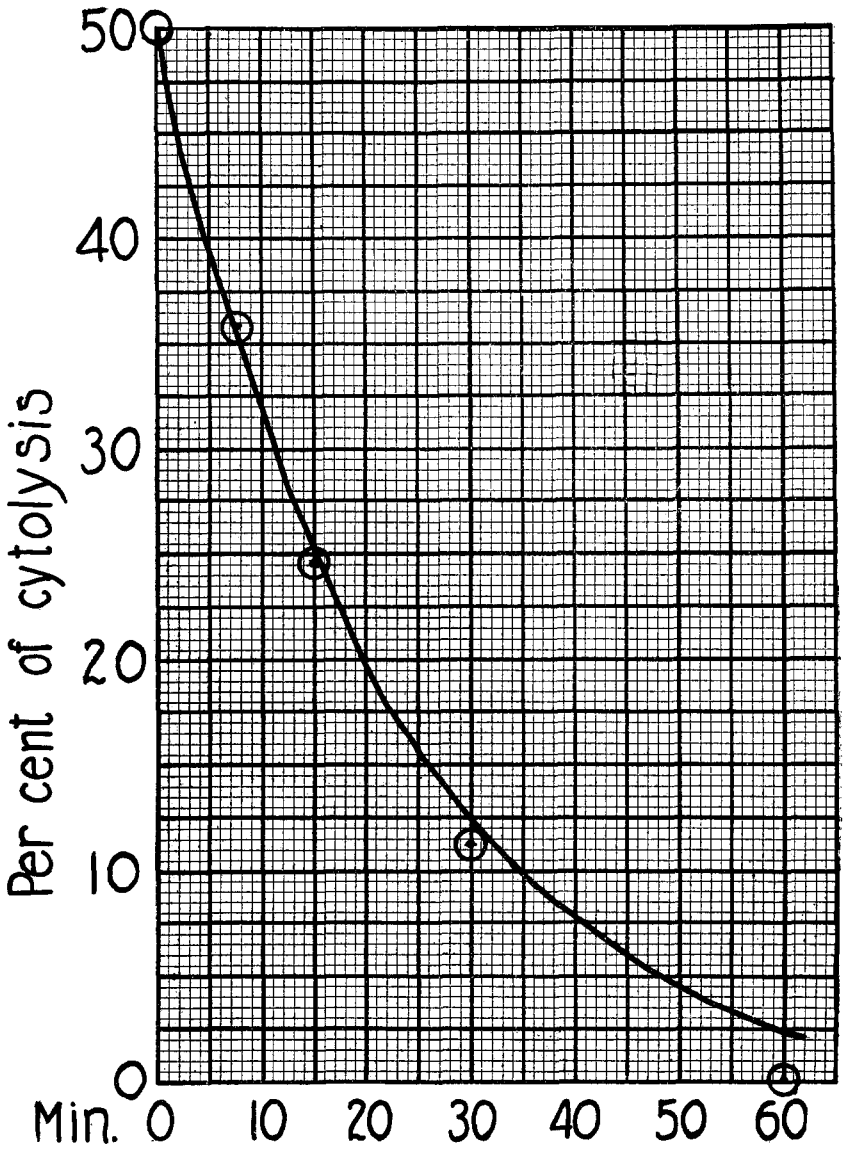

FIG. 1. Graphic representation of the recovery of *Paramecium caudatum* from fluorite radiation as a function of time. The per cents of cytolysis are plotted as ordinates and the intervals of time between the two 4 second exposures are plotted as abscissæ. The curve is a graphic representation of the equation $X = X_0\epsilon^{-Kt}$, in which K equals 0.0473, X_0 equals the per cent of cytolysis when the time interval is zero, and X the per cent of cytolysis after the intervals of time, t. ϵ is the base of the natural system of logarithms.

mass reactions. I have plotted for comparison the curve for the monomolecular reaction formula

$$X = X_0 \epsilon^{-Kt}$$

when K equals 0.0473, X_0 equals the per cent of cytolysis when the time interval is zero, and X the per cent of cytolysis after the intervals of time, t. The calculated number of cytolized organisms is given in Table I. It will be seen that the observed percentages fall very close to the theoretical curve.

When a number of organisms are exposed to fluorite rays they are not all killed by the same length of exposure, but, owing to individual idiosyncrasies and unknown variations in the experimental conditions,

TABLE II.

Relation between the Frequency of Cytolysis and Length of Exposure to Fluorite Rays.

Length of exposure.	No. of organisms exposed.	No. of organisms cytolized.	Cytolysis.
sec.			*per cent*
6	51	1	2
8	100	29	29
10	106	48	46
12	114	79	69
14	120	109	91
16	105	99	94

some organisms are cytolized by a shorter exposure than others. These differences in susceptibility to the influence of rays may affect the shape of the recovery curve. The nature of the effect will depend entirely upon the relative frequency of cytolysis for various exposure times.

The results of some experiments upon the relation between the length of exposure and the frequency of cytolysis are given in Table II and are represented graphically in Fig. 2. It was not possible in these experiments to duplicate all of the conditions such as light intensity, etc., of the recovery experiments; thus a 4 second exposure did not cause any of the organisms to cytolize. The experimental results are, however, significant in connection with the recovery ex-

periments, for as will be seen by an inspection of Fig 2, for exposures between 6 and 14 seconds duration, with but a single exception,

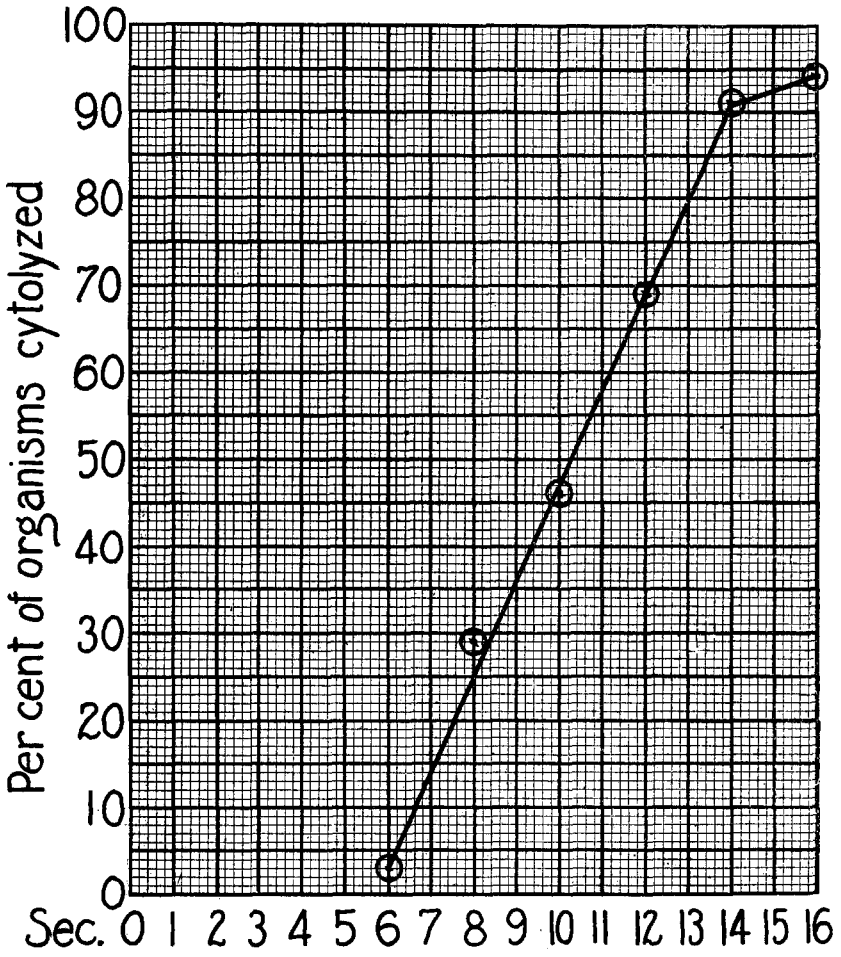

FIG. 2. Graphic representation of the frequency of cytolysis of *Paramecium caudatum* as a function of the length of exposure to fluorite radiation. The per cent of organisms cytolized is plotted as ordinates, the length of exposure as abscissæ.

the curve expressing the relation between the frequency of cytolysis and the length of exposure is practically a straight line. That is, within the limits of experimental error, there are just as many organ-

isms in the class requiring 10 seconds as there are in the classes requiring 12 or 14 seconds exposure to produce cytolysis. The point at 8 seconds is the only one which does not fall upon the straight line and this point could be brought very close to the line by omitting the results of the experiments of 1 day in which the death rate was abnormally high. Such a distribution of susceptibility will have no effect upon the shape of the recovery curve, and we may conclude that the correspondence of the recovery rate with an exponential function of time is not entirely accidental but is the result of an orderly occurrence of the processes involved in the recovery from fluorite radiation.

We may find a cause for such uniformity if we assume that cytolysis occurs when a certain amount of some toxic photoproduct has been formed. (The fact that no cytolysis occurs until the length of exposure is increased to 6 seconds is in harmony with such an assumption.) Recovery then, depends upon the removal of the toxic substance. This removal is accomplished by orderly processes.

Since the rate of recovery is so nearly represented by the monomolecular reaction formula, processes of a chemical nature suggest themselves. Other processes, however, may be conceived. If for example, the toxic substance were removed by diffusion out of the organism, the rate of recovery might correspond very closely to an exponential function of time, especially if the rate of diffusion in the outer limiting membrane were slow as compared with the rate within the cytoplasm. For in this case, since the concentration outside of the organism would, due to the ciliary action, always be zero, the amount of toxic substance diffusing across the membrane in a given time would be proportional to the concentration of the toxic substance remaining within.

Wood and Prime[3] advanced a similar explanation for their observation that it required a much longer exposure to kill carcinoma tissues *in vivo* than *in vitro*. They say[4]: "The constant supply of fresh nutriment to the cells by the blood and the removal of any chemical products formed by the radium in the tissue, must account for this dif-

[3] Wood, F. C., and Prime, F., Jr., The action of radium on transplanted tumors of animals, *Ann. Surgery*, 1915, lxii, 751.

[4] Wood and Prime, *Ann. Surgery*, 1915, lxii, 759.

ference." This suggestion makes the diffusion of toxic substances out of the radiated cell of interest in connection with the toxemias which often follow large doses of either ultra-violet, Roentgen, or γ-radiations.

However, we cannot arrive at very certain conclusions regarding the processes of recovery until these experiments have been repeated with various exposure times, with various radiation intensities, and at various temperatures. Until the data from such experiments are available we may best leave the subject with the simple statement that during the interval of time between the two exposures the organisms recover from the effects of the first 4 second exposure so that when the second 4 second exposure is added the total effect is less than that of a single 8 second exposure. The rate of recovery corresponds very closely to an exponential function of time.