CXXIV. THE EFFECT OF γ -RAY IRRADIATION UPON THE GROWTH AND NITROGENOUS METABOLISM OF THE PROTOZOON, *BODO CAUDATUS*.

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DATA concerning the retardation of the multiplication of growing cultures of *Bodo caudatus* when exposed to γ -radiation were recorded in a previous communication by one of us [Robertson, 1932]. The biological reactions to this radiation, the nature of this inhibiting effect and a consideration of the sensitive period in the life of the cell and of its behaviour upon removal from the radium, form the subject of a detailed study which will be recorded elsewhere [Robertson, 1935]. The reader is referred to this paper for particulars of the technique used for the culture, counting and measurement of the organism.

The purpose of the present communication is to record certain effects of γ -radiation upon the nitrogenous metabolism of *Bodo caudatus* and to correlate these effects with the morphological changes which were simultaneously produced. It has been shown in a previous communication [Lawrie, 1935] that the chief end-product of nitrogenous metabolism of a culture of *Bodo caudatus* fed upon a suspension of bacteria in an inorganic medium is ammonia. The method of estimation of ammonia by deproteinisation and nesslerisation there described has been used in the present work. The rate of production of ammonia has been taken as an index of the rate of nitrogen metabolism in the cultures.

There is little previous work which bears directly upon our experiments, because previous studies of the biological and biochemical effects of irradiation have usually been concerned with the effects following the irradiation rather than with those contemporaneous with the irradiation.

Crabtree [1932] irradiated Jensen Rat Sarcoma from radium so screened that the tissues received mainly, but not quite exclusively, γ -rays. She found that exposures of at least ten hours were required to produce a regular response. With periods of irradiation of ten hours, respiration, but not aerobic glycolysis, was depressed. When eighteen hours' exposure was employed both respiration and aerobic glycolysis were depressed. A similar result was obtained with normal animal tissues. Krontowski [1933], using a similar source of radiation, found that an exposure sufficient to produce delayed death of his tissue cultures was insufficient to impair glycolysis. Krontowski was, however, studying effects subsequent to irradiation. Holmes [1933] studied the effect of γ -radiation upon the metabolism of embryonic kidney tissue. An exposure of 14 hours was allowed and the culture fluid analysed after two days' development of the culture. Thus the effects here measured were effects subsequent to irradiation combined with the effects contemporaneous with the irradiation. This treatment

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resulted in a 50–60 % inhibition of carbohydrate breakdown but had no effect upon protein breakdown. With smaller doses results suggestive of stimulation of carbohydrate breakdown were obtained.

EXPERIMENTAL.

Every irradiated culture was studied simultaneously with normal control cultures taken from the same freshly inoculated material as the irradiated culture and differing from it only in as much as they were at no time exposed to γ -radiation. Thus a typical experiment carried out to observe the effect of irradiation from the 8th to the 11th hour would involve the following observations. After eight hours' growth one Petri dish would be taken for counting and estimation of ammonia content. A second Petri dish would be placed on radium at this time. At eleven hours this culture on radium and also a second normal control culture would be taken for counting and analysis. Thus the gain in numbers and ammonia content could be found for (a) a normal culture developing from the 8th to the 11th hour and (b) for an exactly similar culture undergoing irradiation during this period. The assumption, which other experiments have proved to be justified, is made, that the culture set to develop on radium is, at the 8th hour, very nearly identical with the other normal cultures.

The γ -radiation was applied from the following source. The radium element was enclosed in a soldered silver plaque. It amounted to 400 mg. divided over an area of 38.5 cm.² and was contained in cells of 0.1 mm. thickness of platinum further screened with 1.0 mm. of silver. The plaque may be considered as representing a concentration of 10.38 mg. per cm.², and the distance of the bodos from the radium was 0.5 to 0.75 cm.

DISCUSSION OF RESULTS.

Our results are summarised in Table I.

The unit of area employed in measuring the cells is 0.25 micron².

In the first column of the table the periods during which irradiations were carried out and the age of the culture when these irradiations were applied are tabulated. The irradiated bodos were invariably larger than the bodos in the corresponding normal control cultures. In the third column, the effects of the irradiations upon the total number of cells in the cultures are given. In each case the figures express the number of bodos in the irradiated culture per 100 bodos in the normal culture. In the fourth column, the figures for the increase in the number of bodos in the normal and irradiated cultures during the period of irradiation are presented in the same way. The morphological changes produced by the irradiation are summarised in the fifth column. A classification of the periods of irradiation on a rather broader basis than that used in the first column is given in the sixth column, and the influence of these irradiations upon the rate of ammonia production is tabulated in the seventh column. The method by which these figures, termed here the "ammonia ratio," were obtained is as follows. The ammonia produced during any given period was measured in both irradiated and control cultures. This quantity of ammonia was divided by the number of bodos present in the culture at the end of the period. This ratio represents the nitrogenous metabolism, calculated on a "per bodo" basis, associated with the development of the culture from an initial state, common to both irradiated culture and control, to the final state found in the culture at the end of the period of observation. Finally, this ratio, calculated for the irradiated

Table I. *The effect of γ -ray irradiations of different durations upon the size, numbers and ammonia production of the cells in actively growing cultures of Bodo caudatus.*

I	II	III	IV	V	VI	VII
Duration of exposure	Disparity in the mean cell size of irradiated compared with normal cultures at the end of the period of exposure Units of area	Average discrepancy in total numbers present at end of exposure Normal/Irrad.	Average ratio of increase in numbers Normal/Irrad.	Nature of cell response in the irradiated culture	Duration of exposure	Ammonia ratio
2 hours, from 5th to 7th hour	4.35	100 : 95.7	100 : 80.0	Slight check in division. No significant rise in the size of the cells. Slight response	2-4 hours, between 5th and 14th hours	30 % decrease
3 or 4 hours, between 5th and 14th hours	9.03	100 : 76.2	100 : 50.0			
5 hours from start	14.55	100 : 65.1	100 : 25.4	Marked forcing up of the mean size of the cells. Check in division, merging gradually into acceleration of division	5-9 hours, between start and 9th hour	102 % increase
7½ and 9 hours from start	14.33	100 : 72.5	100 : 50.3			
15 hours, from 7th to 22nd hour	12.65	100 : 65.6	—	Maintenance of the enhanced size of the cells and the establishment of a rate of multiplication not far removed from the normal	15-22 hours	42 % increase
22 hours from start	18.52	100 : 62.2	100 : 56.2			

culture, is expressed as a percentage increase above, or decrease below, the same ratio calculated for the normal control culture. It is these percentages which appear in column VII.

It is seen that γ -irradiation can produce very different effects upon the nitrogenous metabolism according to the duration of the exposure and its morphological effects. Thus, progressively increasing periods of irradiation give first an inhibition in the rate of ammonia production per bodo, then a marked increase and finally a less marked increase. The inhibition of ammonia production coincides with the least marked effects upon the size and rate of division of the cells. The greatest stimulation of ammonia production, on the other hand, coincides roughly with the most marked effects upon the size and rate of division of the cells. Finally, an effect upon the ammonia production of an intermediate degree—*i.e.* a moderate stimulation—coincides with a well-marked effect upon the size of the cells, appearing in the absence of any marked effect upon their rate of division. This last morphological effect was well illustrated in the graph showing the growth rates of normal and irradiated cultures published in 1932 [Robertson, 1932]. The resumption of the normal rate of division and fall in the “ammonia ratio” coincided with the development of the second generation of cells produced in the presence of the radium. It would seem that the capacity for the resumption of growth at this normal rate in irradiated cultures is connected with, or conditioned by, the physiological and morphological changes taking place during the period of the greatest stimulation of ammonia production, which is also the period of the evolution of the first generation of cells exposed to the γ -rays.

It is important to note that the same type of effect is produced upon the total ammonia production of the cultures by the different durations of irradiation

described above as is produced upon the ammonia production per bodo. Thus the increased value of the "ammonia ratio" in the longer irradiations is not merely an expression of the fact that there are fewer and larger bodos in the irradiated cultures.

We have no means of deciding from our data as to whether the effects upon ammonia production are secondary to the morphological effects, or *vice versa*, or whether all these effects are directly produced by the γ -radiation.

The qualitative contrast between the effects of very small and of large doses of γ -radiation is, of course, in accordance with the biological effects of many toxic agents.

SUMMARY.

Cultures of the protozoon *Bodo caudatus* have been exposed to γ -radiation. The effects of varying the duration of this exposure upon the cell size, rate of multiplication and the rate of ammonia production in the culture are described.

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