LVII. METABOLISM OF TISSUES GROWING IN CULTURE.

V. EFFECT OF RADIUM ON THE METABOLISM OF CULTURES OF EMBRYONIC KIDNEY TISSUE.

By BARBARA ELIZABETH HOLMES.

From the Biochemical Laboratory, Cambridge.

(*Received March 1st, 1933.*)

In previous papers we have described our technique for estimating the nitrogen and the sugar metabolism of rat-kidney tissue in culture, and also the effect of various sugars in preventing the formation of urea and ammonia by the cultures [Holmes and Watchorn, 1927; Watchorn and Holmes, 1927; 1931]. In recapitulation here it is only necessary to say that "growing" tissues show a formation of urea and ammonia which is not found in tissues which are not growing, that the presence of glucose and fructose in the medium always and that of galactose sometimes prevents the formation of urea and ammonia (although accelerating the growth), while xylose has no such effect. Glucose, fructose, galactose and xylose are all broken down by the tissue, as shown by estimation of reducing substances at the beginning and end of the culture period. Later work has shown that glucose is the most extensively used and that xylose breakdown is sometimes very small.

In addition to this, recent work has brought out one new and interesting point. The increase in area of the cultures of kidney tissue, which we have always spoken of as "growth" of the cultures, we had formerly considered to be due to cell division as well as cell out-wandering. Lately, however, attempts at counting mitotic figures have shown that no division is taking place. It is possible that cell division continues for a few hours after the planting of the culture, but there is very little doubt that by far the greater part of the increase in area is due to the wandering of cells away from the fragment planted. It is therefore interesting to note that, in the absence of suitable carbohydrate, cell movement is apparently supported by the breakdown of nitrogen-containing substances. A recent paper by Clark *et al.* [1931] has shown that the beating heart oxidises protein as well as carbohydrate and produces urea and ammonia, and it seems likely that the production of urea and ammonia by the wandering cells is due to a similar process. In the absence of out-wandering these substances are not produced by the culture. Clark, however, finds that protein and carbohydrate are oxidised at the same time, whereas in these cultures the presence of carbohydrate in the ordinary physiological concentrations inhibits the protein breakdown.

Experiments with radium.

The effect of radium upon tissue cultures is well known but puzzling, and very little is known which might help to explain it. It was felt that a knowledge of the metabolism of irradiated cultures might be helpful. The source of

irradiation used was a 300 mg. plaque, the radium container being of platinum 0·5 mm. in thickness, this ensuring that only γ-rays reach the tissues. The tissue was planted as usual on strands of cotton-wool in a pyrex flask containing a little embryo extract, and was arranged so that as far as possible all the tissue should lie immediately over the radium container, which was 0·5 cm. distant from the bottom of the flask.

A very small dose of γ-radiation is known to cause a temporary stoppage of cell division, but a very large dose is needed to cause the death of the cells in culture. Spear has shown [1930] that an enormous dose of γ-rays will prevent immediately any out-wandering from a culture, which is then spoken of as dead, and that rather smaller doses will cause death after a latent period, the latent period becoming longer as the doses become smaller. In cultures in which delayed death is going to occur, the appearance of the cells shortly after irradiation is fairly normal and occasionally mitoses are seen, but these soon cease and the cells appear progressively more abnormal. Out-wandering, however, may continue for several days or even weeks until eventually the culture dies. The actual cause of death is unknown, but many suggestions have been made, among others, that slow self-poisoning of the cells may be taking place owing to an abnormal metabolism.

The duration of exposure to radium in the experiments quoted below was 14 hours. Shorter exposures were first tried, but although similar results were obtained they were irregular and therefore unsuitable for work of this kind, in which the results of one experiment have to be compared with the results of another experiment carried out at another time.

Supposing that the kidney tissue was equal in sensitivity to the fibroblasts used by Spear, the death of the cultures would not occur for about 10 days, whereas these cultures were taken for estimation after 2 days.

With an exposure of 14 hours it was regularly found that the breakdown of both glucose and galactose was cut down, usually to about 50 or 60 % of the breakdown found in the control. Table I gives typical examples.

Table I. *Mg. sugar present after 2 days.*

Original amount	Non-growing tissue	Control growing tissue	Irradiated growing tissue	Percentage inhibition of sugar breakdown
		Glucose		
1·91	1·29	1·1	1·50	50
2·26	1·82	1·40	1·68	33 (only part of the tissue could be irradiated)
		Galactose		
1·31	1·03	1·03	1·15	43
1·31	—	0·95	1·11	44

As one of the theories of the action of γ- and X-rays on tissues postulates the damage of the colloid structure of the cell, we thought it might be worth while to injure the cell structure by freezing below $-7°$ and compare the effect on carbohydrate breakdown of this freezing with that obtained after radium. We accordingly froze one culture containing glucose to $-12°$, which should cause the formation of ice-crystals inside the cells and injure the structure by so doing. In this case, whereas 0·63 mg. of glucose was broken down by the control, only 0·12 mg. was broken down by the frozen culture. This is a similar effect to that produced by radium, though more extreme.

The effects of radium on protein metabolism had to be tested in cultures to which no glucose or galactose had been added, since these sugars themselves prevent protein breakdown. This is unfortunate, as it has been suggested by some authors [Frik and Posener, 1926; Mayer, 1926] that *in vivo* the injection of large amounts of glucose can sensitise the tissues (tumour) of an animal to irradiation. However, the sugar contents of the glucose- and galactose-containing cultures were rather lower than the ordinary physiological level of the body fluids, so that any effect of excessive amounts of sugar would not come into consideration. Moreover it was possible to add xylose as a control in the nitrogen experiments, as this sugar had been found to have no effect on protein metabolism, and in most cases xylose was present in the experiments given in Table II.

Table II. *Mg. NH_3- and urea-nitrogen.*

Non-growing	Control growing	Increase	Irradiated growing	Increase	Remarks
0·127	0·127	0	0·142	0·015	—
0·114	0·144	0·030	0·175	0·061	4 kidneys; more tissue than usual
0·090	0·126	0·036	0·109	0·019	Known to have been badly planted
0·093	0·118	0·025	0·118	0·025	—
0·070	0·106	0·036	0·093	0·023	—

There is no doubt that under the conditions of these experiments the protein breakdown was far less easily affected by irradiation than the carbohydrate breakdown. The figures given show that the radium had probably no effect at all, and that only the ordinary variation due to varying extents of out-wandering in the cultures was recorded. At most, there is an occasional, irregular inhibition of protein breakdown such as could be produced with much smaller doses of γ-radiation in the case of carbohydrate breakdown. The protein metabolism is estimated by the increase in ammonia- and urea-N in the "growing" cultures, as compared with cultures which could not "grow" because the fragments were floating freely in the medium (see earlier papers).

It will be seen that the irradiated tissues twice gave a higher answer, once the same answer, and twice a lower answer. Moreover, one of these low answers could be fully accounted for by bad planting of the tissue.

Several experiments show that no autolytic formation of urea and ammonia takes place in cultures after irradiation. In the following experiments growth was for various reasons not found in the cultures, and in these the irradiated cultures, like the controls, show no ammonia or urea formation (Table III).

Table III. *Mg. NH_3- and urea-nitrogen.*

Non-growing	Control "growing"	Irradiated "growing"
0·090	0·095	0·092
0·097	0·094	0·091
0·088	0·088	0·088
Non-growing	Control "growing"	Frozen "growing"
0·089	0·108	0·085

The non-formation of autolytic urea and ammonia does not prove that injury to the structure of the cell has not taken place, since freezing to $-12°$, which almost certainly injures the structure, entirely prevents growth (Table III) but does not cause the appearance of autolytic ammonia and urea during the 2 days experiment. Obviously, however, any injury due to the radium must

be far less gross than any due to the freezing, since the radium does not prevent a normal protein breakdown in wandering cells. The apparently selective action of radium does not support the theory that its effect is merely one of damage to colloidal structure, since this affects both types of metabolism equally.

Further support is given to the idea that the protein metabolism is very insensitive to the action of γ-rays by the fact that microscopical examination of stained cultures shows that out-wandering is just as extensive after irradiation as it is in the controls. We have always considered that the amount of protein breakdown (in the absence of carbohydrates) is determined by the amount of out-wandering or "outgrowth," so that the microscopical findings confirm the chemical, and show that no depression of protein metabolism is likely to be found during the first 2 days after the 14 hours' exposure to γ-rays.

Acceleration of carbohydrate breakdown.

In earlier experiments, when lower intensities of irradiation were being used, quite definite acceleration of galactose breakdown was obtained on two occasions out of four or five experiments. There is enough variation in the sensitivity of cultures from one experiment to another to make it difficult to repeat a result of this sort, and more time could not be spent in the attempt.

Unfortunately the exact intensity of the irradiation received by the tissues in these particular experiments is difficult to determine, but it was certainly very much less than that required to decrease sugar breakdown or to bring about death without a very long latent period occurring after the exposure to radium.

Increases of metabolism after irradiation have been reported by several observers, but it is usually thought that these represent responses to slight injury rather than real stimulation of the cells. It is difficult to picture any type of injury to these cultures which could produce an increase in carbohydrate breakdown.

Table IV. *Mg. of galactose disappearing.*

Non-growing		Growing	
Control	Irradiated	Control	Irradiated
0·36	0·54	—	—
0·21	0·37	0·25	0·43
—	—	0·35	0·52

Table IV gives the figures for the two experiments mentioned, showing the increased disappearance of galactose under the influence of radiation.

Discussion.

A search of the literature shows that the facts already known about the effects of radiation on cell metabolism are contradictory and difficult to reconcile with one another. Crabtree [1932], using surviving tissues in a glucose-bicarbonate Ringer's solution, found that the respiration of the tissues was very much reduced by radiation long before the glycolysis was affected. Krontowski [1933] also found that, with tissue cultures suffering a delayed death after irradiation, the glycolysis continued almost unaltered at the end of 10 days, although all out-wandering had ceased; considerably higher doses of radium were needed to produce an alteration in glycolysis than to produce delayed death. Apart from any question of the effect of the radiation, Krontowski's

results are very difficult to understand, since we have found that actively wandering cultures have a higher carbohydrate metabolism than cultures that are not out-wandering. This is surely the result that would be expected, and, if true for tissues which though not showing out-wandering are perfectly healthy, is still more likely to be true of cultures which have ceased their cell movement owing to the delayed injurious effect of irradiation.

Frik and Posener [1926], on the other hand, found that, *in vitro*, glycolysis was far more easily affected by X-rays than was the respiration of the tissues. The most striking example of this was given by the retina, which proved to be very sensitive to radiation, and in which glycolysis could be completely inhibited by exposures which left the respiration unaltered. Wels [1924] irradiated yeast and other cells and found the respiration rather insensitive; exposures which affected the viable count of staphylococcus cultures did not cut down the respiration.

The results recorded in this paper would presumably agree with those of Frik and Posener, since glycolysis is stopped but protein oxidation continues and could take the place of carbohydrate oxidation.

It is obviously difficult to reconcile these conflicting results with one another, but there are possible explanations of the differences found.

Crabtree and Krontowski both used radium containers which would allow of the passage of a certain amount of β-radiation. Though the proportion of β- to γ-radiation was very small, the influence of β-radiation on biological material is so very great that its effects would have been apparent. It is not at all certain that the effects of β-radiation on metabolism would be the same as the effects of γ-radiation and X-rays. In Crabtree's interesting experiments the tissues were surviving but not growing in culture, and it is possible that the striking recovery of cells from the immediate effects of γ-rays, which is found in tissue cultures, might not be present in his preparations. In this case no doubt different answers would be given by tissue culture and by surviving tissue experiments.

A further clue to the reason for the many discrepancies is given by the work of Frik and Posener already quoted. These authors found that although total glycolysis is much cut down and respiration unaltered, aerobic glycolysis may actually be greater in the irradiated tissues; in other words that although oxidation continues, it is not oxidation of the products of carbohydrate breakdown. For some tissues under some conditions the cutting down of carbohydrate oxidation would mean a large decrease in the whole oxygen uptake, whereas under other conditions and with provision of suitable substrates, oxidation of protein and other substances might take the place of carbohydrate oxidation. Tissues in culture are provided with plenty of utilisable protein substances, and kidney tissue in particular probably oxidises substances other than carbohydrate quite readily.

The different components of the rather complex enzyme system by which glycolysis is brought about in cells are known to vary quantitatively even if not qualitatively from one tissue to another. This is likely to make the glycolytic systems of some tissues more sensitive to radium than those of other tissues. Comparison of the effects of radium on different tissues as well as a study of its action on as many enzyme systems as possible should show whether there is really any selective action, and if so what is the most vulnerable part of the cell organisation.

SUMMARY.

1. In the absence of suitable carbohydrate, cell movement appears to take place at the expense of protein breakdown.

2. Fourteen hours' exposure at a distance of 0·5 cm. to the γ-rays from 300 mg. radium produces a 40 or 50 % inhibition of carbohydrate breakdown. Smaller doses (*e.g.* 9 hours) produce this effect only irregularly, and small doses may actually produce an acceleration of breakdown.

3. Fourteen hours' exposure produces no effect or at most a very irregular effect on protein breakdown, which therefore appears to be much less sensitive to γ-radiation.

4. Fourteen hours' exposure on several occasions produced no visible effect on the increase in area of the culture.

5. The possible significance of the contradictory results obtained by several workers in this field is discussed.

A great deal of this work was carried out with the co-operation of Miss E. Watchorn, who was only prevented by lack of time from continuing it.

My most grateful thanks are due to Dr Spear for his continued advice and help, without which the work would not have been possible.

I have to thank the Medical Research Council for a personal grant.

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