CCXCVIII. AMMONIA FORMATION IN IRRADIATED TISSUES.

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SINCE cells obtain their energy principally by the oxidation and splitting of sugars, the effect of radiation on their carbohydrate metabolism has been most widely investigated.

Holmes [1933], using cultures *in vitro* of embryonic kidneys, has studied the nitrogenous metabolism of irradiated cells by estimating the end-products urea and ammonia. She found that doses of γ -radiation over 14 hours did not produce an unequivocal effect on protein breakdown (as measured 48 hours after the treatment), but that carbohydrate breakdown was inhibited 40–50 % during the same period, showing the greater sensitivity of carbohydrate metabolism to radiation.

Lawrie & Robertson [1935] studied the effect of X-radiation on the growth and nitrogenous metabolism of a protozoon, and found definite effects, which, however, varied according to the duration of the exposure. It was shown earlier [Crabtree, 1935] that the glycolysis of tumour tissue can be almost completely checked by $\beta + \gamma$ -radiation applied for 4 hours at low temperature, respiration remaining intact after similar treatment. It seemed desirable to discover if the suppression of glycolysis was accompanied by the liberation of ammonia, a reaction demonstrated by Warburg *et al.* [1924] as occurring in tumour and other normally glycolysing tissues when deprived of sugar.

The rate of this ammonia formation in the absence of sugar was shown to be roughly parallel with the rate of glycolysis characteristic of a wide range of tissues. That ammonia formation was linked to glycolysis and not respiration was confirmed by Salter & Robb [1934], who also showed that cyanide checked neither glycolysis nor ammonia sparing, but that iodoacetic acid checked both. Watchhorn & Holmes [1927] found that addition of glucose inhibited ammonia formation in cultures of tissues growing *in vitro*. They regarded this as an example of the protein sparing action of carbohydrate made evident *in vitro*. Since many workers have described the formation of lactic acid by cultures *in vitro* of tissues which, in the adult condition, exhibit no aerobic glycolysis, it is possible that the findings of Watchhorn & Holmes provide a further illustration of the relationship between ammonia formation and glycolysis.

Whereas the workers quoted above found ammonia formation to increase when glycolysis was checked by the absence of sugar, this same effect is produced when glycolysis has been suppressed by radiation and sugar is present. Deprivation of substrate or loss of the power to break down this substrate lead to the same end-result, an accumulation of ammonia. The origin of this ammonia is unknown. Warburg *et al.* [1924] suggested and Watchhorn & Holmes [1927] assumed that it was a product of protein breakdown, and that under normal physiological conditions sugar protects protein. Salter & Robb [1934] quote evidence suggesting that purine metabolism may be involved.

The increased ammonia formation accompanying the suppression of glycolytic function by irradiation at low temperature was at first thought to be only a further confirmation of the original finding of Warburg *et al.* [1924]. But further experiments in which irradiation was carried out under different conditions, and in which glycolysis remained unimpaired, showed that the increased ammonia formation observed was not necessarily coupled with glycolysis, but was a characteristic effect of the radiation itself. Irradiation at body temperature, aerobically, anaerobically or in the presence of cyanide, primarily damages the respiratory system whilst glycolysis proceeds normally. Yet under all these conditions radiation leads to a large increase in the rate of ammonia formation, showing that this phenomenon is independent of glycolysis. Under such conditions ammonia formation seems to be linked to respiration. In fact, if suitable normal tissues with low glycolysing powers are irradiated, the rate of ammonia formation increases in all cases, though to a variable extent. If it be assumed that increased ammonia formation implies increased protein degradation, then the generalization arising from this work (from the point of view of induced changes of metabolism) is, that radiation operates primarily by damaging carbohydrate metabolism, and this damage, whether to oxidative or splitting processes, leads to a secondary reaction in which protein is utilized more rapidly than in normal cells, or in isolated tissues preserved in artificial media for periods up to 5 or 6 hours. It is of interest to quote one of the earliest enquiries on the influence of X-rays on metabolic processes. Williams [1906] studied the action of X-radiation upon the excretion of uric acid in a clinical case of myelogenous leucaemia. He found that the amount of uric acid excreted during the period of irradiation was well above the normal limits, and moreover, that the total output of nitrogen exceeded the intake.

Experimental methods and results.

The apparatus used for irradiating tissue slices with $\beta + \gamma$ -radiations has been previously described [Crabtree, 1935]. It was immersed in water at the appropriate temperature and the tissues irrigated with Ringer's solution containing glucose and bicarbonate (R.G.B.), either aerobically, anaerobically or containing $M/600$ HCN. The tumour tissue used was Jensen's rat sarcoma (J.R.S.), and rat liver, brain and testes were used as types of normal tissue, all possessing low aerobic glycolysis but varying in their power of glycolysing anaerobically. After irradiation, the slices were then transferred to small vessels containing 6 ml. of either R.G.B. or the same medium with glucose omitted, attached to manometers, and reincubated at 37.8° with shaking for 1 hour under aerobic conditions. The tissues were then dried and weighed, and ammonia was estimated in the 6 ml. of medium used. Control tissues were preserved, without irradiation, under similar conditions. At first, an initial measurement of the rate of ammonia formation was made to assess the effect of the experimental conditions on this process. This was discontinued when it was found that the difference between the initial rate of ammonia formation and that after 4 hours of preservation without irradiation was negligible.

Ammonia was estimated by the standard method of Folin. The slight precipitate produced by the addition of a few drops of 10% sodium tungstate solution to the R.G.B., followed by acidification with HCl to Congo red, was centrifuged out, and, after making alkaline with Na₂CO₃ solution, the ammonia was displaced by a current of air into 5 ml. of $N/20$ HCl and estimated colorimetrically by Nessler's reagent. Preliminary tests with known amounts of ammonia showed a recovery of 95–97% by the aeration process.

The rate of ammonia formation is expressed in units similar to those used for respiration and glycolysis.

$$Q_{\text{NH}_3} = \frac{\mu\text{l. NH}_3}{\text{mg. dry tissue} \times \text{hours}}$$

Tables I and II include a selection of results obtained after irradiation of tumour and normal tissues respectively.

Table I. *Increased rate of ammonia formation by J.R.S. tissue irradiated under different conditions.*

Time of irradiation hr.	Temp. during irradiation ° C.	Irrigating medium used during irradiation	Irradiated Q_{NH_3}	Control Q_{NH_3}	Increase in Q_{NH_3} due to irradiation %
4	0-5	R.G.B. aerobic	1.10	0.34	220
4	0-5	"	0.87	0.40	120
4.5	0-5	"	0.75	0.32	130
3	0-5	"	0.71	0.36	100
4	0-5	"	0.97	0.66	50
4	0-5	"	0.91	0.49	85
4.5	0-5	"	0.86	0.50	70
4	37.8	"	0.94	0.45	110
4	37.8	"	0.91	0.60	50
4	37.8	"	1.10	0.61	80
4	37.8	"	0.86	0.55	55
3.5	37.8	M/600 HCN in R.G.B.	1.10	0.49	125
3.5	37.8	"	1.32	0.81	65
4	37.8	"	0.62	0.41	50
4	37.8	R.G.B. anaerobic	0.80	0.41	100
4	37.8	"	0.71	0.45	60

Table II. *Increased rate of ammonia formation by irradiated normal tissues.*

Time of irradiation = 4 hr. Temperature during irradiation = 0-5°.			
Rat tissue	Irradiated Q_{NH_3}	Control Q_{NH_3}	Increase in Q_{NH_3} due to irradiation %
Brain	0.87	0.54	60
	0.94	0.71	32
	0.41	0.24	70
	1.35	0.66	105
	0.60	0.38	58
Liver	0.34	0.26	30
	0.40	0.28	43
	0.46	0.31	48
	0.23	0.22	—
Testis	0.37	0.15	145
	0.36	0.16	125
	0.12	0.07	40
	0.26	0.16	63

The first seven results in Table I were obtained with tumour tissue in which the glycolytic process was largely suppressed after irradiation at low temperature, and, it was thought, illustrated the relationship existing between glycolysis and ammonia formation described by Warburg *et al.* [1924]. The rate of ammonia formation was of the same order whether the final incubation was carried out in the presence or absence of glucose, owing to the inactivation of the glycolytic system.

The succeeding experiments, in which radiation was applied at 37.8° under different environmental conditions, and in which glycolysis remained active,

showed that the earlier conception was wrong. Under these conditions, excessive ammonia formation was not coupled with glycolytic failure, but, to judge from earlier work [Crabtree, 1932], with incipient or actual respiratory failure. Ammonia was formed at a similarly increased rate in media with or without glucose, i.e. with glycolysis proceeding actively or not at all. These experiments make it clear that an increased rate of ammonia formation is a characteristic reaction induced by radiation itself and independent of the glycolytic system. The experiments with normal tissues shown in Table II confirm this. It is noteworthy that the rate of ammonia production in these normal tissues varies considerably and may be determined by variations in their functional activity or the age of the animals used. The increased rate of ammonia production induced by radiation is, however, a common and significant factor. Taken in conjunction with the earlier findings that carbohydrate metabolism is primarily damaged by radiation, and assuming that this excess ammonia formation represents increased protein breakdown, it would be anticipated that a fall of respiratory quotient would occur as the damaging effects of radiation progressed. It may be recalled that Bancroft *et al.* [1935] recorded that X-radiation treatment *in vivo* led to a fall of respiratory quotient in the Philadelphia rat sarcoma No. 1.

SUMMARY AND CONCLUSIONS.

1. Irradiation of tissues *in vitro* induces an increased rate of ammonia formation.
2. In the case of tumour tissues this effect is independent of the state of activity of the glycolytic system. Whether the latter is suppressed by irradiation at low temperature or in full activity after irradiation at body temperature, ammonia production increases to a similar extent.
3. Normal non-glycolysing tissues—liver, testes, brain—also produce ammonia more rapidly after irradiation.
4. It is suggested that this increased ammonia production is due to the secondary utilization of protein as carbohydrate metabolism, either oxidative or splitting, is impaired.

REFERENCES.

- Bancroft, Beck & Russell (1935). *Biochem. J.* **29**, 2416.
Crabtree (1932). *Sci. Rep. Cancer Res. Bd, Lond.* **10**, 33.
— (1935). *Biochem. J.* **29**, 2334.
Holmes (1933). *Biochem. J.* **27**, 391.
Lawrie & Robertson (1935). *Biochem. J.* **29**, 1017.
Salter & Robb (1934). *Amer. J. Cancer*, **22**, 87.
Warburg, Posener & Negelein (1924). *Biochem. Z.* **152**, 309.
Watchorn & Holmes (1927). *Biochem. J.* **21**, 1391.
Williams (1906). *Biochem. J.* **1**, 249.