CXXIX. THE EFFECT OF X-RAYS UPON THE METABOLISM OF TUMOUR TISSUE

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IN a previous paper [Bancroft *et al.* 1935] were reported the results of a study made on the effect of X-rays upon the growth and metabolism of transplantable tumours. It was found, particularly with the Philadelphia rat sarcoma No. 1, that the respiratory quotient was lowered whenever there was any effect upon the growth of the tumour. It was not possible to determine whether this was due to a direct effect of the X-rays on the tissue or whether it was due to secondary changes induced by the action of the radiation. The irradiation of tissue slices *in vitro* seemed to offer a better method of studying the metabolic changes without the complications of such secondary effects as X-ray damage to the tumour bed or vascular system. With X-radiation, as with all radiation, there appears to be a latent period before any detectable changes in the metabolism occur, and with tissue slices it was possible by increasing the X-ray dose sufficiently to shorten this period and to permit the study of some of the direct effects of irradiation on metabolism. In this paper are presented the results showing both immediate and delayed effects of large doses of X-rays upon the carbohydrate metabolism of tissue slices.

EXPERIMENTAL

In general, the methods used by Elliott & Schroeder [1934] were employed. These consisted of measuring the oxygen uptake (Q_{O_2}), the respiratory quotient (R.Q.), and the acid change (Q_A), of thin slices of tissue in bicarbonate medium in an atmosphere of 95% O_2 and 5% CO_2 , by means of the Dixon-Keilin apparatus [1933]. Sufficient slices were cut from a tumour to permit the immediate determination of the metabolism of one set (initial control), the irradiation of the slices for another manometer (irradiated), and the submission of a third set to the same manipulations but omitting the irradiation (delayed control). By comparison of the initial control and the delayed control, it was possible to detect injury to the tissue occurring in the (sometimes prolonged) interval before the determination of metabolism. Elliott & Schroeder [1934] showed that the rate of O_2 uptake of rabbit kidney slices fell slowly on standing. It was found that this fall in Q_{O_2} could be minimized if the tissues were kept in a sufficiently large volume of Ringer-Krebs medium containing glucose. Slices cut from the tumours were placed immediately in beakers containing Ringer-Krebs medium through which O_2 - CO_2 gas mixture was continually bubbled. Alternate slices were placed in each of three beakers in order that the samples of tissue in each beaker should be as nearly uniform as possible. The tissues for X-ray and for the delayed control were suspended in approximately 4 ml. of Ringer-Krebs medium containing glucose in tightly-stoppered Erlenmeyer flasks

filled with the O_2 - CO_2 mixture. After X-raying, the tissues were returned to the beakers. Since tumour rapidly metabolizes glucose into lactic acid, even in the presence of oxygen, it was necessary to replace the solutions every 2 or 2.5 hr. so that the acid formed would not lower the pH sufficiently to damage the tissues.

The X-ray apparatus was a special water-cooled tube of the Coolidge type with a copper anode operating at about 85 kV. and 12 mA., using full wave kenetron rectification. The cathode, enclosed in a brass water-jacketed head, was kept at ground potential. Two copper windows, 0.005 in. thick, were symmetrically placed above and below the target at a distance of 3.55 cm. This permitted the irradiation of the tissues through the upper window and the simultaneous determination of the dose through the lower, by means of a cylindrical ionization chamber of 12.9 cc. volume which was connected through an Ayrton shunt to a Leeds and Northrup high sensitivity galvanometer. The galvanometer was calibrated daily against a dry cell of 1.54 V. connected through an external resistance of 50,200 ohms. For maximum accuracy it was found best to use the 0.0001 shunt for calibration, whereas in view of the smaller current generated in the ionization chamber by the X-rays, the 0.1 shunt was used for the actual measurement of the dose. If N is the number of cm. deflexion of the galvanometer on the 0.0001 shunt, 1000 N will be the deflexion on the 0.1 shunt. The current necessary, therefore, to cause 1 cm. deflexion of the galvanometer in E.S.U. per sec. with the 0.1 shunt will be given by the equation $I = \frac{E \times K}{R \times 1000 N}$,

where E is the E.M.F., R the resistance, K the number of E.S.U. per amp. and N is the number of cm. deflexion of the galvanometer on the 0.0001 shunt. Hence, $I = \frac{1.54 \times 3 \times 10^9}{50,200 \times 1000 N}$ or $\frac{92.1}{N}$. The number of r units per cm. deflexion per min.

with the 0.1 shunt is $\frac{1 \times 60}{\text{vol. of ionization chamber}}$ or $\frac{92.1 \times 60}{N \times 12.9} = \frac{428}{N}$. Experiments showed that only 58 % of the rays were transmitted through the pyrex Erlenmeyer flasks used for irradiation of the tissues, so that the factor to be used becomes $248/N$. Thus, when N was determined to be 10.1, the tube would deliver 24.5 r units per cm. per min. and at a deflexion of 18 cm. would be equivalent to approximately 15,000 r units in 34 min. The amounts of X-rays from the two windows were measured separately and found to be the same, so that the ionization chamber actually measured the dose given to the tissues.

Although irradiation of tumour tissue *in vivo* causes metabolic changes only after a latent period of some hours, it seemed possible that this might be a function of the relatively small dose given and that a massive dose might produce a measurable change immediately. Preliminary experiments were therefore made upon tissue slices suspended in Ringer phosphate solution of pH 7.5. The O_2 uptake after irradiation was determined in simple Barcroft manometers filled with oxygen and containing rolls of filter paper moistened with alkali to absorb CO_2 . The tissue slices, after weighing, were suspended in Ringer phosphate medium, oxygenated and irradiated with varying doses of X-rays. Table I gives the results obtained.

Table I. *Effect of X-rays upon the O_2 uptake of Philadelphia No. 1*

r units of X-ray	6400		8600		11,500		15,280		17,200	
	Control	Irrad.	Control	Irrad.	Control	Irrad.	Control	Irrad.	Control	Irrad.
$-Q_{O_2}$	12.6	12.1	9.2	9.2	11.1	12.5	15.8	12.3	8.3	8.2
$-Q_{O_2}$	11.4	11.4	10.8	10.5	—	—	11.2	11.5	—	—

Even with doses as high as 17,000 *r*, no apparent change in the O₂ uptake is evident immediately. Crabtree [1932] was also unable to find any immediate change after irradiation with radium.

It is well known that the results obtained with phosphate buffers are often not comparable with those obtained with bicarbonate-containing medium. In the above case, Ringer phosphate was used in which the phosphate concentration was nearly isotonic, and the results in the above table agree well with those of later experiments in which bicarbonate-containing medium was used. Indeed, the metabolism of tumour slices which had been X-rayed in Ringer phosphate solution was no different from that of slices which had been kept in Ringer-Krebs medium, as shown in Table II.

Table II

	Control			Irradiated		
	R.Q.	-Q _{O₂}	Q _A	R.Q.	-Q _{O₂}	Q _A
Ringer-Krebs	0.82	10.1	20.6	—	—	—
Ringer phosphate	0.83	10.9	20.1	0.85	10.1	21.8

For the experiments illustrated in Table II slices were taken from the same tumour, the metabolism of one set was determined immediately in Ringer-Krebs medium, and the other two sets were suspended in Ringer phosphate and one of the latter two sets was X-rayed with a dose of 15,000 *r*. At the completion of the X-ray treatment, the metabolism of the two sets was determined in the Dixon-Keilin manometers in the usual way. No difference in the metabolism due to the influence of Ringer phosphate could be detected.

Crabtree & Cramer [1933] reported that tumour tissue was more sensitive to the action of radium radiation when the respiration was partially inhibited by various conditions such as cold, although Crabtree later [1936] found that the radiation did not directly alter the respiration rate. We found no immediate effect on the Q_{O₂} of the Philadelphia No. 1 sarcoma after 15,000 *r*, even when the temperature was below 5° during irradiation. All subsequent irradiation was carried out at room temperature.

RESULTS

The results obtained at varying times after the irradiation of Philadelphia No. 1 tumour tissue with 15,000 *r* units of X-ray are shown in Table III.

Table III shows that there is no immediate effect of X-rays upon the metabolism which is clearly outside the experimental error, and that up to 4.5 hr. after X-ray there is no difference in O₂ uptake between the irradiated and the delayed control. There is, however, almost always some lowering in the O₂ uptake due to standing, and this should be distinguished from the X-ray effect. A definite lowering of the R.Q. which increases with time to a maximum, and a rise in the total acid formation (Q_A), are alterations in the metabolism which may be ascribed to the effect of X-rays. In an experiment with 23,000 *r*, the R.Q. dropped from 0.90 to 0.78 and the Q_A increased from 20.4 to 27.4, in 1 hr. after irradiation. This about corresponds to the effects observed after 2.5 hr. with 15,000 *r*, indicating that the latent period is considerably shortened by increasing the dose. It should be noted that the rise in the total acid formation parallels the fall in the R.Q. In this connexion, it is interesting that Crabtree [1932] found no change in the aerobic glycolysis under continuous radium radiation. However, he determined only the total O₂ uptake and the total CO₂ evolution from his bicarbonate-containing medium. On the assumption that the

Table III. *Changes in the metabolism of tumour tissue at varying times after irradiation*

Time hr. after X-ray		R.Q.	$-Q_{O_2}$	Q_A	R.Q.	$-Q_{O_2}$	Q_A
0	Control	0.80	11.2	15.4	0.87	12.0	19.9
	Irradiated	0.79	10.3	22.3	0.84	11.3	21.4
1	Control	0.80	9.2	23.8	0.89	9.8	17.2
	Irradiated	0.74	9.4	23.4	0.83	9.8	19.7
2	Control	0.91	10.5	22.0	—	—	—
	Irradiated	0.78	11.6	31.7	—	—	—
2.5	Control	(0.93)	(10.4)	(19.3)	(0.88)	(12.3)	(22.8)
	Control	0.92	9.7	20.5	0.81	10.3	22.6
	Irradiated	0.70	9.5	22.4	0.69	11.8	29.3
3.5	Control	(0.87)	(11.2)	(22.3)	—	—	—
	Control	0.96	10.2	19.6	0.90	10.9	19.2
	Irradiated	0.75	12.9	25.6	0.69	10.6	25.8
4.5	Control	(0.87)	(13.0)	(22.2)	(0.90)	(9.7)	(18.3)
	Control	0.93	10.4	20.3	0.92	9.2	20.1
	Irradiated	0.76	11.7	25.2	0.72	11.2	25.3

The numbers in parentheses represent values for the metabolism of the initial controls.

R.Q. remained unity throughout the experiment, he calculated that the aerobic glycolysis remained unchanged. We have found with X-rays that the respiratory CO_2 evolution falls to about the same extent as the amount of CO_2 liberated by acid formation rises. It is possible that a similar situation occurs with radium radiation and that only a method which differentiates between respiratory CO_2 and CO_2 liberated from bicarbonate in the medium would show the increased rate of acid formation.

In Table IV are given some results obtained by the irradiation of normal rat kidney tissue.

Table IV. *The effect of 15,000 r radiation on the metabolism of rat kidney tissue*

	Time hr.	Control			Irradiated		
		R.Q.	Q_{O_2}	Q_A	R.Q.	Q_{O_2}	Q_A
No glucose	1.5	(0.85)	(21.4)	—	—	—	—
		0.86	16.6	—	0.90	16.9	—
Glucose	2.5	(0.84)	(21.4)	—	—	—	—
		0.89	20.8	—	0.88	23.8	—

The values for the initial controls are given in parentheses.

Irradiation with 15,000 r of X-rays appears to have no measurable effect upon the metabolism of kidney tissue either in the presence or absence of glucose. In 2.5 hr., the R.Q. of tumour shows a fall of about 20 %, whereas the R.Q. of kidney remained quite constant. The 1.5 hr. experiment, done in the absence of glucose, shows from the fall in the O_2 uptake of the delayed control that considerable damage to the tissue has occurred. This damage, however, affects only the O_2 uptake and is of a different character from that done by X-rays. It may be due to the death of a certain percentage of cells, whereby the O_2 uptake per unit weight is reduced, whereas X-rays alter the type of metabolism and may not change the total number of active cells. In the longer experiment, the tissues were kept in glucose-containing medium and the loss in respiring ability was much smaller.

Tumour tissue is a highly glycolysing tissue even in the presence of oxygen, whereas kidney is not. If the lowered R.Q. of tumour tissue after irradiation were due to damaged carbohydrate oxidation, it was thought possible that similar effects might appear in kidney tissue and be more marked in tumour tissue when some intermediary product of carbohydrate metabolism was added to the tissue. Experiments were therefore carried out using pyruvic acid for a substrate. The results are shown in Table V.

Table V. *Metabolism of pyruvic acid by kidney and tumour tissue*
2.5 hr. after radiation with 15,000 r

	R.Q.	$-Q_{O_2}$	Q_A	R.Q.	$-Q_{O_2}$	Q_A
Kidney	1.4	31.0	-22.5	1.2	32.9	-17.8
Kidney	1.3	33.6	-20.0	1.3	29.2	-15.2
Kidney	(1.4)	(33.7)	(-23.0)	—	—	—
	1.4	32.6	-20.4	1.3	33.5	-18.8
Tumour	(1.1)	(15.3)	(-1.9)	—	—	—
	1.1	16.2	-2.5	1.0	16.8	-1.0

The values for the initial controls are given in parentheses.

The high R.Q. and the disappearance of acid groups, as shown in the table, indicate that some metabolism of pyruvate occurs in tumour tissue, and that much more occurs in kidney tissue. No effect of X-rays on this metabolism was observed even after 2.5 hr.

DISCUSSION

The results obtained with the irradiation of Philadelphia No. 1 sarcoma tissue *in vitro* are similar to those obtained previously [Bancroft *et al.* 1935] *in vivo*, and indicate that the fall in R.Q. is a direct effect of the radiation on the tissue itself rather than a secondary one due to damage to the tumour bed or vascular system. It appears that the X-ray effects can be clearly distinguished from the gradual loss of activity of surviving slices on standing, for the latter is characterized by a fall in the total O_2 uptake and the former is primarily an effect upon the R.Q.

The rise in acid formation which appears consistently with irradiation of tumour tissue slices was not observed after irradiation *in vivo*. At present, no explanation can be given to account for this. With testis, an increased glycolysis has been observed after irradiation, *in vitro* by Frik & Posener [1926] and *in vivo* by Adler [1930]. Crabtree's [1932] failure to observe this rise with Jensen rat sarcoma after irradiation with radium may be due to his assumption of a constant R.Q.; a lowering of the R.Q. would mask the rise in the aerobic glycolysis in the manometric method used by him. On the other hand, tumours differ widely in their responses to a given dose of X-rays and the mechanism of X-ray action may be very different with different tumour strains. Holmes [1933] with small doses of radium showed an increased breakdown of galactose by cultures of embryo kidney. Larger doses inhibited carbohydrate breakdown but did not affect protein oxidation. Crabtree [1936] has recently shown that ammonia formation is increased by the action of radiation both in normal and tumour tissues and that this increase is not solely due to damage to carbohydrate metabolism but is partly due to a definite stimulation of protein metabolism. The extent of this ammonia formation is not sufficient to mask an increased aerobic glycolysis, for he found a maximum of Q_{NH_3} 1.1, whereas the acid increase is of the order of Q_A 5.0.

It might be thought that the lowered R.Q. was due to a partial but incomplete oxidation of carbohydrate resulting in the formation and accumulation of acids other than lactic acid. However, in one of the 3.5 hr. experiments, where the Q_A increased after radiation from 19.6 to 25.6, the lactic acid was determined chemically and it was found that the Q_{LA} increased from 19.4 to 23.6, which indicates that the increased acid is nearly all lactic acid. A possible explanation would be that normal tumour does oxidize carbohydrate to some extent [see Elliott *et al.* 1935] and that this carbohydrate oxidation is inhibited by the X-rays; while increased protein oxidation [Crabtree, 1936] maintains the O_2 uptake at its normal level, though with a lower R.Q. The fact that the R.Q. of tumour tissue is unaffected with pyruvic acid as a substrate, and that the R.Q. of kidney, which metabolizes pyruvic acid readily, is not affected, suggests that X-rays attack some process other than that of intermediate carbohydrate oxidation. That is to say, since pyruvic acid is oxidized in the normal manner, the damage to the carbohydrate oxidation must occur before this stage is reached, possibly, for example, in the oxidation of lactic to pyruvic acid.

SUMMARY

1. Following the methods of Elliott & Schroeder [1934], a study has been made of the immediate and delayed effects of large doses of X-rays upon carbohydrate metabolism of tumour tissue slices.
2. X-rays produce a definite lowering in the R.Q. of Philadelphia rat sarcoma No. 1 *in vitro*, similar to that previously observed *in vivo*.
3. A parallel increase in aerobic acid formation, which appears to be mostly lactic acid, indicates that the lowered R.Q. is not due to incomplete oxidations resulting in the formation and accumulation of acids other than lactic acid.
4. Under conditions which produce a pronounced lowering of the R.Q. of tumour tissue, X-rays produce no measurable effect upon the metabolism of rat kidney slices.
5. Since irradiated tumour and kidney slices metabolize pyruvic acid in a normal manner, X-rays probably act upon some process other than intermediate carbohydrate oxidation, possibly attacking carbohydrate oxidation before the pyruvic acid stage.

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