CCLXXXVII. THE EFFECT OF X-RAYS UPON THE METABOLISM AND GROWTH OF TRANSPLANTABLE TUMOURS.

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THE study of the primary action of X-rays upon tumour tissue *in vivo* has been handicapped by the inability to detect small differences in metabolism because of large variations from animal to animal. The small changes in metabolism that might indicate a primary effect of radiation could not be estimated from irradiation *in vivo*. Attempts have been made to avoid this difficulty; for example Crabtree [1933] found that continuous radiation with radium produced a lowering of the oxygen uptake by tumour slices before any effect on the aerobic glycolysis was evident. However, in living tissue such radiation may be largely effective through indirect means which could not be detected by such a method. We have found that the metabolisms of two tumours inoculated in similar situations into the same animal are very similar and do not show the variations characteristic of tumours in different animals. By shielding one tumour from the direct action of X-rays, it is possible to use this tumour as a control for the radiated one. This technique has made it possible to detect small characteristic changes in respiration and glycolysis caused by X-rays.

EXPERIMENTAL.

Two transplantable rat tumours were used in these experiments; Philadelphia 1, a fibrotic sarcoma, and Walker 256, a rapidly growing cellular carcinoma. A complete histological description of these tumours has been given already by Waldschmidt-Leitz, McDonald and co-workers [1933]; both have been maintained in the laboratory over several years by reinoculation and have been found to be quite consistent in their characteristic growth and histological appearance. The tumour fragments used for inoculation were obtained under as nearly sterile conditions as possible immediately after the animal was killed by a blow on the head. The tumour was then transferred to a sterile Petri dish and moistened with Ringer solution and the healthy portions cut into fragments of appropriate size. For the double inoculations, these pieces were divided as nearly as possible into two equal cubes of 1-2 mm.³ and inoculated with a trochar subcutaneously into both flanks after sterilising the skin with alcohol. It is important in this connection that the two pieces should be nearly identical as to size and quality of tissue in order that the tumours may grow at the same rate. Care should also be taken lest the tumour penetrate the body wall, since Crabtree [1929] has shown that the metabolism is much lower in such conditions than it is when inoculated subcutaneously. By inoculating fifteen animals, it was usually possible to get ten to twelve animals with two tumours that would grow at the same rate. Only male rats weighing 80-120 g., of the Germantown Albino strain, were used and they were fed on a standard diet consisting of lettuce, bread, a mixture of boiled

cereal grains and some milk. The Walker 256 tumours were large enough for measurement about 10 days after inoculation and the Philadelphia 1 tumours after about 17–20 days.

Each experimental animal was weighed and the approximate size of the tumour measured with calipers every 2–3 days for the Walker 256 and every 3–4 days for the Philadelphia 1, and drawings were made according to these measurements to record the growth. In order to facilitate the tabulation of these measurements, the area in cm^2 was determined by means of a Keuffel and Esser planimeter. After several measurements to make sure that the two tumours were growing at approximately the same rate, the animals were subjected to treatment. If there was any slight difference in size of the two tumours, the larger one was always irradiated.

The strength of the X-rays used was measured during radiation by the usual ionisation chamber-galvanometer arrangement, the ionisation chamber having been calibrated previously with a standard ionisation chamber as described by Taylor and Singer [1930]. It was found after some experimentation that the total dose of X-rays given over varying periods of time was not as good a measure of the strength of the X-rays as the saturation method of measurement of Pfahler [1928]. This method depends upon the fact that the biological effects of each dose of X-rays decrease with time, the rate of decrease following a logarithmic curve. When applying successive doses, the average effectiveness over the period covered by the treatment is not equal to the sum of X-ray units actually applied, but to the average of the residual effects of the successive doses. For example, to maintain a level of X-rays of 400 R, 150 R units would be administered after the effectiveness of the preceding dose had dropped to 350 R. The loss of the effectiveness of the X-rays was calculated from the curve given by Pfahler for X-rays of 127 KV through 3 mm. of aluminium. For radiation, the rats were immobilised by wrapping in cheesecloth, fastened down to $\frac{3}{4}$ inch boards of suitable size with drawing pins through the gauze and covered with heavy lead arches through which a hole had been cut slightly larger than the size of the tumour to be irradiated. The position of the animal was such that the control tumour was well protected by lead and only a small portion of the body of the rat was in the path of the X-ray beam through the radiated tumour. The calibration of the X-ray apparatus gave a factor of 1.59 R units per minute per cm. deflection of the galvanometer. Thus at a deflection of 20 cm. for 15 minutes the animal would receive $1.59 \times 20 \times 15 = 477$ R units.

At the completion of the X-ray treatments, or after a suitable time, the rat was stunned by a blow on the head and bled from the neck and the tumours were removed and covered with Ringer-Locke solution. Slices were cut immediately for manometric determination of the oxygen uptake, *R.Q.* and aerobic glycolysis of the two tumours in the Dixon-Keilin [1933] apparatus. The methods and precautions described by them as modified by Elliott and Schroeder [1934] were followed exactly. At the same time sections were taken from near the periphery to correspond as closely as possible with those used in the manometers for histological examination. These were fixed in Zenker's solution and then stained with haematoxylin and eosin. These sections after mounting were examined as to size and form of cells, relative abundance of tumour cells and the number of cells showing mitotic figures.

In order to check the metabolism of different tumours in the same animal a number of experiments were carried out with untreated animals. The results of these experiments with the Philadelphia 1 sarcoma and the Walker 256 carcinoma are given in Table I.

Table I. *Metabolism of sets of two tumours inoculated into each animal.*

		R.Q.		$-Q_{O_2}$		Q_A	
		Left	Right	Left	Right	Left	Right
Philadelphia 1	Without glucose	0.77	0.73	11.7	11.2	—	—
	Without glucose	0.85	0.81	10.6	10.6	—	—
	With glucose	0.86	0.83	10.2	10.4	20.6	20.2
Walker 256	With glucose	0.82	0.80	11.2	10.0	13.2	12.7
	With glucose	0.79	0.76	11.1	10.3	15.8	18.6

Elliott and Baker [1935] have shown that there may be an error of 0.03 in the R.Q. measurements so the agreement between the R.Q. measurements may be considered satisfactory. The determination of the aerobic glycolysis in twin tumours does not yield as close checks as the other measurements and indeed Elliott *et al.* [1935] have found it difficult to obtain close checks on the aerobic glycolysis even from slices taken from the same tumour.

RESULTS.

In Table II, the effect of different levels of radiation upon the growth and metabolism of the Walker 256 carcinoma is shown. The effect of X-rays seems to be more or less proportional to the dose as calculated by Pfahler's saturation method. With the higher levels of X-ray, the effect is always a regression in the size of the tumour and a reduction in the oxygen uptake of the tissue with occasional effect upon the R.Q. but without any appreciable effect upon the aerobic glycolysis (Q_A). These determinations with the higher levels of X-ray were made after considerable regression had taken place, so it is not possible to say whether the reduction in respiration caused the reduction in growth or *vice versa*. However, in view of Exps. 22 and 20 in which the rate of growth was affected without any significant changes in the metabolism, it seems probable that the lower oxygen uptake, observed in the other cases, was a result of the reduction in growth rather than a causative factor. The practically complete elimination of tumour cells as a result of the radiation in Exps. 25 and 35 with the extreme drop in the oxygen uptake as well as the loss of the power to glycolyse aerobically should be noted. The close agreement in the type and extent of the changes in the metabolism between Exps. 14 and 15 and between 20 and 22 when treated similarly even though not at the same time shows that it is possible to obtain consistent results if the conditions are maintained the same.

Although the number of experiments is not sufficient definitely to establish a regular sequence of events after irradiation, there seems to be a correlation between the effects on growth and the characteristic changes in the metabolism and histology of the tumours. The first histological effect of radiation observed in this work was a reduction in the number of mitoses which was often accompanied by incomplete cell division and the formation of large or giant cells. At the same time the growth of the radiated tumour was slowed up but without any consistent changes in the oxygen uptake or the aerobic glycolysis. A reduction in the number of tumour cells in any given area followed and was usually accompanied by cessation of growth and a small decrease in the oxygen uptake, the aerobic glycolysis remaining unaffected. During the last stage, the number of tumour cells was decreased further and an infiltration of connective tissue appeared which gradually smothered the remaining tumour cells. This was accompanied by a regression in the size of the tumour, a further reduction in the oxygen uptake and a gradual decrease in the aerobic glycolysis.

Table II. *Effects of X-radiation on Walker 256 carcinoma.*

Exp.	Average X-ray dose*	Supplementary treatment	Effect on growth of tumours	R.Q.		-Q _{O₂}		Q _A		Tumour cells		Histological effects†	
				Control	Rad.	Control	Rad.	Control	Rad.	Control	Rad.	Control	Rad.
15	1000 R												
	(2565)	Normal	Marked regression	0.72	0.69	11.7	9.9	16.7	17.2				
	(2851)	Normal	Marked regression	—	—	—	—	—	—	946	146	73	7 (6)‡
14	(1913)	Normal	Marked regression	0.70	0.67	11.6	9.5	18.2	17.8				
11	900 R												
	(1736)	Starved	Regression	—	—	—	—	—	—	846	704	66	25 (7)‡
	(1595)	Normal	Regression	—	—	—	—	—	—				
	(1525)	Normal	Regression	0.73	0.58	11.3	8.6	24.5	25.1				
25	(1589)	Normal	Regression	—	—	—	—	—	—				
	800 R												
	(1721)	Insulin	Marked regression	—	—	12.2	2.8	28.6	4.7	592	6	54	0
	(1535)	Insulin	Growth stopped	0.80	0.79	10.4	11.3	15.2	15.9				
22	(1425)	Insulin	Growth stopped	0.84	0.80	10.5	11.8	11.1	12.8	720	810	43	30
28	700 R												
	(2012)	Normal	Growth stopped	0.82	0.70	9.6	8.4	21.9	18.0	386	291	35	9 (8)‡
39	(1377)	Normal	Growth stopped	—	—	—	—	—	—				
	600 R												
	(1836)	Normal	Growth stopped	—	—	—	—	—	—				
	(1469)	Normal	Growth stopped	—	—	—	—	—	—				
	(1326)	Normal	Growth retarded	—	—	—	—	—	—				
	(1080)	Normal	Growth stopped	—	—	—	—	—	—				
	(983)	Normal	Growth stopped	0.79	0.65	9.9	9.0	16.7	18.0				
500 R													
	(1036)	Insulin	Growth stopped	—	—	—	—	—	—				
	(1036)	Insulin	Growth stopped	—	—	—	—	—	—				
	(1040)	Normal	Growth retarded	—	—	—	—	—	—				
400 R	(1014)	Normal	Growth retarded	—	—	—	—	—	—				
35	(805)	Starved	Marked regression	0.85	—	10.1	1.8	18.0	6.5	—	0	—	0
38	(874)	Normal	Growth retarded	0.82	0.64	10.4	10.5	17.5	20.7	336	348	26	20

* The average X-ray dose is the average effective amount of radiation as calculated from Pfahler's curves for maintaining a saturation dose, assuming that 100% saturation for 127 KV through 3 mm. of Al is 550 R. The numbers in parentheses indicate the sum of the individual doses of X-rays given to each animal.

† The number of tumour cells was determined by actual counts of 10 microscope fields taken at random from the stained sections and the number of mitoses from counts of 50 such fields.

‡ The figures in parentheses indicate the number of abnormal mitoses.

Table III. *Effects of X-radiation on Philadelphia 1 sarcoma.*

Exp.	Average X-ray dose*	Supplementary treatment	Effect on growth	R.Q.		-Q _{O₂}		Q _A		Histological effects			
				Control	Rad.	Control	Rad.	Control	Rad.	Tumour cells		Mitoses	
										Control	Rad.	Control	Rad.
950-1000 R													
23	(2674)	Starved	Marked regression	(0.73)	(0.74)	11.2	6.2	16.0	0.9	1000	376	25	0 ¹
21	(1800)	Starved	Marked regression	0.81	0.52	10.9	8.7	14.0	14.7				
16	(1840)	Insulin	Regression	0.78	0.51	8.9	8.1	12.7	17.6				
17	(1780)	Insulin	Growth stopped	0.94	0.70	10.2	10.9	18.1	20.0				
900 R													
18	(2310)	Insulin	Marked regression	—	—	—	—	—	—				
	(1765)	Insulin	Regression	0.72	0.60	8.2	8.0	17.1	17.3				
	(2310)	Insulin	Regression	—	—	—	—	—	—				
800 R													
36	(2810)	Starved	Growth stopped	0.76	0.55	11.4	7.5	15.8	15.5	872	598	32	14 ²
	(2810)	Starved	Growth stopped	—	—	—	—	—	—				
	(2350)	Insulin	Growth stopped during X-ray	—	—	—	—	—	—				
	(2350)	Insulin	Growth stopped during X-ray	—	—	—	—	—	—				
	(3109)	Normal	Growth stopped	—	—	—	—	—	—				
37	(3109)	Normal	Growth stopped during X-ray	0.72	0.57	10.5	9.1	19.1	17.3	676	616	10	12 ³
	(1621)	Insulin and glucose	Regression	—	—	—	—	—	—				
	(1621)	Insulin and glucose	Regression	—	—	—	—	—	—				
	(1621)	Insulin and glucose	Regression	—	—	—	—	—	—				
700 R													
41	(2310)	Normal	Growth stopped during X-ray	—	—	—	—	—	—				
	(2310)	Normal	Growth retarded	—	—	—	—	—	—				
	(1498)	Insulin and glucose	Growth stopped	—	—	—	—	—	—				
42	(1796)	Adrenaline	Marked regression	0.88	—	9.7	2.0	16.7	5.5	—	—	—	— ⁴
	(1796)	Adrenaline and glucose	Growth unaffected	0.76	0.78	9.5	10.3	14.3	16.7	—	—	—	— ⁵

32	600 R											
	(1541)	Starved	Regression	—	—	—	—	—	—	—	—	19
	(1541)	Starved	Regression	—	—	—	—	—	—	—	—	25
	(1960)	Insulin	Growth retarded	0.81	0.65	10.2	7.1	14.9	13.9	900	532	15
31	(2450)	Insulin	Growth retarded	0.78	0.69	10.6	8.1	15.3	18.3	890	950	27
	(1910)	Normal	Growth stopped	—	—	—	7.9	14.1	12.6	1100	1136	37
500 R												
40	(2408)	Starved	Growth stopped	—	—	—	—	—	—	—	—	6 ⁸
	(1695)	Starved	Growth stopped	—	—	—	—	—	—	—	—	
	(1307)	Starved	Growth stopped	—	—	—	—	—	—	—	—	
	(1307)	Starved	Growth retarded	0.78	0.73	8.7	7.3	13.2	10.2	1102	616	27
32	(1151)	Starved	Growth stopped	—	—	—	—	—	—	—	—	6 ⁷
	(1870)	Insulin	Growth stopped	0.74	0.66	9.2	8.3	17.5	14.7	980	320	30
	(1460)	Insulin	Growth unaffected	0.79	0.78	10.3	10.8	15.7	20.5	860	601	36
	(2600)	Insulin	Growth retarded	0.75	0.66	8.8	7.3	13.0	10.8	940	392	31
34	(1671)	Normal	Growth stopped during X-ray	—	—	—	—	—	—	—	—	6 ⁸
	(1671)	Normal	Growth stopped during X-ray	—	—	—	—	—	—	—	—	
26	(1760)	Normal	Growth retarded	0.85	0.67	10.0	10.2	13.6	17.9	1006	810	13
	(1400)	Normal	Growth retarded	0.82	0.72	10.1	10.2	14.7	17.2	900	610	11
	(1320)	Adrenaline	Growth stopped during X-ray	—	—	—	—	—	—	—	—	
	(1320)	Adrenaline and glucose	Growth stopped during X-ray	—	—	—	—	—	—	—	—	
400 R												
30	(1870)	Starved	Growth retarded	0.88	0.65	8.2	7.5	13.9	14.2	918	712	20
	(1350)	Starved	Growth retarded	—	—	—	—	—	—	—	—	23 ⁹
	(1350)	Starved	Growth retarded	—	—	—	—	—	—	—	—	
	(1425)	Insulin	Growth retarded	—	—	—	—	—	—	—	—	
(1012)	(1425)	Insulin	Growth retarded	—	—	—	—	—	—	—	—	
	(1012)	Adrenaline	Growth retarded	—	—	—	—	—	—	—	—	
(1012)	(1012)	Adrenaline and glucose	Growth retarded	—	—	—	—	—	—	—	—	
	(1012)	Adrenaline and glucose	Growth retarded	—	—	—	—	—	—	—	—	

¹ The radiated tumour contained mostly enlarged cells with much connective tissue.

² Some connective tissue was beginning to appear in the radiated tumour.

³ Many of the radiated tumour cells were enlarged.

⁴ The tumour cells were nearly all replaced by connective tissue.

⁵ Necrosis made cell counts of doubtful value.

⁶ The amount of connective tissue present was increased.

⁷ Most of the cells were abnormal and the amount of connective tissue was greatly increased.

⁸ All the mitoses in the radiated tumour were abnormal.

⁹ The connective tissue was increased and there were many somewhat enlarged cells.

* The numbers in parentheses indicate the sum of the individual doses of X-ray administered to each animal.

In preliminary work, even high doses of X-rays failed to produce much effect upon the metabolism of the Philadelphia 1 sarcoma. Eichholtz *et al.* [1934] reported that subcutaneous injection of insulin seemed to increase the sensitivity of certain rat tumours to X-radiation, owing, he believed, to its effect upon the glycolysis. Since the aerobic glycolysis is practically unaffected by X-ray treatment until after considerable degeneration of the tissue has taken place, it seemed possible that the effect might be a result of changes in the blood sugar level which altered the amount of blood sugar available for tumour growth. A set of animals therefore was injected subcutaneously with one unit of insulin per kg. 2 hours before irradiation. A large number of blood sugar analyses were made, the blood being taken from the femoral artery by means of a small incision, but no consistent correlation was found between the blood sugar level and the sensitivity of the tumours towards irradiation, even though the amount of sugar was reduced considerably. Moreover, subcutaneous injections of adrenaline and glucose, which raised the blood sugar level enormously, did not decrease the sensitivity of the tumours. The results of irradiating the tumours of these two sets of animals as well as a set of untreated (normal) animals are summarised in Table III.

The effects of different levels of radiation upon the Philadelphia 1 tumour are rather variable although, in general, they are proportional to the dose of X-rays given. This variability may depend, in part, upon some unknown factor which influences the sensitivity. The differences in sensitivity towards radiation produced by the different treatments of the animals are not great, but the effect of radiation on the tumours may be slightly higher for the starved animals than for those treated with insulin, and slightly greater for the latter than for the normal animals.

The histological changes seem to follow the same course under the influence of X-rays as was shown with the Walker 256 carcinoma, but the results are more indefinite; the proportion of enlarged cells after irradiation is always smaller, the infiltration of connective tissue is less pronounced and a complete disappearance of tumour cells has never been observed so far even with heavy doses of X-rays.

The effect of X-rays upon the metabolism shows a small but fairly regular decrease in the oxygen uptake (Q_{O_2}) but no significant change in the aerobic glycolysis except in the final stages of regression of the tumour. There is, however, an almost constant lowering of the R.Q. with the Philadelphia 1, which is also common with the Walker 256. In Table III there are only three apparent exceptions to this change in the R.Q. all of which are readily understandable. In Exps. 29 and 42 X-rays had no apparent effect either on the growth or on the metabolism so that no change in the R.Q. would be expected. The R.Q. of Exp. 23 was also normal, but since the histology showed that practically all the tumour cells had been replaced by connective tissue cells the comparison is really between the metabolisms of two different tissues and not between radiated and non-radiated tumour tissues.

DISCUSSION.

Warren [1928] states that the injury to cell structures of body tissues by radiation is roughly proportional to the amount of radiation administered, although the extent of the injury may vary with different tissues and different species. The effect of radiation upon mitoses and the formation of enlarged cells has been observed also by many workers (see, for example, Ludford [1932]). The infiltration of connective tissue, after radiation by radium has caused considerable damage to the tumour cells, has been observed by Crabtree. This is in agreement with the results obtained here with X-rays. The aerobic glycolysis seems to

be quite resistant to the action of X-rays or radium (see Crabtree) and is affected only after the radiation has caused considerable degeneration of the tumour tissue. On the other hand Adler [1930] found a gradual increase in the aerobic glycolysis with small amounts of X-rays. The decrease in the oxygen uptake observed also by Cramer after continued irradiation with radium is possibly a secondary effect. However, the decrease in the R.Q. which usually occurs when there is any effect upon growth seems likely to be an immediate effect of radiation and indicates a definite alteration in the chemical activity of the cell. This alteration in the metabolism may be important as a clue to the mechanism of the action of radiation upon cell metabolism.

SUMMARY.

1. When two tumours are implanted in one animal their growth rates and metabolisms usually correspond closely. It has been found practicable to irradiate one of such duplicate tumours and to observe the effects of X-radiation on the growth, histology and metabolism of the treated tumour, using the untreated tumour as a control.

2. The sequence of histological effects of X-rays seems to be a reduction in the number of mitoses or a formation of enlarged cells, a gradual degeneration of tumour cells and finally an infiltration of connective tissue which smothers the remaining cells.

3. The aerobic glycolysis continues unimpaired during treatment with X-rays until after a very considerable amount of degeneration of tissue has occurred.

4. There is a lowering of the oxygen uptake, which may be a secondary effect of damage done to the tissues by radiation.

5. With the Philadelphia 1 sarcoma, X-ray treatment always lowers the R.Q. Since this occurs whenever the growth has been affected in the least, it is possibly a primary effect of X-rays upon the metabolism.

6. The Philadelphia 1 sarcoma is much less sensitive to the action of X-rays than the Walker 256 carcinoma. There are indications that 24-hour starvation or injection of insulin increases this sensitivity although such an increase in sensitivity does not appear to be directly associated with a lowered blood sugar level.

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