CCLXXVII. THE DIFFERENTIAL EFFECT OF RADIUM RADIATION ON THE CARBOHYDRATE METABOLISM OF NORMAL AND TUMOUR TISSUES IRRADIATED AT LOW TEMPERATURE.

By HERBERT GRACE CRABTREE.

From the Imperial Cancer Research Fund, London.

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THE action of short-wave radiation in affecting the rate of growth or causing the regression of an established tumour is a complex process which may be visualised in three phases.

1. The primary physical effects of atomic excitation, ionisation and disintegration, which induce physico-chemical and chemical changes.

2. The primary biological effects such as altered permeability of cell membranes or changes in the viscosity, p_H, state of protein aggregation and enzymic activity of the cell contents.

3. The final response of the affected tissues, both normal and cancerous, which is shown by visible changes in cell morphology and a general interaction between stroma and parenchyma.

The work reported here deals with the second phase only, namely with the changes induced in the enzymic activity of cells irradiated under different environmental conditions.

Many attempts have been made to attribute the action of radiation to its predominating effect on some single structure or physico-chemical equilibrium within the cell. Any such specific action seems improbable on general grounds, but it seems clear that damage to certain vital structures, with low powers of recovery, would lead to general impairment of cell function. In this sense it is possible to speak of selective damage to definite cell systems of paramount importance in cell economy.

With this conception in mind, the changes produced by radiation in the carbohydrate metabolism of tumour cells were examined [Crabtree, 1932]. It was thought possible that the abnormal metabolism of tumour cells might be related to their supposed greater vulnerability to radium. It was found that the two energy-yielding processes of respiration and glycolysis were not equally vulnerable to radiation. A selective diminution of respiration occurred whilst aerobic glycolysis remained relatively unimpaired. Though radiation affected two cell processes in a differential manner, no support was given to the idea that tumour cells were inherently more sensitive than normal cells.

This selective effect was utilised as a basis for a more extensive study of the possibility of influencing the vulnerability of tumour cells to radiation by varying the physiological condition of the respiratory system. In a series of papers [Crabtree and Cramer, 1934, 1, 2; Crabtree, 1934] it was shown that the radio-sensitiveness of tumour cells was a function of the chemical condition of the iron-containing, oxygen-transporting factor of the respiratory system. With this factor in a reduced condition (either in free state in N_2-anaerobiosis or in partial

combination in CO-anaerobiosis) tumour cells were less radio-sensitive than when it was functioning aerobically. Conversely, when the iron, in oxidised condition, was "fixed" by HCN, tumour cells were more radio-sensitive. Any simple interference with the functional capacity of the glycolytic system, such as treatment with iodoacetic acid, sodium fluoride, variations in p_H or in glucose concentration, produced no detectable effects on the radio-sensitivity of the tissue. Tumour tissues suspended in phosphate media were more radio-sensitive than when suspended in bicarbonate media, a fact which was attributed to the damaging effect of phosphate media on their respiration. All these results supported the original finding that the vital and labile respiratory system was more vulnerable to radiation than the glycolytic system and played a dominant part in the biological response of irradiated tumour cells.

One result was obtained which was not explicable in terms of the above hypothesis. Tumour cells at low temperature were more radio-sensitive than at body temperature. The energy-supplying chemical systems are at a standstill at low temperature, and it is impossible to describe them in terms of functional condition. Yet one suggestive result was obtained. By irradiating tumour tissue under anaerobic conditions at low temperature, it was found that the effect of cold in increasing the radio-sensitiveness under aerobic conditions could be eliminated. This result conformed with the conception that the respiratory system was primarily concerned in the variations of sensitivity found.

The results summarised above were obtained in experiments where tumour tissues were irradiated *in vitro* under physiological conditions with known modifications and subsequently transplanted. The capacity for and rate of growth were the indicators used in assessing the effects of the treatment. This technique had the obvious limitation of being solely applicable to tumour tissue. It was decided to use a technique which was also applicable to normal tissues, and in particular to investigate the changes in metabolism induced by irradiation at low temperature. The results indicate that radiation produces a differential effect on the metabolism of normal and tumour tissues, which depends on the fact that tumour tissues have a characteristic high glycolysis.

The belief in a special vulnerability of tumour tissue to radiation has often been expressed in the literature of radiology. The basis of this belief was questionable, since a distinction was not drawn between the effects of radiation on tumour cells and on the tumour as a whole when in living association with its host. All the work carried out in this laboratory had supported the idea that normal and tumour cells were equally vulnerable. The present work shows that that conception was premature.

Technique.

Previous experience had shown the essential importance of the time factor in experiments carried out *in vitro* with surviving material. Making allowance for variations in individual tissues, it was shown [Crabtree, 1932] that little effect on metabolism was detectable up to 6–7 hours of continuous γ-irradiation. This increased the difficulty of assessing results, since such time periods were of the same order as those during which surviving control tissues could be maintained in a condition of maximum functional activity. The only way to speed up the experiments was to use more intensive radiation and shorten the latent period as much as possible. The justification of this is given in the discussion. $(\beta+\gamma)$-Radiation from two radium applicators was used throughout this work. Each applicator, 23 mm. square, contained 110 mg. of $RaBr_2$, $2H_2O$, the equivalent of Ra element being 58 mg. The lids of the applicators were of silver 0·12 mm. thick.

Apparatus for irradiation.

This is shown in Fig. 1. The tissue slices were placed in a cell built up with many layers of mica sealed together with a solution of celluloid in acetone.

The cell had a depth of 1·5 mm., and was shaped to allow an easy flow of the irrigating medium during irradiation. The tissue slices were held in position

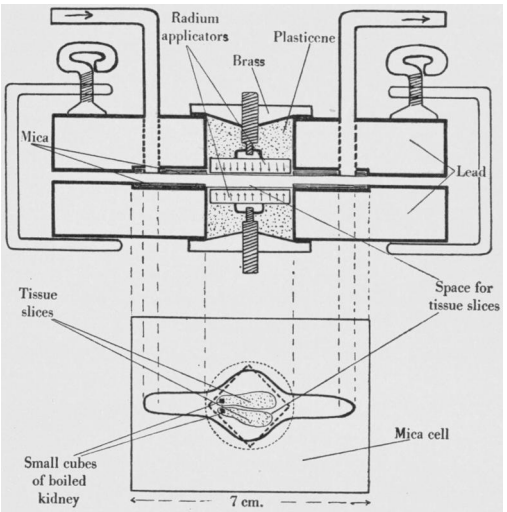

Fig. 1. Apparatus for irradiation and irrigation of tissue slices.

by small cubes of boiled kidney, which were firmly gripped between the two halves of the apparatus when closed. The only obstacle between the unscreened applicators and the tissue slices was a thin sheet of mica *ca.* 0·015 mm. thick, and the liquid layer of medium 1·5 mm. deep. The central rectangular mica sheet containing the cell was fitted into a hollow outer sheet of brass, rectangular in shape and of the same thickness as the mica. This brass was soldered on to a lead plate 1·5 cm. thick containing a central circular hole to receive an applicator. The upper half of the apparatus was similar in construction, the mica portion having a flat surface which formed a lid to the cell. Suitable glass tubes sealed with wax in the upper lead plate and opposed to the two ends of the cell provided an inlet and outlet for the irrigating medium. Plasticene effected the final sealing of the applicator holders and the two halves of the apparatus, which were tightly gripped together with heavy screw-clips. The whole apparatus, thus sealed, was placed in water in a tank at the appropriate temperature, and the medium, after passing through a glass coil immersed in the tank water, was run through at a suitable rate. This ensured constancy of environmental conditions during irradiation, an important factor often neglected in work with radiation in the biological field. Non-irradiated controls were treated similarly in all experiments, and no adverse effect on these controls was ever detected. The times of irradiation were 3–7 hours.

Metabolism measurements.

These were carried out by standard methods. Generally ten bottles were used, five for the controls and five for the irradiated tissues.

Respiration, aerobic and anaerobic glycolysis were measured by Warburg's

method, and two additional measurements of respiration were made in phosphate-Ringer media. In the tables of results the original symbols introduced by Warburg for expressing the magnitude of respiration (Q_{O_2}), aerobic glycolysis ($Q_M^{O_2}$), anaerobic glycolysis ($Q_M^{N_2}$) and total CO_2 evolved ($Q_{CO_2}^{O_2}$), are used.

The last symbol is used only in Table III, which deals with normal tissues having a low aerobic glycolysis. The expression $Q_{CO_2}^{O_2} - Q_{O_2} = Q_M^{O_2}$ shows the relationship of these magnitudes, assuming R.Q. unity.

Results.

Irradiation of tumour tissue under aerobic conditions or in presence of M/*600 HCN at 37·5°.*

The tumour mainly used was Jensen's rat sarcoma (J.R.S.), though a few experiments have been made with tumour strains growing in mice. The latter are

Table I. *Comparison of changes in the metabolism of tumour tissue irradiated under different conditions.*

1–6, in R.G.B. at 37·5° aerobically.
7–18, in *M*/600 HCN in R.G.B. at 37·5° aerobically.

No.	Time of irradiation (hours)	Irrigating medium used during irradiation	Time of measurement of metabolism (hours)	Control				Irradiated			
				Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	Q_{O_2} (phosph.)	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	Q_{O_2} (phosph.)
1	4	R.G.B.	2	11·1	22·9	33·6	9·9, 8·9	9·4	24·0	31·2	9·1, 8·2
2	4	,,	2	14·0	20·5	31·3	10·1, 9·6	11·5	21·4	33·0	9·8, 7·9
3	5	,,	1	13·6	24·3	34·1	9·8	15·4	26·1	33·0	8·9
4	5	,,	1	12·2	25·0	32·7	10·5, 8·9	9·8	26·8	31·9	9·1, 8·6
5	6	,,	1	10·0	23·5	34·1	8·2, 8·1	5·5	22·9	30·8	4·2, 5·3
6	6	,,	1	11·3	26·3	39·1	9·2, 9·6	7·8	25·0	37·3	6·7, 5·4
7	3	*M*/600 HCN in R.G.B.	2	17·6 ↓ 13·5	24·2 ↓ 20·9	36·3 ↓ 32·1	10·8 ↓ 9·2	18·2 ↓ 2·0	25·9 ↓ 18·9	35·2 ↓ 22·3	10·9 ↓ 4·3
8	3	,,	3	16·3 ↓ 15·1	24·4 ↓ 25·3	38·1 ↓ 36·2	10·6 ↓ 8·3	15·7 ↓ 0	21·9 ↓ 14·3	35·3 ↓ 23·4	9·9 ↓ 2·8
9	3	,,	4	14·8 ↓ 13·8	25·4 ↓ 25·1	37·0 ↓ 34·0	12·1 ↓ 10·9	7·5 ↓ 0	23·5 ↓ 14·9	35·0 ↓ 21·0	7·0 ↓ 3·5
10	3	,	3	14·0 ↓ 11·2	31·0 ↓ 28·4	39·0 ↓ 37·2	11·0 ↓ 9·8	12·6 ↓ 5·6	30·6 ↓ 22·6	39·8 ↓ 28·3	10·5 ↓ 3·5
11	4	,,	3	16·4 ↓ 12·9	34·1 ↓ 29·0	42·0 ↓ 38·0	10·2 ↓ 10·0	14·7 ↓ 6·0	31·2 ↓ 24·0	40·6 ↓ 32·0	9·8 ↓ 7·2
12	4	,,	2	12·7 ↓ 11·9	30·2 ↓ 29·3	38·3 ↓ 36·1	9·8 ↓ 8·6	6·3 ↓ 0	15·2 ↓ 9·2	17·2 ↓ 12·0	3·4 ↓ 2·2
13	4	,,	4	12·9 ↓ 9·6	27·9 ↓ 25·5	35·2 ↓ 33·8	11·0 ↓ 9·4	12·8 ↓ 4·2	23·6 ↓ 12·5	30·0 ↓ 18·3	9·6 ↓ 2·0
14	4	,,	1	17·7	30·7	40·1	12·3	0·6	7·5	11·0	1·1
15	5	,,	1	17·0	25·3	36·0	8·6	0	6·9	3·4	0·9
16	5	,,	1	10·9	24·8	31·2	11·2	0	5·8	6·2	0
17	6	,,	1	15·2	31·8	40·3	12·4	0	6·2	7·8	0
18	6	,,	1	12·9	25·3	38·5	11·3	0	4·9	8·0	0

↓ In Exps. 7–13 the initial and final values of the metabolism quotients are given, the arrows indicating a progressive change from one to the other during the time of measurement of metabolism.

always unsatisfactory in work on tissue metabolism, since homogeneous slices of adequate size are difficult to obtain. All the results quoted in the Tables refer to experiments with J.R.S., though the mouse tumours behaved in a similar manner.

The irrigating medium was either Ringer-glucose-bicarbonate (R.G.B.) alone, or $M/600$ HCN in R.G.B. The composition was described earlier [Crabtree, 1928], and the gas phase was 5% CO_2 in oxygen. Table I contains some typical results and shows the times of irradiation and of the subsequent measurement of metabolism.

The general result confirms previous work. Tumour tissue under the influence of radiation at 37·5° suffers a selective diminution of its respiration during a period when its glycolysis, aerobic and anaerobic, is relatively unaffected. When the respiration is almost completely "fixed" with HCN, radiation produces a similar effect, but in considerably greater degree over corresponding periods of treatment. As was found by the transplantation technique described in the introduction, tumour cells treated with HCN and irradiated at body temperature are more vulnerable to radiation than when the respiration is functioning normally. No doubt other inhibitors of respiration would yield similar results, but since it is difficult to see any useful clinical application arising from this idea, this type of experiment has not been extended. Moreover the technique cannot be applied to normal tissues dependent for survival on respiration alone. The value of these experiments is in showing that the increased vulnerability of tumour cells with "fixed" respiration is made evident when two widely different criteria for assessing radiation effects are used, *viz.* transplantability in the animal after small exposures, and inhibition of metabolism *in vitro* after large exposures.

Irradiation of tumour tissue at different temperatures.

Table II contains a summary of typical results obtained. They have been selected at random.

The generalisation that irradiation at body temperature primarily affects respiration and irradiation at low temperature primarily affects glycolysis holds for every experiment carried out, the variations, considered quantitatively, being attributed to inherent variations in individual tissues. Such individual differences are more apparent when irradiation is carried out at body temperature and respiration is the point of attack. At low temperature there is greater uniformity of result; with a suitable dosage a clean-cut inhibition of glycolysis is possible whilst respiration is maintained unimpaired, the tumour tissue temporarily metabolising like a typical non-glycolysing tissue, *e.g.* liver.

One interesting point was noticed frequently. After irradiation at low temperature, metabolism measurements showed that anaerobic glycolysis was smaller than aerobic glycolysis. Using Warburg's two-bottle method for measuring respiration and aerobic glycolysis together, the accuracy of the calculated values of these two quantities depends upon their relative magnitude; a high glycolysis with a low respiration implies that the respiration may be inaccurately determined and *vice versa*. That the anomaly is not explicable on this basis is shown by the addition of HCN, which increases glycolysis to a figure somewhat greater than either the calculated aerobic glycolysis or the anaerobic glycolysis measured in nitrogen directly after irradiation. It is clear that the effect depends upon the power of recovery under different conditions; in oxygen this recovery is significant but not great; in nitrogen it is negligible.

Table II. *Changes in the metabolism of tumour tissue (J.R.S.) irradiated at different temperatures.*

Time of irradiation (hours)	Temperature during irradiation °C.	Irrigating medium used during irradiation	Control				Irradiated			
			Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	Q_{O_2} (phosph.)	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	Q_{O_2} (phosph.)
4	37·5	R.G.B. aerobic	11·1	22·9	33·6	9·9, 8·9	9·4	24·0	31·2	9·1, 8·2
5	,,	,,	9·8	18·1	28·5	9·7, 9·1	10·9	20·1	27·0	8·9, 7·6
5	,,	,,	12·2	25·0	32·7	10·5, 8·9	9·1	26·8	31·9	7·8, 7·5
6	,,	,,	10·0	23·5	34·1	8·2, 8·1	5·5	22·9	30·8	4·2, 5·3
4	19	,,	12·2	22·6	30·2	9·5	11·2	13·5	17·3	7·9, 7·9
5	,,	,,	13·1	25·0	33·6	10·0, 9·8	8·6	15·0	28·0	6·3, 8·0
5	,,	,,	10·2	23·1	31·6	8·9, 9·6	9·0	17·1	26·0	7·3, 6·1
5	20	,,	11·4	26·0	35·6	10·0, 8·6	7·9	20·1	30·6	6·1, 7·4
4	10	,,	10·9	22·9	35·8	10·9, 11·2	8·0	11·2	22·9	8·8, 9·1
5	,,	,,	8·3	19·3	28·0	10·1, 9·4	10·4	6·6	3·6	8·1, 9·0
5	,,	,,	11·3	24·3	35·6	10·0, 9·3	10·1	4·6	6·1	8·6, 9·0
4	0–4	,,	11·9	21·3	29·3	8·0, 8·6	9·3	10·1	15·5	7·4, 7·9
5	,,	,,	9·9	20·0	31·9	8·9, 7·9	8·6	2·1	5·8	7·9, 7·9
5	,,	,,	13·0	20·8	32·9	8·1, 9·2	12·9	4·5	7·6	7·6, 8·5
6	,,	,,	16·9	27·4	38·2	15·7, 12·6	5·5	0	2·5	8·3, 6·9
6	,,	,,	10·1	18·1	25·9	8·8, 8·4	9·3	5·8	6·8	7·7, 6·8
3	0–4	R.G.B. anaerobic	14·6	24·2	34·0	11·0, 9·7	13·6	13·6	21·8	11·9, 9·2
4	,,	,,	9·4	23·9	37·5	11·1, 8·8	6·7	10·6	8·3	12·1, 7·0
4	,,	,,	7·4	25·0	33·9	7·9, 9·1	9·1	11·7	15·1	7·7
5	,,	,,	8·2	23·3	30·5	9·4	11·4	4·6	2·5	8·1, 9·0
5	,,	,,	11·6	25·3	36·3	10·0, 8·9	10·4	3·0	4·0	8·0
3	0–4	*M*/600 HCN in R.G.B.	11·6	26·2	33·7	7·4, 7·1	11·1	13·4	7·0	8·6, 7·0
4	,,	,,	10·2	20·7	32·0	10·2, 7·5	9·4	6·0	9·5	7·1, 6·0
5	,,	,,	11·3	22·8	36·1	9·8, 7·9	10·9	6·5	1·6	10·6, 7·4

Tumour slices have been irradiated at two intermediate temperatures (10° and 20°) with a view to finding the upper limit of the low temperature effect. At 10° the results are indistinguishable from those obtained at 0–4°. This may prove a useful practical consideration should the low temperature effect be utilised clinically. At 20° the effect is mixed, both the partially functioning respiration and glycolysis being inhibited. This observation may have a bearing on the conflicting results obtained by other workers who have measured the effects of radiation on metabolism.

If, instead of irradiating in the cold under aerobic conditions, HCN is added to the irrigating medium or N_2 replaces O_2, no difference is found in the results obtained. Destruction of glycolysing power and maintenance of respiratory power are the invariable effects. Probably this reflects the limitations of the technique, as it contrasts with results already recorded in which the transplantability of tumour tissue was used to assess the effects of radiation. When dealing with artificially induced tissue degeneration, which only occurs after many hours of treatment, as in radiation experiments, differential effects can only be detected when they are of considerable magnitude. Evidently the relatively rapid damage to the glycolysing system at low temperature exceeds the attack on the respiratory system, whatever its condition, under the circumstances of these experiments.

Fig. 2 shows a graphical summary of all the results obtained by irradiating tumour tissue under different conditions. It does not represent a series of individual experiments, but a composite picture of the general nature of the differential effects observed.

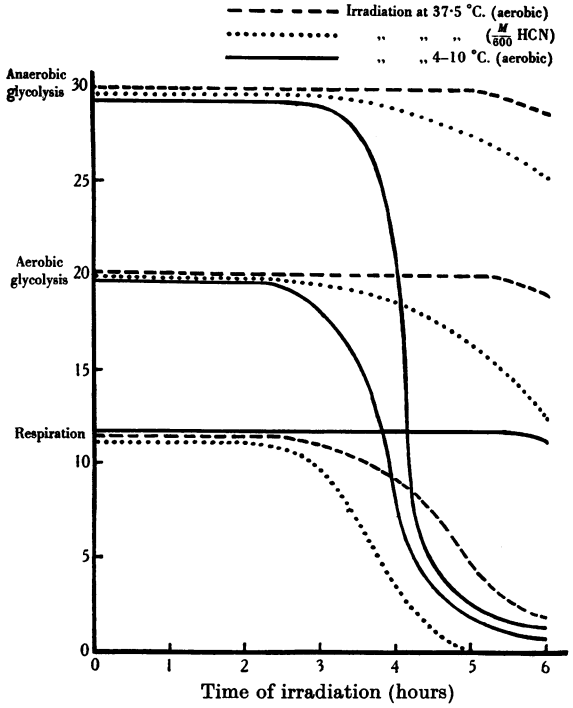

Fig. 2. Effect of radium irradiation on the carbohydrate metabolism of tumour tissue surviving under different conditions.

Table III. *The metabolism of some normal tissues after irradiation at low temperature* (0–4°).

Tissue	Time of irradiation (hours)	Control			Irradiated		
		Q_{O_2}	$Q^{O_2}_{CO_2}$	Q_{O_2} (phosph.)	Q_{O_2}	$Q^{O_2}_{CO_2}$	Q_{O_2} (phosph.)
Rat kidney	3	21·2	20·3	17·3, 18·9	20·9	19·6	18·0, 17·9
,,	4	18·6	18·1	17·3, 18·3	18·9	17·3	17·0, 16·1
,,	4	17·9	16·9	17·3, 15·7	17·7	17·5	20·6, 16·0
,,	5	—	—	14·3, 14·7	—	—	15·2, 14·8
,,	5	14·2	14·0	14·6, 13·5	15·0	15·3	13·6, 14·7
,,	6	15·6	15·1	15·0, 14·8	16·3	16·0	14·5, 15·1
,,	6	11·3	11·0	10·3, 10·3	8·6	9·0	10·3, 8·4
,,	6	18·6	18·9	16·2, 15·3	14·6	15·0	11·4, 12·6
Rat liver	6	6·6	7·3	8·3, 7·7	8·3	8·8	7·4, 7·9
,,	6	8·2	9·9	8·3, 6·9	8·9	10·7	8·0
,,	6·5	9·3	10·4	9·0, 8·7	8·0	9·0	8·2
,,	6	11·1	12·0	10·2, 9·0	10·1	12·0	8·9, 7·0
Rat testis	6	8·5	10·5	7·9, 7·6	7·8	9·3	9·2, 7·4
,,	6	9·3	11·4	8·8, 8·6	9·0	10·1	9·0, 7·9
,,	7	9·0	11·1	8·4, 8·3	7·0	8·2	6·3, 5·3
Rat spleen	5	10·3	15·1	9·3, 9·9	9·2	11·3	8·6, 9·0
,,	5	13·2	18·6	12·2, 10·1	9·2	11·3	10·4, 9·1
,,	6	8·9	12·6	8·0, 9·0	6·0	7·1	6·3, 6·1

Irradiation of normal tissues at low temperature.

It is impossible to apply the technique used for tumour tissues to normal tissues irradiated at body temperature. During the minimum latent period of 3–4 hours which elapses before radiation effects are detectable, normal control tissues at 37·5° degenerate too rapidly. At low temperatures this spontaneous degeneration does not occur. In Table III are collected typical results of experiments in which normal tissues were irradiated under conditions which produced a large or complete inhibition of glycolysis in tumour tissue. No significant effect on the respiration of any of these tissues was found. Any effects produced on the low glycolysis of some of the tissues used were not detectable with certainty, as they were within the margin of error of the technique employed.

Discussion.

Few workers have studied the effect of radiation on tumour metabolism, and the results recorded have been conflicting. Crabtree [1932] and Holmes [1933] have drawn attention to some of these but failed to reconcile the different findings. It is possible that the results recorded here offer a partial explanation. Many workers with radiation in the biological field concentrate upon accuracy of dosage and do not clearly define the environmental conditions of their material during irradiation. Since the dosages required to obtain a certain result, *e.g.* inhibition of the capacity for growth on transplantation, may vary by 100 % according to the external conditions during irradiation, it is clear that the latter are of equal importance in obtaining significant results.

Two criticisms may be directed against this work, *viz.* that the results have been attained with enormous doses of $(\beta+\gamma)$-radiation, and that the experimental conditions, when compared with those found clinically, are too simple. In answer, it may be pointed out that when surviving tissues are irradiated *in vitro* the effects produced are the same for $(\beta+\gamma)$- as for γ-radiation, when the smaller intensity of γ-radiation is compensated by a longer period of treatment. Experiments on metabolic changes, transplantability and regression of tumours in the animal all support this view. In clinical work γ-radiation and X-rays are obligatory on account of their greater penetrating powers and the greater ease of obtaining more uniform dosage. In the experiments described here the maximum intensity of radiation available was deliberately used in order to shorten the experimental period and prevent adverse effects on surviving control tissues, which might confuse the results.

That the effects described are not entirely dependent on the large doses used is suggested by two facts.

1. A few experiments were carried out using γ-radiation only and slightly increasing the time of irradiation. The same type of effect was produced but to a lesser degree. The technique used only permits reactions to be followed over a limited time, but it is probable that once reactions are initiated, provided the dose applied has been sufficient to prevent reversibility, they proceed to the same end-point in a time which is a function of the dose.

2. The results of the metabolism experiments, where they are reproducible at all, run parallel with the results of the transplantation experiments. In the latter case comparatively small doses were used (30–40 min. of $(\beta+\gamma)$-, 3–4 hours of γ-radiation alone). This again suggests that large and small doses initiate processes which are similar in type, the size of the dose only determining the time before the end-effect is reached.

The hypothesis put forward in earlier communications, that the functional condition of the respiratory system determines the response of living cells to radiation and that the glycolytic mechanism is not primarily concerned, is only true when irradiation is carried out at body temperature. Respiration is a process common to all mammalian tissues; to attempt to make a differential attack on normal and tumour tissues by modifying this process did not promise anything of clinical value.

High aerobic glycolysis differentiates pathological overgrowths from almost all normal tissues. Irradiation at low temperatures might provide a method of using this difference to damage the tumour cell selectively by inhibiting its secondary mechanism for obtaining energy. Glycolysis in tumour cells is probably helpful in aiding their survival under conditions of restricted oxygen supply. A badly vascularised tumour, where regions in a state of partial anaerobiosis must exist, is notably insensitive to radiation applied at ordinary conditions of temperature. It is suggested that irradiation at low temperature might make such a tumour more sensitive by selectively damaging its glycolytic mechanism.

Whether irradiation at low temperature can be used to enhance the damage to tumour cells with a concomitant sparing of adjacent normal tissues in clinical cases remains to be proven. Experiments to test this conception are in progress.

Mottram [1924] recorded a relatively increased skin reaction after exposure to radiation at low temperature. This enhanced effect on normal tissues is not incompatible with the results recorded here. In Mottram's work the reactions of normal skin at different temperatures were compared. This work contrasts the reactions of normal and tumour tissues under the same condition of low temperature and shows a differential effect which is favourable to the normal tissue.

Summary and Conclusions.

1. The effect of radium radiation on the carbohydrate metabolism of normal and tumour tissues, irradiated *in vitro* under different environmental conditions, has been studied.

2. "Fixing" the respiration of tumour tissue with HCN at 37·5° makes it more sensitive to radiation.

3. Irradiation of tumour tissue at body temperature, either under aerobic or anaerobic conditions or with HCN present, causes a selective lowering of respiration whilst glycolysis remains relatively unaffected. The effects produced vary in degree, but are similar in character; respiration is primarily damaged at this temperature.

4. Irradiation of tumour tissue at low temperature (0–10°), either under aerobic or anaerobic conditions or with HCN present, causes a selective lowering of glycolysis, whilst respiration remains relatively unaffected. This selective damaging of glycolysis is much more pronounced than the converse effect on respiration at body temperature. It is possible to effect a clean-cut elimination of tumour glycolysis, leaving respiration intact.

5. Irradiation of normal tissues (spleen, liver, kidney, testis) at low temperature, under identical conditions of time and environment, produces little (or no) effect on their metabolism, since their respiration is not accompanied by aerobic glycolysis.

6. This differential damaging of tumour cells at low temperature by way of their characteristic glycolytic process may be of clinical value.

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