CCXXX. THE SEPARATION OF GLYOXALASE ACTIVITY AND GLYCOLYTIC ACTIVITY BY MEANS OF RADIUM RADIATION.

By HERBERT GRACE CRABTREE.

From the Imperial Cancer Research Fund, London.

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INVESTIGATIONS of the changes induced in the metabolism of cells under the action of radium or X-radiation have been few, and have led to conflicting results. Differences of technique are probably responsible for this lack of harmony. Radiation has been applied to cells or their products existing in four different conditions: as extracts, surviving slices, cultures *in vitro* or intact in the organism. Whether the results obtained by each of these methods are really comparable is an open question.

Certainly cell extracts containing potent enzymes are relatively insensitive to radiation. Hussey & Thompson [1923-26], using radium emanation, reported a destructive action on the hydrolytic enzymes trypsin, pepsin and invertase. Willcock [1906] found tyrosinase insensitive to large doses of β -radiation. Crabtree [1932] and Havard [1935] showed that succinoxidase partially lost its activity after heavy radiation, but Havard [1934, 1935] found indophenol oxidase, lactate, citrate and glucose dehydrogenases quite insensitive. It seems doubtful if any conclusion as to the mode of action of radiation on the intact cell can be drawn from a study of its effects on such isolated systems.

Using surviving tissue slices Frick & Posener [1926] described a checking effect of X-rays on the anaerobic glycolysis of rat and mouse tumours, fowl embryos and rat testes, and an inhibition of the Pasteur effect in the case of rat testes. Crabtree [1932] found that tissues under constant irradiation *in vitro* gradually lose their power of using oxygen during a period when glycolytic processes remain unimpaired. This finding, that respiration is more vulnerable to radiation than glycolysis, was confirmed in a series of papers [Crabtree & Cramer, 1934, 1, 2; Crabtree, 1934] where the possibility of varying the sensitivity of tumour tissue to radiation by interference with the respiratory system is described. Later Crabtree [1935] showed that the relative vulnerabilities of these two energy-yielding processes depended on the temperature of the tissue at the time of radiation; at body temperature respiration was primarily affected, at low temperature glycolysis could be inhibited almost completely whilst respiration was unaffected. It was suggested that the differential damaging of tumour cells at low temperature by way of their characteristic glycolytic process might be of clinical value.

Holmes [1933, 1935], using cultures *in vitro* of embryonic rat kidney, found that γ -radiation inhibited their rate of breakdown of carbohydrate, but had little or no effect on the breakdown of protein. Under similar conditions no effect was detected on two special enzyme systems, arginase and lactic dehydrogenase, but the latter enzyme was more easily damaged in minced tissues of young than of old embryos.

Irradiation of normal tissues and transplanted tumours *in vivo*, followed by measurements of metabolism at suitable intervals have led to uncertain and

divergent results. Adler [1930] irradiated rat testes with X-rays and radium and described the metabolic and structural changes induced. He found a progressive diminution of respiration with increasing tissue degeneration, accompanied by a corresponding rise of aerobic and anaerobic glycolysis. Löw-Beer & Reiss [1931], using X-rays, and measuring the metabolism of Jensen's rat sarcoma 1-4 days after irradiation, claim to have completely suppressed respiration without any detectable effect on anaerobic glycolysis or the Pasteur reaction. This contrasts sharply with the results of Crabtree [1932] who found some fall in respiration of the tissues of J.R.S. similarly irradiated with radium *in vivo*, but this was accompanied by the appearance of abnormally high aerobic glycolysis. These results were put forward with diffidence because of the difficulty of obtaining sections which had responded equally to the radiation damage and possessed an equal intensity of metabolism. Frick & Posener [1926] likewise were unable to obtain homogeneous slices and were sceptical of the significance of any results obtained by irradiation *in vivo*.

Since the simple procedure of lowering the temperature of tumour tissue during irradiation causes selective damaging of the glycolytic mechanism, leaving respiration unaffected, it was considered of interest to attempt to analyse this effect. It seemed possible that one stage of the reactions which occur in the breakdown of carbohydrate to lactic acid might be particularly sensitive to radiation, since the inhibition of glycolysis by irradiation at low temperature occurs more quickly and completely than any other biochemical process so far studied. Of the two principal schemes suggested as intermediary phases of carbohydrate breakdown, that of Neuberg, Dakin & Dudley involving methylglyoxal was more suitable for investigation, since the Embden-Meyerhof scheme was evolved from a study of tissue extracts which, owing to their insensitiveness to radiation, increase the technical difficulties to be overcome.

In this paper it is shown that radium radiation applied to highly glycolysing tissues at low temperature can suppress total glycolysis almost completely whilst glyoxalase activity is unaffected.

Methods.

After suitable irradiation of tissue slices, manometric measurements have been made of respiration (Q_{O_2}), anaerobic glycolysis ($Q_M^{N_2}$) and anaerobic glyoxalase activity. Q_{O_2} and $Q_M^{N_2}$ have the significance given in the original work of Warburg [1926], and anaerobic glyoxalase activity is similarly expressed as a function of dry weight of tissue, i.e.

$$\frac{\mu\text{l. CO}_2 \text{ evolved by lactic acid formation in N}_2}{\text{hours} \times \text{mg. dry tissue}}.$$

The methylglyoxal used was prepared by the method of Riley *et al.* [1932]. Fractional distillation at low pressure yielded a product containing less than 3% acid and this was used in sugar-free bicarbonate media in a concentration of 0.01 *M*. The results were similar whether the methylglyoxal was added to the Ringer-bicarbonate before placing the manometers in the thermostat, or tipped it from a side-bulb after equilibrium at 37.5° had been attained.

Experiments with untreated tissues.

Jowett & Quastel [1934] found that glyoxalase activity was proportional to the surface area rather than the weight of the tissue, being apparently determined by the small depth to which methylglyoxal diffuses into the slices. They

were unable to determine if there was a limit to glyoxalase activity as the slice-thickness decreased. As a preliminary to the radiation experiments, the glyoxalase activities of a series of tissues, including many tumours, were measured in order to check this finding. The results confirm the work of these authors to a large extent, but it was found unnecessary to calculate the activity per unit area rather than per unit weight if the slices were cut as thin as possible, i.e. approximately 0.2 mm. thick. Since this approaches the limit of thinness of tissue-slices which are obtainable in practice with a free-handed razor, and consistent values were invariably found, it suggests that the maximum glyoxalase activity is closely approached in measurements on such thin slices. In the case of mouse tumours which generally contain necrotic patches, which on removal give healthy sections of irregular shape, the error introduced by measurement of the surface area must be very considerable, and hence the simpler procedure of weighing has been used throughout this work.

In Table I a series of typical results are collected.

Table I. *Glyoxalase activity of different tissues.*

Ringer's solution containing 0.025 M NaHCO₃ and 0.01 M methylglyoxal.
Temp. = 37.5°. Gas phase = 5% CO₂ in N₂.

Tissue	Time of measurement (min.)	Dry wt. of tissue (mg.)	Thickness of slice (mm.)	Surface area (both sides) (cm. ²)	Anaerobic glyoxalase activity (based on wt.)	Anaerobic glyoxalase activity (based on surface area)
Rat liver	60	2.08	0.21	1	101.0 → 76.2	210 → 158
	"	4.11	0.41	1	46.0 → 31.0	189 → 128
	"	6.20	0.62	1	31.3 → 27.6	194 → 171
Rat liver	45	2.01	0.2	1	67.8 → 46.1	136 → 92
	"	4.41	0.22	2	73.5 → 44.1	162 → 96
	"	7.48	0.25	3	61.2 → 48.2	152 → 119
Rat kidney	60	2.34	0.23	1	72.7 → 46.3	170 → 108
	"	2.18	0.22	1	76.1 → 40.2	166 → 87
	"	3.34	0.33	1	52.7 → 30.0	176 → 100
	"	5.92	0.59	1	36.1 → 19.4	214 → 115
Rat spleen	60	3.00	0.30	1	59.2 → 42.1	178 → 126
	"	3.18	0.32	1	54.0 → 40.9	172 → 131
	"	4.93	0.49	1	54.3 → 36.0	268 → 178
	"	6.01	1.20	0.5	16.7 → 11.0	200 → 132
Rat pancreas	45	7.09	—	—	6.9 → 5.9	—
	"	14.28	—	—	15.1 → 10.6	—
	"	12.55	—	—	9.7 → 7.3	—
	"	33.22	—	—	7.6 → 6.5	—
Jensen's rat sarcoma	60	2.01	0.20	1	40.5 → 38.1	81 → 76
	"	1.93	0.19	1	40.9 → 36.1	79 → 70
	"	4.30	0.43	1	20.1 → 16.1	86 → 69
	"	6.30	1.26	0.5	16.4 → 11.4	103 → 71
Jensen's rat sarcoma	90	2.06	0.21	1	34.1 → 29.6	70 → 61
	"	2.82	0.28	1	28.6 → 25.3	81 → 72
	"	3.85	0.38	1	21.6 → 17.3	83 → 66
Tar carcinoma 2146	30	2.06	0.21	1	41.9 → 38.3	87 → 79
	"	1.81	0.18	1	47.2 → 44.0	85 → 79
Crocker sarcoma	30	1.95	0.20	1	44.6 → 43.2	87 → 84
	"	2.01	0.20	1	41.8 → 40.4	84 → 81
37 sarcoma	30	1.96	0.20	1	40.7 → 30.5	80 → 60
	"	2.05	0.21	1	38.4 → 31.2	79 → 64
Glycogen carcinoma 113	30	2.56	0.26	1	30.9 → 26.2	79 → 67
	"	2.68	0.27	1	32.6 → 25.5	87 → 68
Adeno-carcinoma 27	30	2.62	0.26	1	38.3 → 29.1	100 → 76
	"	2.41	0.24	1	43.5 → 32.5	105 → 75
Tar carcinoma 173	30	1.89	0.19	1	28.2 → 25.6	53 → 48
	"	2.10	0.21	1	33.5 → 28.1	70 → 59

→ indicates that the values were not constant during the time of measurement, only the initial and final values being given.

In the case of normal tissues the degree of activity is of the order found by other workers and no comment is necessary except in the case of pancreas. This tissue cannot be satisfactorily sliced owing to its soft consistency, and the measurements were made on relatively thick masses, with the ratio surface area/weight of a low and incalculable order. Yet a very considerable activity is shown, the anti-glyoxalase obtained from extracts of pancreas apparently not functioning, or at any rate ineffectively when the cells are intact. In the case of tumour tissues Jowett & Quastel [1934] found the activity of glyoxalase to be of the same order as that of total glycolysis. This contrasts with the results of Platt & Schroeder [1934] who reported very low glyoxalase activity in Philadelphia sarcoma No. 1, and Walker carcinoma No. 256. The results with mouse and rat tumours included in Table I are in agreement with those of Jowett & Quastel, showing a high glyoxalase activity in every tumour strain measured, and moreover being almost identical with the high total glycolysis characteristic of transplantable tumours. Platt & Schroeder see no evident relationship between the high glycolysis of tumour tissue and the low glyoxalase activity which they found. If the rate of transformation of methylglyoxal into lactic acid determines the high rate of glycolysis in tumour tissues, the results given here are not inconsistent with the conception that glyoxalase participates in the glycolytic process.

A noticeable feature was the different behaviours in the change of reaction rate of normal and tumour tissues. Jowett & Quastel record a rapid fall in glyoxalase activity during incubation, and a similar effect was observed in these experiments with normal tissues. But tumour tissues, in most cases, retained approximately their initial activity for long periods, in two experiments up to 5 hours.

Experiments with irradiated tissues.

The technique of irradiation has been previously described [Crabtree, 1935]. In order to obtain effects as quickly as possible, mixed $\beta + \gamma$ radiation was used from two applicators each containing 110 mg. of $\text{RaBr}_2 \cdot 2\text{H}_2\text{O}$ spread over an area 23 mm. square. Radiation was applied at low and body temperatures for suitable periods, after which the respiration (in phosphate buffer), anaerobic glycolysis and glyoxalase activity were measured. Some typical results obtained with tumour tissues are collected in Table II.

Irradiation of tumour tissue at body temperature for 4–5 hours has no damaging effect on the glycolytic or glyoxalase systems. Irradiation at low temperature largely suppresses total glycolysis, but leaves glyoxalase activity unimpaired. Respiration of tissues irradiated at body temperature was not measured, since previous work had shown that effects on respiration are only detectable with certainty after the lapse of a longer period than 4–5 hours.

The dose required to produce a large inhibition of total glycolysis can be considerably lessened by the method of divided doses over 5 hours. With the radiation intensities available it has been found impossible to shorten the period during which this effect is produced, but applications of radiation during the first, third and fifth hours, with the tissue at low temperature throughout, produce an effect of similar magnitude to that following continuous irradiation. Platt & Schroeder [1934] emphasize the necessity of maintaining the co-enzyme glutathione in adequate concentration during measurements of glyoxalase activity in extracts. It is clear that the prolonged treatment *in vitro* with an irrigating artificial saline medium and heavy radiation have no effect on cell glutathione, since glyoxalase activity remains constant for many hours and is not enhanced by the addition of glutathione.

Table II. *Respiration, glycolysis and glyoxalase activity of tumour tissue after irradiation at low and body temperatures.*

Tumour	Time of irradiation t_r (hr.)	Temp. during irradiation ° C.	Control						Irradiated			
			Q_{O_2} (phosph.)		$Q_M^{N_2}$		Anaerobic glyoxalase activity		Q_{O_2} (phosph.)	$Q_M^{N_2}$	Anaerobic glyoxalase activity	
			Initial	After t_r	Initial	After t_r	Initial	After t_r				
Jensen's rat sarcoma	4	0-5	8.2	7.0	34.3	35.6	30.2	31.0	6.8	8.6, 11.2	28.3, 30.6	
"	4	"	8.0	8.2	29.3	26.4	27.6	28.3	7.6	7.1	25.9, 30.6	
"	5	"	10.2	8.9	38.9	35.6	27.9	29.3	8.0	5.1	28.0, 24.9	
"	5	"	9.3	9.6	30.6	31.0	26.0	29.0	7.9	6.0	30.1, 25.3	
"	5	"	6.4	7.0	37.0	35.1	30.2	26.8	8.0	3.4	24.0, 33.0	
"	5	"	11.8	9.0	34.0	30.2	24.0	30.2	9.2	8.3	28.4, 27.5	
"	5	"	10.0	9.1	36.0	33.0	32.3	30.0	8.7	4.9	35.0, 27.1	
"	4	37.5	—	—	41.2	42.0	26.3	28.2	—	36.0	26.8, 30.5	
"	4	"	—	—	36.3	33.1	34.7	30.6	—	29.3	28.6, 33.4	
"	5	"	—	—	30.2	31.6	33.5	26.0	—	29.4	26.5, 28.0	
"	5	"	—	—	40.4	35.4	35.8	30.0	—	35.1	30.0, 24.8	
Adeno-carcinoma 27	4	0-5	14.1	12.6	30.1	31.3	38.4	36.5	11.8	13.1	33.6, 30.2	
"	5	"	10.0	11.1	32.6	30.0	34.6	35.0	9.4	7.1	33.0, 28.9	
"	5	"	9.6	9.3	35.4	33.0	30.3	28.1	11.3	5.0	34.0, 30.9	
37 sarcoma	4	"	14.6	12.3	34.6	33.0	34.0	36.1	12.6	6.6	33.7, 33.8	
"	5	"	10.2	11.6	40.1	38.6	36.3	35.2	10.6	4.2	34.0, 35.1	
"	5	"	11.9	9.9	43.2	41.4	34.1	35.1	11.0	3.8	32.0, 36.8	

That the glyoxalase activity of tumour tissue is not exceptional in its insensitivity to radiation, is apparent from results obtained by irradiating some normal tissues. Table III shows that irradiation at low temperature with large doses fails to affect the respiratory system and has little effect on glyoxalase. In the case of liver, and to a less extent of testes, the experimental conditions alone cause a noticeable fall of glyoxalase activity. The added effect of radiation is negligible except in one case of kidney tissue irradiated for 6 hours. Whether the glyoxalase activity which then falls spontaneously in liver tissue can be restored by added glutathione was not tested.

Table III. *Respiration and glyoxalase activity of some normal tissues after irradiation at low temperature.*

	Time of irradiation at 0° C. t_r (hr.)	Control				Irradiated	
		Q_{O_2} (phosph.)		Anaerobic glyoxalase activity		Q_{O_2} (phosph.)	Anaerobic glyoxalase activity
		Initial	After t_r	Initial	After t_r		
Rat tissue							
Kidney	4	12.3	14.1	73.3	68.8	12.2	52.3, 46.4
"	4	14.1	12.3	59.6	63.6	11.1	54.5, 64.5
"	6	15.1	13.6	62.7	62.7	16.1	38.2, 34.1
Liver	4	6.9	6.9	65.2	40.6	6.5	36.8, 40.0
"	4	5.3	5.6	48.0	34.0	5.7	30.3, 26.4
"	5	7.0	6.5	51.0	30.3	5.3	26.4, 28.1
"	5	5.5	5.6	44.3	21.4	4.8	17.4, 25.2
Testes	4	9.3	9.0	27.6	20.5	9.1	19.6, 17.3
"	5	9.2	8.5	24.2	22.5	8.8	17.3, 14.8
"	6	8.6	8.7	23.8	18.4	8.2	15.0, 16.0

Some experiments were carried out with rat retinas to test if the above results with tumour tissues were true for another highly glycolysing tissue. This proved to be true, and it was found that retina was exceptionally sensitive to radiation. It is possible to obtain complete inhibition of glycolysis by irradiating retina at low temperature for only 2 hours, and 60-70% inhibition in one hour. This

represents the most rapid effect as yet demonstrated on any biochemical process in living tissue. It is technically almost impossible to irradiate retinas at body temperature and then draw conclusions as to metabolic changes. As was shown by Crabtree [1934] retina is exceptionally sensitive to the damaging effect of bicarbonate media at 37°, the rapid fall of respiration and glycolysis making experiments with radiation of questionable significance. At low temperature, however, no metabolic change is obvious after irrigation with bicarbonate media for periods up to 4 hours. The general result was the same as with tumour tissues, a complete suppression of the sensitive total glycolysis leaving the insensitive glyoxalase system intact. Respiration again suffered no damage. Some results are given in Table IV.

Table IV. *Changes in the metabolism of rat retinas after irradiation at low temperature.*

Time of irradiation (hr.)	Control			Irradiated		
	Q_{O_2} (phosph.)	$Q_M^{N_2}$	Anaerobic glyoxalase activity	Q_{O_2} (phosph.)	$Q_M^{N_2}$	Anaerobic glyoxalase activity
4	—	65.8	84.5	—	6.3, 11.6	76.3, 80.2
4	15.9	56.3	67.2	13.3	7.5, 6.0	61.8, 56.3
3	—	68.1	82.5	—	11.6, 8.9	74.6, 78.3
3	—	49.3	86.4	—	5.4, 7.1	80.3, 74.9
2	—	47.8	86.8	—	3.2, 9.1	92.0, 84.0
2	20.9	77.1	78.3	18.1	4.5, 9.4	80.1, 73.6
2	—	67.4	82.3	—	7.3, 3.1	84.0, 74.6
2	23.6	55.4	85.0	24.2	6.0, 4.3	80.0, 78.0
2	—	60.2	76.3	—	3.2, 5.7	69.3, 79.1
1	—	62.0	68.9	—	18.5→5.8	69.4, 73.5
1	19.4	68.4	73.2	17.3	25.6→3.5	70.1, 67.8
1	—	89.3	96.1	—	27.3→7.3	86.2, 89.0

→ indicates that the values were not constant over the time of measurement ($\frac{1}{2}$ hr.). Initial and final values only are given.

DISCUSSION.

It has been shown that it is possible by means of radiation to separate various biochemical mechanisms in cells. Respiration and glycolysis, two energy-yielding processes normally allied in a relationship determined by the Pasteur effect are differentially attacked, respiration being more vulnerable at body temperature, glycolysis at low temperature. Glyoxalase activity, until recently conjectured to be essential as a step in the breakdown of hexose to lactic acid, is unaffected by radiation which almost completely suppresses total glycolysis. The conception that radiation produces a generalized damage to cells, leading to slow degeneration, can no longer be held, and the analysis of the varying vulnerability of fundamental biochemical processes seems a hopeful method of approaching the clinical problem of selectively sensitizing tumour cells in the host. Some workers have utilized the findings of metabolic changes induced *in vitro* as a basis of attempts to destroy tumours *in vivo* more readily. Moppett & Harker [1936] on the basis of experiments by Crabtree & Cramer [1934, 1, 2] injected sub-lethal doses of cyanides into living animals bearing transplanted tumours, and claimed that this treatment enhanced the sensitivity of tumours to radiation. Franks *et al.* [1934] injected the maximum dose of iodoacetic acid which could be tolerated directly into transplanted tumours intact in the host and found that growth was inhibited and more regressions occurred, the inhibitions being more pronounced

when this process was combined with radiation. For cases of superficial tumours, it seems more hopeful to make use of the suppression of glycolysis at low temperature as a basis of differential attack on the tumour.

Since glycolysis is extremely vulnerable at low temperature it would be reasonable, by analogy with the known effects of other inhibitors, to anticipate an attack on some link in the chain of reactions ending in lactic acid. If methylglyoxal occupies the prominent position in this chain which has been attributed to it, it is clear from the results recorded here that some previous stage, possibly the reaction involving phosphorylation of hexoses or the splitting of the hexose molecule to the 3-carbon stage, is the primary point of attack of radiation under the conditions used. Attempts to demonstrate this have led to no conclusion. Hexosephosphates are metabolised at a low level by tumour tissues *in vitro*. After irradiation, no detectable effect on the rate of this reaction was measurable, the low values found by other workers [Boyland & Mawson, 1934; Harrison & Mellanby, 1930] persisting.

The separation by radiation of the glycolytic and glyoxalase systems in intact cells is unlike the simultaneous effects produced by the well-known chemical inhibitors on these systems.

Meyerhof [1925] found that the glyoxalase activity of brain tissue was largely checked when the medium was saturated with heptyl alcohol, and compares this with the total checking of glycolysis of tumour tissues by the same agent. Fluoride and iodoacetic acid check both systems in varying degrees. Dickens [1933] emphasized the difference in the concentrations of iodoacetic acid required to check glyoxalase activity in crude and purified liver extracts, the dialysed extracts requiring a much lower concentration, of the same order as that required to produce the effect in liver slices. Some unsuccessful experiments were made during the course of this work to test the possibility of separating glyoxalase activity and glycolysis with different concentrations of iodoacetic acid. Iodoacetic acid poisoning is a time reaction, and though the rate of checking of glyoxalase activity was perhaps a little slower than that of total glycolysis, concentrations of 2 to $5 \times 10^{-4} M$ brought both reactions to a standstill in 20 min., and lower concentrations were ineffective in separating the two systems during the experimental period.

Jowett & Quastel [1934] used the fact that various chemicals and pancreatic extracts inhibit glyoxalase action and glycolysis in a similar manner as a supporting argument in favour of methylglyoxal occupying a key position in the main glycolytic process. The experiments outlined here show that argument to be invalid, and would harmonize equally well with the scheme of carbohydrate breakdown postulated by Embden & Meyerhof in which methylglyoxal plays no part.

The rapid fall of glyoxalase activity *in vitro* observed by previous workers encouraged the hope that irradiation at body temperature, or in the presence of inhibitors of respiration, might adversely affect glyoxalase activity in a selective manner and lead to the reverse way of separating it from glycolysis, but this was not accomplished. Glyoxalase activity survived intact in tumour tissue under constant irrigation with various media and heavy radiation over many hours.

SUMMARY.

1. The anaerobic glyoxalase activities of normal and tumour tissues have been measured. All tissues, including pancreas, show a considerable activity, and in the case of tumours this is high, and of the same order of magnitude as anaerobic glycolysis.

2. Rat retina, irradiated at low temperature, is the most sensitive tissue so far examined. Suppression of glycolysis can be effected in 1–2 hours.

3. Total glycolysis and glyoxalase activity can be almost completely separated by irradiation at low temperature, since the enzyme glyoxalase is insensitive to radiation.

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