CLXXV. THE SUPPOSED EFFECT OF TUMOUR EXTRACTS ON GLYCOLYSIS¹.

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It has been stated by Waterman [1924, 1925], that tumour extracts accelerate the glycolysis of rat and mouse kidney. Such an effect on glycolysis of normal tissues would be of great significance, and we have therefore undertaken experiments to test this finding.

Waterman gives little experimental detail in his earlier paper; a manometric method was used. In this author's later work [1925] the experimental conditions and methods of measuring glycolysis were very different from those of Warburg [1924].

Employing Warburg's methods, of which we have already made use in another connection [1928], we have been unable to find any effect of tumour extracts on glycolysis.

EXPERIMENTS WITH TUMOUR EXTRACTS.

The tumour extracts were prepared in a similar manner to those of Waterman [1925]. Fresh, healthy tumour tissue was sliced, minced with scissors, and rubbed with sand and 0.16 *M* sodium chloride to form a suspension. After standing in the ice-chest the suspension was centrifuged, and the turbid supernatant fluid taken as the extract. Details of the extracts used are given in Table I. They are more concentrated than those of Waterman, who used 0.05–0.1 g. of tumour per cc. of saline, and extracted for 2–12 hours.

Table I. *Tumour extracts.*

Exp. No.	Tumour	g. tumour per cc. saline	Extraction time in hours
1	Flexner rat carc.	0.075	3½
2	" "	0.2	2
3	" "	0.3	3
4	" "	0.15	20
5	Human carc. groin	0.3	18
6	Rat sarcoma	0.14	2½
7	"	0.2	3½

In some of the experiments, the tissues of which the glycolysis was studied were used in the form of thin slices, as recommended by Warburg, in other

¹ This investigation was undertaken on behalf of the Liverpool Medical Research Organisation: Director, Prof. W. Blair Bell, of the University of Liverpool.

experiments the tissues were further cut up with scissors (as was done by Waterman). Where minced kidney was used, about 150 mg. of the tissue was suspended in 3 cc. of Ringer's solution in each manometric vessel, and to one or more of the vessels 0.3 cc. of the tumour extract was added. The same ratio of extract to Ringer's solution was kept in experiments where the tissue was in the form of slices, of which the wet weight varied from about 20 to 50 mg.

In measuring the effect of the extract on the glycolysis of tissue slices the technique was that described in our earlier paper. When minced tissue was employed the extract was usually added to some of the minces before measurements began. The glycolysis of the extract alone in Ringer's solution was also measured: this was small, but just measurable when the extraction time was brief (cells and cell fragments were present), and was subtracted from the glycolysis of the system (tissue + extract).

In Table II the percentage effects of the tumour extracts on the rate of anaerobic glycolysis of several rat tissues are given.

Table II. *Effect of tumour extracts on rate of anaerobic glycolysis of rat tissues.*

Exp. No.	Tissue	Form	Percentage effect of extract
1	Testis	Slices	- 2
2	Kidney	"	+ 1
3	"	"	- 4
	Spleen	"	0
4	Kidney	Minced	- 2
5	"	Slices	+ 10
	"	Minced	- 1
6	Spleen	Slices	+ 6
7	Kidney	"	- 5

The glycolysis of kidney tends to be irregular, both in amount and in rate of decrease with time. In all cases the effect of the extract on glycolysis lies within the experimental error.

EFFECT OF WASHING THE TISSUES ON THEIR SUBSEQUENT GLYCOLYSIS.

It was stated by Waterman [1925] that, if minced kidney tissue be washed twice with Ringer's solution followed by centrifuging, its glycolytic power disappears completely.

We have repeated the experiment. Waterman used 100 mg. of tissue and 2 cc. of a Ringer's solution. We washed 180 mg. of minced rat kidney twice with 4 cc. of Ringer's solution, and found that the rate of anaerobic glycolysis shown by the washed tissue was 93 % of that shown by the same weight of unwashed tissue. The difference from 100 % is within the experimental error.

Any other result would have been surprising, since, when working with Warburg's technique, a tissue slice which has been shaken in Ringer's solution for some time may be transferred to fresh Ringer's solution without appreciable effect on its glycolysis.

DISCUSSION OF METHODS.

Whilst we are unable to offer any individual explanations to account for the effects observed by Waterman but not observed by us, we venture to think that this author's experimental conditions were unsatisfactory.

Waterman incubated 3 cc. of a Ringer-kidney mince, with or without 0.3 cc. of a tumour extract, for 2 hours in a 50 cc. conical flask containing air. The aerobiosis may have been incomplete and glycolysis may have taken place to some extent, as this author supposed.

We have made one or two experiments under somewhat similar conditions. To about 150 mg. of minced kidney were added 3 cc. of a Ringer's solution, with a concentration of bicarbonate similar to that used throughout by Waterman (0.0027 *M*, instead of the 0.02 *M* used by us unless otherwise stated), and previously saturated with nitrogen containing 5 % CO_2 . The vessel containing the mince was bound to a manometer and an atmosphere of nitrogen (containing no CO_2) introduced. After having been shaken for 10 minutes in the thermostat the manometers were read, and allowed to remain stationary for nearly 2 hours; they were then shaken for 10 minutes before being read again.

In both these and Waterman's experiments the p_{H} is ill-defined, and the bicarbonate concentration too low for full glycolytic power to be manifested. In addition, with such a large weight of tissue and such a low bicarbonate concentration, the system will show retention of carbon dioxide owing to the buffering power of the tissue, as is the case for serum [Warburg, 1925]. Both the manometric and acid-titration methods will on this account give results which are too low under Waterman's conditions.

The values derived by us for the glycolysis under these conditions ($Q_{\text{CO}_2}^{\text{N}_2} = 0.2-0.3$) were about ten times smaller than those derived by Waterman under his not dissimilar conditions with the use of his acid-titration method. The p_{H} in Waterman's experiments may have been higher than in ours, but nevertheless the large difference in the results suggests that Waterman's titration method is in error. It appears, in fact, doubtful to what extent this method was properly tested and controlled. Thus, for instance, Ringer's solution seems to have been used as a "no glycolysis" control for a system containing tissue. It must be pointed out, however, that the bicarbonate content of the tissue should not be neglected when the Ringer's solution has a very low bicarbonate content.

Several experiments under the usual conditions of Warburg's technique, except in regard to the treatment of the tissue, showed that minced kidney usually glycolyses only at 30-50 % of the rate of sliced kidney, $Q_{\text{CO}_2}^{\text{N}_2}$ having values of about 2 instead of 4-6. This is probably due to mechanical injury of the tissue in mincing. Respiration appears to behave in a similar way [Minami, 1923]. Furthermore, the rate of glycolysis of minced kidney decreases more rapidly with time than does that of sliced tissue.

Thus we have reason to believe that Waterman studied the behaviour of tissues which were damaged, and under conditions much more remote from the physiological than those of Warburg.

When to these two facts is added the use of an inaccurate, and perhaps insufficiently controlled, method of measuring glycolysis, it seems unlikely that results so obtained have any application to physiological conditions. It should perhaps be mentioned that we [1928] have been unable to confirm Waterman's finding [1925, 1926] that calcium salts affect glycolysis, nor could Krebs and Kubowitz [1927], working in Warburg's laboratory, confirm Waterman's statements [1926] that various sera affect glycolysis.

SUMMARY.

Saline extracts of malignant tumours have no effect on the anaerobic glycolysis of normal rat tissues. Washing tissues with a suitable Ringer's solution does not affect their glycolysis. Experimental conditions are discussed.

ADDENDUM.

(*Note added November 10th, 1928.*)

Kraut and Bumm have recently claimed [1928] that extracts of tumours and other tissues accelerate greatly the rate of glycolysis of kidney tissue. In the only detailed experiment given (Table I) it is evident, however, that these authors, following Warburg's [1923] earliest and only approximate technique for measuring anaerobic glycolysis, have misinterpreted their manometric changes. Glycolysis is erroneously taken as given by the difference between manometric changes shown by tissue slices in Ringer's solution (*a*) with, and (*b*) without, glucose. Furthermore, in calculating this difference no allowance appears to have been made for the different weights of the tissue slices used.

No increase in glycolysis due to the tissue extract is present, although claimed, in the experiment discussed, in which moreover the manometric changes recorded seem so small that the results cannot be accurate.

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