CCXVII. GLUCOSE AND HEXOSEDIPHOSPHATE BREAKDOWN IN TUMOUR TISSUE

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THE work described in this paper was carried out as a preliminary to an investigation of the effect of X- and γ-rays on anaerobic glycolysis of tissues. In view of the interesting discovery made by Crabtree [1935] that glycolysis in tissues can be readily inhibited by radium treatment in the cold but is resistant to irradiation at 37°, it is plainly worth while trying to discover at what point in the reactions by which glucose is broken down to lactic acid the irradiation interferes. Anything that can be determined on this point must help to give precision, which is very much needed, to our ideas concerning the action of rays on cells and may contribute something to knowledge of the glycolytic process.

The recent work of Needham & Lehmann [1937] has once more brought into prominence the idea originally put forward by Ashford & E. G. Holmes [1929] and encouraged by the results obtained by Geiger [1935] that glucose breakdown can proceed along a path not involving phosphorylation and therefore not the same path as that followed by glycogen or hexosediphosphate. Needham & Lehmann were able to show definitely that in embryonic tissues there is a very active non-phosphorylating glucose breakdown and a very feeble phosphorylating mechanism dealing with glycogen and hexosediphosphate. This latter mechanism is defective in four distinct places, and added hexosediphosphate is converted into triosephosphate and accumulates as such. The glucose breakdown is activated in a specific manner by glutathione.

Boyland *et al.* [1937] have shown that when suitable methods are used, a large breakdown of hexosediphosphate to lactic acid can take place in extracts from tumour tissue. On the other hand, Mendel [1929] found that glyceraldehyde inhibited lactic acid formation from glucose in tumour tissue and it is known from the observations of E. G. Holmes [1934] that glyceraldehyde does not inhibit lactic acid formation from starch in muscle extracts.[1] There is therefore the possibility that tumour tissue possesses the mechanism for carbohydrate breakdown by both of these routes. The following experimental data support the idea that this is indeed so.

It cannot be claimed from the results of this investigation that the breakdown of glucose and the breakdown of hexosediphosphate by tumour tissue are two completely distinct processes, and that no part of the mechanism of breakdown is the same. The fact, that when chopped tumour tissue is used the hexosediphosphate glycolysis is generally much smaller than the glucose glycolysis, makes comparison difficult and makes it difficult to assess the importance of experiments which show a differential action of certain substances in accelerating or inhibiting the glycolysis of hexosediphosphate and that of glucose.

[1] This author also showed that glyceraldehyde had no effect on the breakdown of glucose in muscle extracts in the presence of hexokinase. In these circumstances the glucose is converted into hexosediphosphate and then broken down to lactic acid.

It is clear, however, that in some respects there are definite differences in tumour tissue between the catalytic system by which glucose is broken down and that by which hexosediphosphate is broken down.

Experimental

Boyland *et al.* used the Crocker mouse tumour for their experiments, as this tumour does not destroy adenylic acid and cozymase as quickly as do some others. The Crocker tumour was accordingly used in the following experiments except when otherwise stated. The glycolysis experiments were carried out in the Warburg apparatus in bicarbonate-CO_2 buffer at *p*H 7·4. The results were checked and confirmed by occasional chemical estimations of lactic acid.

Although it was shown by Needham & Lehmann that glucose breakdown is connected with cell structure, while hexosediphosphate breakdown proceeds in extracts, it was thought best in this case to use tissue for all experiments, in order that a direct comparison might be obtained. This is especially necessary for experiments with irradiation, as it is not satisfactory in our present state of ignorance to compare the results of irradiating extracts with those obtained when whole tissue is irradiated.

It was unfortunately seldom found possible to show hexosediphosphate glycolysis in tissue slices, so that tissue chopped with scissors had to be used. This must presumably be due to difficulty of approach of substrate to enzyme at some stage of the breakdown, so that damaged cells are more effective than whole ones. With the degree of chopping employed glucose glycolysis may be somewhat reduced, but is still very large.

The capacity of the tissue to glycolyse hexosediphosphate is very variable, and old slow-growing tumours are particularly lacking in it. It was found advisable to chill the tissue before chopping and to keep it cold throughout the chopping and measuring. Measurement was sometimes carried out by means of a cold tissue syringe rather than by weighing for this reason.

For the purpose of irradiating *in vitro* it is naturally necessary to keep the tissue for some time after the tumour is taken from the animal before it can be put into the Warburg apparatus, and some of the following experiments were done on tissue which had been kept for 4 hr. in the ice-chest, in order to determine the optimum conditions for glycolysis in such tissues. These experiments are included here as they gave some interesting results.

Cozymase and adenylic acid were always added to the medium in the experiments with hexosediphosphate. There are no indications in any of the experiments carried out here that cozymase and adenylic acid increased glucose glycolysis. This distinction between the two systems is not one that can be stressed, since there is probably always some cozymase and adenylic acid remaining inside the intact cells and the glucose can penetrate these intact cells. It seemed, however, worth making one experiment in which the two systems were directly compared. The tissue used had been kept for 4 hr. in the ice-chest. This treatment reduces glucose glycolysis to less than half its original level, so that it might be expected that any activating effect of cozymase and adenylic would be detectable. Plainly, an activating effect is only found in the case of hexosediphosphate glycolysis.

The figures given in Table I are total amount of CO_2 evolved; since chopped tissue is used and not slices, there was no object in using the $Q_{N_2}^{CO_2}$ notation. The weight of tissue used was nearly always 0·1 g. wet weight, and during the period mentioned in the tables there was no falling off in rate of CO_2 output.

Table I

Experiment lasting 1 hr. Total CO_2 output given (μl.)

Substrate	Without cozymase	With cozymase
Na hexosediphosphate	17·5	101·4
Glucose	130·7	136·2
	Without adenylic acid	With adenylic acid
Na hexosediphosphate	26·6	63·6
Glucose	80·4	83·0

The experiments with pyruvic acid and glyceraldehyde, these being substances which can presumably pass in and out of cells, are probably of more significance.

Experiments with pyruvate

Even when all precautions are taken the glycolysis of hexosediphosphate by fresh Crocker tissue is very variable in extent and after keeping the tissue for 4 hr. in the ice-chest this glycolysis is often entirely absent. It was found that if pyruvate were added to the medium, acid formation proceeded once more. The experiments reported in Table II were carried out with such tissue:

Table II

The amount of pyruvate used is 0·1 ml. of $M/2$ or $M/5$ in the 3 ml. of fluid in the cup. CO_2 evolved (μl.)

		Hexosediphosphate		Glucose	
Duration min.	Pyruvate alone	Without pyruvate	With pyruvate	Without pyruvate	With pyruvate
(1) 35	23	40·3	150	—	—
(2) 35	10	37	150	—	—
(3) 40*	16	67	120	—	—
(4) 50	—	69	98	83	92
(5) 80	—	18·5	44	70	66
(6) 60	—	37	101	134	147

* Fresh tissue.

It will be seen that the pyruvate affects the hexosediphosphate glycolysis only; it does not restore glucose glycolysis even when this has also fallen to a low level as a result of storage of the tissue in the ice-chest (see Exps. 4 and 5).

It seems that tumour tissue after it has been kept resembles embryo tissue in this respect. It has still the power of causing dismutation between triosephosphate and pyruvate (this is sometimes, not always, found in embryo), but it cannot form pyruvic acid at great enough speed. Probably, when pyruvate is not added, an accumulation of triosephosphate occurs, as Needham & Lehmann have described for embryo tissue. It has been shown by Lehmann [1935] that during the ageing of muscle extract some part of the system transforming phosphoglyceric acid into phosphopyruvic acid is easily lost, thus preventing the formation of lactic acid, and it may be that the same thing occurs, though far more rapidly, when tumour tissue is kept at 4°. The extra acid formed in the presence of pyruvate estimates as lactic acid (pyruvate is included in the controls in order to allow for the possibility that some of the pyruvate estimates as lactic acid). When fresh tissue is used and no pyruvate added, it appears that only part of the acid formed is actually lactic, suggesting that part may also be phosphoglyceric, formed by dismutation of 2 molecules of triosephosphate. The

exact details of these occurrences will be worked out in connexion with the irradiation experiments. Meanwhile the experiments with pyruvate suggest that there must be a difference between the glucose glycolytic system and the hexosediphosphate glycolytic system, even if it is only a difference in the relative speeds of reaction at the various stages of breakdown.

Two irradiation experiments which have already been carried out show that the production of lactic acid from hexosediphosphate with added pyruvate can still continue after a dose of $\gamma+\beta$ rays which greatly inhibits glucose glycolysis. The effects of *dl*-glyceraldehyde make an even sharper distinction.

Glyceraldehyde experiments

The known inhibitory effect of *dl*-glyceraldehyde on glucose breakdown could be demonstrated on chopped Crocker tumour, on Jensen rat sarcoma slices and on Walker carcinoma slices. In all cases the expected 80–90 % inhibition occurred; in the case of the Crocker tumour the glycolysis was slow because the tissue had been kept for 4 hr. in the ice-chest, and the inhibition by glyceraldehyde was 100 %.

It has not been possible to show that *dl*-glyceraldehyde has any effect on hexosediphosphate glycolysis.

Table III

	Amount of CO_2 evolved		
Duration min.	With hexosediphosphate	With hexosediphosphate + *dl*-glyceraldehyde 0·1 %	Remarks
(1) 90	50	52·7	—
(2) 60	44·5	49·3	—
(3) —	120	101·4	In this experiment pyruvate was present. Glycolysis without pyruvate and glyceraldehyde was 67 μl.

This confirms on tissue the work on muscle extract. The extra lactic acid formation caused by the addition of pyruvate is slightly cut down in the example given, but it is probable that this does not always happen and the effect is at all events slight.

Competition experiments

Experiments were carried out with glucose and hexosediphosphate together in order to see if there was any competition between these two substrates. The results are given below. Fresh tissue was used.

Table IV

	CO_2 evolved (μl.)				
Duration min.	No substrate	Glucose	Hexosediphosphate	Glucose + hexosediphosphate	Remarks
(1) 55	23·7	163·5	87	201·5	Pyruvate used to activate hexosediphosphate glycolysis
(2) 65	—	248	111	303	No pyruvate
(3) 30	7·5	41	25	57	No pyruvate. Done on small scale in small manometers on slices of mouse tumour 113

It will be seen that if the "no substrate" value is subtracted from each of the others, the glycolysis of hexosediphosphate and glucose together is not much smaller than the sum of the two separately. Since the origin of this small blank value is not known, it is impossible to say whether one can correctly do this. It is in any event plain that competition between the two substrates is slight, since there is certainly considerable summation although both systems are saturated (the concentration of glucose is 0·3 %, the concentration of hexosediphosphate is equivalent and the curves of CO_2 output are straight lines throughout the period mentioned in Table IV). This again, of course, suggests that the systems are, at least in part, distinct.

Glutathione experiments

It was typical of the glucose glycolytic system described by Needham & Lehmann that glutathione acted as a specific activator. The glutathione originally present was removed from the tissue brei by dialysis. It was not possible to carry out experiments of this sort on tumour tissue, as even the glucose glycolysis in this tissue seems to be exceptionally easily destroyed by keeping and it is impossible to prepare an active brei of this sort. A washed residue can be made, if the tumour is chopped, washed with distilled water, ground with sand, washed with saline and centrifuged down, which will still give a small glycolysis (about 1/5 or less of the original). On this washed residue glutathione will sometimes show an activating effect, giving 30–100 % increases in glucose glycolysis. The Jensen tumour was always used for this work, as the amount of glutathione originally present in it is small, but even with this tissue it is not at all easy to be sure that enough glutathione has been washed out, and the results are irregular. It was found to be better to use rather small amounts of glutathione (say 1 or 2 mg. in the 3 ml.) as larger amounts can have an inhibitory effect on tissue which already contains glutathione.

It is noticeable that both glycolytic systems in tumour tissue are readily destroyed by keeping, even in the ice-chest, particularly if the tissue is chopped.

One other experiment made with the washed residue is worth mentioning. It is sometimes possible to wash the residue sufficiently to remove all adenylic acid while still leaving some glucose glycolytic activity in it. This can be shown by the fact that phosphopyruvic acid is not dephosphorylated by the residue unless adenylic acid is added. There was not sufficient phosphopyruvic acid available to repeat this experiment many times but on one occasion the result was so striking that the figures are given here. The glycolysis was not increased by the addition of adenylic acid.

Table V

Glucose glycolysis during 1½ hr. of 0·3 g.	Percentage dephosphorylation* in 2 hr.
70 μl. CO_2	Without adenylic acid 6
	With M/1000 adenylic acid 53
	With M/100 adenylic acid 66·5

* The dephosphorylation is estimated by the amount of inorganic phosphate appearing. These estimations were carried out by Dr Lehmann.

It is not easy to secure that all the adenylic acid is washed away while still leaving a residue with sufficient glycolytic power, so that the experiment is not easily repeatable. On this occasion there was undoubtedly some glycolysis without transport of the phosphoric group from pyrophosphate to adenylic acid, and one other result confirms this.

SUMMARY

1. Chopped tumour tissue (Crocker mouse tumour) can form acid in the Warburg manometer from both glucose and Na hexosediphosphate.

2. The hexosediphosphate glycolysis is lost by keeping the tissue some hours in the ice-chest, but is restored by adding pyruvate. Pyruvate has no effect on glucose glycolysis under the same conditions.

3. *dl*-Glyceraldehyde inhibits glucose glycolysis but has no effect on hexosediphosphate glycolysis.

4. Some comparisons are made between glycolysis in the embryo and in tumour tissue.

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