XXIX. GLYCOLYSIS IN MUSCLE AND OTHER TISSUES.

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As has been pointed out by Meyerhof [1927], the rate at which glucose is utilised by the mammalian muscle enzyme system is very much smaller than that at which glycogen is broken down to lactic acid. This is true whether minced muscle or muscle extract be employed, but in the latter case the difference is much more marked; in fact it is commonly found that an extract highly active for glycogen is practically without action upon glucose in similar concentration. In presence, however, of an activator whose preparation from yeast is described by Meyerhof, such an extract acquires the power of dealing with glucose and other fermentable hexoses.

It is well known that certain tissues other than muscle, *e.g.* brain and kidney, habitually metabolise glucose with formation of lactic acid; this has been demonstrated in the case of brain by Warburg, Posener and Negelein [1924], Loebel [1925] and by Holmes and Holmes [1925 and other papers], and in the case of kidney by Irving [1927, 1928]. In view of such observations the interesting possibility arises that these tissues may contain an activator similar in nature to the substance which can be obtained from yeast. The work reported in this paper comprises a number of observations which arose out of experiments originally undertaken with the purpose of testing this view.

Preliminary results appeared to indicate that brain did contain a factor which could be extracted and which was capable of activating the system (muscle + glucose) with production of lactic acid; and the same seemed to be true, in lesser degree, for kidney. After a series of failures to obtain active extracts, however, it became apparent that the power of activation was dependent upon the presence of intact tissue in the so-called extracts; and when cell-free preparations were used, or when the tissues were finely ground during the process of extraction, in no case was any activating power demonstrable. This finding is in concordance with the state of affairs in muscle tissue itself, in that whole muscle is able to a certain extent to utilise glucose for the formation of lactic acid—a property which is in large degree lost on mincing, and almost completely lost in an aqueous extract.

It would appear then that at least in the instances of muscle, brain and kidney the first stage in the fermentation of glucose is a process involving intact cells, an observation which has been made by Irving for kidney tissue

and for red blood-corpuscles. Although there is no reason to suppose that the series of reactions leading to the production of lactic acid from carbohydrate is the same for muscle and other tissues, the fact that these other tissues are able to activate glucose in such a way that it can be attacked and fermented by the muscle enzymes supports the possibility that this first stage at least may be identical in all three cases.

Methods.

The suspensions of the various tissues employed were made, except where otherwise stated, by chopping the material finely with scissors and razor, after which the chopped mass was thoroughly mixed with water, or more frequently with Ringer's solution, in such a way that 5 g. tissue were equivalent to 30 cc. of suspension. After allowing the coarser particles to settle the suspensions were used without further treatment. At first these operations were carried out at 0°, but this was later found to be quite unnecessary and indeed to have a somewhat detrimental effect on the subsequent activity of the preparations. Fresh tissue was always employed, the brain and kidney being taken from the same rabbit as was used for the making of the muscle extracts. In the case of the kidney the cortex only was used.

Muscle extracts were made exactly as described in a former paper [Case and McCullagh, 1928].

Experimental tubes were set up as follows:

25 cc. muscle extract,
10 cc. 2 % glucose,
10 cc. phosphate buffer (as in former work),
5 cc. activating fluid (or water for controls).

The muscle extract was always added last of all; 15 cc. samples were then mixed with 45 cc. of trichloroacetic acid, and the remainder incubated for 2 hours in a water-bath at 27°. At the end of this period further samples were taken in the same way. After filtering off the precipitated proteins, 30 cc. of each filtrate were treated with 10 cc. of 10 % lime suspension and 5 cc. of 10 % $CuSO_4$ to remove interfering carbohydrates. Lactic acid was then estimated on 5 or 10 cc. of the copper-lime filtrate according to the method of Friedemann, Cotonio and Shaffer [1927].

Where phosphates were measured, the Briggs modification of the Bell-Doisy method was used.

Table I.

	mg. lactic acid in 15 cc. sample			
	Before incubation		After incubation	
	Schenk	Trichloroacetic	Schenk	Trichloroacetic
1.	5·7	5·9	18·0	18·8
2.	8·1	7·9	27·5	28·3
3.	11·0	12·2	23·5	23·6
4.	6·4	6·6	24·0	25·1

The method of deproteinisation by means of trichloroacetic acid indicated above has been checked against the more laborious Schenk procedure and has been found to give entirely satisfactory results. This is shown by the typical figures given in Table I. In each case the samples were taken from incubation mixtures of muscle extract, buffer and starch.

These are enough to show that the differences which exist between the values obtained by the two methods lie for the most part within experimental error.

Comparison of brain, kidney, etc. with yeast activator.

Table II demonstrates the production of lactic acid from glucose in presence of brain and of kidney. In tubes 5 and 6 the muscle extract had previously been heated at 80° for 5 minutes, so that these tubes are controls showing that the quantities of brain and kidney employed did not alone give rise to the formation of appreciable amounts of lactic acid.

Table II.

	mg. lactic acid in 15 cc. sample		
	Before incubation	After incubation	Change
1. Water control	8·5	9·2	0·7
2. Yeast activator	8·9	23·0	14·1
3. Brain	9·2	17·0	7·8
4. Kidney	8·6	15·2	6·6
5. Brain (+heated muscle) ...	8·0	8·0	0·0
6. Kidney (+heated muscle)	8·2	8·3	0·1

Various other tissues were next tried under the same conditions as brain and kidney. The results are given in Table III.

Table III.

	mg. lactic acid in 15 cc. sample		
	Before incubation	After incubation	Change
1. Water control	9·6	10·2	0·6
2. Brain	9·6	25·3	15·7
3. Kidney	9·1	18·9	9·8
4. Liver	9·5	11·3	1·8
5. Spleen	9·0	9·7	0·7
6. Testis	9·7	10·0	0·3
7. Lung	9·0	10·2	1·2
8. Muscle	10·0	14·2	4·2
9. Blood	9·9	12·8	2·9
10. Brain (+heated muscle) ...	10·1	10·9	0·8
11. Kidney (+heated muscle)	10·4	11·3	0·9
12. Blood (+heated muscle) ...	10·2	10·1	−0·1

Other than brain and kidney, the only tissues which here bring about activation are muscle, as would be expected, and blood. This latter is of interest in view of Irving's [1926] investigations on the glycolysis of red blood-corpuscles. The experiment was repeated, using a suspension of blood-corpuscles which had been centrifuged and washed with saline. The blood was obtained

from a rabbit killed by a blow on the neck, as it was feared that amytal might exert a disturbing influence.

Table IV.

	mg. lactic acid in 15 cc. sample		
	Before incubation	After incubation	Change
1. Water control	8·4	8·6	0·2
2. Blood-corpuscles	8·7	10·9	2·2
3. Blood-corpuscles (+heated muscle)...	8·9	9·2	0·3

The activation by the blood-corpuscles, though not very marked in extent, is nevertheless quite definite.

Table V gives typical results of experiments which were done to illustrate the deleterious effect of grinding and filtering, etc.

Table V.

	mg. lactic acid in 15 cc. sample		
	Before incubation	After incubation	Change
1. Water control	11·3	11·8	0·5
2. Brain chopped (+Ringer)	11·7	28·8	17·1
3. „ chopped (+water)	11·4	19·6	8·2
4. „ Ringer suspension centrifuged ...	11·7	14·1	2·4
5. „ Ringer filtered through muslin ...	11·9	17·7	5·8
6. „ ground lightly with sand	12·0	14·6	2·6
7. „ well ground with sand	11·8	12·0	0·2
8. Kidney chopped as usual	12·4	21·9	9·5
9. „ „ and centrifuged	11·8	12·5	0·7
10. „ ground with sand	12·0	11·7	−0·3

It is evident from this that the activation which is brought about by brain and kidney is not dependent upon a soluble or extractable substance, as is the case with yeast; for any process which destroys or removes intact cells has a corresponding effect in diminishing the activating power of the tissue.

Attempts which have been made to prepare alcoholic precipitations and to bring about separation by means of various other precipitants have all proved fruitless.

The effect of cyanide on the activation.

The glycolysis of brain tissue is known not to be affected by the presence of cyanide; in fact this substance is usually added to the buffer solutions used in investigations concerning brain carbohydrate metabolism, in order to prevent oxidation of lactic acid which otherwise takes place to a large extent. Nor does cyanide exert any influence on the production of lactic acid from carbohydrate by muscle extract.

On the other hand, it has been shown by Irving [1927] that the utilisation of glucose by kidney tissue can be completely inhibited by potassium cyanide in small concentration. It seemed of interest therefore to ascertain whether

cyanide would inhibit the activation of glucose + muscle by kidney tissue and not by brain.

First of all the following experiment was performed in order to confirm the fact that while the glycolysis of brain itself is not inhibited by cyanide, that of kidney is.

Tubes were set up thus:

1. 5 g. chopped brain +10 cc. 2 % glucose +20 cc. phosphate buffer
2. 5 g. chopped brain „ „ „ +*M*/500 KCN
3. 5 g. chopped kidney „ „ „
4. 5 g. chopped kidney „ „ „ +*M*/500 KCN

Table VI.

	mg. lactic acid in 15 cc. sample		
	Before incubation	After incubation	Change
1.	2·8	1·9	−0·9
2.	2·9	5·6	+2·7
3.	2·0	5·5	+3·5
4.	2·3	2·6	+0·3

Table VII presents the results of a typical experiment performed with the object of investigating the influence of cyanide on the activations of yeast, brain and kidney.

The incubation tubes were as follows:

1. 25 cc. muscle + 10 cc. glucose + 10 cc. buffer + 5 cc. H_2O
2. „ „ „ 5 cc. yeast activator
3. „ „ „ „ „ +*M*/500 KCN
4. „ „ „ 5 cc. brain
5. „ „ „ „ +*M*/500 KCN
6. (heated) „ „ „
7. (heated) „ „ „ +*M*/500 KCN
8. 25 cc. muscle „ „ 5 cc. kidney
9. „ „ „ „ +*M*/500 KCN
10. (heated) „ „ „
11. (heated) „ „ „ +*M*/500 KCN

Table VII.

	mg. lactic acid in 15 cc. sample		
	Before incubation	After incubation	Change
1.	5·5	6·4	0·9
2.	5·5	18·2	12·7
3.	5·6	19·0	13·4
4.	5·4	13·3	7·9
5.	5·5	14·0	8·5
6.	5·0	6·2	1·2
7.	5·1	6·5	1·4
8.	5·7	15·7	10·0
9.	5·6	15·4	9·8
10.	5·2	5·4	0·2
11.	5·3	5·4	0·1

From the figures in the foregoing two tables the following facts of importance emerge.

(*a*) Brain glycolysis is not inhibited by *M*/500 KCN.

(*b*) Kidney glycolysis on the other hand is prevented by this concentration of cyanide.

(*c*) The activation of the system muscle + glucose by either yeast, brain or kidney is unaffected by cyanide.

The conclusion must be drawn from this that the stage at which cyanide inhibits glycolysis by kidney tissue alone is one subsequent to the preliminary process of activation. This is what might be expected, in that there is no reason to suppose that the mechanism of activation involves any oxidative process.

The influence of fluoride.

It is well known that when fluoride is present in an incubated mixture of starch or glycogen and muscle extract, the breakdown of hexosephosphates but not their formation is prevented; consequently no lactic acid appears, and free phosphate rapidly disappears.

In the course of some experiments with yeast activator it was found that the addition of fluoride to the system (muscle + glucose + activator) suppressed not only the formation of lactic acid, but in addition the synthesis of hexosephosphate, so that no change in either carbohydrate, free phosphate or lactic acid was observable at the end of the incubation.

The effect of fluoride on the changes occurring in presence of brain and kidney was therefore tried, with the results shown in Table VIII.

The contents of the tubes were as follows:

1. Muscle + starch + buffer + 5 cc. H_2O
2. „ „ „ „ + *M*/50 NaF
3. „ glucose „ 5 cc. yeast activator
4. „ „ „ „ „ + *M*/50 NaF
5. „ „ „ 5 cc. brain
6. „ „ „ „ + *M*/50 NaF
7. „ „ „ 5 cc. kidney
8. „ „ „ „ + *M*/50 NaF

(The usual controls with heated muscle extract were performed, but are omitted for the sake of clarity.)

In addition to lactic acid determinations, free phosphate was estimated before and after incubation in 3 cc. of the trichloroacetic acid filtrate.

Table VIII.

	mg. lactic acid in 15 cc. sample			mg. P as free phosphate in 3 cc. sample		
	Before	After	Change	Before	After	Change
1.	7·8	28·2	20·4	0·82	0·77	−0·05
2.	7·8	8·1	0·3	0·83	0·12	−0·71
3.	7·9	25·9	18·0	0·94	0·62	−0·32
4.	7·8	8·2	0·4	0·94	0·92	−0·02
5.	8·0	17·5	9·5	0·85	0·80	−0·05
6.	7·9	7·6	−0·3	0·86	0·39	−0·47
7.	8·1	16·0	7·9	0·84	0·72	−0·12
8.	8·0	8·2	0·2	0·85	0·25	−0·60

This demonstrates that the action of fluoride, when muscle extract is utilising glucose in presence of brain or kidney, is similar to its effect in the case where muscle extract alone is breaking down starch or glycogen; *i.e.* the hydrolysis of phosphoric esters is prevented: whereas when yeast acti-

vator is employed, fluoride prevents even the synthesis of hexosephosphate from glucose.

The effect of phloridzin etc. upon yeast activation.

Dann and Quastel [1928] showed that the zymin fermentation of glucose was greatly retarded by phloridzin and the corresponding phenol, phloroglucinol; glucosides other than phloridzin did not exhibit this phenomenon.

It seemed conceivable that phloridzin and phloroglucinol might also exert this inhibitory effect upon the activation of glucose by yeast prior to glycolysis. If this were found to be the case it would be evidence that the first change undergone by glucose is the same in the cases of alcoholic fermentation and glycolysis. The following experiment however shows the entire absence of any effect of these substances. In addition to the usual contents, each incubation vessel contained 10^{-3} gram-molecule of the material under test.

Table IX.

	mg. lactic acid in 15 cc. sample		
	Before incubation	After incubation	Change
1. Water control	5·3	5·7	0·4
2. Yeast activator	5·5	26·2	20·7
3. „ + phloridzin	5·2	26·9	21·7
4. „ + phloroglucinol	5·7	27·4	21·7
5. „ + amygdalin	5·1	25·5	20·4
6. „ + salicin	5·5	26·0	20·5

From these results one must conclude either that the first or activating stage differs in the two processes or that the inhibition observed by Dann and Quastel occurs at some later period in the fermentation.

The effect of tumour tissue on muscle glycolysis.

It has been stated by Waterman [1924, 1925] and by Kraut and Bumm [1928] that cell-free extracts of tumours are capable of accelerating the glycolysis of certain normal tissues; these claims have however been criticised on various grounds by Brooks and Jowett [1928]. It was thought worth while to investigate the influence, if any, of such extracts upon the fermentation of glucose by muscle extracts.

Experiments which were carried out with the Rous chicken sarcoma indicated that, in common with the other tissues that have been tried, cell-free extracts of this tumour are not able to activate the utilisation of glucose by muscle extract; whereas suspensions of the comparatively uninjured sarcoma tissue do possess this power.

The following are typical of a number of experiments which were made:

1. Muscle + glucose + buffer + H_2O
2. „ „ „ + chopped tumour suspension
3. „ „ „ + „ „ centrifuged
4. „ „ „ + tumour ground with Ringer's solution in mortar
5. „ „ „ + „ „ „ and centrifuged
6. „ „ „ + tumour ground with sand
7. (heated) „ „ + chopped tumour suspension

The tumours were always obtained from recently-killed fowl. Lactic acid estimations before and after incubation are given in Table X.

Table X.

	mg. lactic acid in 15 cc. sample		
	Before incubation	After incubation	Change
1.	6·8	7·9	1·1
2.	7·2	28·0	20·8
3.	7·2	9·7	2·5
4.	7·2	13·5	6·3
5.	7·0	9·2	2·2
6.	7·0	8·3	1·3
7.	6·7	7·4	0·7

In this connection it is pertinent to note that, in common with other tissues, hashing of cancer tissue diminishes its glycolytic activity, while freezing, grinding or extraction destroys this property entirely [Barr, Ronzoni and Glaser, 1928].

Discussion and summary.

It is shown that in the presence of small amounts of brain and kidney tissue, a muscle enzyme preparation which alone is incapable of any appreciable degree of glycolysis is enabled to ferment glucose with production of lactic acid. This activation is compared with that brought about by addition to the system of the substance obtained by Meyerhof from yeast. Evidence is further adduced to show that, contrary to what occurs in the case of the yeast preparation, the activation of glucose by brain and kidney is associated with the presence of intact cell-structures. It is not suggested that this property is one specific for the tissues mentioned; these were chosen because they are among the tissues with the highest glycolytic activity, and it is probable that their activating power differs from that of other tissues only in degree.

The apparent correlation that exists between glycolysis and the presence of uninjured cells is in harmony with the views generally held concerning glucose metabolism. It is by no means certain that from any tissue a cell-free extract can be obtained which has the power of fermenting glucose, though at different times claims of this nature have been made; Stiven [1928], for example, has recently described the preparation of a cell-free extract of muscle which is stated to produce lactic acid from glucose. Even so, however, the fact remains that in the cases of all tissues that have been examined, partial or complete destruction of the cell-structure is concomitant with corresponding diminution in glycolytic power, from which the inference may be drawn that these structures are essential for at least a major part of the glycolytic activity possessed by the tissues.

A brief study of one particular type of tumour tissue, viz. the Rous sarcoma, has indicated that here also the intact tissue is necessary for the

activation of glucose, and it has not been found possible to extract any substance which possesses this property.

As to the nature and significance of the activation brought about by these tissues or by yeast activator, there is little that can be said. There is at present no explanation for the fact that a muscle extract which vigorously breaks down glycogen to lactic acid should be practically inactive when the substrate is glucose, other than the postulate that the hexose molecules which form the structure of the polysaccharide are present in some hypothetical "active" condition in virtue of which they are accessible to the muscle enzymes. A discussion of this aspect of the question cannot here be entered upon, but the subject is clearly relevant to a study of glucose activation.

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