LXXXV. CONTRIBUTIONS TO THE STUDY OF BRAIN METABOLISM.

V. RÔLE OF PHOSPHATES IN LACTIC ACID PRODUCTION.

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We have endeavoured to investigate the part played by inorganic phosphate in the production of lactic acid from carbohydrate by surviving brain tissues. Winterstein and Hecker [1923] have studied the phosphorus metabolism of the isolated spinal cord of the frog, and Gerard and Wallen [1929] have investigated that of peripheral nerve, both amphibian and mammalian. While, however, previous work had demonstrated the ready production of lactic acid by brain tissues, both *in vitro* [Warburg, Posener and Negelein, 1924] and *in vivo* [Holmes and Holmes, 1925, 1926, 1927], we believe that no serious attempt has previously been made to study the part played by phosphate in the process. It was natural to suppose that it would be a prominent one and would follow, more or less closely, the lines already established by Harden [1923], Embden [1926], Meyerhof [1926, 1] and many other workers, for the metabolism of yeast and of striped muscle.

This supposition has turned out to be erroneous; it appears that brain tissue possesses two mechanisms for the production of lactic acid, and that of these the one that has the greater quantitative significance is independent of the presence of phosphate.

Methods.

Rabbits were used for all experiments. They were killed by a sharp blow on the back of the neck, the brains excised as quickly as possible, and chilled by being placed in a glass basin surrounded by ice. When cold, they were freed from membranes and adherent blood-clot, chopped, and sampled. Portions weighing 0·5–2·0 g. were weighed out as rapidly as possible. For "resting" or "initial" values, the tissue was at once transferred to chilled 20 % trichloroacetic acid (B.D.H. "A.R."). It was ground with sand, after standing 1–2 hours or sometimes overnight, and filtered through a small filter-paper into a 50 cc. volumetric flask. After three further extractions with 10 % trichloroacetic acid, the whole process being spread over 1½ hours,

the contents of the flask were made up to 50 cc. with water. Phosphates were estimated by the Briggs [1924] method. "Phosphagen" therefore appeared as inorganic phosphate. For the estimation of lactic acid, 30 cc. of the filtrate were taken: the Van Slyke copper-lime precipitation was carried out, and the lactic acid estimated by the method of Friedemann, Cotonio and Shaffer [1927]. Blank distillations were regularly performed, the figures obtained varying between 0·1 and 0·3 cc. of 0·005 *N* iodine. Our yields against zinc lactate were 93 % [see Friedemann and Kendall, 1929].

To observe the effect of various substances on lactic acid formation and phosphate liberation, the chopped tissue was suspended either in Ringer's or buffer solution contained in a boiling-tube. This was closed with a rubber stopper, fitted with glass exit tube, rubber tubing, a clip, and sealed with paraffin wax. The tubes were immersed in a water thermostat at 37°. They were evacuated, and sometimes filled with nitrogen, but the presence or absence of nitrogen did not affect the result.

In many of the experiments quoted in this paper we have suspended the tissue in borate buffer. We have also carried out a number of experiments in which Ringer's solution was used as the suspension medium, and the results have been quite similar to those quoted. It also appears to be immaterial (so far as liberation of phosphate is concerned) whether strictly anaerobic conditions are maintained (as in these experiments) or whether the tissue is merely incubated in an open beaker. Both the maintenance of strictly anaerobic conditions and the use of borate buffer are, however, of importance with regard to experiments described later, and we have therefore, for the sake of uniformity and simplicity, quoted for the most part experiments where similar conditions prevailed.

We deliberately chose first to investigate the possibility of hexosephosphate formation from glucose rather than from glycogen. We were primarily interested in its rôle in connection with the production of lactic acid, and, while brain tissue produces lactic acid very freely from glucose, its production from glycogen is much less impressive.

Table I shows the liberation of free phosphate by chopped brain tissue at 37° under anaerobic conditions, and the effect of glucose on this process. The liberation of phosphate is a well-marked phenomenon, and it is inhibited, to a slight, but quite definite extent, by the presence of glucose. This fact might be interpreted as meaning that there is a certain formation of hexosephosphate from glucose by chopped brain tissue. The inhibition of phosphate liberation amounts to about 4 mg. per 100 g., and this would correspond to the formation of 24 mg. hexosemonophosphate per 100 g. tissue, in the space of 2 hours. Controls using boiled brain show no increase of lactic acid on incubation with glucose. Inorganic phosphate, however, increases—5 mg. per 100 g. in one case and 9 mg. per 100 g. in another. In each case the weighed sample was dropped into boiling buffer solution and subsequently placed on a boiling water-bath for some minutes.

Table I.

All values in mg. phosphorus per 100 g. fresh tissue. Anaerobic.

	No glucose					Glucose 0·4 %				
Exp.	Initial	Final	Difference	Time of incubation (hrs.)	Incubated in	Initial	Final	Difference	Time of incubation (hrs.)	Incubated in
1	48·0	62·0	14·0	2	Ringer	34·6	47·8	13·2	2	Ringer
2	41·6	55·0	13·4	2	,,	37·3	49·7	12·4	2	,,
3	43·0	67·0	24·0	2·25	,,	39·2	56·2	17·0	2	Borate buffer
4	40·8	60·6	19·8	2	Borate buffer	48·9	59·0	10·1	2	p_H 8·2
5	41·2	64·8	23·6	2	p_H 8·63	38·3	59·2	20·9	2	,,
	39·0	64·0	25·0	2	p_H 9·03	40·8	55·8	15·0	2	Borate buffer
						41·2	59·4	18·2	2	p_H 8·63
	31·0	63·4	32·4	3	p_H 8·63	31·0	54·6	23·6	3	,,
	44·2	68·5	24·3	3	,,	44·2	64·9	20·7	3	,,
						40·0	56·4	16·4	2	,,
	31·0	40·3	9·3	Boiled	,,					
	44·2	49·2	5·0	,,	,,					

The possibility of demonstrating hexosephosphate synthesis in this way depends, of course, upon the assumption that the breakdown of hexosephosphate is a slower process than its synthesis. Thus, Meyerhof [1926, 1, 2], using the muscle enzyme and starch or glycogen, was able to demonstrate a very definite disappearance of free phosphate during the early stages of incubation. Our failure to observe a more marked disappearance might, therefore, have been due to the fact that, after the lapse of 2 hours, the process of breakdown had outstripped that of synthesis. Table II shows two experiments where the liberation of free phosphate, both in the presence and absence of glucose, was followed at short intervals of time. There is, however, no evidence of esterification of phosphate. From these experiments, therefore, we have obtained, at best, evidence that may be interpreted as indicating hexosephosphate synthesis to only a very limited extent.

Table II. *Time of liberation of inorganic phosphate by brain tissue, with and without glucose.*

Mg. phosphorus per 100 g. fresh tissue. Tissue suspended in Ringer's solution.

	Time (mins.)	With glucose	Without glucose
1.	0	44·7	44·7
	10	53·4	—
	40	57·3	56·4
	80	70·2	72·8
2.	0	45·0	45·0
	10	49·1	46·7
	20	51·2	51·1
	40	56·8	48·6

Our next step was, therefore, to examine the effect of adding sodium fluoride to the preparations. Embden and Hayman [1924] were in this way able to demonstrate the synthesis of hexosephosphate by muscle from added

glycogen, and we hoped that we should be able in a similar manner to inhibit the breakdown of any hexose ester that might be formed from glucose.

We were at once struck by the very feeble inhibiting effect exerted, even by high concentrations of fluoride, on the liberation of free phosphate. In the presence of concentrations of fluoride, such as 0·05 *M*, the effect was only occasionally observed. Even with the highest concentrations, inhibition was never complete, nor was it constant from experiment to experiment.

We next observed the effect of different fluoride concentrations both on lactic acid production and on phosphate liberation. The results of such an experiment are shown in Table III. It is obvious that the two phenomena bear no quantitative relationship to each other; inhibition of lactic acid production is marked, and at concentrations of fluoride as low as 0·002 *M* it amounts to 64 %. The data in Table IV tell the same story; in addition, another point becomes clear. If the inhibition of lactic acid production depends on the formation of hexosephosphate, and its stabilisation by fluoride, then for every two molecules of lactic acid, the production of which is inhibited, a definite amount of phosphorus should change from inorganic to organic combination. This amount should be either one or two atoms, according as the mono- or di-phosphate is the substance involved. The atomic weight of phosphorus being 31, and the molecular weight of lactic acid 90, the amount of phosphorus disappearing—or, at least, prevented from appearing as inorganic phosphate—would accordingly be either one-sixth or one-third of the amount of lactic acid inhibited. Now it is perfectly clear that in no case does the inhibition of phosphate approach the figure required to account for the observed inhibition of lactic acid, even on the basis of monophosphate formation. This seems to make the conclusion inevitable that, in the case of lactic acid formation by brain tissue from glucose, the inhibition by fluoride must depend on some mechanism other than the stabilisation of hexosephosphate, which is that usually postulated to account for the inhibition occurring in other tissues.

Table III. *Effect of varying concentrations of sodium fluoride on inorganic phosphate and lactic acid production of chopped brain incubated anaerobically for 2 hrs. with 0·4 % glucose in borate buffer p_H 8·2.*

Mg. of phosphorus and lactic acid per 100 g. fresh tissue.

	Phosphorus	% inhibition	Lactic acid	% inhibition
Initial value	39·2	—	157	—
NaF 0·1 *M*	53·9	13	185	88
„ 0·02 *M*	56·2	0	191	85
„ 0·01 *M*	58·6	0	202	81
„ 0·005 *M*	59·0	0	222	72
„ 0·002 *M*	59·6	0	240	64
No NaF	56·2	—	391	—

So far, the only piece of evidence that we could bring forward in favour of hexosephosphate formation was the slight decrease in the amount of phosphate liberated when brain was incubated in the presence of glucose.

Our experience with fluoride certainly told heavily in the opposite direction. We tried to attack the problem, therefore, from another side, and endeavoured to demonstrate the formation of lactic acid by brain tissue in circumstances in which free phosphate had been eliminated as an active participant. We first tried, unsuccessfully, to render brain tissue free from phosphate by repeated washings with warm Ringer's solution. We next attempted to immobilise the free phosphate by addition of $CaCl_2$ at alkaline reaction. Two samples of chopped brain tissue were suspended in 4 cc. borate buffer, p_H 8·15, containing 1 cc. of 1·2 % $CaCl_2$ per g. tissue. They were allowed to stand for 30 minutes at room temperature. Glucose was then added to make the final concentration 0·4 %, and both samples were incubated for 2 hours, anaerobically. They were then removed from the incubator, and the lactic acid formation in one of them determined by the usual method. The other was centrifuged, the supernatant fluid, which was still turbid, was withdrawn, and the precipitate ground with sand and washed at the centrifuge with a little borate buffer; the washings were added to the fluid, and acid ammonium molybdate added in quantity slightly greater than that employed in the Briggs estimation. The fluid was rapidly filtered, and to the clear filtrate the remaining Briggs's reagents were added in proper proportions. In several such experiments either there was no development of colour whatever, or a very faint tinge of green appeared on long standing.

Table IV. *Effect of fluoride on liberation of phosphate by brain tissue.*

Mg. of phosphorus or lactic acid per 100 g. fresh tissue, incubated at 37° anaerobically.

Phosphorus								Lactic acid			
Incubation time (hrs.)	Amount glucose %	Initial	Final, no NaF	Final, NaF present	Inhib.	Concentration NaF	Suspension medium	Initial	Final, no NaF	Final, NaF present	Inhib.
2	Nil	41·6	55·0	52·4	2·6	0·1 *M*	Borate buffer p_H 8·2	—	—	—	—
3	0·4	41·7	59·2	49·9	9·3	0·5 *M*	Ringer	124	232	125	108
2	0·4	37·8	49·7	47·4	2·3	0·1 *M*	,,	131	332	133	199
2	0·4	34·6	47·8	39·7	8·1	0·1 *M*	,,	162	356	161	195
2	0·25	40·8	55·8	55·0	0·8	0·1 *M*	Borate p_H 8·63	132	430	143	287
2½	0·25	41·2	59·4	57·9	1·5	0·1 *M*	,,	136	331	144	187
3	0·25	31·0	54·6	45·8	8·8	0·1 *M*	,,	143	470	165	305
3	0·25	44·2	64·9	61·8	3·1	0·1 *M*	,,	129	330	134	196
2	0·25	40·0	56·4	53·1	3·3	0·1 *M*	,,	138	397	153	244

This was accepted as evidence that the preparation was free from soluble phosphate. The tissue was ground with sand, so there can be no question of phosphate being precipitated from the fluid medium, but remaining inside the cells, in soluble form; to assure ourselves on this point, in several experiments the tissue was ground up with sand before being incubated with glucose.

It will be seen from Table V that these preparations form lactic acid in amounts quite comparable to those formed in other circumstances; in other

words, lactic acid is still formed by brain tissue even when there is no inorganic phosphate present in soluble form. This seems to make it quite certain that there can be no intermediate formation of hexosephosphate as a step in the process. It is of interest that borates do not interfere with the formation of lactic acid from glucose by brain tissue, since it has been stated that they do prevent lactic acid production in muscle [Chrzaszezewski and Mozolowski, 1928].

Table V. *Lactic acid production by brain tissue from glucose, in medium rendered free from phosphate, in 2 hours' incubation at 37° anaerobically.*

Mg. lactic acid per 100 g. fresh tissue.

Procedure	Final lactic acid content	p_H of buffer
Tissue chopped	283	8·15
,,	233	8·15
Tissue chopped and ground with sand	240	8·15
,, ,, ,,	235	8·15
,, ,, ,,	218	8·15
Tissue chopped	260	8·89
Average initial value	130	

In an endeavour to clinch the point, we performed experiments to see whether the addition of extra phosphate to preparations previously rendered phosphate-free by the addition of calcium, would have any effect on the velocity of lactic acid formation. The procedure was as follows. Four samples consisting of equal weights of chopped brain tissue were put up in borate buffer at various p_H containing calcium (1 cc. 1·2 % $CaCl_2$ per g. tissue) as before. They were allowed to stand, sometimes at room temperature, sometimes in ice, for 30 minutes. To two of them were then added amounts of Na_2HPO_4 solution calculated to precipitate all the calcium (1 cc. 2·6 % Na_2HPO_4, $12H_2O$ per g. tissue) and to leave a definite excess of phosphate. The addition of the phosphate and precipitation of calcium naturally caused a shift in p_H towards the acid side. The two samples were therefore balanced against each other on a hydrogen electrode apparatus and the p_H of the sample to which phosphate had been added was brought back to its original value by the addition of alkali; the dilution which resulted being compensated by the addition of distilled water to the other sample. Glucose was then added to bring the concentration to 0·4 %, and all four samples were incubated anaerobically, two for 1 hour, the other two for 2 hours.

The results shown in Table VI are not completely regular, but they seem to show that the presence of phosphate causes only a very slight increase in the rate of lactic acid formation either in the first or second hour. The probable reason for this small effect of phosphate will be discussed immediately; meanwhile it appears that, so far as glucose is concerned, the part played by phosphate in lactic acid formation is negligible.

While there seems to be no doubt that the glucose-lactic acid mechanism is independent of the intervention of phosphate, we wished to explore the possibility of the existence of a separate mechanism for lactic acid formation,

involving glycogen and hexosephosphate, and going forward on a much smaller scale.

Table VI. *Velocity of lactic acid formation from glucose, (a) in the absence, (b) in the presence of phosphate. Incubated anaerobically.* p_H *of borate buffer as indicated.*

Mg. lactic acid per 100 g. fresh tissue.

		Phosphate absent					Phosphate present			
Exp.	p_H	Initial	End of 1st hr.	End of 2nd hr.	Formed in 1st hr.	Formed in 2nd hr.	End of 1st hr.	End of 2nd hr.	Formed in 1st hr.	Formed in 2nd hr.
—	8·73	137	157	190	20	33	163	193	26	30
—	8·89	126	175	256	54	81	179	270	58	91
70	8·83	140	156	228	16	72	181	255	41	74
71	8·83	124	203	245	79	42	213	246	89	33
72	8·83	126	182	215	56	33	188	232	62	44
75	9·03	128	164	189	36	25	161	221	33	60
—	9·03	138	173	201	35	28	185	204	47	19
Average		131	173	218	42	45	180·5	232	50·5	51·5

It is notoriously difficult to demonstrate the degradation of added glycogen by surviving tissues (as opposed to tissue extracts) even in the case of such organs as muscle, which are known to produce lactic acid freely from glycogen stored in the cells themselves. If chopped brain is incubated in buffer or Ringer's solution containing 0·4 % glycogen, very little more lactic acid is produced than by the brain tissue alone. This observation is in accordance with that of Loebel [1925]. Thus, the average initial value for lactic acid in brain tissue was found to be 112 mg. per 100 g., which rose to 136 mg. on incubation without glycogen, Meyerhof's method [1920] being used for lactic acid estimation. The rise in this case was 22 mg. per 100 g. Table VII shows that when glycogen is added in concentrations as high as 0·4 %, the increase is only 30 mg. per 100 g. The experiments (see Table VIII) on the inhibition of phosphate liberation by sodium fluoride when brain is incubated with glycogen show that the phosphate which fails to appear (a much larger quantity than is the case when glucose is added instead of glycogen (Table IV)) is more than sufficient to account for the observed inhibition of lactic acid formation by the stabilisation of hexosephosphate.

Table VII. *Production of lactic acid by brain tissue incubated with* 0·4 % *glycogen.*

Mg. lactic acid per 100 g. fresh tissue.

	Initial	Final	Medium
	122	162	Ringer
	137	193	,,
	138	146	,,
	130	143	Borate p_H 8·63
	126	142	,, 8·63
	125	144	,, 8·63
	125	183	,, 8·63
Average	129	159	

Table VIII. *Brain tissue incubated anaerobically with* 0·4 % *glycogen in Ringer's solution or borate buffer* p_H 8·63 *for* 2 *hours.*

All values in mg. per 100 g. fresh tissue.

Phosphorus (inorganic)				Lactic acid				
Initial	Incubated, no fluoride	Incubated, with 0·1 *M* NaF	Inhibition of P	Initial	Incubated, no fluoride	Incubated, plus 0·1 *M* NaF	Inhibition of lactic acid	Inhibition of P required to account for formation of diphosphate
37·8	50·7	38·5	12·2	122	162	131	31	10·1
45·0	66·4*	57·8	8·6	120	141	121	20	6·6
40·7	69·0†	57·5	11·5	126	163	141	22	7·3
40·7	68·5	57·5	11·0	—	—	—	—	—
39·0	62·5‡	51·0	11·5	126	162	134	28	9·3
39·0	61·0	50·8	10·2	126	172	129	43	14·0

*	Inc. without glycogen		70·0
†	„		75·7
	„	+NaF	68·3
‡	„		65·5
	„	+NaF	57·0

Table IX.

Lactic acid, mg. per 100 g. fresh tissue.

	Initial	Incubated with glycogen	Incubated with glycogen and $CaCl_2$ ("phosphate-free")	Incubated with glycogen, $CaCl_2$ and phosphate (phosphate)	p_H of buffer (borate)
	126	142	136	162	8·63
	125	144	129	144	8·63
	125	183	136	163	8·63
	130	143	127	134	8·63
Average	126	153	132	151	

Finally, we determined the effect of incubating brain tissue with glycogen, in the absence of soluble inorganic phosphate, and after the addition of Na_2HPO_4, just as was done in the case of glucose. The results, presented in Table IX show that, when soluble inorganic phosphate is "immobilised" by calcium, the production of lactic acid is completely inhibited, while the addition of excess of phosphate restores the power of lactic acid formation almost to its original condition. This offers a sufficiently striking contrast to the state of affairs which exists in the parallel experiments when glucose, and not glycogen, is the substrate, and it appears to offer an explanation of the slightly greater velocity of lactic acid formation in the presence of glucose when phosphate is replaced, for the tissue itself must be presumed to contain a certain amount of glycogen or hexosephosphate, or both, and the conversion of this into lactic acid would then be checked by the presence of calcium, and would proceed normally in the experiments in which phosphate was restored. We have on one occasion observed a production of lactic acid from sodium

hexosediphosphate. Under the same conditions a rise of 127 mg. per 100 g. was observed with glucose at the same hexose concentration, while the diphosphate gave a rise of 55 mg. per 100 g. (p_H 8·85 at 37° for 2 hours, anaerobically).

Lactic acid, therefore, can be produced by brain tissue in two distinct ways: from glucose, by some mechanism which does not involve the intervention of phosphate, and from glycogen, by a mechanism in which phosphate plays an essential part, and which is, presumably, akin to that already made familiar by studies on muscle.

Both processes are inhibited by sodium fluoride, and the production of lactic acid from glucose is evidently a mechanism which is very sensitive to the action of this substance. If, however, our findings are correct, this inhibition can no longer be explained on the lines at present current, namely the formation and stabilisation of a hexose ester, since no hexose ester is formed.

We have no certain knowledge of the origin or meaning of the phosphate production which occurs. Holmes and Holmes [1926] have previously shown that both a pentose and hypoxanthine can be demonstrated in brain tissue, and it seems clear that nucleotides play a part in the metabolism of nerve tissue, as they do in that of muscle [Embden, 1926; Parnas, 1929]. Gerard and Wallen [1929] have shown that pyrophosphates are present in nerve, while the possibility of phosphates arising from some of the many phosphorus-containing lipins of brain is almost unexplored. There is therefore no need, at present, to assume that phosphate liberation has necessarily any connection with carbohydrate metabolism.

The direct evidence that we can bring to bear on the question of the origin of the phosphate which is liberated is chiefly negative in character, and falls under four headings.

(1) The amount of pyrophosphate in brain tissue, as estimated by the method of Lohmann [1928] is small, and Table X shows that it can account for only a trifling amount of the rise in orthophosphate.

(2) A single experiment was performed with rabbit gut phosphatase prepared by the method of Kay [1928]. A preparation which was very active towards hexosediphosphate failed to cause any rise in inorganic phosphate when incubated with brain tissue.

Table X.

Pyrophosphate-phosphorus of brain, mg. P per 100 g. fresh tissue.

		Before hydrolysis (HCl)	After hydrolysis	Difference (pyrophosphate) P
Resting		47·5	53·0	5·5
Incubated	Glucose	54·4	58·4	4·0
3 hrs. p_H 8·85	No glucose	58·2	61·2	3·0
Ditto	Glucose	63·5	65·3	1·8
for 18 hrs.	No glucose	92·5	93·2	0·8

(3) Estimations of organic, acid-soluble phosphorus by incineration with H_2SO_4 and "perhydrol" show a change of the order, and in the direction, which would be anticipated from the small decrease in the amount of inorganic phosphorus liberated in the presence of fluoride as compared with that liberated in its absence both with, and without, glucose.

(4) Some experiments were performed on tissues which had been extracted with (*a*) acetone only, and (*b*) with acetone followed by ether. The ether was changed once a day for 14 days. These preparations have entirely lost their power of forming lactic acid; but Table XI shows that they had by no means lost their power of liberating inorganic phosphate. It will be seen from the table that the additional treatment with ether lowers both the "initial" and incubated values by about 20 %.

Table XI.

Mg. inorganic P per 100 g. tissue (wet).

In results it is assumed that 1 g. wet tissue = 0·2 g. dry.

Exp.		Acetone-dried	Acetone-ether-dried
1	Initial	58·2	47·5
2		65	—
3		62	61
	Incubated:		
1	2·5 hrs. at 37° with 2·5 % glucose, p_H 8·89	70	62
2	Ditto, p_H 8·43	82 with glucose 82 without glucose	65·5 with glucose 68·1 without glucose
3	3 hrs., p_H 8·43	75 without glucose 77 ,, +0·1 *M* NaF 79·5 + glucose 74·5 + ,, +0·1 *M* NaF	66 without glucose

Discussion.

Our experiments lead us to believe that brain contains two mechanisms for the production of lactic acid, one from glycogen, requiring the presence of phosphate, and therefore presumably following the same lines as in muscle, *i.e.* by way of hexosephosphate, and the other from glucose which is quantitatively more important, and does not require the presence of inorganic phosphate. In this connection it is of interest that Stiven [1929] has observed a much smaller degree of ester formation by muscle extract during lactic acid formation in the presence of glucose, than in the cases when glycogen was acting as a substrate. Case [1929] reports that muscle extract + brain suspension will produce lactic acid from glucose and that this process uses phosphate, in that inorganic phosphate disappears in the presence of fluoride. In this process it appears, therefore, that the glucose molecule undergoes some change prior to the hexosephosphate stage. The brain activator in some way renders the molecule accessible to the muscle enzymes, which are then capable of behaving towards it as they do towards glycogen. In contrast to this, we have shown that in the production of lactic acid from glucose by

brain alone, a hexosephosphate stage does not occur. In that case the glucose is broken down presumably after the intervention of the activator, through stages of which we have no knowledge at present, but not involving a hexosephosphate. Of the nature of the "activated" glucose, or as to how the activation is brought about, we are as yet ignorant.

SUMMARY.

1. Inorganic phosphate is liberated from brain tissue both anaerobically and aerobically, and in the presence as well as in the absence of glucose. No evidence of hexosephosphate synthesis has been found at any stage in the process of formation of lactic acid, although the tissue is capable to a small extent of performing this synthesis.

2. Both phosphate liberation and lactic acid production from glucose by brain tissue are inhibited by sodium fluoride, but, whilst the former is affected only by a high fluoride concentration, the latter is sensitive to very high dilutions of the salt. No quantitative relationship can be traced between the amounts of phosphate and lactic acid which are prevented from appearing by fluoride.

3. Lactic acid is freely formed from glucose, even when all available phosphate is immobilised. The velocity of lactic acid formation from glucose is not increased by the replacement of phosphate.

4. Much less lactic acid is formed from glycogen than from glucose; the process is inhibited by fluoride and by immobilising phosphate. It can be restored by replacing phosphate.

5. It is concluded that brain tissue possesses two mechanisms of lactic acid formation: one, involving glucose, is quantitatively the more important, and is independent of phosphate; the other is much smaller, involves glycogen, and depends on the availability of phosphate.

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