III. INHIBITION BY FLUORIDE OF GLUCOSE BREAKDOWN IN TUMOUR TISSUE AND RETINA EXTRACTS

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THE process by which glucose is broken down to lactic acid in animal tissue is not yet known in detail. The possibility still remains that the production of lactic acid proceeds, in some tissues, without the intermediate formation of phosphate compounds [Ashford & Holmes, 1929; Needham & Lehmann, 1937]. The following estimations of inorganic phosphate in tumour brei during glycolysis may therefore be of interest, although it cannot be claimed that they afford definite proof of a "non-phosphorylating" glucose breakdown in this tissue.

Fluoride was used in the ordinary way to prevent the formation of lactic acid and allow of the accumulation of phosphate esters. In the earlier experiments concentrations as high as $N/20$ or $N/40$ NaF were used, but experiments using $N/100$ or $N/200$ NaF were later carried out and gave exactly similar results. All experiments were done in the Warburg apparatus at 37° . A control manometer, in which no fluoride was present, was frequently included in order to determine the amount of lactic acid that could be made by the tissue in the conditions of the experiment. This gave a guarantee that the tissue was capable of active glycolysis and gave some indication of the amount of hexose ester that might be expected to accumulate in the presence of NaF.

Some experiments were carried out in N_2 and others in 95% N_2 + 5% CO_2 , the latter with the usual $NaHCO_3$ buffer. A single tumour was used for each experiment, obviously necrotic tissue being removed and 0.25 or 0.3 g. of tissue taken for each Warburg cup after chopping and mixing.

The figures given in Table 1 show that no disappearance of inorganic phosphate occurred with the tumours named in column 1 under anaerobic conditions

Table 1

Tissue and gas phase	Inorganic P (mg.)			Lactic acid (mg.)	
	Resting value	With glucose and NaF	No glucose no NaF	Resting value	With glucose no NaF
Tumour 113; N_2 + CO_2	0.68	0.67	—	0.38	1.12
Crocker tumour; N_2 + CO_2	1.82	1.82	1.95	0.70	2.0
Crocker tumour; N_2 + CO_2	1.72	1.74	1.86	0.9	2.64
Crocker tumour; N_2	1.39	1.54	1.58	1.32	3.89
Crocker tumour; N_2	1.60	1.51	1.62	0.97	3.3
Tumour 113; N_2 + CO_2	0.60	0.65	—	0.31	1.51
Crocker tumour; N_2	1.20	1.14	1.26	—	2.58

in the presence of glucose and NaF. Considerable amounts of lactic acid were formed by the control samples with NaF omitted (Table 1, columns 5 and 6), which suggests that, if phosphate esters were formed, they should have been present in large enough amounts for the disappearance of inorganic phosphate

to be easily detectable. These results are in agreement with those of Ashford & Holmes [1929], who found no evidence of any ester formation from glucose in brain.

The possibility had to be considered that glucose might have been phosphorylated by organic phosphate compounds which were not rephosphorylated under the conditions of the experiment and thus did not cause disappearance of inorganic phosphate. In order to test this as far as possible, control samples were subjected to the same treatment as the others, but were kept in medium containing neither glucose nor NaF. It might be expected that under these conditions and in an experiment lasting 2–3 hr. such organic compounds would break down and an increase of inorganic phosphate would be detected. It can be seen, however (Table 1, column 4), that such increases are always very small. If organic compounds exist in the tumour tissue which are capable of phosphorylating glucose they must be compounds which are not broken down by the tissue phosphatases. (The phosphate estimations were kindly carried out by Dr Lehmann.)

Some experiments were also carried out to compare the effects of very dilute NaF, $N/1000$, on the breakdown of glucose and of hexosediphosphate. The experiments were done in 0.26 % NaHCO_3 buffer in presence of 95 % N_2 + 5 % CO_2 and the production of acid was followed on the manometer. The inhibition of acid production was greater in the case of glucose, but when estimations of lactic acid were made at the end of the experiment it was found that all the acid formed from glucose was lactic acid whereas a considerable amount of the acid formed from hexosediphosphate was not lactic acid. It has been shown by Needham & Lehmann [1937] that in chick embryo even dilute NaF inhibits the change of phosphoglyceric acid into phosphopyruvic acid. The unknown acid, which was recorded in the manometer readings but could not be estimated as lactic acid, was, therefore, probably phosphoglyceric acid.

The figures are given in Table 2. They represent the total acid and lactic acid made from glucose and hexosediphosphate during the experiment, the resting value and the small amount of acid formed by the tissue without added

Table 2. *Lactic acid formed from glucose or from hexosediphosphate*

Amounts expressed in mg.

Exp.	Additions	Glucose	Glucose + NaF	H.D.P.	H.D.P. + NaF
(1)	Lactic acid found	0.30	0.21	1.1	0.30
	Acid calculated from CO_2	0.34	0.21	1.0	0.65
(2)	Lactic acid found	1.0	0.71	—	0.23
	Acid calculated from CO_2	0.95	0.83	0.98	0.80
(3)	Lactic acid found	0.9	0.57	0.6	0.20
	Acid calculated from CO_2	0.76	0.40	0.5	0.43
(4)	Lactic acid found	0.63	0.37	0.72	0.34
	Acid calculated from CO_2	0.45	0.33	0.78	0.53
(5)	Lactic acid found	0.38	0.20	0.85	0.36
	Acid calculated from CO_2	0.36	0.16	0.82	0.60
(6)	Lactic acid found	0.50	0.21	0.76	0.20
	Acid calculated from CO_2	0.38	0.23	0.68	0.50

substrate having been subtracted. The inhibition of lactic acid formation from hexosediphosphate is not complete and if the experiment is continued too long, i.e. after the production of acid as shown by the manometer readings has become very slow, this intermediate acid will not be found. No doubt after its further

formation has ceased the intermediate acid is slowly converted into lactic acid without significant change of pH or evolution of CO_2 .

The description of hexosediphosphate glycolysis in tumour tissue is given by Boyland *et al.* [1937] and also by Holmes [1937; 1939]. Mouse tumour 113, which was used in the experiments just described, is exceptionally active in this respect. Cozymase and adenylic acid must, however, always be added as activators. These activators were tipped into the main part of the Warburg flask just before taking the initial manometer reading. The values of acid measured manometrically and lactic acid estimated chemically should therefore have been in good agreement and in the flask without NaF were so. Lactic acid formation from glucose, which did not require the addition of activators, must have proceeded during the setting up of the experiment and, as expected, the manometric estimation of lactic acid was usually a little lower than the chemical estimation. The contrast with the figures given in the last column of Table 2 is marked.

The figures are all fairly small, since one tumour of this type yields only small amounts of living tissue. It should be pointed out that the evolution of a small amount of CO_2 produces a large excursion on the manometer of the specially small (vol. 6–7 ml.) Warburg apparatus employed. The amount of lactic acid present was ample for duplicate estimations by the method of Friedemann *et al.* [1927].

One possible explanation of the figures given in Tables 1 and 2 is that an early stage of glucose breakdown is inhibited by NaF, so that intermediate compounds are not formed and therefore not detectable. It is perhaps not possible to know from experiments with NaF whether phosphorylation of glucose does, in fact, occur. If it does, the process of phosphorylation must differ from that of glycogen phosphorylation in its sensitivity to NaF, since this can be used to detect the intermediate products of glycogen breakdown.

Extracts of retinae [Kerly & Bourne, 1940; Süllman & Vos, 1939] can form lactic acid from both glucose and glycogen. Preliminary experiments with such extracts suggested that, even in this case, glucose glycolysis was inhibited by lower concentrations of NaF than was glycogen glycolysis. Furthermore, it could be shown with these extracts as well as with the tumour brei, that when lactic acid formation was inhibited by NaF all disappearance of inorganic phosphate was also inhibited. On the other hand the finding of Süllman, that inorganic phosphate disappears from the extracts in the presence of glucose and without the addition of NaF, was confirmed. In such extracts lactic acid is rapidly formed and inorganic phosphate disappears. The addition of $N/200$ NaF inhibited both these processes. The results are given in Table 3 and include an experiment in which zinc was added as inhibitor. It had been shown by Lehmann [1939] that zinc does not inhibit the formation of Cori ester (glucose-1-phosphate) from glycogen by muscle extract. It is obvious that, in this experiment, it inhibited the formation of any hexose ester from glucose.

In Table 3 are included two experiments with tumour tissue, which showed that when glycolysis was wholly or partially inhibited by dilute NaF, the glucose which was not broken down to lactic acid remained as glucose. It is therefore not surprising that there is no disappearance of inorganic phosphate in similar conditions (Table 1). It seems that the only conclusion that can be drawn from these experiments with tumour and retina in the presence of NaF is that the first stage of glucose breakdown is particularly sensitive to this inhibitor. The work of Ashford & Holmes [1929] and Ostern *et al.* [1939] included similar experiments with NaF: brain and liver tissues were used; no disappearance of

Table 3

Exp.	Retina extract	Glucose mg.	Inorganic P mg.	Lactic acid (calc. from manometer readings) mg.
1	Resting value	3.3	0.60	—
	No inhibitor	0.73	0.31	1.2
	N/2000 ZnSO ₄	3.0	0.60	Nil
2	Resting value	2.72	0.34	—
	No inhibitor	0.74	0.0	1.2
	N/400 NaF	2.4	0.30	0.30
3	Resting value	4.5	—	—
	No inhibitor	0.74	—	2.1
	N/200 NaF	4.1	—	Nil
4	Resting value	—	0.51	—
	No inhibitor	—	0.23	1.75
	N/120 NaF	—	0.50	Nil
	Crocker tumour brei			
5	Resting value	2.3	—	—
	No inhibitor	1.36	—	0.81
	N/400 NaF	1.91	—	0.41
6	Resting value	2.09	—	—
	No inhibitor	1.03	—	1.2
	N/120 NaF	2.03	—	0.36

inorganic phosphate occurred in the presence of glucose and NaF. Here again, the explanation may be that the whole process of glucose breakdown was inhibited.

Süllman & Vos [1939] do not make any suggestion as to the nature of the phosphate compounds formed when inorganic phosphate disappears during glycolysis in retinal extracts without NaF. This matter is now under investigation. It is almost certain that some part of this phosphate is used in phosphorylating the adenylic acid which has to be added to the extracts as activator¹ but the fate of the rest of it is as yet unknown.

SUMMARY

1. Experiments with tissue from the Crocker tumour and mouse tumour 113 showed that no disappearance of inorganic phosphate occurred in the presence of glucose and NaF. No evidence could be obtained of phosphate ester formation from glucose.

2. It was found that the disappearance of glucose in these preparations was inhibited by the addition of NaF.

3. It was thus plain that the presence of NaF inhibited glucose breakdown at the first stage and prevented the formation of any intermediate compounds.

4. Experiments with extracts of pigs' retinæ gave similar results. The disappearance of inorganic phosphate which occurs during glucose glycolysis was inhibited by the presence of NaF, as was also the disappearance of the glucose itself.

5. Dilute NaF (N/1000) inhibited the formation of lactic acid from hexose-diphosphate by tumour tissue and caused the accumulation of some acid other

¹ On one occasion this occurred even in the presence of NaF. It led to disappearance of phosphate which was not accompanied by any disappearance of glucose. The phosphate could all be recovered from the adenylyl pyrophosphate fraction.

than lactic acid. This did not occur when glucose was used instead of hexose-diphosphate.

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