CXX. THE GLUCOSE METABOLISM OF KIDNEY TISSUE *IN VITRO*. II.

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IN a previous communication [Irving, 1927] it was shown that the disappearance of glucose from phosphate buffer in which chopped kidney cortex was suspended was markedly inhibited or completely stopped when the incubation was carried out under anaerobic conditions or in the presence of small amounts of HCN. In this paper, experiments are recorded which deal with the fate of the glucose utilised.

EXPERIMENTAL.

The technique employed was in all respects the same as that previously described. The kidneys were perfused till free from blood, and the cortex was cut off and chopped with a razor into small cubes which were well shuffled. In most experiments 2 g. were placed in 8 cc. of phosphate buffer, p_H 8.2. This suspension was then treated as described below. The tubes, which were incubated at 37.5° , were continually shaken by a mechanical device. The precautions as regards sterility were as described in the earlier paper. The glucose added was Kahlbaum's purest, and the sugar estimations were carried out by Hagedorn and Jensen's method. Incubation in no case exceeded 2 hours.

The writer had suggested previously that the glucose utilised by kidney tissue might be going, in part at least, to form polysaccharides. Further work has shown that this does not take place. Hydrolysis of the buffer solution in which the kidney cortex had been suspended after incubation with and without glucose showed small and equal rises in reducing power in both cases. Glycogen estimations undertaken on the kidney tissue before and after incubation showed that this substance fell in amount, and synthesis did not take place. The method of estimation used was shown to give quantitative figures on pure glycogen and also on glycogen added to kidney tissue. Typical figures are shown in Table I.

Table I.

2 g. kidney cortex. 8 cc. phosphate buffer. 8 mg. glucose. 2 hours' incubation at 37.5° .		
Glucose utilised (mg.)	Glycogen content (mg.)	
	Before	After incubation
5.52	1.36	1.06
10.0	1.91	0.96
None added	1.26	0.87

Lactic acid estimations were also undertaken. Clausen's method [1922] and that recently described by Friedmann, Cotonio and Shaffer [1927] were employed. The claims made for the latter method by the authors were found to be fully substantiated. Whereas Clausen's method gave only 85 % of the value on pure lactic acid, the newer estimation gave 97 to 100 % recovery.

After the necessary glucose samples had been taken, the entire suspension was cooled in ice and ground in an ice-cold mortar with cold tungstic or trichloroacetic acid. It was then made up to a known volume and centrifuged. An aliquot part of the supernatant fluid was saturated with ammonium sulphate and acidified with phosphoric or sulphuric acid. The lactic acid was then extracted with specially purified ether, either by using a continuous extractor as devised by Clausen or by shaking five times with three volumes of ether as described by Fletcher and Hopkins in their original paper. This method was found to give theoretical figures with pure lactic acid solutions, but slightly (about 8 %) less with kidney tissue. The necessary corrections have been made in all the figures quoted. The ether gave no blank value.

In the presence of glucose either ether extraction or the copper-lime procedure must be adopted. In the course of some work undertaken by Dr H. D. Kay and the writer, it was found, however, that protein-free kidney filtrates contain some substance which reduces permanganate very strongly, and which thus interferes with the estimations. This substance is still present after treatment with the copper-lime reagents, but is not soluble in ether. The ether extraction method is thus the only one applicable to the kidney.

The results obtained on the kidney suspension incubated with, and without, glucose were that the bulk of the sugar utilised was converted into lactic acid, positive thiophen tests showing that the figures obtained were not due to some substance simulating lactic acid. In order to confirm the results previously obtained, pairs of experiments were undertaken simultaneously on the same kidneys, one sample being aerobic, the other either anaerobic or in presence of HCN. In the case of the second sample, the glucose utilisation and the lactic acid production were always found to be greatly diminished. Typical results are given in Table II. In the cases shown, allowance has been made for the production of lactic acid from its precursors in the kidney tissue. In most cases it was found that the lactic acid maximum had been reached before incubation was begun. HCN, in the concentration present in the final estimation, was found to have no effect upon the lactic acid values.

It will be seen that the percentage transfer of glucose to lactic acid in each pair of samples was fairly constant, though it differed widely from experiment to experiment. This would suggest that the same processes were taking place aerobically, and to a less extent, anaerobically. The fact that with more rapidly acting kidneys the transfer was lower might be evidence that the lactic acid formed was being further oxidised, as shown by Meyerhof and Lohmann [1926], but experiments carried out to test this have given no decisive answer. The main conclusion which can be reached from these figures is that the formation of lactic acid from glucose was markedly inhibited under anaerobic conditions.

Table II.

2 g. kidney cortex.
8 cc. phosphate buffer.
10-15 mg. glucose.
2 hours' incubation at 37.5°.

Exp. no.	Control sample			Experimental sample			Conditions
	Glucose utilised (mg.)	Lactic acid formed, net. (mg.)	% formation of lactic acid	Glucose utilised (mg.)	Lactic acid formed, net. (mg.)	% formation of lactic acid	
1	13.88	7.01	51	8.18	5.33	65	Anaerobic
2	7.76	5.99	77	2.64	1.82	69	"
3	5.93	5.79	98	3.08	2.57	84	"
4	12.08	7.63	63	3.90	2.03	52	HCN <i>M</i> /500
5	7.95	2.06	26	2.78	0.99	36	"
6	4.24	3.79	90	1.84	1.88	102	"

Simpson and Macleod [1927] have found that under certain conditions the lactic acid produced in cat muscle is lower in amount than the glycogen disappearing, thus indicating the production of intermediate substances. Meyerhof [1927] has shown that during the degradation of carbohydrates by the muscle enzyme a relatively stable phosphoric ester accumulates, which he considers is hexosediphosphoric acid. On analogy with this, the possibility existed that in this case the excess glucose had become esterified, and experiments were undertaken to test this.

Owing to the small amount of tissue available, phosphorus estimations at the end of 2-hour periods only have so far been attempted. The kidney cortex was incubated in isotonic sodium carbonate solution at a p_H of about 8.3 in two tubes, one with, and one without, glucose. Samples for glucose estimations were taken and the whole suspension was then extracted as usual with 4 % trichloroacetic acid and centrifuged. Free phosphorus determinations were then made directly on one portion of the supernatant fluid by Briggs's method, and another portion was extracted for lactic acid determinations.

In all the experiments, a slight fall in the inorganic phosphorus content was found in the tube which had contained glucose, as compared with the control tube. In some cases the fall was sufficient to account for all the glucose not turned to lactic acid if it was assumed that hexosediphosphoric acid was formed, while in some there was still glucose not accounted for. This might be due to the formation of a mixture of mono- and di-phosphoric esters. Typical figures are given below.

Table III.

2 g. chopped kidney cortex.
8 cc. isotonic Na_2CO_3 solution ($p_H=8.3$).
8-10 mg. glucose.
2 hours' incubation at 37.5°.

Exp. no.	...	1	2	3
Glucose utilised (mg.)	...	4.35	5.78	2.78
Lactic acid produced (mg.)	...	3.82	4.85	1.74
Glucose unaccounted for (mg.)	...	0.53	0.93	1.04
Fall in free phosphorus (mg.)	...	0.19	0.22	0.11
Phosphorus required for hexose-diphosphoric acid (mg.)	...	0.18	0.27	0.36

It was also observed that the rate of utilisation of glucose in carbonate solution was as a rule slower than in phosphate buffer. It is thus very probable that organic phosphorus compounds act as intermediaries in this reaction.

The fact that this mechanism in the kidney differs so markedly from that in muscle is no doubt bound up with its special function as a secreting organ. The writer has as yet no evidence as to the part which this metabolic reaction plays in that function, but, as he is unable temporarily to investigate the subject further, it has appeared advisable to report the progress made.

SUMMARY.

Glucose is changed in large part to lactic acid by kidney tissue *in vitro*. A small amount is esterified with phosphoric acid. The utilisation of glucose and the production of lactic acid are strongly inhibited *in vacuo* or in the presence of HCN.

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