CCLXXIX. THE CARBOHYDRATE METABOLISM OF THE KIDNEY.

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THE chemical changes leading to the formation of lactic acid from carbohydrate in animal tissues have been extensively investigated. The evidence now seems to be fairly complete for the sequence of chemical reactions, embodied in the well-known scheme of Embden *et al.* [1933], leading to lactic acid formation in normal skeletal muscle; nevertheless it is believed by some that the older methylglyoxal theory is not yet entirely discredited and that it must be regarded as one which represents an alternative path of glycolysis in muscle. Lactic acid formation in frog heart has been studied extensively by Gaddie & Stewart [1934] and by others; Ashford & Holmes [1929] and Ashford [1933; 1934] have made similar studies using brain tissue. The conclusion reached was that brain tissue may form lactic acid in either of two ways, namely by the breakdown of glycogen with the intervention of phosphate and by the degradation of glucose without the participation of phosphate. The work of Jost [1934] on the formation of lactic acid in ox kidney brei led him to conclude that carbohydrates in the kidney are transformed into lactic acid by the same series of reactions as that postulated by Embden for muscle. Jost demonstrated the anaerobic formation of lactic acid in kidney brei from glyceraldehydephosphoric acid, from glyceraldehyde, from a mixture of glycerophosphoric acid and pyruvic acid and from a mixture of glycerophosphoric acid and phosphoglyceric acid. Under the conditions of his experiments pyruvic acid, when added alone to kidney brei, was reduced to lactic acid but the amount of the latter was much less than that formed from a mixture of pyruvic acid and glycerophosphoric acid. He was unable to decide whether the kidney possesses an alternative mechanism by which lactic acid may be formed from carbohydrate without the formation of intermediary phosphorylated compounds.

The present investigation was designed to throw some further light on the mechanism or mechanisms controlling lactic acid formation from carbohydrate by surviving kidney cortex. The paper deals with (a) the effects of fluoride, cyanide and iodoacetic acid on both lactic and phosphoric acid production in the excised kidney; (b) the glycogen content of the kidney; (c) the effect of arsenate on carbohydrate breakdown to lactic acid by kidney tissue; (d) pyruvic acid as an intermediary in kidney glycolysis.

Methods.

Normal male rabbits, averaging 2.35 kg., were used. Unless otherwise stated, the animals were killed by a sharp blow on the back of the neck, the kidneys were excised as quickly as possible, and the cortex was rapidly clipped off. Approximately equal portions (2–3 g.) of the tissue were dropped into the following weighed vessels. The time elapsing between the death of the animal and the "initial value" averaged 1–1.5 min. The experimental vessels were set up as follows:

I. "Initial value." 50 ml. weighing bottle containing 5 ml. cold 25% trichloroacetic acid. The lactic acid found in this mixture was termed the "initial value".

II. 50 ml. weighing bottle containing 5 ml. suspension medium.

III. 50 ml. weighing bottle containing 5 ml. isotonic saline.

The vessels were re-weighed and the weights of tissue calculated. Vessels II and III were then incubated at 37.5° for 2 hours without antiseptic.

Immediately after samples Nos. II and III had been placed in the water-bath, the contents of No. I were finely ground with sand. The thin brei was transferred quantitatively to a 25 ml. cylinder, made up to known volume (15 or 20 ml.) with 6 % trichloroacetic acid and filtered through a Whatman No. 30 filter-paper into a small flask which was stoppered tightly and placed in the refrigerator.

At the end of the incubation period samples II and III were removed from the water-bath and 2 ml. of 25 % trichloroacetic acid added to each. The contents of each tube were then treated as described for sample I. The three filtrates thus obtained were analysed for lactic and phosphoric acids.

The lactic acid was extracted with ether as follows. To 5 ml. of the protein-free filtrate in a 50 ml. glass-stoppered cylinder were added 2.5 g. of ammonium sulphate, 2 ml. of 85 % phosphoric acid and five volumes of purified ether; the contents of the cylinder were shaken vigorously for 5 min. The ethereal layer was drawn off with a 15 ml. rubber-bulb pipette and filtered through a Whatman No. 50 paper into a 500 ml. flask. The above process was repeated five times; the stopper, pipette, filter-paper and funnel were washed with small portions of ether which were added to the main extract. Two ml. of 10 % sodium hydroxide solution were then added and the ether was distilled. After removal of the last traces of ether from the flask by aeration, the residue was dissolved in water and the solution made up to volume in a 50 ml. flask.

Lactic acid estimations were then carried out in duplicate on 15 ml. of this solution according to the method of Friedemann *et al.* [1927]. The concentration of sulphuric acid was reduced and that of manganese sulphate increased in accordance with the suggestion of Friedemann & Kendall [1929].

The colorimetric method of Briggs [1922] was used to determine inorganic phosphorus in the trichloroacetic acid filtrates. Total acid-soluble phosphorus in these filtrates was estimated by the method of Kay & Byrom [1927] and the precautions emphasized by Kay [1931] were carefully observed.

EXPERIMENTAL.

It was decided in the beginning to investigate the effect of sodium fluoride on lactic acid production and on phosphate liberation in the excised kidney, since at that time (1932) it was agreed by most workers in the carbohydrate field that, for skeletal muscle at least, the inhibition of lactic acid formation by sodium fluoride was due to the stabilization of hexosephosphate.

It was found that lactic acid production was completely inhibited by $M/5$ fluoride but there was no marked inhibition of phosphate liberation. Moreover Kay (private communication) has shown that the rate of breakdown of the naturally occurring phosphoric ester in the kidney is entirely independent of that of the production of lactic acid. These observations support the view that the precursor of lactic acid in the kidney is a non-phosphorylated compound. In this connexion it is of interest that Ashford & Holmes [1929] and Ashford [1933; 1934] have shown that while phosphates may play a part in the formation of lactic acid from glycogen, the major portion of the lactic acid production of surviving brain tissue is independent of the intervention of phosphate.

Experiments were next performed to determine the effect of cyanide on the two processes and it was found that $M/100$ cyanide at pH 7.0 has no appreciable effect on phosphate liberation and lactic acid production.

The effect of sodium iodoacetate at pH 7.0 on the liberation of inorganic phosphate and on lactic acid formation in the kidney was next studied. Lohmann [1932], Jowett & Quastel [1933] and Dickens [1933] have shown that in skeletal muscle iodoacetate inhibits glyoxalase activity by interacting with glutathione which functions as the coenzyme of glyoxylase. Since this enzyme is known to be present in both skeletal muscle and kidney and since both of these tissues possess the ability, under certain experimental conditions, to convert hexosephosphate into methylglyoxal, it was reasonable to suspect that lactic acid formation normally proceeds by way of methylglyoxal and that iodoacetate blocks glycolysis at the methylglyoxal stage. However the well-known experiments of Lohmann who demonstrated the formation of lactic acid from glycogen by muscle extracts devoid of glutathione revealed the possibility of another series of chemical changes, representing at least an alternative route, leading to the formation of lactic acid in muscle. The changes embodied in the scheme of Embden *et al.* [1933] represent such a route. It has been shown [Meyerhof, 1935] that iodoacetate inhibits the oxidation of triosephosphate to phosphoglyceric acid by yeast preparations; furthermore Embden & Deuticke [1934] observed that muscle poisoned by bromoacetate is no longer able to convert hexosediphosphate into a difficultly hydrolysable ester or to transform pyruvic acid into lactic acid even in the presence of glycerophosphate as hydrogen donor. They concluded that the halogenacetic acids may inhibit glycolysis by preventing these oxidation-reduction transformations.

The results of several experiments are set out in Table I. It will be seen that iodoacetate (pH 7.0) stops all lactic acid formation in the excised kidney. This drug has, however, no significant inhibitory effect upon the liberation of phosphate. These observations are in conformity with the view that the main path of carbohydrate degradation to lactic acid by kidney tissue is one which does not involve the participation of inorganic phosphate. It is of interest to note that in every case except Exp. A of Table I the lactic acid content of the tissue decreased during incubation in the presence of iodoacetate. These results are in accord with those of Haldi [1932] who noted a disappearance of lactic acid in kidney tissue incubated in the presence of iodoacetate, but not in brain tissue under comparable conditions. A loss of lactic acid in excised muscle was

Table I. *Effect of iodoacetate on phosphate liberation and lactic acid production by kidney cortex incubated at 37.5°.*

All values are expressed as mg. per 100 g. of freshly chopped tissue.

	Time of incubation hours	Free P				Total acid				Lactic acid			
		A	B	C	D	A	B	C	D	A	B	C	D
Tissue plus 25% trichloroacetic acid "Initial value"	0	28	43	29	33	112	119	96	114	78	101	72	89
Tissue plus sodium iodoacetate* (pH 7.0)	2	81	104	64	91	123	130	101	133	82	67	51	52
Tissue plus isotonic saline	2	80	104	71	86	125	132	124	134	127	—	125	152

* Concentration of iodoacetate in Exps. A and B, 0.01 M ; and in C and D, 0.02 M .

observed by Lundsgaard [1930] and by Meyerhof & Himwich [1924] who attributed the loss to oxidation. The observed loss of lactic acid in kidney cortex might be likewise explained.

In search for the source of lactic acid, the first which suggested itself was glycogen. A review of the literature relating to the glycogen content of this organ has been made by Irving [1928]. Irving determined the glycogen content of rabbit kidney cortex but views his own results with suspicion, inasmuch as he believes that they are affected to a large extent by non-sugar substances. It was necessary, therefore, to reinvestigate the problem.

Estimation of glycogen.

The estimations of the glycogen content of the kidney cortex were made on material obtained from normal male rabbits anaesthetized with amytal. 45 mg. of amytal per kg. of body weight were dissolved in 3 ml. of water and injected intravenously. When anaesthesia was complete, one kidney was exposed without undue handling and carefully removed. Approximately 3 g. of cortex were rapidly clipped off into a weighed 15 ml. pyrex centrifuge-tube equipped with a rubber stopper and a straight glass condenser and containing 2 ml. of hot 60 % potassium hydroxide. The time taken for this operation averaged 18 sec. After the tissue had been thoroughly mixed with the alkali the tube was returned to the boiling water-bath. The second kidney was treated similarly.

After 30 min. in the water-bath the tubes were cooled, re-weighed and returned to the bath for a total of 3 hours, after which they were removed and allowed to cool slightly but not to form a gel. Absolute alcohol was added until the final concentration was 66 %. The vessels were stoppered, shaken gently and allowed to stand overnight in the refrigerator. The material was centrifuged at 2500 r.p.m. for 15 min. and the supernatant fluid was carefully removed from the precipitate with a fine capillary pipette attached to a pump. The precipitate was washed by triturating with 66 % ethyl alcohol saturated with sodium chloride, and, after centrifuging, the supernatant solution was removed. This process was repeated until the supernatant fluid was clear, 3 to 4 washings being sufficient.

The glycogen was hydrolyzed in the same tube with 12 ml. of 2.2 % hydrochloric acid for 3 hours at 100° and, after cooling, the solution was neutralized with 40 % sodium hydroxide. It was rendered just acid with hydrochloric acid, filtered into a 25 ml. volumetric flask and, after rinsing the centrifuge tube and filter-paper with water, made up to volume. Dr G. A. Grant kindly estimated the fermentable sugar in the hydrolysate, employing the procedure of Harding & Grant [1933]. This eliminated all errors due to non-carbohydrate reducing substances which appear to be relatively high in kidney tissue.

Six determinations of the glycogen content of the kidney cortex of different animals yielded results equivalent to 9, 10, 11, 11, 26, 28 mg. glycogen per 100 g. fresh tissue. The right and left kidneys of the same animal contained equal amounts of glycogen.

Table II shows the glycogen, free phosphorus, total acid-soluble phosphorus and lactic acid contents of kidney cortex before and after incubation for 2 hours at 37.5°. It is clear that the low glycogen content of the cortical tissue is insufficient to account for more than a very small portion of the lactic acid formed in 2 hours at 37.5°.

The formation of lactic acid from several carbohydrates and the effect of arsenate on the process were investigated, with the hope that further information might be obtained regarding the precursor of lactic acid. Ashford [1933] showed that the rate of glucose breakdown by brain tissue was entirely unaffected by the

Table II. *Glycogen, free phosphorus, total acid-soluble phosphorus and lactic acid contents of kidney cortex before and after incubation for 2 hours at 37.5°.*

All values are given in mg. per 100 g. freshly chopped tissue.

	Free P	Total acid-soluble P	Lactic acid	Glycogen
Tissue plus 25% trichloroacetic acid "Initial value"	29	87	40	—
Tissue plus isotonic saline	65	98	127	—
Tissue plus absolute alcohol "Initial glycogen value"	—	—	—	9.0
Tissue plus isotonic saline "Final glycogen value"	—	—	—	0.24

presence of arsenate. On the other hand it has been known for many years that arsenate accelerates the rate of breakdown of hexosediphosphate by yeast preparations; moreover, recent investigations of Pett & Wynne [1934] have supported the suggestion that the stimulating effect of arsenate ions on the liberation of inorganic phosphate from hexosephosphate is exerted, not directly on the phosphatase system, but rather on the glycolytic system. The weighing bottles were set up as follows:

I. Tissue plus 5 ml. of the carbohydrate solution.

II. Tissue plus 5 ml. of the carbohydrate solution plus arsenate.

III. Tissue plus 5 ml. of isotonic saline.

Fresh solutions of Pfanstiehl monosaccharides were prepared on the morning of each experiment. From the barium salt of synthetic glucose-6-monophosphoric acid, kindly presented by Dr P. A. Levene, a solution of the sodium salt was prepared by treatment, at 80°, with the calculated amount of sodium sulphate. The mixture was placed in the refrigerator overnight and the next morning, just before using, was centrifuged for 35 min. The supernatant solution was carefully poured off and tested for freedom from barium ions. Barium hexosediphosphate, prepared from yeast, was ground in a mortar with the calculated amount of sulphuric acid in dilute solution; the mixture was centrifuged and the solution of the sodium salt was poured off and tested for freedom from barium ions. Before using, it was adjusted to pH 7.0 with solid disodium hydrogen phosphate.

The colorimetric method devised by Pett [1933] was employed for the estimation of inorganic phosphate and total acid-soluble phosphate in the presence of arsenate ions.

The results in Table III show that glycolysis in kidney tissue is very marked and is practically unaffected by the presence of $M/500$ arsenate. Fructose is much more slowly converted into lactic acid than is glucose. Kidney tissue is apparently unable to convert mannose and galactose into lactic acid, either in

Table III. *Percentage conversion of carbohydrates (0.1%) into lactic acid by kidney cortex in 3 hours at 37.5° in the presence and absence of 0.002 M arsenate.*

Glucose	Glucose + arsenate	Fructose	Fructose + arsenate	Mannose	Mannose + arsenate	Galactose	Galactose + arsenate	Glycogen	Glycogen + arsenate
58	64	14	15	0	0	0	0	51	37
49	63	27	27	4	0	0	13.6	43	41
52	49	18	16	2	1	0	0	20	21
63	62	—	—	—	—	—	—	27	31
44	50	—	—	—	—	—	—	—	—
61	60	—	—	—	—	—	—	—	—

the absence or the presence of arsenate. Glycogen gives rise to lactic acid but the amount formed is smaller than that produced from glucose.

Other compounds tested were hexosediphosphate and glucose-6-monophosphate. The results with hexosediphosphate were somewhat variable but they suggest (Exps. 3 and 4, Table IV) that added hexosediphosphate can, at least partially, be transformed into lactic acid. Glucose-6-monophosphate gave variable results (Table V). The irregularity of the results with the hexosephosphates is difficult to explain; in no case was the amount of extra lactic acid formed as great as in the glucose experiments.

Table IV. *Percentage conversion of hexosediphosphate into lactic acid by kidney cortex in 3 hours at 37.5°.*

Hexosediphosphate added in each case was equivalent to 5 mg. lactic acid.

Experiment	1	2	3	4	5
% conversion	0	4	13	17	0

Table V. *Phosphate liberation and lactic acid production from glucose-6-monophosphate in the presence and absence of 0.002 M arsenate.*

Phosphorus values are given in mg. per 100 g. freshly chopped tissue.

	Time in hours	Free P					Total acid soluble P					% conversion of added ester to lactic acid					
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	F
Tissue plus isotonic saline	2	69	65	73	88	83	93	113	113	131	123	—	—	—	—	—	—
Tissue plus 5 ml. hexosemono- phosphate = 5 mg. lactic acid	2	84	80	89	104	93	125	122	144	164	146	0	7	45	5	4	41
Tissue plus 5 ml. hexosemono- phosphate = 5 mg. lactic acid plus arsenate. Total concen- tration of arsenate, M/500	2	—	—	—	106	89	—	—	—	155	159	—	—	—	10	9	—

It is of interest to note that even when the tissue was incubated for 4 and 5 hours with hexosemonophosphate, no lactic acid in excess of the relatively small amounts produced during 2-hour incubation periods was observed.

The results, while variable, tend to substantiate the view that the greater portion of lactic acid produced by the cortical tissue of the kidney arises from glucose by some pathway independent of phosphate. It is not unlikely, however, that the small amount of glycogen in the kidney is broken down to lactic acid via hexosediphosphate.

On incubating cortical tissue with glucose and hexosediphosphate it was found that the latter did not appreciably increase the rate of lactic acid formation from glucose. Meyerhof [1918] showed that the induction period frequently observed on incubating maceration extract of yeast with sugar could be abolished by adding a small amount of hexosediphosphate. However, no such catalytic effect of this substance on the formation of lactic acid in either muscle or kidney has been observed.

The formation of lactic acid from pyruvic acid.

Sodium pyruvate solution (pH 7.0) in a concentration of 0.1 M and in the presence of rabbit kidney cortex was observed to give rise to appreciable amounts of lactic acid in excess of that formed in control experiments. As in the other experiments, no special precautions were taken to ensure anaerobic conditions. The results supplement those of Jost [1934] whose experiments were conducted anaerobically.

DISCUSSION.

The work of Jost [1934] suggests that under anaerobic conditions ox kidney brei converts carbohydrates into lactic acid by the same series of reactions as those postulated by Embden for skeletal muscle. Definite evidence of an alternative path was not obtained by Jost. The present experiments indicate that in rabbit kidney there may be an alternative system of glycolysis in which, at any rate under aerobic conditions, phosphate is not primarily involved. In support of this view are the following observations. (1) Lactic acid production and liberation of inorganic phosphate appear to be independent phenomena. (2) Hexosediphosphate and glucose-6-monophosphate fail to yield uniformly positive results with kidney whereas they are converted by muscle into lactic acid. (3) Arsenate does not appreciably accelerate the rate of lactic acid formation from carbohydrates by rabbit kidney. In view of the fact that Meyerhof [1927] and Meyerhof & Lohmann [1927] have shown that arsenate increases the rate of lactic acid formation, by fresh extracts of rabbit muscle, from glucose in the presence of hexokinase, from hexosediphosphate, from the Robison ester and from polysaccharides, the present results suggest that glycolysis in muscle and in kidney may normally proceed by different routes.

SUMMARY.

1. Sodium fluoride, in the concentration used, completely inhibits lactic acid formation in the excised kidney. No appreciable inhibitory effect is noted on phosphate liberation. The inference is that the two mechanisms are entirely independent.
2. The mechanisms controlling the liberation of phosphoric acid and the production of lactic acid are not significantly affected by $M/100$ cyanide.
3. Sodium iodoacetate inhibits lactic acid formation in kidney cortex. This drug has, however, no appreciable effect upon the liberation of inorganic phosphate. A loss of lactic acid is observed in the cortical tissue after incubation for 3 hours at 37.5° in the presence of iodoacetate.
4. The total glycogen content of the cortical tissue is not sufficient to account for the lactic acid formed when such tissue is incubated for 2 hours at 37.5° .
5. Kidney cortex produces large amounts of lactic acid from glucose, smaller amounts being formed from glycogen and even smaller quantities from fructose. Galactose and mannose give negative results. The results with hexose-mono- and diphosphates are variable.
6. Arsenate ions do not accelerate glycolysis in the cortical tissue of the kidney.
7. Pyruvic acid is readily reduced to lactic acid by kidney cortex and may, therefore, function as an intermediary in glycolysis.

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