CLXXXVII. A NOTE ON THE LACTIC ACID AND GLYCOGEN CONTENT OF KIDNEY CORTEX.

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DURING the course of work already described [1928] some interesting observations were made on the metabolism of the rabbit's kidney which appeared to justify further investigation. The present experiments have been undertaken with the object of comparing the initial values of the glycogen and lactic acid content of the cortex with those obtained after the tissue had been incubated. The methods employed and the results obtained are described below.

EXPERIMENTAL.

*Glycogen*¹. The method of estimation used was a micro-modification of that of Pflüger. 2 g. of kidney tissue were heated in a small centrifuge tube at 100° with 2 cc. of 60 % potassium hydroxide for 2 hours. At the end of that time, the tube was cooled and 6.2 cc. of absolute alcohol and a pinch of NaCl were added; the precipitate, usually fairly bulky, was centrifuged down, and the supernatant fluid decanted and discarded. The precipitate was then usually teased up in a small amount of 70 % alcohol with a thin glass rod, the end of which was ultimately broken off into the suspension to avoid any loss. The precipitate was again centrifuged down, and the supernatant fluid poured away. 6 cc. of a 2.2 % hydrochloric acid solution were now added and the tube was heated in a boiling water-bath for 4 hours. The contents were then neutralised and filtered, and the filtrate was made up to 20 cc.; sugar estimations were carried out on 2 or 5 cc. by the method of Hagedorn and Jensen.

The following table shows the degree of accuracy of this method on pure glycogen and on glycogen added to kidney tissue.

Table I. *Estimation of pure glycogen.*

	mg.	mg.
Amount taken	0.95	1.77
Amount recovered	0.91	1.71

Recovery of added glycogen from kidney cortex.

	mg.	mg.	mg.
Glycogen content of 2 g. cortex	1.36	0.90	1.26
Glycogen content of cortex plus added glycogen	2.33	1.70	1.93
Glycogen added	0.95	0.82	0.75
Glycogen recovered	0.97	0.80	0.67

¹ The writer is indebted to Prof. C. Lovatt Evans, F.R.S., and to Dr W. K. Slater for much useful information on the properties and estimation of glycogen.

The use of one tube for the whole estimation avoided what had been found a frequent source of error, namely, adhesion of the precipitated glycogen to the walls of the flasks previously employed. The figures given below all express glycogen in terms of glucose.

Lactic acid. The lactic acid was extracted and estimated by a similar routine to that previously described [1928]. A weighed amount of cortex (usually about 2 g.) was ground with sand in a mortar containing 5–7 cc. of 6 % trichloroacetic acid as protein precipitant. The resulting suspension was made up to 10 or 15 cc. and centrifuged; lactic acid was extracted from 5 cc. of the supernatant fluid with ether and the estimation was carried out by the method of Friedmann, Cotonio and Shaffer [1927].

Fig. 1. The production of lactic acid and free phosphate in incubated kidney cortex.

The initial values were obtained as follows. The rabbit was killed by a blow on the back of the skull, and the left kidney, as being the most accessible, was dissected out as rapidly as possible and dropped into freezing mixture at -15° . The time taken by this operation varied from 22 to 36 seconds. After the kidney had frozen solid, one-half of the cortex was removed and placed in a cooled weighed beaker containing 5–7 cc. of frozen trichloroacetic acid, and the rest of the cortex was dropped into a cooled weighed centrifuge tube containing 1.5 cc. of frozen 60 % potassium hydroxide. In both samples the cortex was mixed as thoroughly as possible with the frozen acid and potash. After the vessels had been again weighed, that containing the sample for glycogen estimation was plunged into boiling water and, after the tissue had disintegrated, more potassium hydroxide if necessary was added. The lactic acid sample, still frozen solid, was transferred from the beaker to a cooled

mortar and rapidly ground with sand. When the hash had completely melted it was transferred to a small cylinder and made up to the requisite volume, the procedure being then as described above.

The final values were obtained from the remaining kidney. This was removed from the animal immediately after the left kidney had been placed in the freezing mixture. Slices of the cortex were put in a small stoppered weighing bottle and incubated at 37.5° for 2 hours. The procedure was then as described above.

In order to show that the above technique was justified, final values were determined on the right and left kidneys of the same animal. The results in Table II show that for this purpose the two kidneys appear to be identical.

Table II. *Comparison of the final values of lactic acid and glycogen of the right and left kidneys.*

	Right	Left
Glycogen (mg. per 100 g.)	37	34
Lactic acid (mg. per 100 g.)	142	146

RESULTS.

The production of lactic acid. During the work previously described [1928], it was frequently noted that the production of lactic acid was a matter of some rapidity and it was soon found that the size of the initial value depended entirely on the speed with which the kidney was removed from the body. In this respect this organ closely resembles muscle; Exp. 7 in Table III and Fig. 1, the latter the result of an experiment undertaken with Dr H. D. Kay, clearly illustrate this fact. The initial values obtained are shown in Table III but it is very improbable that these represent the true resting lactic acid content: this value must in the normal kidney be but a mere trace [cf. Davenport and Davenport, 1927].

The final values obtained after incubation were usually fairly constant, varying between 130 and 160 mg. per 100 g., provided that comparable techniques as regards the handling of the animals were used. Thus Exp. 5 is of interest as showing the effect of ether anaesthesia and the resulting muscular activity on, presumably, the blood-lactic acid.

Glycogen. A perusal of the literature has shown that little is known concerning the glycogen content of the kidney. Its occurrence in this organ was first noted by Claude Bernard, and a few writers [e.g. Erlich, 1883; Paschutin, 1884] also identified it by histological methods. More recently Brown and Greene [1918] reported its presence but gave no figures. Fichera [1904], however, found none in normal tissue, and small amounts only after phloridzin or experimental glycosuria. Junkersdorf and his co-workers [1924, 1925] are, to the writer's knowledge, the only authors who quote quantitative results, but they worked solely with phloridzinised dogs. The values they reported varied between 39 and 180 mg. per 100 g.

The values obtained by the present writer are given in Table III. It will be seen that the initial figures show a lack of constancy and that, with one exception, the differences between the initial and final values are small, or else no difference exists. Since it is not unlikely that these small differences are due to flaws in the technique, it may be doubted whether the values obtained represent glycogen at all. The glycogen content of other tissues (*e.g.* muscle [Simpson and Macleod, 1927]) falls very rapidly under similar conditions; the behaviour of kidney-glycogen, however, appears to resemble more closely that of brain [Holmes and Holmes, 1926].

Table III. *Lactic acid and glycogen content of kidney cortex before and after incubation at 37.5° for 2 hours.*

Exp. no.	Lactic acid (mg. per 100 g.)		Glycogen (mg. per 100 g.)		Lactic acid increase (mg. per 100 g.)	Glycogen decrease (mg. per 100 g.)
	Initial	Final	Initial	Final		
1	68.5	161	103	57	92.5	46
2	60	129	102	84	69	18
3	82	170	40	44	88	0
4	45	132	50	40	87	10
5	145*	233	33	38	88	0
6	52	142	68	51	90	17
7	133†	144	—	—	11	—

* Animal anaesthetised with ether. Kidney frozen *in situ* with ethyl chloride.

† Experiment to illustrate the effect of slowness in removing kidney.

Of greater interest is the evidence derived from a comparison of these figures with those obtained for lactic acid. The discrepancy which exists between the lactic acid produced and the glycogen changes observed suggests that (with the exception of Exp. 1) very little if any lactic acid comes from this source: the remaining acid presumably being derived from some non-glycogen precursor.

In seeking for other precursors of lactic acid, the first which suggested itself was free sugar. The easiest way to test this was to make use of a fact already described [Irving, 1927] that kidney tissue after freezing and subsequent thawing has completely lost its power of degrading glucose. Experiments were therefore carried out as follows: the left kidney was removed as quickly as possible and frozen, while the right kidney was removed at the same time but not cooled. The left kidney, when frozen solid, was cut in half; the cortex from one half was treated as usual for the initial value, while that from the other half was incubated for 2 hours. The cortex from the right kidney was simultaneously incubated as a control. The results which are shown in Table IV indicate that a small part of the lactic acid formed may have its origin in glucose, but the major part comes from some other precursor.

Table IV. *Lactic acid content (mg. per 100 g.) of normal and previously frozen kidney cortex before and after incubation at 37.5° for 2 hours.*

Exp. no.	Initial	Final	
		Frozen cortex	Normal cortex
1	51	115	145
2	49	108	—

The most probable of these might be supposed to be a lactacidogen-like substance; but, as stated by Kay [1928], experiment has shown that such an origin is extremely improbable, the rate of breakdown of the naturally occurring phosphoric esters appearing to be entirely independent of that of the production of lactic acid. This is shown in Fig. 1. It is of course possible that the large and unrelated changes seen in the free phosphate content of incubated kidney tissue may obscure possible changes in free phosphate connected with lactic acid production, but at present no decisive answer is available.

In conclusion, it is interesting to speculate on the significance of the rapid production of lactic acid which occurs on removal of the kidney from the body. The similar changes which occur in muscle under these conditions are the deranged chemical transformations which are normally associated with contraction. To what process in the normal kidney this chemical change is related is as yet completely unknown; but there can be no doubt that the rapid production of lactic acid in the excised kidney must represent the first onset of autolysis and may be but a caricature of any process in the normal organ. It is of interest to note, without in any way implying a teleological argument, that McCance [1924] has found that the optimum reaction for certain autolytic changes is in the neighbourhood of p_H 6.0.

The production of lactic acid from preformed compounds in the kidney, on analogy with the changes in muscle, is probably an intermittent phenomenon; it is therefore likely that this transformation is associated with some definite specialised function, while the utilisation of glucose, a regular linear process, is related more to the continued metabolism and existence of the cells themselves.

SUMMARY.

The production of lactic acid in excised kidney cortex is, like that in muscle, a reaction of some rapidity. Since under these conditions the glycogen content changes but little, it is deduced that the major part of the lactic acid must be formed from some other source. It is likely that glucose may be one of the precursors, but the origin of the bulk of the acid is unidentified.

It is probable that the glycogen figures obtained are contributed, to a large extent, by non-glycogen substances.

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