CCLI. THE METABOLISM OF LACTIC AND PYRUVIC ACIDS IN NORMAL AND TUMOUR TISSUE.

I. METHODS AND RESULTS WITH KIDNEY CORTEX.

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THE formation and breakdown of lactic acid have been very extensively studied in muscle tissue and bacteria; in animal tissues other than muscle the metabolism of lactic and pyruvic acids has been investigated chiefly by feeding, injection and perfusion experiments on whole animals and organs.

The introduction by Warburg of the surviving tissue slice technique with manometric measurements has given us a considerably more precise means for studying local intermediary metabolic processes, and lately the usefulness of such methods has been demonstrated by Krebs and Dickens and their collaborators and others.

The apparatus recently developed by Dixon and Keilin [1933] has provided a means whereby the oxygen uptake, respiratory quotient and acid formation or disappearance can be measured simultaneously on the same sample of tissue in the presence of a bicarbonate-containing medium. The work described in this series should demonstrate the utility of this apparatus and, we hope, throw considerable light on the activity of the various tissues in metabolising lactic and pyruvic acids, including the intermediate stages in the breakdown of these substances. Combined with the manometric experiments, chemical estimations are carried out on the manometer contents to determine formation or disappearance of lactic acid, pyruvic acid and glycogen.

In this paper we describe the details of our methods and the results obtained with kidney cortical tissue, chiefly of the rabbit. Though this tissue does not form or metabolise lactic acid to any great extent, it appears [Krebs, 1933] to be the chief seat of the oxidative deamination of amino-acids and would be expected therefore to have a high power for dealing with keto-acids. Later papers will deal with other tissues amenable to the method in the hope that light may be thrown on differences of behaviour in tissues such as tumour, wherein lactic acid is rapidly formed, brain and diaphragm which have the capacity for forming it anaerobically and liver wherein glycogen appears to be produced from lactic and pyruvic acids [see *e.g.* Meyerhof *et al.*, 1926]. The question of the metabolism of keto-acids other than pyruvic acid may be studied later.

I. Definition of terms.

Throughout this work we use the terms proposed by Warburg for oxygen uptake and CO_2 output, namely,

$$-Q_{O_2} = \mu l. \ O_2 \text{ taken up per mg. dry weight of tissue per hour.}$$
$$Q_{CO_2} = \mu l. \ CO_2 \text{ given out per mg. dry weight of tissue per hour.}$$
$$\text{(All gases measured at N.T.P.)}$$

In place of his term for "glycolysis" we propose to introduce two more precise terms, namely,

$$Q_A = \mu l. \ CO_2 \text{ given out by any acid formation per mg. dry weight of tissue per hour.}$$
$$Q_{LA} = \mu l. \ CO_2 \text{ given out by lactic acid formation per mg. dry weight of tissue per hour.}$$

The manometric results will thus give Q_A, the acid formation (or, when negative, disappearance). We suggest the term "acidogenesis" for this quantity. The results of chemical estimation of lactic acid will give Q_{LA} (1 mg. lactic acid = 249 $\mu l.$ CO_2). Q_A will thus represent the algebraic sum of the formation and removal of lactic acid, pyruvic acid and any other acids, or their neutralisation by, for instance, NH_3 production.

In order to reduce all other quantities measured to equivalent terms, two more terms, for pyruvic acid and for glycogen, will be used. That for pyruvic acid will be,

$$Q_{Pyr} = \mu l. \ CO_2 \text{ liberated by the production of pyruvic acid, per mg. dry weight of tissue per hour.}$$

This quantity will be obtained from chemical estimation of pyruvic acid (1 mg. = 255 $\mu l.$ CO_2).

Though changes in amount of glycogen do not affect the manometric readings, for uniformity and convenience in discussing results the following term will be used,

$$Q_{Ps} = \frac{\text{mg. of glycogen formed} \times 277}{\text{mg. dry weight} \times \text{hours}};$$
$$\text{(Ps = Polysaccharide)}$$

when glycogen is estimated as glucose, the factor becomes 249 instead of 277. This factor is arrived at by considering the glycogen as $2n \ (C_3H_5O_{2.5})$, and regarding it as a 3 carbon compound similar to the lactic or pyruvic acid.

As a general term to describe the units used thus in manometric work, we propose the term M.E., *i.e.* Manometric Equivalent.

II. Methods.

Apparatus. The manometers used were of the Dixon and Keilin [1933] type and fulfilled the specifications made by them in every respect. The dimensions of the manometers were determined as described by Dixon and Keilin and the constants for O_2 and CO_2 calculated from their formula. Three sets of constants, however, were calculated for use according to the contents of the vessels. Constants A were for use when only 3 ml. of fluid were present; for B, 200 $\mu l.$ were deducted from the gas volume to allow for hanging cups (see below) and about 200 $\mu l.$ were added to the fluid volume and deducted from the gas volume to allow for tissue present, and constants C were for cases when tissue, but no hanging cups, was present. Constants for readings taken at 10° were also calculated for use in certain experiments.

In the case of rapidly metabolising substrates it was necessary to have the substrate, dissolved in the proper medium, in a small cup hooked by a short platinum wire to the alkali tube as described by Keilin [1929]. These, present in both sides, could then be shaken down after passing gas, equilibrating and turning acid into the left cup. These "danglers" were large enough to contain 0·3 ml. of liquid and were made with oval cross-section. This shape made them easier to introduce with forceps, and as they could not roll about there was less danger of damage to the tissues. Experiments with coloured liquid showed that the contents very rapidly washed out uniformly into the rest of the medium. To ensure their dropping easily the wire hook was bent so that they only just hung, and the paper in the alkali-tube was bent back away from the hook. Unequal measurement into the two vessels of the bicarbonate-containing medium can produce large errors. For this purpose, therefore, a well-cleaned 5 ml. burette graduated in 0·01 ml. and connected directly to the tonometer containing the medium was used.

For the displacement upwards of the strong alkali, glass rods and not mercury were always used. With these there is no danger of splashing out mercury when shaking down the danglers and there is the advantage that, providing excess grease has been removed from the inside tube, they never stick; and the fact that they have gone down properly is known since the alkali tap cannot be turned again. The rods were of pyrex glass about 26 mm. long and 5 mm. in diameter and interchangeable, all being of equal volume.

It is essential that after filling the strong alkali into the well in the bottom stopper, the latter should be turned until it is only just closed off. Otherwise changes of temperature and pressure cause globules of alkali to work up through the grease into the vessel giving premature absorption of CO_2. Elastic bands can be strained on so that the stop-cock remains in this position.

A smooth rubber-containing grease was used for the bottom stopper and was also found necessary for the bored side-stoppers unless they were very perfectly ground. Anhydrous lanoline was used for all other joints and stop-cocks. Care should be taken that no grease gets on the edge of the side-bulb as otherwise the acid may fail to flow into the vessel when the bulb is turned. The stop-cocks on the manometer itself must be well cleared so that a proper equilibrium may be reached between the two vessels after passing gas and before starting the experimental period. The readings for h_1 and h_2 were not taken until equilibrium was reached. For h_1, that is after turning acid into the experimental vessel, about 25 minutes were allowed. The absorption of CO_2 is rather slower than mentioned in Dixon and Keilin's paper and it is rarely safe to take the final reading of h_2 until one and a half to two hours after introduction of the alkali. At the end of the experiment the manometer taps should be opened without moving the apparatus, and after about ten minutes a zero reading taken to see whether any correction is to be applied to h_1 and h_2.

The vessels etc. should be cleaned soon after using so that the time of contact of some parts with strong alkali is minimised. For cleaning, the following series seemed best: wipe with paper, rinse in xylene, alcohol, strong chromic acid for 30 minutes, cold and hot tap-water, distilled water. If, after the chromic acid, black patches remain, these are best removed with a soapy pipe cleaner and cold water.

The bath employed was of the ordinary type used with the Barcroft differential manometer; it was of such a size that three simple manometers or two Dixon-Keilin apparatus could be worked with on either side of the stirrer. The apparatus for passing gas into the vessels is shown in Fig. 1. It is similar to that

used by Dixon and Keilin but includes a system for purifying and leading a 95 % N_2–5 % CO_2 gas mixture for anaerobic experiments. The two wash bottles for saturating the gas with water vapour stand in the water-bath at 37°. The right-hand distributor and wash bottle are used only for anaerobic experiments and when not in use are kept closed to prevent oxygen from dissolving in the water.
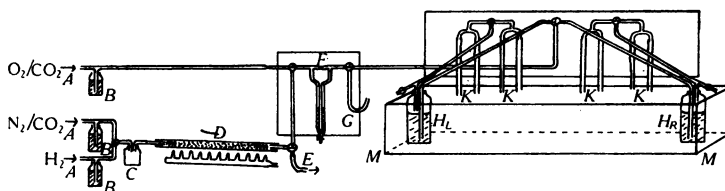

Fig. 1. Diagram of gas-leading system. *A*, gases from cylinders; *B*, mercury safety bottles to release excess pressure; *C*, trap to prevent mercury reaching copper; *D*, tube containing reduced copper wire, heated to dull redness to remove oxygen in anaerobic experiments; *E*, escape for H_2 when reducing copper oxide; *F*, flow-meter; *G*, side-tube for saturating bicarbonate-Ringer with gas mixture; *H*, bottles standing in bath for saturating gases with water vapour, H_L for O_2/CO_2, H_R for N_2/CO_2; *K*, thick-walled rubber tubes connecting with manometers; *MM*, ordinary large Barcroft tank. Stirring, shaking, heating and thermo-regulating apparatus not shown. All tubes except *K* are of copper or glass, joined with thick-walled rubber tubing.

Solutions. The medium used throughout was that described by Krebs [1932] containing Na, K, Ca, Mg, Cl, PO_4, SO_4, HCO_3, CO_2, in proportions closely approaching those in serum. Glucose (0·2 %) was added only where mentioned. The medium was saturated with O_2/CO_2 and kept in tonometers. About 260 ml. were prepared at a time and used up within a few weeks.

Lactic acid solution was prepared by dilution of 70 % Na lactate with Krebs medium to give a stock solution of the necessary strength. For pyruvic acid 1 ml. of a 22·5 % solution of redistilled pyruvic acid was brought to p_H 7·6 by the addition of about 2·5 ml. of Krebs medium which had been saturated with $NaHCO_3$. It was then diluted to 10 ml. with Krebs medium. Addition of 0·25 ml. of this to 2·75 ml. of medium in the experiment gave a concentration of roughly 0·02 N. All the other substrates used were weighed out and then neutralised and made up to 10 ml. as with the pyruvic acid. With succinate, fumarate, malate and oxaloacetate the normality was calculated on half the molecular weight. In the case of *dl*-malate and *dl*-lactate, experiments were also done with solutions of twice this strength.

Using solutions made up in this way, dilution of the medium was negligible and the only difference from the control experiment consisted in the presence of the substrate and the Na ions necessary to neutralise it.

Oxaloacetic acid was prepared from malic acid by the method of Fenton and Jones [1900], M.P. 144°. Solutions of oxaloacetate had to be made up from the solid immediately before use since in neutral solution this substance appears to decompose to pyruvic acid completely within two days in the ice-box.

General procedure. It was found possible to work simultaneously with three of the Dixon-Keilin apparatus. These were usually prepared the evening before making an experiment, following the instructions of the authors with the added precautions mentioned above.

The animals were stunned by a blow on the back of the neck and bled from the throat. The capsules were at once removed from the kidney, and then slices were cut with a well-sharpened dissecting razor wetted with ordinary Ringer-Locke solution. The surface slice was discarded and it was usually found that

three or four homogeneous slices could be cut from each of several parts of the kidney before the medullary part appeared. Each slice as cut was dropped into a small beaker containing Krebs medium through which O_2/CO_2 was continually bubbled. Sufficient slices for both vessels of one manometer were put into one beaker, the slices necessary for each manometer being kept separate. This precaution was necessary when glycogen estimations were to be made, so that the tissue in two vessels of each manometer was in the medium for about the same length of time. A distributor was used so that gas bubbled simultaneously through each of the beakers containing tissue. All the slices for the three manometers were cut from one kidney.

The well-mixed Krebs medium was then run accurately into both vessels, taking 3, 2·75 or 2·5 ml. according to the addition to be made. The slices, which were handled with flat-bladed coverslip forceps, were rinsed in Ringer-Locke solution, dried gently on smooth filter-paper and weighed. Usually between 110 mg. and 160 mg. wet weight were used, and the amounts placed in the two vessels agreed to 1 or 2 mg. The slices were weighed on a gold wire platform using a specially adapted type of chainomatic balance, which was as rapid as a torsion balance.

In the case of the experiments with minced tissue the whole of both kidneys was minced using a Latapie mincer. It was impossible to weigh the mince into the vessels but it was found very simple to inject a definite volume, 0·3 ml. This was done using a Bashford syringe with the needle attachment removed. This syringe consists of a cylindrical 0·5 ml. barrel calibrated in 0·05 ml. and a glass plunger which goes right to the end. If the plunger is moistened with Ringer solution, the mince can be sucked up out of a crucible, the level accurately adjusted and the desired volume ejected into the medium in the manometer vessel. Two samples are similarly ejected into weighed crucibles and dried for a few hours; their dry weight agrees within 2 mg. in 70 mg.

In the case of lactate experiments the lactate solution was usually run directly into the vessel with an accurate 0·5 ml. pipette. With the other substrates, 0·25 ml. of the solution was measured, using a capillary pipette with its end ground to a point, into danglers, and these were carefully hung in the vessels.

The manometer was joined up and placed in position in the bath and shaking started. When danglers were being used, after passing gas for ten minutes and allowing more than five minutes for equilibration, the manometer stoppers were closed, the apparatus was removed from the bath, acid tipped into the left cup, and then both danglers were shaken down, and the apparatus was replaced in the bath. The remainder of the experiment proceeded as usual. In general we have used an experimental period of 90 minutes before tipping acid into the right cup. Readings were taken after 10, 15 and 30 minutes and every 15 minutes thereafter.

After the final zero reading had been taken, the vessels were removed from the manometers, the side-bulbs and the glass rod, paper and bottom stopper removed and the slices carefully taken out and dropped into about 6 ml. of water in a crystallising dish. The slices were rinsed in two more lots of water and finally placed in a weighed crucible, or, when glycogen estimations were being made, they were put into a weighed centrifuge-tube and covered with about 8 drops of 15 % ammonia. The slices were dried at about 105° for at least 1 hour in crucibles and 3 hours when ammonia was added.

The first wash was added to the vessel contents which were then poured through a funnel into a 25 ml. volumetric flask; the other two washings were

used to rinse out the vessel and the whole made up to 25 ml. This solution was used for the chemical analyses.

Reliability of the manometric measurements. Duplicate experiments given in Table I illustrate the accuracy of the results obtained using this apparatus with the above precautions.

Table I. *Rabbit-kidney cortex in glucose-containing medium.*

	Rabbit no.	Experi-mental period min.	Wet wt. mg.	Dry wt. mg.	$-Q_{O_2}$	R.Q.	Q_A	Q_{LA} (by estimation)
A	4	60	187	32·3	14·9	0·84	+1·5	+0·86
			101	18·5	14·75	0·85	+1·4	+0·94
B	16	90	85	17·4	13·3	0·83	+1·9	+1·5
			97	19·7	13·6	0·85	+1·5	+1·3

In each case different manometers were in use for the duplicates. In A it should be noted that widely different amounts of tissue were used. In B a considerably smaller amount of tissue was used than was usual, so that small absolute errors give larger proportionate effects.

When working with three manometers it was impossible to start them all simultaneously. About an hour elapsed from the death of the animal until the beginning of the experimental period with the first manometer and a further hour before the start of the last. In order to know what effect this delay might have on the results, the following experiments were done.

Table II. *Rabbit-kidney cortex.*

Experiment	Animal no.	Length of time waiting in bicarbon-ate-Ringer at room temperature	Dry wt. mg.	$-Q_{O_2}$	R.Q.	Q_A	Q_{LA} (by estimation)
A. Normal, glucose* present	13	½ hr.	25·1	14·4	0·85	+1·6	—
		2 hrs.	28·5	13·0	0·85	+1·7	—
B. Normal, no glucose	12	10 mins.	25·1	12·4	0·70	+0·3	—
		1 hr.	27·3	11·4	0·72	+0·3	—
		2¼ hrs.	32·5	10·5	0·725	+0·15	—
C. $M/50$ lactate, no glucose present	10	10 mins.	23·2	17·75	0·87	−3·1	—
		2 hrs.	16·0	16·4	0·925	−3·25	—
D. $M/50$ lactate, no glucose	11	10 mins.	22·4	16·5	0·86	−3·8	−3·9
		2¼ hrs.	31·4	15·45	0·86	−3·4	−3·9

* 0·2 % glucose was present both in the manometer vessel and in the medium in which the tissue waited.

These experiments show that if there is any considerable delay a diminution in respiration of from 3 to 8 % may occur; no conclusions should be drawn when differences as small as this are observed. The R.Q. remains almost unaltered.

In view of these results duplicate experiments were not made, but in each series two experiments with different additions and one without addition were made. Experiments were always repeated completely with tissue from a different animal and often with fresh solutions.

III. CHEMICAL ESTIMATIONS.

Determination of glycogen.

This determination involved two main difficulties. First, there was considerable diffusion of glycogen out of the slices into the medium, so that it was necessary to determine glycogen not only on the tissue itself but also on an aliquot of the manometer fluid; also the slices placed in the two manometer vessels should have been waiting in the bicarbonate medium for about the same length of time. Second, drying the slices in the ordinary way caused a large destruction or hydrolysis of glycogen. Further it was necessary to make sure that no hydrolysis of glycogen occurred during the experiment, since the tissue in the left-hand vessel is shaken in the presence of nearly $0.3 N$ HCl at $38°$ for up to four hours.

The method of estimation used was in principle that of Pflüger [1909] with suitable modifications. After hydrolysis the Hagedorn-Jensen [1923] method for titration of glucose was used.

Determination in the slices. It was found that by making the slices rather strongly alkaline the loss of glycogen on drying could be avoided. By using 15 % ammonia for this purpose the dry weight was unaffected and could be determined before proceeding with the estimation. After drying and weighing in a centrifuge-tube (18×90 mm.), 1 ml. of 60 % (by weight) KOH is added to the tissue, and after fixing on a 12-inch glass tube with a rubber stopper to act as a reflux condenser, the liquid is heated just to boiling on a free flame and the tube placed in a boiling water-bath for 2 hours, shaking occasionally[1]. At the end of this period, 2 ml. of water, 7 ml. of 95 % alcohol and 0.5 ml. of 1 % Na_2SO_4 are added, and the mixture is stirred with a thin glass rod. The latter is finally rinsed with an additional 1 ml. of alcohol. After standing overnight the tube is centrifuged for 10 minutes and the liquid decanted. The precipitate is treated with a further 10 ml. of alcohol (without stirring), and the centrifuging and decanting are repeated. The tube is dried at $110°$ for 15 minutes to drive off the alcohol which appears to interfere with the Hagedorn-Jensen titration.

The tube is rinsed with 2 ml. of water and 2 ml. of $2 N$ HCl, and heated for 2 hours (with condenser) in a boiling water-bath. After cooling and adding a drop of phenol red[2], the liquid is neutralised with $2 N$ NaOH, the final adjustment to a faint red colour being made with $0.1 N$ NaOH. The solution is poured into a large test-tube and made up with washings from the small tube to a 15 ml. calibration mark. The Hagedorn-Jensen [1923] glucose estimation and blank then follow in the ordinary way, the glucose values being converted into glycogen by multiplication by the factor 0.927.

Determination in the fluid. The vessel contents having been made up to 25 ml., 5 ml. are measured into a centrifuge-tube (18×90 mm.), a drop of phenol red is added and N NaOH[3] drop by drop until a distinct alkaline reaction is obtained. A cork with glass inlet and exit tubes is inserted and the tube placed in an oven at $110°$ for evaporation, a gentle current of air being passed through the tube, but not through the liquid, to hasten the evaporation. In practice a distributor and frame were used so that a battery of six tubes could be dried simultaneously. Usually about 3 hours are required.

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[1] Since strong alkali causes the tube to lose 4 to 5 mg. in weight, it is necessary to weigh the tube before each determination to obtain the dry weight of tissue.

[2] Alcoholic solutions of phenolphthalein gave high values.

[3] Neutralisation with Na_2CO_3 gave correct results, but 1 drop of 60 % KOH without indicator gave low glycogen values, probably due to destruction by strong alkali present on evaporation.

After the evaporation, 1 ml. of water and 1 ml. of 60 % (by weight) KOH are added, and the mixture is heated for 2 hours under an air-condenser in a water-bath. After cooling 1 ml. of water (not 2 ml. as for the tissue), 7 ml. of 95 % alcohol and 0·5 ml. of 1 % Na_2SO_4 are added and the rest of the procedure is exactly as described for the tissue.   •

Determination in liver. The above details apply to kidney tissue. Because of the large amount of glycogen in liver it is necessary to change the procedure slightly since the Hagedorn-Jensen micro-method covers only the range 0·002 to 0·385 mg. glucose.

Instead of making up the volume to 15 ml. and oxidising the whole amount, the volume is made up to 25 ml. in a volumetric flask and a suitable aliquot, to be determined by preliminary tests, is oxidised with ferricyanide. Usually 2 to 5 ml. of the solution from the tissue and 5 to 10 ml. of the solution from the vessel fluid are suitable. The proper aliquot is made up to 15 ml. and the usual procedure followed.

Experiments on the glycogen methods.

Estimation of glycogen in solution. Hagedorn-Jensen titrations of solutions containing 0·1 and 0·2 mg. of pure glucose gave 100 % recovery. Similar accuracy was obtained in estimations of pure glycogen solutions. Further, heating 1 ml. of the glycogen solution with 1 ml. of 60 % KOH for 2 hours in a boiling water-bath and carrying the whole procedure through as described above, caused no serious decrease in accuracy. (Required, 0·500 mg. Found: 0·501, 0·501, 0·509 mg.) To test the effect of the long period in the presence of dilute HCl during the manometric experiment, 3 mg. of pure glycogen were dissolved in 6 ml. of bicarbonate Ringer solution and 0·8 ml. of 3·03 N HCl were added. (In actual experiments only 0·3 ml. of acid are added to 3 ml. solution.) Samples of 1 ml. were taken at once and after 4 hours' shaking at 38° and the glycogen was estimated, 0·46 and 0·45 mg. being found (taken, 0·45 mg.).

Estimation of glycogen in slices. These experiments were done on slices of rat-liver, usually from unfed animals.

(a) The following results show the agreement between estimations of glycogen in slices dropped directly after weighing into 60 % KOH.

	Wet wt. of tissue	mg. glycogen found	mg. glycogen per 100 mg. wet wt.
A	147	1·72	1·17
	172	2·03	1·18
B	173	3·94	2·27
	163	3·66	2·25

(b) When tissues are dried after rinsing, but without other treatment, losses of glycogen up to 40 % are observed. These losses are practically eliminated by treatment with 15 % ammonia, a slight loss of 3 % or less would be compensated for since the two sides of the manometer contain equal weights of tissue. Weaker ammonia is not so effective.

Found, mg. glycogen per 100 mg. of wet tissue

Without drying KOH at once	Drying with 15 % ammonia
7·94	7·76
3·41	3·34

Accuracy of the combined estimations. Rat-liver was sliced in the usual way, and 100 to 200 mg. were placed in 3 ml. of Krebs medium, and 0·4 ml. of 3 N HCl was added (*i.e.* slightly more acid·than normally). After 4 hours' shaking at 38° the tissue was rinsed and dried with ammonia, and the liquid and rinsing water were made up to 25 ml. A sample of the tissue was dropped immediately into KOH. The glycogen was then determined in the tissue, fluid and the control.

	mg. glycogen found				
	In tissue	In solution	Total	Total per 100 mg. wet wt.	Total per 100 mg. dry wt.
Control* 11 mg. wet	—	—	3·71	3·34	—
					(15·0)†
Exp. (*a*) 132 mg. wet	2·41	1·60	4·01	3·04	15·2
Exp. (*b*) 164 mg. wet	2·72	2·13	4·85	2·96	14·7

* Fresh tissue dropped directly into KOH.
† Calculated on the wet wt./dry wt. ratio of (*a*) and (*b*).

The estimations after treatment similar to that in an actual experiment agree within 3 % but the results are about 10 % lower than the control and the absolute error is 0·34 mg. In the case of kidney with a lower glycogen content the absolute error is much smaller and the percentage error is also smaller seeing that larger aliquots can be used for titration. Examples of results with kidney appear in the rest of this paper. It is unlikely that the method can be further improved, since determination of the wet weight of the tissue slices cannot be made very accurate without causing damage in the drying on filter-paper, and the dry weights have some uncertainty due to irregular diffusion out of glycogen and other substances and to slight disintegration.

Determination of lactic and pyruvic acids.

For the determination of lactic acid the method of Friedemann *et al.* [1927] as recently modified by Friedemann and Graeser [1933] was followed exactly. Taking a 10 ml. sample (of the 25 ml. of made up manometer fluid) sugars were removed by the copper sulphate-calcium hydroxide method, but as experiments with the Folin-Wu tungstic acid protein precipitant showed that only traces of protein were present, this precipitation was omitted. The sugar precipitation procedure removes all tissue particles and also a part of any proteins in solution.

Since we were making determinations on solutions which in some cases contained succinic, fumaric and malic acids, we tested the extent to which these substances can affect the estimations under the conditions of the method. On titrating a final aliquot of 4/25 of a solution containing 5·3 mg. lactic acid in 25 ml., there were required 9·42 ml. 0·01 N iodine. On similar estimation of a solution of the same normality (3·95 mg. in 25 ml.) of malic acid, 2·24 ml. of iodine were required. When the distillation was carried on for 20 mins. instead of 10 mins., 2·44 ml. were needed. With one-fifth of this concentration of malic acid, 0·47 ml. was required. This and other results showed that the titre was roughly proportional to the amount of malic acid and that it gave a titration of about one-fourth of that of an equivalent of lactic acid.

Similar experiments showed no effect of fumarate or succinate on the titration.

Pyruvic acid was estimated on 5 ml. of the made up manometer fluid by the method of Clift and Cook [1932]. Proteins were not removed with trichloroacetic acid, since sufficiently sharp titration end-points were obtained without that

procedure. In the case of anaerobic experiments, where considerable tissue breakdown occurred, and in the experiments with mince, the end-points were less definite.

RESULTS.

In this section results of comparative experiments are presented and interpreted as far as possible. Details of a few typical experiments are shown in the protocols. All the figures were obtained with rabbit-kidney cortex except where otherwise stated. The term "normal" implies that no substrate other than glucose was added. When glucose is stated to have been present, it was present in both the "normal" and in the experiment with substrate addition. The experimental period was in all cases 90 minutes. Except for an unusual experiment which is commented on, all the curves of the movement of the manometer fluid during the experimental period were almost linear, showing that no appreciable changes in the rate of metabolism were occurring. It should be mentioned that complete disappearance of a substrate initially present in $0.02\ N$ concentration in 3 ml. of medium would correspond to about 30 M.E. with the weight of tissue slices usually used.

Table III. *Lactate.*

Rabbit-kidney cortex		$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Pyr}	Total glycogen (mg.) in		Q_{Ps}
							Control cup	Experimental cup	
Glucose present	Normal	14·3	0·90	+1·7	+0·8	0·0	0·34	0·63	(+3·0?)
	dl-Lactate (a)	17·7	0·87	−3·1	−3·1	+0·9	0·37	0·34	−0·3
	0·02 M (b)	16·4	0·93	−3·3	−1·3?	+0·5	0·36	0·32	−0·4
No glucose	Normal	12·4	0·76	+0·25	+0·15	0·0	0·33	0·33	0
	dl-Lactate (a)	16·5	0·86	−3·9	−3·9	+0·65	0·37	0·44	+0·5
	0·02 M (b)	15·5	0·86	−3·4	−3·9	+0·45	0·35	0·35	0
No glucose	Normal	11·15	0·76	+0·45	+0·3	0	0·40	0·38	−0·1
	dl-Lactate 0·04 M	13·95	0·85	−3·0	−1·65	+0·4	0·38	0·41	+0·2

Lactate (Table III). In the "normal" experiments we see that lactic acid production (Q_{LA}) does not account for the whole acid formation (Q_A). This is more marked in liver, as will be shown in a later paper.

Compared with the other substrates mentioned below, added lactate does not appear to be very rapidly metabolised, even when the amount of lactate is doubled, as was done to give a concentration of the active form comparable with that of substrates mentioned later.

The presence or absence of glucose does not appear to affect the rate of lactic acid breakdown. However in the absence of glucose the R.Q. is, as would be expected, raised by the lactic acid oxidation. The fact that the acid disappearance is as great as or greater than the lactic acid removal would seem to indicate that the lactic acid removed is all oxidised to something other than acid, and the results below make it likely that this means complete oxidation to CO_2 and H_2O.

If this is so we may apply the following consideration. In the first experiment the lactic acid removed in presence of excess is $(0.8+3.1=)\ 3.9$ M.E. This amount for complete combustion requires $(3.9 \times 3 =)\ 11.7$ M.E. of O_2. The oxygen uptake is certainly increased (from 14·3 to 17·7) but not to the extent of 11·7 M.E. Hence it appears that the lactic acid oxidation can replace the oxidation of other substrates, *i.e.* it has a sparing action on their metabolism. The same consideration applies even to those experiments where the lactic acid consumption is smaller.

In these and numerous other experiments the results of the glycogen estimations have convinced us that there is no glycogen synthesis from lactic acid in kidney cortex.

In the above experiments the formation of a small amount of bisulphite-binding substance was observed when lactate was added. This substance seems not to be destroyed by heat in alkaline solution, and we believe it to be pyruvic acid and have entered it as such in the tables. The amount formed is very small, corresponding to a titration of only about 0·12 ml. of 0·002 N iodine in a 5 ml. sample of the total 25 ml., after deducting the blank of 0·11 ml. Nevertheless it has been found absolutely invariably when lactic acid was added. Further, practically the whole amount of this small titre is obtained when the experiment is allowed to run for 30 minutes, instead of the usual 90 minutes. We think it probable therefore that the first stage in lactate oxidation in the kidney is to pyruvic acid. This however accumulates only to a slight extent, since, as appears in the next section, pyruvic acid is much more rapidly dealt with than lactic acid.

Table IV. *Pyruvic acid.*

Rabbit-kidney cortex		$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Pyr}	Total glycogen (mg.) in Control cup	Experimental cup	Q_{Ps}
Glucose present	Normal	13·7	0·85	+1·45	+1·3	+0·1	0·33	0·30	−0·2
	Pyruvate 0·016 M	14·4	1·17	−3·9	(+2·1)	−7·1	0·28	0·29	+0·1
No glucose	Normal	13·1	0·74	−0·15	+0·2	0	0·26	0·30	+0·2
	Pyruvate 0·017 M	15·75	1·07	−5·9	(+1·45)	−7·95	0·30	0·32	+0·2

Pyruvic acid (Table IV). These and other entirely similar experiments show that pyruvic acid is metabolised by kidney cortex about twice as fast as lactic acid (compare e.g. Q_{Pyr} −7·95 with Q_{LA} −3·9), and again the presence or absence of glucose has no important effect. The lactic acid determination shows a certain increase. This may be due to reduction of a portion of the excess pyruvic acid to lactate. It appears probable, however, that it may be partly accounted for by the production of malic acid. It is seen that the oxygen uptake is not increased very much. Considering that only that part of the pyruvic acid disappearance which is accounted for by removal of acid is completely oxidised, we have in the first experiment 3·9 M.E. pyruvic acid oxidised. This requires $(3·9 \times 2\frac{1}{2} =)$ 9·7 M.E. of O_2. As with lactic acid we see again that pyruvic acid must have a replacing or "sparing" action on other metabolites. Since the R.Q. of the combustion of pyruvic acid is 1·2, the raised R.Q. in its presence is understandable and lends support to the belief that a large amount is completely burnt.

Again there is definitely no indication of the formation of glycogen from pyruvic acid in kidney cortex.

The results of an experiment with rat-kidney cortex are presented in Table V for comparison with the activity of rabbit tissue.

Table V.

Rat-kidney cortex		$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Pyr}
No glucose	Normal	18·6	0·75	+ 0·9	0	0
	With pyruvate 0·012 M	27·2	1·24	−14·2	+5·0	−18·7

The experimental period in this case was only 60 minutes and small amounts of tissue were used. The same type of result is obtained, except that the tissue from the smaller animal is much more active.

A blank experiment, in which pyruvic acid was present in the medium but no tissue, showed that no spontaneous change in the pyruvic acid occurred, *i.e.* no oxygen uptake, CO_2 output, acid change, change in the titre of pyruvic acid or appearance of "lactic acid" occurred.

The question of acetaldehyde formation. The possibility of decarboxylation of pyruvic acid in animal tissues has often been discussed. Our experiments convince us that not more than possibly a trace of decarboxylation occurs anaerobically. In three experiments made exactly as normally except that N_2/CO_2 gas mixture was substituted for O_2/CO_2, we obtained respectively $Q_{Pyr} - 1 \cdot 0$, $-0 \cdot 4$, and $0 \cdot 0$, with corresponding amounts of CO_2 evolved. In the aerobic experiments it was possible to show in two ways that no free acetaldehyde was produced. Acetaldehyde boils at 21°, so at 38° its presence should affect the manometric readings. The first method, therefore, consisted in removing the manometer to a bath at 10° to take readings of h_1 and h_2 at both temperatures. Applying the proper manometer constants for these two temperatures it was found that the gas volumes had been unaffected. A separate blank test showed that a small amount of added acetaldehyde affects the manometer strongly above 21° and its effect is completely removed on cooling to 10°. The manometer fluid was washed out and made up with cooled water and the bisulphite-binding substances estimated before and after treatment by alkali and heat. No difference in the small change was found between the solutions from the control and experimental manometer cups so it was again clear that no alkali-labile carbonyl-compound (acetaldehyde) had been formed.

During anaerobic experiments considerable disintegration of the tissue takes place, presumably due to autolysis.

In the anaerobic experiment described in Table VI the calculations were made on the dry weights (29·9 and 25·6 mg.) of the left-hand cups. In the right-hand, experimental, cups the undisintegrated tissues remaining had dry weights of only about half the original.

Table VI. *Anaerobic experiment.*

Rabbit-kidney cortex		Q_{CO_2}	Q_A	Q_{LA}	Q_{Pyr}
No glucose	Normal	0	+0·65	+0·25	0
	Pyruvate 0·015 N	0·98	−0·1	+1·2	−1·0

The formation of a little lactic acid from pyruvic acid under anaerobic conditions makes it seem more probable that the "lactic acid" found in the aerobic experiments with pyruvate is at least in part actually lactic acid, produced from pyruvic acid. The reaction lactate ⇌ pyruvate seems to be to some extent reversible.

Succinate, malate, fumarate and oxaloacetate (Table VII). These are all typical experiments. That these substances are even more rapidly metabolised than is pyruvate can be judged from the high acid disappearance and their effect on the respiratory quotient. The R.Q. of complete combustion for succinate is 1·14, for fumarate and malate it is 1·33 and for oxaloacetate 1·6. The addition of these substrates has raised the total R.Q. considerably, the effects running roughly parallel to the R.Q. of the substrate having regard to the extent of acid disappearance in each case. The results of some lactic acid determinations are shown

Table VII. *Succinate, malate, fumarate and oxaloacetate.*

Rabbit-kidney cortex		$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Pyr}	mg. glycogen in Control cup	mg. glycogen in Experimental cup	Q_{Ps}
Glucose present	Normal	11·8	0·88	0	—	0	—	—	—
	Succinate 0·02 N	17·2	1·21	− 16·9	—	(+ 1·0)	—	—	—
Glucose absent	Normal	10·4	0·76	− 0·85	+ 0·2	0	0·39	0·36	− 0·2
	Succinate 0·02 N	17·0	0·94	− 11·6	(+ 3·4)	(+ 1·3)	0·40	0·34	− 0·4
	l-Malate 0·02 N	14·15	1·57	− 16·9	(− 4·3)	(+ 1·1)	0·44	0·44	0
Glucose absent	Normal	10·6	0·69	− 0·4	+ 0·3	0	—	—	—
	dl-Malate 0·04 N	14·15	1·44	− 13·7	(− 5·8)	(+ 1·25)	—	—	—
Glucose absent	Normal	12·2	0·75	+ 0·6	+ 0·3	—	—	—	—
	Fumarate 0·02 N	15·4	1·42	− 15·5	—	(+ 1·4)	—	—	—
Glucose absent	Normal	11·5	0·76	− 0·65	+ 0·15	0	—	—	—
	Oxaloacetate 0·02 N	14·45	1·54	− 11·15	+ 1·35	− 5·6*	—	—	—

* Bisulphite-binding compounds estimated after alkali and heat treatment.

in brackets since it is probable that mainly malic acid was giving the effect in each case and it should be remembered that if it were all malate these figures should be multiplied by about four. With succinate, then, we see a large increase in malic acid which would be due to oxidation of succinate to fumarate, which is then converted to malate. With malate as added substrate, there is a disappearance mainly due to oxidation. With succinate, malate and fumarate definite production of bisulphite-binding substances is found (entered in brackets under Q_{Pyr}). These substances are probably oxaloacetate and pyruvate.

Again we find in the case of all the above substrates the phenomenon of "sparing" the oxidation of other substances. In the case of succinate and malate estimations showed that there was no sign of glycogen synthesis.

As will be seen in the discussion, we consider that the metabolism of pyruvic acid consists in a reaction circuit in which the last step is conversion of oxaloacetic acid back to pyruvic acid.

The results of the experiment with oxaloacetate illustrate this step in the metabolism well. Loss of CO_2 by decarboxylation involves at the same time the disappearance of acid. The acid disappearance measured, $Q_A − 11·2$, is then partly made up simply by conversion of oxaloacetic acid into pyruvic acid, and partly by the further combustion of the pyruvic acid. For the estimation of pyruvic acid the solutions were treated with alkali and heated so that all oxaloacetic acid would be converted to pyruvic acid. We see that about 5·6 M.E. of pyruvate have disappeared which would represent mainly that part of the pyruvic acid which is further oxidised. A part, however, is seen to have been reduced to lactate just as pyruvate is reduced when it is added directly as substrate.

Decarboxylation of oxaloacetate does not involve oxidation and, as would be expected, it occurs anaerobically as is shown in the experiment described in Table VIII.

Table VIII. *Anaerobic experiments with 0·02 N oxaloacetate.*

Rabbit-kidney cortex	Q_{O_2}	Q_{CO_2}	Q_A	Q_{LA}
Glucose absent	0	6·3 to 15·0	− 6·4 to − 15·2	+ 1·25 to + 3·0
Glucose absent	0	15·4 to 20·9	− 15·3 to − 20·7	+ 6·10 to + 8·25

In view of considerable anaerobic disintegration of the tissue in the experimental cup the results shown are calculated on the dry weights of the tissue in the left- and right-hand cups respectively. It will be seen later that disintegration of tissue diminishes the activity of the decarboxylating mechanism.

Again we see, as with pyruvic acid added directly, some reduction to lactic acid.

There has been much discussion in the literature concerning the occurrence of carboxylases. These experiments show clearly that there is in intact kidney cortex an active β-decarboxylating mechanism while the α-carboxylase activity is negligible.

Table IX. *Formate.*

Rabbit-kidney cortex		$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Pyr}
No glucose	Normal	12·4	0·76	+1·1	+0·2	0
	Formate 0·02 N	12·1	0·76	−0·4	+0·2	0
	Formate 0·02 N	11·4	−0·76	+0·1	—	—

Formate (Table IX). Though the R.Q. of formate combustion is 2, its presence is without effect on the R.Q. of the tissue. The slight decrease in acid is nearly within experimental variation. It is clear therefore that formate is not metabolised by kidney cortex to any appreciable extent.

Table X. *Acetate.*

Rabbit-kidney cortex		$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Pyr}
No glucose	Normal	12·2	0·75	+0·65	+0·25	—
	Acetate 0·02 N	16·3	0·90	−4·7	+0·25	+0·1

Acetate (Table X). Assuming, as seems reasonable, that the acid disappearance is a rough measure of acetic acid oxidation, it appears that this substance is less actively metabolised than pyruvic, succinic, fumaric, malic and oxaloacetic acids. Its metabolism differs from that of the other substances in that there is no formation of lactate (or malate) or pyruvic acid. Oxidation of 4·7 M.E. of acetic acid involves the uptake of 9·4 M.E. of O_2. Subtracting these amounts from the observed total we get 6·9 M.E. of O_2 taken up by other substrates at R.Q. 0·75. Acetate therefore competes to some extent with other substrates but appears not to alter their type of metabolism since their R.Q. is unchanged.

Table XI. *β-Hydroxybutyrate.*

Rabbit-kidney cortex		$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Pyr}
No glucose	Normal	12·15	0·76	−0·45	+0·2	0
	β-Hydroxybutyrate 0·04 N	11·1	0·76	−0·75	+1·4	(+1·7)

β-Hydroxybutyrate (Table XI). This and similar[1] experiments show that the presence of β-hydroxybutyrate has practically no effect on the respiration. There is, however, a definite formation of a bisulphite-binding substance, the amount

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[1] One experiment with an old and impure sample of β-hydroxybutyrate showed a rapid formation of acid which gradually came to an end after 60 minutes. It was thought at first to be oxidation to acetic acid, but experiments with pure material did not show this acid formation, so it was probably due to the splitting of polymerisation products.

of which was not altered on treatment with alkali and heat. This substance may be acetoacetic acid, which on the alkali treatment would give acetone. The formation of a substance estimated as lactate is noteworthy. If this actually is lactate it would denote an α-oxidation of the β-hydroxybutyric acid.

In any case this substrate does not behave as a possible intermediary in pyruvic acid breakdown.

Table XII. *Experiments with minced tissue.*

All these experiments were made with minced whole kidneys of rabbits; the dry weight taken was 60–70 mg. No glucose was present in the medium.

Kidney mince	$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Pyr}
Normal	1·72	0·62	+0·42	0	0
dl-Lactate 0·03 N	1·74	0·62	+0·45	−0·03	+0·02
Pyruvate 0·03 N	1·41	0·65	+0·34	(+0·17)	−0·05
Normal	1·84	0·58	+0·38	—	0
dl-Malate 0·04 N	2·19	0·64	+0·38	—	(+0·30)
Normal	3·18	0·68	+0·52	+0·97	0·09
l-Malate 0·02 N	2·91	0·77	−0·21	(−0·19)	(+0·17)
Fumarate 0·02 N	3·62	0·69	+0·25	(+2·23)	(+0·30)
Normal	2·02	0·65	+0·39	+0·13	0
Acetate 0·02 N	1·75	0·59	+0·25	+0·06	0
Oxaloacetate 0·02 N	1·79	1·64	−1·79	+0·32	−1·0

Experiments with minced tissue (Table XII). These experiments are not exactly comparable with those with slices since whole kidney and not pure cortex was used. Nevertheless cortical matter constitutes a large fraction of the whole organ so that the conclusions drawn seem valid. It is seen that not only is the respiration, as is well known, greatly diminished by mincing but the power to oxidise lactate, pyruvate, malate, fumarate, acetate and oxaloacetate is almost completely abolished. In the case of fumarate we have an increase in the amount estimated by the lactic acid determination and with malate a decrease (which is quite notable compared with the high lactic acid formation in the corresponding normal). These changes, which are fairly large considering that as malate the figures should be multiplied by four, are presumably due to the enzyme fumarase which establishes an equilibrium between fumarate and malate and which seems to retain its activity on mincing. The decarboxylation of oxaloacetic acid is still evident after mincing but occurs at a greatly diminished rate.

The case of succinate is different and interesting. For convenience in discussion the actual volumes, in μl. of gas measured, are included in brackets with the manometric equivalents in Table XIII showing a typical experiment.

Table XIII.

Kidney mince	$-Q_{O_2}$	Q_{CO_2}	R.Q.	Q_A	Q_{LA}	Q_{Pyr}
Normal	2·14 (206)	1·62 (155)	0·75	+0·25	+0·12	0
Succinate 0·02 N	5·45 (524)	1·44 (138)	0·26	(< +1·0)	(+1·46) (≡2·26 mg. malate)	+0·23

There is very little change in the CO_2 output and we can apply the following consideration. If, as seems reasonable, the ordinary R.Q. is scarcely changed in

the presence of succinate we would have (138/0·75=) 184 μl. of O_2 taken up in the ordinary way. This leaves (524−184=) 340 μl. of O_2 extra taken up by the succinate. This corresponds almost exactly with the amount of O_2 (332 μl.) required to oxidise to fumaric acid the whole of the 3·5 mg. of succinate added. The curve of the total gas volume changes during the experimental period (see Fig. 2) indicates a very rapid oxygen uptake which comes to an end after about
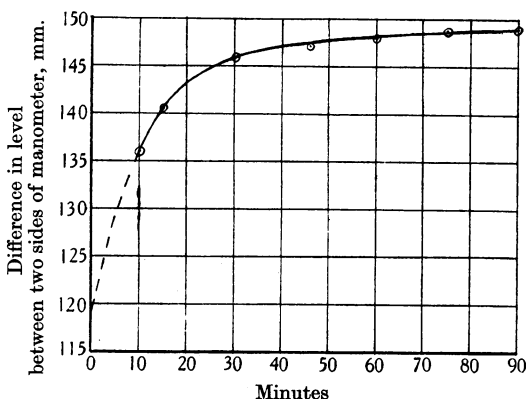

Fig. 2. Minced tissue with succinate. Curve showing change in level of manometer fluid due to resultant of O_2 uptake, respiratory CO_2 evolution and CO_2 displaced or absorbed by acid formation or disappearance.

50 minutes. In view of the rapid change in the curve it is impossible to extrapolate it back to zero time so that the Q_A cannot be estimated properly but it is certainly not negative, i.e., there is no acid disappearance. The lactic acid titration shows an increase of 1·34 M.E.; it is most probable that this increase is all due to malic acid and would then correspond to the formation of about 1·8 mg. malate leaving about 1·7 mg. of fumarate. Slight formation of "pyruvate" is observed due perhaps to a small further metabolism of malate.

It can thus be concluded that in minced tissue the succinic dehydrogenase is still strongly active, as also is fumarase. The further oxidation of malate and fumarate, in confirmation of the previous experiments, is almost completely prevented by mincing.

PROTOCOLS.

The following are the details of a typical manometric experiment.

Example 1. Experiment on kidney cortex slices with pyruvate.

Medium in Manometer No. 20	3 ml. of glucose-free Krebs bicarbonate medium
Medium in Manometer No. 21	2·75 ml. of glucose-free Krebs bicarbonate medium and in "danglers" 0·25 ml. of the same Ringer containing 4·5 mg. of neutralised pyruvic acid. The danglers were shaken down immediately after turning the acid into the left cup at zero time.
Rabbit killed at 10 a.m	Temperature of bath 38°

Manometer		No. 20	No. 21
Wet weight of tissue in L cup		159 mg.	143 mg.
,, ,, R cup		159 ,,	144 ,,
Zero time		11.15 a.m.	11.50 a.m.
Difference in level of mano-	10 mins.	− 109·0	− 119·4
meter fluid after tipping	15 ,,	109·9	120·3
acid into left cup	30 ,,	112·6	123·7
	45 ,,	114·8	126·3
	60 ,,	116·8	129·3
	75 ,,	118·6	131·7
	90 ,,	120·6	133·5
Value of h (obtained by extrapolating curves to zero time)		− 13·5	− 16·4
Reading h_1. Time after	15 mins.	− 13·1	+ 0·1
turning acid into R cup	25 ,,	− 13·1	+ 0·9
(h_1 corrected for final zero)		(− 13·1)	(+ 0·6)
Reading h_2. Time after	1½ hrs.	− 44·0	− 48·7
turning KOH into both	2 ,,	43·7	49·0
sides	2½ ,,	43·8	49·3
(h_2 corrected for final zero)		(− 43·8)	(− 49·6)
Final zero reading		0·0	+ 0·3
Dry weight (w) of tissue in L cup		32·9 mg.	25·4 mg.
,, ,, R cup		29·9 ,,	27·8 ,,

$$-Q_{O_2} = \frac{h_2 \times k_{O_2}}{w} \times \frac{60}{t \text{ mins.}} \qquad = \frac{43\cdot8 \times 13\cdot45}{29\cdot9} \times \frac{60}{90} = 13\cdot13 \qquad \frac{49\cdot6 \times 13\cdot21}{27\cdot8} \times \frac{60}{90} = 15\cdot73$$

$$Q_{CO_2} = \frac{(h_1 - h_2)k_{CO_2}}{w} \times \frac{60}{t} \qquad = \frac{30\cdot7 \times 14\cdot18}{29\cdot9} \times \frac{60}{90} = 9\cdot71 \qquad \frac{50\cdot2 \times 13\cdot93}{27\cdot8} \times \frac{60}{90} = 16\cdot77$$

$$\text{R.Q.} = \frac{Q_{CO_2}}{-Q_{O_2}} \qquad = \qquad 0\cdot74 \qquad\qquad 1\cdot07$$

$$Q_A = \frac{(h - h_1)k_{CO_2}}{w} \times \frac{60}{t} \qquad = \frac{-0\cdot4 \times 14\cdot18}{29\cdot9} \times \frac{60}{90} = -0\cdot13 \qquad \frac{-17\cdot0 \times 13\cdot93}{27\cdot8} \times \frac{60}{90} = -5\cdot68.$$

ESTIMATIONS.

The manometer fluids were made up to 25 ml.

Lactic acid. The final aliquot titrated corresponded to 8/25 of the total fluid. A reagent blank of 0·18 ml. has been deducted from all titres.

Manometer cup	Corrected titre ml. of 0·002 N iodine	Lactic acid in total fluid mg.	Difference mg.	CO_2 liberated by lactic acid μl. (1 mg. $\equiv$ 249 μl.)	$\frac{Q_{LA}}{w} \times \frac{60}{90}$
20 L	0·22	0·062	+0·034	+8·5	+0·19
20 R	0·34	0·096			
21 L	0·49	0·138	+0·258	+64·2	+1·43
21 R	1·41	0·396			

Pyruvic acid. 5 ml. of the total 25 ml. were titrated. A reagent blank of 0·11 ml. has been deducted from titres.

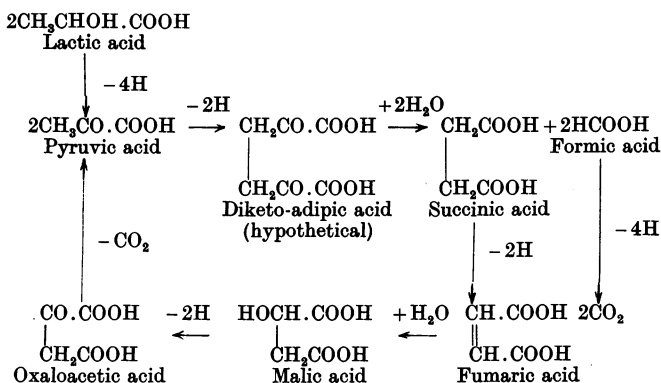| Manometer cup	Corrected titre ml. of 0·002 N iodine	Pyruvic acid in total fluid mg.	Difference mg.	CO_2 liberated by pyruvic acid μl. (1 mg. $\equiv$ 255 μl.)	$\frac{Q_{Pyr}}{w} \times \frac{60}{90}$
20 L	0·0	0	0	0	0
20 R	0·0	0			
21 L	10·24	4·51	−1·30	−332	−7·95
21 R	7·49	3·21			

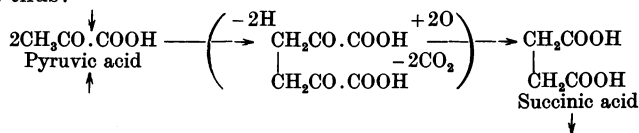*Glycogen.* Figures given are in terms of glucose from glycogen.

Manometer cup	Glycogen in fluid mg.	Glycogen in tissue mg.	Total glycogen mg.	Difference mg.	Equivalent volume of gas μl. (1 mg. $\equiv$ 249 μl.)	$\frac{Q_{Ps}}{w} \times \frac{60}{90}$
20 L	0·19	0·07	0·26	+0·04	+10	+0·22
20 R	0·23	0·07	0·30			
21 L	0·23	0·07	0·30	+0·02	+ 5	+0·12
21 R	0·25	0·07	0·32			

DISCUSSION.

Needham [1932] has summarised the evidence for the following scheme of lactic acid oxidation in muscle.

$$2CH_3CHOH.COOH$$
Lactic acid

$$\downarrow -4H$$

$$\begin{array}{ccccc} & & -2H & & +2H_2O \\ 2CH_3CO.COOH & \longrightarrow & CH_2CO.COOH & \longrightarrow & CH_2COOH + 2HCOOH \\ \text{Pyruvic acid} & & & & \text{Formic acid} \end{array}$$

$$CH_2CO.COOH \qquad CH_2COOH$$
Diketo-adipic acid   Succinic acid
(hypothetical)

$$-CO_2 \qquad\qquad -2H \qquad -4H$$

$$\begin{array}{ccccc} CO.COOH & \xleftarrow{-2H} & HOCH.COOH & \xleftarrow{+H_2O} & CH.COOH \quad 2CO_2 \\ CH_2COOH & & CH_2COOH & & CH.COOH \\ \text{Oxaloacetic acid} & & \text{Malic acid} & & \text{Fumaric acid} \end{array}$$

We feel that a similar scheme explains satisfactorily all the complex series of observations mentioned in this paper. In one particular, however, the scheme does not apply. Since we find no sign of rapid oxidation of formate, the hydrolytic breakdown of the hypothetical $\alpha\alpha'$-diketo-adipic acid apparently does not occur and this section of the scheme must be left more indefinite and should be represented thus:

$$2CH_3CO.COOH \longrightarrow \left(\begin{array}{c} -2H \qquad\qquad +2O \\ \longrightarrow CH_2CO.COOH \longrightarrow \\ CH_2CO.COOH \qquad -2CO_2 \end{array} \right) \longrightarrow \begin{array}{c} CH_2COOH \\ CH_2COOH \\ \text{Succinic acid} \end{array}$$

Pyruvic acid

The fact that lactic acid is first oxidised to pyruvic acid has been indicated frequently in the literature, and our invariable finding of a trace of what is presumably pyruvic acid when lactate is present in excess bears this out. Only a trace is found, however, since, as we find, pyruvate is more rapidly removed than lactate. Succinate, fumarate and malate and oxaloacetate are all even more rapidly removed, and pyruvate, succinate and fumarate all give indications of the formation of malate and traces of keto-compound which may be oxaloacetate or pyruvate which appear later in the cycle.

These substances all appear to "spare" the oxidation of other metabolites, and they appear to be completely oxidised in the end with no sign of glycogen synthesis from them in the kidney.

On destroying the structure of the tissue by fine mincing the mechanism of the cycle appears to be destroyed except for the two stages succinate → fumarate and fumarate → malate. The enzymes mainly responsible for these two steps, succinic dehydrogenase and fumarase remain very active. These facts illustrate the danger of drawing, from experiments on tissues in which the structure is not intact, conclusions such as those of Gözsy and Szent-Györgyi [1934] on the part played by intermediaries. The loss of activity of the enzymes may be due simply to a dilution of the necessary co-enzymes by diffusion from the minced tissue into the medium. Andersson [1934] has shown that while the dehydrogenases of lactate and malate are activated by cozymase or the co-enzyme of Banga et al. [1932], succinic dehydrogenase does not seem to require a co-ferment.

Acetic and β-hydroxybutyric acids have also been considered in the literature as possible intermediaries in lactic acid breakdown. Acetate does not appear to be metabolised at quite as high a rate as the above substances, and there seems no place in the scheme where it would occur.

The experiments with β-hydroxybutyrate would indicate that it has no place in the breakdown of lactic and pyruvic acids; rather does it appear to be a possible precursor in lactic acid formation.

SUMMARY.

1. Supplementary and more precise terms for use in describing manometric results are defined.

2. The reliability of the Dixon-Keilin apparatus, when certain precautions are followed, is demonstrated, and a technique is described for using the apparatus to follow the rapid metabolism of various substances by tissue slices and mince.

3. A method for the determination of glycogen changes in tissue slices and medium is described.

4. A study has been made of the metabolism in rabbit-kidney cortex of lactate, pyruvate and various other substances. Measurements have been made of the changes caused by the presence of these substances in determinations of the following: O_2 uptake, R.Q., and total acid, lactic acid, pyruvic acid and glycogen formation or disappearance.

5. Lactate is metabolised slowly by kidney cortex. There is an indication that pyruvic acid is the first stage in its oxidation.

6. Pyruvate is more rapidly oxidised than lactate; and succinate, fumarate, malate, and oxaloacetate are still more rapidly oxidised. In the cases of all these substances there are indications that the one is converted into the other in the foregoing order in the course of its breakdown. Formate is not oxidised.

7. Though the presence of all these substances causes an increase in the rate of respiration, they exert a "sparing" action on the oxidation of other substances present in the tissue. No glycogen is formed from any of them in kidney cortex.

8. Acetate is also fairly rapidly metabolised though there is no suggestion that it is a step in the breakdown of pyruvate. β-Hydroxybutyrate does not affect the respiration appreciably; there are indications that lactate, as well as acetoacetate, is a product of its metabolism.

9. Mincing the tissue almost completely destroys its ability to metabolise the above substances, except that the succinic dehydrogenase and fumarase remain strongly active, and the β-carboxylase acting on oxaloacetic acid remains slightly active.

10. No anaerobic decarboxylation of pyruvic acid occurs in kidney slices, nor can the formation of acetaldehyde be detected in aerobic experiments. Oxaloacetic acid however is decarboxylated to give pyruvic acid.

[*Note added, October 8th, 1934.*] Krebs has pointed out that in the case of the substances shown in Table VII the total oxygen uptake is not sufficient to account for complete oxidation of the acids disappearing. Also the R.Q. is in some cases higher than could be accounted for by complete oxidation. It would seem that there is another course of metabolism of these substances which also involves disappearance of carboxylic acid. A similar observation was made by Ashford and Holmes [1931] in their study of the oxidation of lactate in brain tissue.

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