# CXXXII. THE METABOLISM OF LACTIC AND PYRUVIC ACIDS IN NORMAL AND TUMOUR TISSUES

## III. RAT LIVER, BRAIN AND TESTIS

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(*Received 6 April 1937*)

In the first two papers of this series [Elliott & Schroeder, 1934; Elliott *et al.* 1935] methods were described for the study of the course of breakdown of lactate and pyruvate by tissue slices, using a combination of manometric and analytical techniques. In the first paper, it was indicated that in rabbit kidney cortex, lactate is oxidized to pyruvate, and that most of the pyruvate removal occurs in a cyclic series of reactions whereby pyruvate (2 mol.) is converted successively into succinate, fumarate, malate and oxaloacetate, the latter compound decomposing to yield pyruvate again (1 mol.) in half the original amount. In the second paper, it was shown that in rat kidney a similar cycle of reactions occurs, but that in two strains of transplantable rat tumours the reactions lactate → pyruvate, succinate → fumarate, and malate → oxaloacetate scarcely occur at all. Pyruvate is partially oxidized by tumour but without a corresponding disappearance of acid groups. In papers shortly to be published further evidence for the occurrence of the reaction cycle in kidney will be given by showing definitely the production of succinate from pyruvate, and it will be shown that synthesis of carbohydrate[1] accounts for part of the lactate, pyruvate, succinate etc. which disappears with kidney slices and part of the lactate and pyruvate disappearing with liver.

The methods described by Elliott & Schroeder with the improvements described by Elliott *et al.* have now been applied, without further modification, to rat liver, brain and testis, and the results which are given in the present paper show that in none of these tissues does the cycle of reactions observed in kidney occur to any extent.

In future work we plan to study the behaviour of muscle, retina, embryo tissue and yeast.

### Experimental

As in previous papers the concentrations of substrates in the medium were, unless otherwise stated: *dl*-lactate, $M/25$; pyruvate and acetate, $M/50$; succinate, fumarate, *l*-malate and oxaloacetate, $N/50$ or $M/100$. Keilin danglers were not used for making substrate additions, except in stated cases.

The terms used for expressing results are those defined in the previous papers. Figures in brackets under $Q_{LA}$ refer to estimations which were given wholly or partly by malate [see Elliott *et al.* 1935]. In the tables below, figures for $Q_{Keto}$

[1] Total carbohydrate. Elliott & Schroeder [1934] and Elliott *et al.* [1935] showed that kidney cortex does not synthesize glycogen.

have been omitted in experiments with oxaloacetate since it was found that the presence of tissue extracts interferes with the estimation of oxaloacetate and the results are quite unreliable.

The movement of the manometer fluid during the experimental period is the resultant of $O_2$ uptake, $CO_2$ output and acid change; for convenience the graph of this movement against time will in future be referred to as "the composite curve". When metabolic events are proceeding steadily, the composite curve is a straight line.

It is difficult to decide whether it is more significant to calculate results in terms of the dry weight of the tissue at the end of an experiment ("final dry weight") or in terms of a dry weight deduced from the initial wet weight of the tissue slices placed in the vessel and the dry weight/wet weight ratio of a separate sample of the slices ("initial dry weight"). The initial dry weight should more truly represent the amount of tissue taken, though it is not very accurate unless the tissue has been dried so thoroughly on filter-paper as to risk damaging it.

Table I. *Wet weight/dry weight ratios of tissue slices*

| | | Wet wt./initial dry wt. | | Wet wt./final dry wt. | |
|---|---|---|---|---|---|
| Tissue | | Dried directly after weighing | Rinsed in $H_2O$ before drying | Aerobic experiments | Anaerobic or malonate-inhibited |
| Kidney | Mean | 4·5 | 4·7 | 6·1 | 7·9 (7·0) |
| | Usual range | 4·1–5·2 | | 5·2–6·7 | 7·2–9·0 (5·9–7·8) |
| | Examples* | 4·4, 4·5 | 4·7 | | |
| | | | | 5·4, 5·9, 5·5, 5·8 | |
| | | | | 6·5, 6·4 | (7·8, 7·6) |
| | | | | | 8·7, 9·0, 8·2 |
| Liver | Mean | 4·35 | 4·45 | 6·3 | 6·8 |
| | Usual range | 3·9–5·0 | | 5·3–7·7 | 5·3–8·9 |
| | Examples* | 4·6, 4·5 | 4·8, 4·6 | | |
| | | 4·5, 4·7 | 4·6, 4·5 | | |
| | | 4·4, 4·4 | | 6·4, 6·5, 6·4, 6·3 | |
| | | 4·2 | | 6·7, 6·5, 7·7, 7·1 | |
| | | 4·0, 4·0 | | 6·0, 5·9 | 7·3, 7·0 |
| | | 4·2, 4·3 | | | 6·8, 5·7, 5·2, 6·2 |
| Brain | Mean | 8·1 | 8·8 | 12·0 | 12·1 |
| | Usual range | 7·1–8·9† | 8·0–9·7 | 10·6–14·2 | 10·1–14·1 |
| | Examples* | 7·6, 7·8, | 8·3, 8·5 | | |
| | | 8·9, | 9·4 | 12·3, 12·0, 12·9, 11·8 | |
| | | 8·3 | | 11·3, 12·0, 11·7, 12·8 | |
| Testis | Mean | 7·4 | 8·3 | 13·8 | 14·50 |
| | Usual range | 7·2–7·7 | 7·2–10·1 | 11·6–16·3 | 11·7–16·7 |
| | Examples* | 7·7, 7·7 | 8·23, 8·27, | | |
| | | 9·5, 9·7‡ | 10·2, 9·9‡ | | |
| | | 7·4 | | 14·5, 13·5, 13·4, 13·1 | |
| | | 7·6 | | 15·6, 14·3, 14·7, 14·7 | |
| | | 7·6 | | | 16·3, 15·2, 15·3 |
| Phila. No. 1 sarcoma | Mean | 6·0 | 6·15 | 7·6 | 7·5 |
| | Usual range | 5·7–6·2 | 5·7–6·5 | 7·0–8·4 | 7·2–8·9 |
| | Examples* | 6·2 | 6·3 | | |
| | | 5·8 | 5·85 | | |
| | | | | 8·4, 8·1, 7·4, 7·5 | |
| | | | | | 7·5, 6·7, 6·8 |

* Typical examples of determinations in sets of experiments on one sample of tissue.
† Exceptional values of 5·3 and 10·4 have been obtained.
‡ Exceptionally high ratios.

The final dry weight is usually considerably less than the initial dry weight since some solids have been lost by disintegration of cells or have diffused out of the tissue, and it may depend on the extent to which the tissue has been damaged in slicing. Even in a single set of experiments, the wet weight/final dry weight ratio for the tissue in different manometer vessels varies widely. In anaerobic experiments or where respiration has been inhibited, by malonate for instance, kidney tissue disintegrates considerably so that the final dry weight is particularly low. Possibly the final dry weight is the best measure of the intact tissue present during the experiment, and as it is known that the metabolism of damaged tissue is in most respects very small [see e.g. Elliott & Schroeder, 1934], the final dry weight may be the best basis on which to compute the activity of the tissue. In the tables given below, as in previous papers, the quantities are calculated from the final dry weight, and in a few cases, the values calculated from the initial dry weight are given in small type. In general, the conclusions given in the text apply to both sets of values. Of course, to estimate the metabolism per unit weight of fresh organ, the ratio fresh weight/dry weight of the organ must be used. The figures in Table I refer only to the ratio with prepared, rinsed slices and are higher than that of directly dried tissue.

Table I shows wet weight/dry weight ratios found, and variations observed, with slices prepared in the usual way from various tissues. Since it seemed likely that the salts in any Ringer solution adhering to the moist tissue might appreciably affect the dry weight, the slices were in many cases rinsed in distilled water before drying. Experiments showed that, in the case of brain, where the tissue is drained only on perforated disks, a difference of about 8–9 % is found; with testis, which cannot be drained very thoroughly, the difference was 5–7 %, but with the other tissues, well-drained on filter-paper, the difference never exceeded 5 % and was usually inappreciable. Duplicate determinations of initial dry weight agreed within 5 % or less in all cases.

In order to give some idea of the way in which tissues may vary in activity, the results of all our experiments are given in the following tables without selection. The values given for R.Q. and $Q_{CO_2}$ are corrected for the blank error described by Elliott & Baker [1935].

## Liver

The effects of lactate and pyruvate on the metabolism of liver tissue have been studied by various workers and especially by Meyerhof and his collaborators. Below are given the results of more comprehensive experiments to determine the fate of these substances with liver slices. These results are in agreement with those of previous workers as far as they went.

For these experiments slices were prepared from three lobes of the liver and roughly the same relative amount of tissue from each lobe was put in each manometer vessel, the total weight of moist tissue for each vessel being about 200 mg. Results obtained with liver slices are shown in Tables II, III and IV.

The results of duplicate experiments with liver (Table II) show that while the agreement between R.Q. values is close, a variation up to about 10 % in the $Q_{O_2}$ values should be expected. The metabolic events occurring in liver in the course of an aerobic manometric experiment with no substrate addition other than glucose deserve closer study since it is often observed that the composite curve is not a straight line but bends as though glycolysis occurs for the first 20–30 min. of the experimental period and gradually ceases. In nearly all experiments with livers of fed rats a certain amount of lactate, about 0·3 mg., was found in the left-hand vessel, indicating a quite rapid aerobic glycolysis

Table II. *Rat liver. Duplicate experiments*

| Substrate | Glucose | $-Q_{O_2}$* | | R.Q. | $Q_A$ | $Q_{LA}$ | |
|---|---|---|---|---|---|---|---|
| No addition | + | 11·3 | 8·3 | 0·89±0·02 | +0·8 | +0·2 | |
| ,, | + | 10·5 | 8·2 | 0·87±0·02 | +0·7 | +0·2 | |
| ,, | + | 12·1 | | 0·93±0·03 | +2·3 | — | |
| ,, | + | 13·0 | | 0·93±0·02 | +1·6 | — | |
| ,, | + | 13·8 | 8·7 | 0·98±0·01 | +2·2 | | Usual wt. of tissue, 200 mg. moist |
| ,, | + | 14·0 | 9·4 | 0·97±0·02 | +3·0 | | Larger wt. of tissue, 312 mg. moist |

* Figures in small type are calculated on the basis of initial dry weights.

during the initial 18 min. of treatment with gas and equilibration. This behaviour of liver is similar to that of brain (see below). With starved livers only about 0·1 mg. was found. (Elliott & Baker [1935] noted that livers of some well-fed animals showed a high anaerobic glycolysis, which fell off rapidly.) It will be noticed in many of the experiments without added substrate that the lactic acid found ($Q_{LA}$) does not account for the whole acid formation ($Q_A$); probably some of the lactic acid formed undergoes further metabolism with the formation of other acid products (see below).

The metabolism of liver tissue varies considerably, but from the results shown in Tables III and IV it will be seen that its behaviour with the various substrates tried is quite different from that of kidney tissue [Elliott *et al.* 1935], and there is no evidence that the cycle of reactions carried out by kidney occurs to any extent in the liver. The effects observed with each substrate will be considered separately.

*Lactate.* Meyerhof *et al.* [1925; 1926] showed that the addition of lactate or pyruvate causes some increase in the respiration of liver tissue especially when the rat has been starved for 1 or 2 days. In Tables III and IV the figures vary and the differences are often of the order of magnitude of experimental variation, but in general a small increase in oxygen uptake is found with added lactate. The R.Q. of normal livers is usually scarcely affected, but the negative values for $Q_A$ (also observed by Meyerhof & Lohmann [1926]) and $Q_{LA}$ indicate that lactate is being metabolized, in some cases quite rapidly. Except in the case of one abnormal liver, the rate of lactate metabolism ($-Q_A$ and $-Q_{LA}$) was found to be higher with the livers of starved animals; the normal R.Q. of starved liver is low and the oxidation of added lactate causes a raised R.Q. The first product of the oxidation of lactate by liver, just as by other tissues, seems to be pyruvate; this is indicated by a small increase in keto-bodies. This production of pyruvate, as is to be expected, is often appreciable during the initial period, but, as its removal soon becomes as rapid as its formation, the increase over the experimental 90 min. is small.

In some cases, the acid disappearance ($-Q_A$) is approximately equal to the lactate disappearance ($-Q_{LA}$); in others, the acid group removal is considerably less than the lactate disappearance. Usually a large fraction of the lactate which is removed is synthesized to carbohydrate [Takane, 1926; Benoy & Elliott, 1937]. The synthesis may involve the whole lactate molecule, or after oxidation to pyruvate a part of the molecule may be oxidized to $CO_2$ while the remainder forms carbohydrate, according to an equation suggested by Meyerhof *et al.* [1925]. In either case $-Q_A$ should equal $-Q_{LA}$. A second fraction after oxidation to pyruvic acid is converted into succinic acid [Elliott & Greig, 1937], possibly by a series of oxidations and dismutations involving the intermediate formation of

## Table III. *Livers of well-fed rats*

| Substrate[4] | Glucose | $-Q_{O_2}$[1] | | R.Q.[5] | $Q_A$[5] | $Q_{LA}$[2, 3] | $Q_{Keto}$[3] | $Q_{Ald.}$ |
|---|---|---|---|---|---|---|---|---|
| No addition | + | 10·8 | 7·0 | 0·90 ± 0·02 | +2·6 | +1·3 | + 0·6 | 0 |
| Lactate | + | 11·2 | 7·9 | 0·85 ± 0·02 | −1·4 | −2·0 | + 0·9 [+4·1] | 0 |
| Pyruvate | + | 11·1 | 7·7 | 1·53 ± 0·02 | −5·9 | +5·0 [+11] | −13·8 | +0·2 |
| Succinate | + | 13·7 | 8·6 | 0·66 ± 0·02 | +2·8 | (+2·6) | + 0·3 | 0 |
| No addition | − | 11·9 | 9·3 | 1·07 ± 0·02 | +0·7 | +0·9 | + 0·3 | — |
| Lactate | − | 12·6 | 10·5 | 1·13 ± 0·02 | −3·6 | −6·3 | + 1·0 [+2·2] | — |
| Pyruvate | − | 13·0 | 10·8 | 1·60 ± 0·01 | −5·7 | +4·9 [+9] | −10·6 | — |
| Succinate | − | 14·0 | 12·2 | 0·94 ± 0·01 | +1·2 | (+1·1) | + 0·4 | — |
| No addition | − | 12·2 | 7·7 | 0·93 ± 0·02 | +2·8 | +1·5 | + 0·5 | — |
| Lactate | − | 14·7 | 10·1 | 0·94 ± 0·02 | −3·2 | −6·1 | + 1·0 [+3·7] | — |
| Pyruvate (dan.) | − | 7·6 | 5·8 | 1·76 ± 0·03 | −2·1 | +7·5 | −11·2 | — |
| Succinate (dan.) | − | 14·6 | 10·3 | 0·72 ± 0·02 | +2·2 | (+2·8) | + 0·7 | — |
| No addition | + | 12·5 | 8·8 | 0·87 ± 0·02 | +1·8 | +1·2 | + 0·4 | — |
| Fumarate | + | 8·0 | 5·5 | 0·91 ± 0·03 | +2·7 | (+1·3)[+12] | + 0·2 | — |
| Malate (dan.) | + | 13·0 | 9·0 | 0·80 ± 0·02 | +3·1 | (−0·4) | + 0·6 | — |
| Acetate | + | 16·0 | 10·7 | 0·94 ± 0·01 | −4·2 | +1·2 | + 0·5 | — |
| No addition | − | 10·6 | 6·7 | 0·75 ± 0·03 | +0·1 | — | — | — |
| Fumarate 0·03 *M* | − | 9·5 | 6·3 | 0·78 ± 0·04 | +0·1 | — | — | — |
| No addition | − | 10·7 | 7·6 | 0·88 ± 0·02 | +1·1 | +0·6 | + 0·1 | — |
| Fumarate (dan.) | − | 10·5 | 7·3 | 0·91 ± 0·02 | +1·4 | (+3·9) | + 0·5 | — |
| Malate (dan.) | − | 9·6 | 7·0 | 0·87 ± 0·02 | +1·1 | (−0·2) | + 0·6 | — |
| Acetate | − | 12·3 | 9·1 | 0·92 ± 0·02 | −2·9 | +0·2 | + 0·5 | — |
| No addition | − | 14·8 | 8·9 | 0·91 ± 0·02 | +2·1 | +1·0 | + 0·5 | +0·1 |
| Fumarate | − | 13·7 | 8·8 | 0·87 ± 0·02 | +2·5 | (+0·3)[+18] | + 0·4 | +0·3 |
| Malate | − | 15·9 | 11·6 | 0·87 ± 0·01 | +1·8 | (−0·1) | + 0·7 | — |
| Oxaloacetate | − | 15·6 | 10·2 | 1·64 ± 0·02 | −9·9 | +4·4 | — | +0·6 |
| No addition | + | 11·0 | 7·3 | 0·96 ± 0·02 (587 μl. $CO_2$) | +1·3 (+74 μl. $CO_2$) | +0·8 | + 0·3 | — |
| Oxaloacetate | + | 10·5 | 6·9 | 1·51 ± 0·02 (944 μl. $CO_2$) | −4·4 (−257 μl. $CO_2$) | +2·9 | — | — |
| Oxaloacetate, no tissue | + | — | | (251 μl. $CO_2$) | (−262 μl. $CO_2$) | 0 | 0 | 0 |

Anaerobic

| Substrate | Glucose | $Q_{CO_2}$[5] | $Q_A$[5] | $Q_{LA}$[3] | $Q_{Keto}$ | $Q_{Ald.}$ |
|---|---|---|---|---|---|---|
| No addition | + | 0 (−0·4?) | + 3·9 | + 2·6 | 0 | 0 |
| Pyruvate | + | 1·7 | + 2·7 | + 3·9 [+13] | −1·9 | +0·2 |
| No addition | + | 0 (−0·4?) | + 2·3 | + 1·3 | −0·7 | — |
| Pyruvate | + | 1·5 (91 μl.) | + 2·0 | + 2·5 [+14] | −2·4 | 0 |
| Oxaloacetate | + | 6·2 (310 μl.) | − 3·0 | + 2·1 [+14] | — | — |
| Oxaloacetate, no tissue | + | — (161 μl.) | — | — | — | — |
| No addition | + | 0·0 | + 7·4 (379 μl.) | + 6·1 | 0 | 0 |
| Oxaloacetate | + | 6·7 (381 μl.) | +10·1 (548 μl.) | +17·7 [+11] | — | 0 |
| Oxaloacetate, no tissue | + | — (270 μl.) | — (−269 μl.) | 0 | 0 | 0 |
| No addition | − | 0·0 | + 2·7 | + 2·0 | −0·1 | — |
| Pyruvate | − | 1·6 (82 μl.) | + 5·0 | + 5·6 [+18] | −3·1 | — |
| Oxaloacetate | − | 8·7 (393 μl.) | + 0·9 | + 8·4 [+1?] | — | — |
| Oxaloacetate, no tissue | − | — (374 μl.) | — | — | — | — |
| No addition | − | 0 (−0·6?) | + 3·0 | + 2·8 | 0 | — |
| Pyruvate (dan.) | − | 1·0 (59 μl.) | + 4·0 (+236 μl.) | + 5·7 | −2·7? | — |
| Oxaloacetate (dan.) | − | 8·9 (413 μl.) | − 1·1 (− 52 μl.) | + 8·1 | — | — |
| Oxaloacetate, no tissue (dan.) | − | — (300 μl.) | — (−300 μl.) | — | — | — |

(1) Figures in small type are calculated on the basis of initial dry weights.

(2) Figures in (brackets) under $Q_{LA}$ refer to estimations given wholly or partly by malate.

(3) Figures in [brackets] give the approximate rate during the initial 18 min. of treatment with gas and equilibration before acid was added to the left-hand vessel. They are calculated from the difference between the left-hand vessel with no addition and the left-hand vessel with the substrate, in experiments in which no danglers were used, i.e. when the substrate was present from the start.

(4) In experiments marked (dan.) the substrate was added by means of Keilin danglers after treatment with gas and equilibration.

(5) The figures for μl. $CO_2$, given in brackets under R.Q. or $Q_{CO_2}$, represent the total respiratory $CO_2$ evolution during the experimental period. The figures for μl. $CO_2$ under $Q_A$ represent the total $CO_2$ evolved or absorbed as the result of acid changes.

Table IV. *Livers of rats fasted 20–24 hours*

| Substrate[4] | Glucose | $-Q_{O_2}$[1] | R.Q.[5] | $Q_A$ | $Q_{LA}$[2, 3] | $Q_{Keto}$[3] | $Q_{Ald.}$ |
|---|---|---|---|---|---|---|---|
| No addition | + | 17·0 12·8 | 0·61±0·01 | + 2·4 | + 1·0 | + 1·1 | — |
| Lactate | + | 21·6 15·6 | 0·77±0·01 | −10·6 | −11·8 | + 1·7 [+3·6] | — |
| Pyruvate | + | 19·1 13·9 | 1·29±0·01 | −13·3 | + 3·7 [+8] | −17·9 | — |
| Acetate | + | 18·5 13·6 | 0·71±0·01 | − 2·2 | + 2·3 | + 2·8 | — |
| No addition | − | 12·5 9·0 | 0·34±0·02 | + 3·9 | + 2·3 | + 2·7? | — |
| Lactate | − | 13·6 10·7 | 0·66±0·02 | − 6·1 | −10·7 | + 1·3 [+1·4] | — |
| Pyruvate | − | 16·0 12·1 | 1·19±0·02 | − 9·1 | + 7·2 [+3·4] | −17·1 | +0·7 |
| Succinate | − | 13·0 9·9 | 0·31±0·02 | + 3·1 | (+ 2·9) | + 1·6 | — |
| No addition* | + | 11·3 9·6 | 0·76±0·02 | + 2·6 | + 1·8 | + 0·5 | — |
| Lactate* | + | 12·2 10·8 | 0·76±0·02 | + 0·8* | − 0·8* | + 0·9 [+1·5] | — |
| Pyruvate* | + | 11·1 9·4 | 1·25±0·02 | − 1·6* | + 6·4 [+7] | − 8·3* | — |
| Succinate* | + | 13·5 11·4 | 0·54±0·01 | + 4·0 | (+ 4·2) | − 0·3 | — |
| No addition | + | 10·1 7·2 | 0·60±0·02 | + 1·4 | + 0·6 | + 0·6 | — |
| Fumarate (dan.) | + | 10·2 6·2 | 0·60±0·02 | + 1·6 | (+ 2·7) | + 0·6 | — |
| Malate (dan.) | + | 9·2 7·0 | 0·55±0·02 | + 3·0 | (+ 0·9)? | + 0·6 | — |
| Acetate | + | 9·5 6·3 | 0·66±0·02 | − 0·2 | + 1·1 | + 1·2 | — |
| No addition | − | 12·6 8·6 | 0·53±0·02 | + 2·6 | − 0·8 | + 1·5 | — |
| Fumarate | − | 9·1 7·1 | 0·44±0·03 | + 2·0 | (+ 0·5)[+9·0] | + 1·0 | — |
| No addition | − | 14·2 9·7 | 0·57±0·02 | + 1·9 | + 1·3 | + 1·4 | — |
| Acetate | − | 14·8 9·9 | 0·65±0·02 | − 1·2 | + 2·5 | + 2·4 | — |

Anaerobic

| Substrate | Glucose | $Q_{CO_2}$[5] | $Q_A$ | $Q_{LA}$[2, 3] | $Q_{Keto}$[3] | $Q_{Ald.}$ |
|---|---|---|---|---|---|---|
| No addition | − | 0·1 | +1·7 | +0·5 | 0 | — |
| Pyruvate | − | 1·1 | +2·2 | +1·7 [+9] | −1·5 | — |
| No addition | + | 0·0 | +1·7 | +0·6 | 0 | 0 |
| Pyruvate | + | 1·9 (82 μl.) | +1·6 | +2·6 [+8] | −7·1? | 0 |
| Oxaloacetate | + | 5·8 (266 μl.) | −1·6 | +2·7 [+3] | — | — |
| Oxaloacetate, no tissue | + | — (152 μl.) | — | — | — | — |
| No addition | + | 0·8 (32 μl.) | +1·2 | +0·4 | +0·3 | — |
| Pyruvate | + | 0·3? | +2·3 | +1·7 [+11] | −0·5? | — |
| Oxaloacetate | + | 4·6 (209 μl.)? | −1·9 | +2·1 [+6] | — | — |
| Oxaloacetate, no tissue | + | — (281 μl.) | — | — | — | — |

(1), (2), (3), (4) and (5) see Table III.
* Very pale liver giving low figures, probably unhealthy.

acetic and α-ketoglutaric acids [Krebs, 1936]. (The succinate would be immediately oxidized to fumarate but no further, see below.) This would involve no disappearance of acid groups. Possibly a third fraction may be oxidized completely to $CO_2$ and $H_2O$. In this case again, acid group and lactate disappearance would be equal. It is probable that variations in the relative rates of these three processes occur so that it is not possible to draw any exact conclusions from the $Q_A$ values except that a high negative $Q_A$ indicates high metabolism of the acid.

*Pyruvate.* The disappearance of keto-groups ($-Q_{Keto}$) is large, showing a rapid metabolism of pyruvate. Excess of pyruvate drives the reaction, lactate $\rightleftharpoons$ pyruvate + 2H, to the left and a considerable increase in lactate is found especially during the initial period. The effect on respiration varies and in some cases there is a lowering of oxygen uptake which could be accounted for by the fact that pyruvate is acting as a hydrogen acceptor in place of oxygen to some extent. The very high R.Q. fits in with this; the R.Q. for oxidation of pyruvate alone is 1·2 but other substances are apparently also being oxidized with evolution of $CO_2$ and reduction of pyruvate instead of $O_2$.

The above considerations of the fate of lactate apply also to pyruvate. Benoy & Elliott [1937] found that liver slices synthesize carbohydrate from

pyruvate usually even more rapidly than from lactate. In most cases the pyruvate disappearance ($-Q_{\text{Keto}}$) is nearly accounted for by the sum of the lactate formed from it and the acid disappearance. But there is usually a small excess of pyruvate disappearing which is probably accounted for as α-keto-glutarate and fumarate.

In anaerobic experiments with added pyruvate, the lactate formation is increased especially during the initial period; this is partly reduction of pyruvate but it is partly due to an increased rate of glycolysis, since an increased formation of acid groups is also found. The movement of the manometer fluid showed that this increased glycolysis occurred chiefly during the first 40 min. of the experiment. It is known that traces of pyruvic acid stimulate anaerobic glycolysis in certain tissues [Mendel *et al.* 1931; Rosenthal, 1932; Dickens & Greville, 1933, 1]. In agreement with the results of Meyerhof *et al.* [1925], it was found that a slight evolution of $CO_2$ usually occurs; this may be due to breakdown of pyruvate or it may be "respiratory" $CO_2$ accompanying oxidations (dismutations) in which pyruvate acts as hydrogen acceptor. The latter explanation is more probable. Simple decarboxylation of pyruvic acid would yield acetaldehyde but none was found on analysing the manometer vessel contents. In special experiments it was found that the KOH paper for $CO_2$ absorption could have absorbed, in the usual time, only a small fraction of any small amounts of aldehyde in the medium; separate experiments in which no alkali was introduced showed no trace of aldehyde formation.

*Succinate.* Succinate is rapidly oxidized by liver tissue but apparently only as far as fumarate and malate. The tables show that accompanying an increased $O_2$ uptake there is a considerable lowering of the R.Q., which is to be expected if fumarate and malate are the end products and no $CO_2$ is evolved in the oxidation. There is no disappearance of acid groups. Previous papers of this series showed that with kidney tissue there is a rapid further oxidation causing disappearance of acid groups. These effects are better illustrated in a subsequent paper where greater concentrations of succinate are used. Using $M/100$ substrate, the succinate is completely oxidized before the end of the experimental period.

*Fumarate and malate.* Liver differs sharply from kidney cortex in that fumarate and malate do not appear to be oxidized by it. The enzyme fumarase is present, since the malate, ($Q_{\text{LA}}$), increases when fumarate is added and decreases when malate is added, showing an equilibrium between these two substances. In some cases this equilibrium was found to be nearly reached within the preliminary 18 min. of treating with gas and equilibrating the manometer vessels. But there is no clear effect on the R.Q. nor is there disappearance of acid. The oxygen uptake is sometimes unaffected and sometimes decreased. It might be thought that the decreased $O_2$ uptake is due to fumarate acting as hydrogen acceptor and being reduced to succinate, but, in this case, a raised R.Q. would be expected, and this is not found. Possibly fumarate has a slight toxic effect, but one experiment with a higher concentration of fumarate showed no increased effect.

*Oxaloacetate.* As was shown in the previous paper, oxaloacetate breaks down spontaneously to pyruvate and $CO_2$ under the conditions of these experiments. Judging from the $CO_2$ evolutions found in the anaerobic experiments there may be a slight catalysis of this decarboxylation. Since the decomposition of oxaloacetate in neutralized solution is rapid and dependent on the concentration, the manometer without tissue was always set up first after neutralizing the substrate solution, so that the increased $CO_2$ evolution in the presence of tissue is due to the tissue and not to a higher oxaloacetate concentration. Allowing for the $CO_2$

evolution and acid group removal which decarboxylation involves, the effects in aerobic or anaerobic experiments are about the same as with added pyruvate. It was found that even on acidifying oxaloacetate solutions a slight steady evolution of $CO_2$ occurs. Since this slight evolution goes on in the left-hand vessel during the experimental period, determinations of $CO_2$ evolution with oxaloacetate are rather inaccurate and low, but probably the results with and without tissue are affected to the same extent.

*Acetate.* Of the acid disappearing when pyruvate and lactate are added to liver slices a part is probably oxidized to $CO_2$ and water. It has been shown that succinate is not an intermediate in this process, since no acid disappears when succinate or fumarate is added directly. It is known that pyruvate may be oxidized to acetate by biological systems [see e.g. Barron & Miller, 1932], and the scheme put forward by Krebs [1936] suggests that acetate is a usual product of pyruvate metabolism in animal tissues. It seemed likely that acetate might be the intermediate product in the complete oxidation of lactate and pyruvate by liver. The experiments with added acetate give support to this idea. It is seen that addition of acetate causes a slightly increased $O_2$ uptake, the R.Q. is raised, and a disappearance of acid groups occurs.

*General remarks.* The metabolism of liver tissue is not affected by the presence or absence of glucose in the medium, and with all the substrates tried the same type of result was obtained in either case. The livers of fasted rats behaved qualitatively in the same way as the livers of fed animals. Meyerhof & Lohmann [1926] found a still greater increase in respiration with lactate with the livers of rats starved 36 hr.; the animals used here were starved 20–24 hr.

In a forthcoming paper [Elliott & Greig, 1937) it is shown that liver does oxidize pyruvate to succinate to some extent, and the above experiments show that succinate is readily oxidized to fumarate and malate but that these substances are not further oxidized in the liver. In another paper [Benoy & Elliott, 1937] the synthesis of carbohydrate is studied.

### *Brain cortex*

For experiments with four manometers, slices of grey matter were prepared from the brains of four rats, all the slices from each brain being dropped into one beaker containing Krebs's medium with $O_2/CO_2$ mixture bubbling through. For each manometer roughly equal amounts of tissue, usually two slices, were taken from each of the four beakers. In most of the experiments, with or without glucose, the medium in the beakers contained glucose, since decreasing respiration and irreversible damage to the tissue occur in the absence of glucose. After rinsing in Ringer-Locke solution (this was done twice for experiments in the absence of glucose), the material for each vessel was drained on perforated porcelain disks over filter-paper and weighed (usually 150–220 mg. moist). For experiments with glucose in the medium, the tissue was introduced simultaneously into the left- and right-hand vessels of the manometer to avoid error due to glycolysis starting earlier in one vessel than in the other.

Table V shows the results of experiments with brain tissue. The range within which the results agree in duplicate experiments is shown in the first two experiments. In the next two experiments it is seen that when a very large amount of tissue is in the vessels the $O_2$ uptake is not diminished, showing that diffusion is not limiting the respiration. But when there is a very large amount of tissue present the usual small aerobic glycolysis ($Q_A$ and $Q_{LA}$) appears to be abolished. The explanation of this is interesting. In the usual manometric experiment

Table V. *Brain*

| Substrate | Glucose | $-Q_{O_2}$[1] | | R.Q. | $Q_A$ | $Q_{LA}$[2, 3] | $Q_{Keto}$[3] |
|---|---|---|---|---|---|---|---|
| No addition | + | 14·8 | 11·0 | 0·89 ± 0·03 | +2·1 | +2·3 | +0·1 |
| No addition | + | 16·6 | 11·6 | 0·90 ± 0·03 | +2·5 | +2·6 | +0·5 |
| Lactate | + | 15·2 | 10·8 | 0·95 ± 0·03 | −0·1 | +1·4 | +0·1 [+1·8] |
| Pyruvate | + | 12·3 | 8·0 | 1·18 ± 0·04 | +0·6 | +4·9 [+17] | −3·9 |
| No addition | + | 13·6 | 8·5 | 0·96 ± 0·03 | +1·8 | +1·8 | −0·2 (187 mg. moist tissue) |
| No addition | + | 12·5 | 7·4 | 0·93 ± 0·02 | +1·4 | +1·6 | −0·1 (318 mg. moist tissue) |
| Lactate | + | 13·9 | 8·7 | 0·94 ± 0·3 | +1·5 | +1·3 | +0·1 [+1·1] |
| No addition | + | 14·0 | 10·8 | 0·93 ± 0·03 | +2·2 | +2·1 | −0·3 (216 mg. moist tissue) |
| No addition | + | 15·8 | 12·1 | 0·93 ± 0·01 | +0·2 | 0·0 | −0·2 (438 mg. moist tissue) |
| Lactate | + | 15·2 | 11·6 | 0·98 ± 0·03 | −1·4 | +0·8 | +0·1 [+4·0] |
| No addition | + | 12·0 | 5·6 | 0·93 ± 0·03 | +2·4 | +2·2 | −0·1 (192 mg. moist tissue) |
| No addition | + | 12·6 | 6·0 | 0·96 ± 0·01? | +0·7 | +0·6 | −0·1 (403 mg. moist tissue) |
| Pyruvate | + | 9·8 | 4·5 | 1·22 ± 0·04 | +1·8 | +5·2 [+10] | −3·6 |
| No addition | + | 13·5 | 11·8 | 0·97 ± 0·03 | +1·6 | +1·0 [+12] | 0·0 |
| No addition | − | 9·3 | 7·8 | 0·99 ± 0·04 | −1·6 | −0·9 | 0·0 |
| Lactate | − | 12·4 | 10·2 | 0·95 ± 0·03 | −2·9 | −2·0 | −0·2 [+3·1] |
| Pyruvate | − | 11·6 | 9·0 | 1·22 ± 0·03 | −3·0 | +1·4 [+8] | −4·3 |
| No addition | − | 12·3 | 7·9 | 0·92 ± 0·03 | −2·6 | −1·0 | −0·2 |
| Lactate | − | 15·0 | 10·4 | 0·95 ± 0·03 | −4·3 | −7·8 | 0 [+4·1] |
| No addition | − | 6·6 | 4·8 | 0·98 ± 0·10 | −0·4 | −0·3 | +0·2 |
| Lactate | − | 13·6 | 11·0 | 1·03 ± 0·04 | −3·9 | −2·2 | +0·2 [+4·4] |
| Pyruvate | − | 12·9 | 9·4 | 1·28 ± 0·04 | −4·1 | +0·6 [+14] | −6·2 |
| Acetate | − | 7·3 | 4·9 | 1·01 ± 0·08 | −1·3 | −1·2 | +0·1 [+1·1] |
| No addition* | − | 1·4 | — | — | +1·1 | +0·3 | +0·2 |
| Lactate* | − | 4·1 | — | 0·95 ± 0·10 | 0 | −2·7 | +0·2 [+2·3] |
| Pyruvate* | − | 3·8 | — | 1·3 ± 0·10 | +0·3 | +0·6 [+6] | −1·5 |
| Fumarate* | − | 1·2 | — | — | 0 | (+2·7) | +0·1 [+1·0] |
| No addition | − | 6·6 | 5·0 | 1·06 ± 0·08 | −0·8 | −0·7 | −0·2 |
| Lactate | − | 11·9 | 9·2 | 0·95 ± 0·05 | −2·8 | −4·4 | +0·4 [+2·9] |
| Fumarate | − | 9·8 | 7·1 | 1·01 ± 0·06 | −2·1 | (+5·1) | 0 [+0·3] |
| Malate | − | 9·2 | 7·2 | 0·96 ± 0·05 | −1·8 | (−3·0) | −0·4 [+1·9] |
| No addition | + | 14·0 | 11·8 | 0·95 ± 0·02 | +2·3 | +1·4 | 0 |
| Succinate | + | 17·3 | 14·9 | 0·74 ± 0·02 | +2·1 | (+2·9) | −0·2 [+0·8] |
| Fumarate | + | 13·5 | 10·9 | 1·00 ± 0·03 | +1·2 | (+2·7) | 0 [+1·3] |
| Malate | + | 13·1 | 10·9 | 0·98 ± 0·03 | +1·7 | (+2·0)? | −0·3 [+2·7] |
| No addition | − | 5·8 | 4·1 | 0·95 ± 0·08 | −0·5 | −0·2 | −0·2 |
| Succinate | − | 10·6 | 8·7 | 0·65 ± 0·05 | −0·6 | (+7·2) | +0·1 [−0·9] |
| Malate | − | 6·1 | 5·1 | 1·00 ± 0·08 | −0·8 | (−2·5) | +0·9 [+0·2] |
| No addition | + | 15·7 | 11·6 | 0·96 ± 0·03 | +1·2 | +1·8 | 0 |
| Acetate | + | 15·1 | 11·3 | 0·97 ± 0·03 | +0·1 | +1·3 | −0·3 [0·2] |
| No addition | − | 10·4 | 7·1 | 1·00 ± 0·04 | −1·9 | −0·4 | 0 |
| Acetate | − | 10·6 | 7·4 | 0·94 ± 0·03 | −2·4 | −1·5 | 0 [+0·7] |

Anaerobic

| Substrate | Glucose | $Q_{CO_2}$[5] | $Q_A$[5] | $Q_{LA}$[5] | $Q_{Keto}$ |
|---|---|---|---|---|---|
| No addition | + | 1·0 | +13·9 | +13·3 | −0·8 |
| Pyruvate | + | 2·9 (63 μl.) | +19·2 | +21·5 [+12] | −1·7 |
| No addition | + | 1·2 | +13·7 | +13·0 | 0 |
| Pyruvate | + | 2·3 (61 μl.) | +18·0 | +20·4 [+18] | −1·8 |
| No addition | − | 0 (−1·8?) | + 1·0 | − 0·4 | −0·1 |
| Pyruvate | − | 2·1 | + 0·5 | + 2·5 [+12] | −3·2 |
| No addition | − | 0·2 | − 0·5 | − 0·6 | −0·1 |
| Pyruvate | − | 1·9 | + 0·5 | + 2·1 [+10] | −9·0? |
| No addition | − | 0·0 | + 1·8 | − 0·2 | +0·1 |
| Pyruvate | − | 3·3 (99 μl.) | + 1·3 (+37 μl.) | + 2·4 [+9] | −5·3 |
| Oxaloacetate | − | 10·0 (236 μl.) | − 4·9 (−115 μl.) | + 3·0 | — |
| Oxaloacetate, no tissue | − | — (157 μl.) | — | — | — |
| Oxaloacetate | + | 8·0 (242 μl.) | +23·8 | +27·4 | — |
| Oxaloacetate, no tissue | + | — (165 μl.) | — | — | — |

(1), (2), (3) and (5) see Table III.

* Respiration permanently damaged by keeping the slices in glucose-free medium before use.

about 20 min. elapse between introducing the tissue and killing it in the left-hand vessel at the start of the experimental period. It was usually found that there had been an appreciable lactate formation (0·3–0·6 mg.) in the left-hand vessel and that the amount in the right-hand experimental vessel was only a little larger, corresponding to the small $Q_{LA}$ found. When a large amount of tissue was used, the lactate concentration was built up more quickly and the amount in the left-hand vessel at the start of the experimental period was higher (0·9, 0·9 mg.) and apparently had reached a limiting concentration since no more formed in the right-hand vessel during the 90 min. It was found in separate experiments that slices of brain produced lactate at a rate of $Q_{LA} = 6·0$ and 6·2 in the initial 20 min. under the conditions of an ordinary manometric experiment and probably the rate was considerably higher in the first few minutes. In the presence of $M/25$ *dl*-lactate no lactate increase in the initial 20 min. was found ($Q_{LA} = +0·6$ and $-2·2$). One must conclude that, in the absence of lactate, brain cortex has a definitely high aerobic glycolysis and this glycolysis is inhibited by a small concentration of lactate as though a reversible reaction were involved. Once the lactate has reached a critical concentration, glycolysis proceeds only fast enough to replace lactate oxidized. It seems as though the glucose is split to lactate, and lactate itself, and not an intermediate product of glycolysis, is oxidized. The respiration of brain slices in the presence of lactate alone is just about the same as in the presence of glucose alone. Possibly a similar explanation can be applied to the observation of Elliott & Baker [1935] who found that lower values for aerobic glycolysis of retina were obtained when larger amounts of tissue were used. This will be studied in later work.

*Lactate.* Unlike liver, brain is very dependent on glucose or other added substrate for continuance of its respiration [Loebel, 1925; Dickens & Greville, 1933, 2]. Lactate and glucose are apparently alternative substrates for brain and in the presence of glucose excess lactate addition makes no significant difference in the respiration rate or R.Q.; a little of the excess acid may be used up. In the absence of glucose, the presence of lactate keeps the respiration rate up to normal and the disappearance of lactate and acid groups (also observed by Meyerhof & Lohmann [1926]) shows that lactate is being metabolized. Work by Benoy & Elliott [1937] has shown that carbohydrate is not synthesized from lactate and pyruvate by brain, and Elliott & Greig [1937] find that probably only a small amount of succinate is formed. The increased $O_2$ uptake and $CO_2$ output in the absence of glucose indicate that the lactate is being oxidized. The disappearance of lactate is not large but it is sufficient to account for the increased respiration if most of the lactate is completely oxidized (1 manometric equivalent of lactate requires 3 manometric equivalents of $O_2$). It seems probable that in the case of brain most of the lactate disappearing is oxidized to $CO_2$ and $H_2O$. As with other tissues, it is probable that pyruvate is the first product of oxidation [see Peters, 1936]. In the presence of glucose, a trace of pyruvate (about 0·1 mg.) was always found in both vessels and addition of lactate increased the amount only slightly. In the absence of glucose, practically no keto-body was found but, with added lactate, the keto-concentration in the left-hand vessel, i.e. within the preliminary 18 min., had reached that usually found in the presence of glucose.

*Pyruvate.* In the presence of glucose the main oxidation processes in brain tissue seem to be little affected by added pyruvate, but the actual oxygen uptake is lowered and the R.Q. is raised owing to pyruvate to some extent replacing oxygen as hydrogen acceptor. There is some disappearance of pyruvate ($-Q_{Keto}$) but most of this is simply reduced to lactate, this reduction occurring rapidly during the initial period. The acid formation is lowered slightly showing that

perhaps a little pyruvate is completely oxidized in place of or in addition to glucose and other metabolites. In the absence of glucose, provided that the tissue has not been damaged by previous lack of metabolites, added pyruvate maintains the oxygen uptake at about the same level as is found with glucose plus pyruvate. In this case, while some pyruvate is reduced to lactate during the experimental period, quite a large amount disappears as acid indicating probably complete oxidation. The R.Q. is raised to about the value expected for pyruvate oxidation.

The results of anaerobic experiments are similar to those obtained with liver. As is well known, brain shows a high anaerobic glycolysis in the presence of glucose, and the acid formed is all lactic acid ($Q_A = Q_{LA}$). Addition of pyruvate increases the glycolysis considerably and its action is evidently catalytic since the increased lactate cannot be accounted for by pyruvate disappearance. Most of the pyruvate which does disappear, however, is reduced to lactate which accounts for the excess of $+Q_{LA}$ over $+Q_A$ in the presence of pyruvate. There is a small evolution of $CO_2$ which, as with liver, is probably $CO_2$ evolved by the oxidations in which pyruvate is acting as hydrogen acceptor. The results in the absence of glucose are similar except that there is no glycolysis; the reduction of pyruvate to lactate is, as usual, rapid in the initial period.

*Succinate, fumarate and malate.* The behaviour of grey matter with these substances is very similar to that of liver. Succinate is readily oxidized to fumarate and malate, its addition causing a large increase in $O_2$ uptake and a lowering of the R.Q. Fumarate and malate are interconvertible but there is little further oxidation[1]; small increases in $O_2$ uptake and decreases in acid have been noticed with added fumarate and malate. The inhibitory effect of fumarate sometimes observed with liver was not noticed with brain.

Possibly oxidation of fumarate and malate to oxaloacetate occurs to a very small extent. In the presence of all three substrates a trace of extra keto-body usually seemed to be formed in the initial period. The amounts were too small for accurate determination.

*Oxaloacetate.* Brain slices do not catalyse the decarboxylation of oxaloacetate appreciably since in anaerobic experiments the $CO_2$ evolved does not exceed the sum of the $CO_2$ evolved spontaneously plus that given off when pyruvate is present. The pyruvate formed by the breakdown of oxaloacetate behaves in the usual way.

*Acetate.* Added acetate appears not to be oxidized by brain cortex. There was no effect on the $O_2$ uptake, the R.Q. was not consistently affected and the small increased disappearance of acid groups was all accounted for by disappearance of traces of lactate apparently introduced with the tissue.

*General remarks.* Elliott & Greig [1937] found that whole brain produces a little succinate from pyruvate but considerably less than does liver [see also Weil-Malherbe, 1937]. As with liver, the succinate formed would be oxidized to fumarate and malate and apparently no further. The above results are all compatible with the belief that, in brain cortex, glucose is split to lactate, the lactate is oxidized reversibly to pyruvate, and most of the pyruvate is oxidized to $CO_2$ and $H_2O$, but since acetate is not oxidized, it is not yet possible to suggest what are the intermediate steps in the oxidation of pyruvate. Benoy & Elliott [1937] found no synthesis of carbohydrate from lactate and pyruvate by brain.

[1] Quastel & Wheatley [1932] made similar observations with mashed whole brain of various animals.

*Testis*

For experiments with four manometers, the testes of two or three animals were pulled apart into small bunches of tubules, the material from each testis being dropped into one small beaker containing Krebs's medium through which

Table VI. *Testis*

| Substrate | Glucose | $-Q_{O_2}$ (1) | | R.Q. | $Q_A$ (1) | | $Q_{LA}$ (1, 2, 3) | | $Q_{Keto}$ (3) | $Q_{Ald.}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| No addition | + | 14·1 | 6·9 | 0·97±0·03 | +8·6 | 4·0 | — | | — | — |
| No addition | + | 13·0 | 7·2 | 0·98±0·03 | +7·3 | 4·0 | — | | — | — |
| No addition | + | 11·3 | 7·1 | 0·98±0·03 | +5·9 | 3·5 | + 5·5 | 3·5 | + 0·3 | — |
| No addition | + | 12·3 | 7·1 | 1·01±0·03 | +5·2 | 2·9 | + 5·7 | 3·3 | + 0·5 | — |
| No addition | + | 12·8 | 7·3 | 0·94±0·04 | +8·3 | 4·6 | Usual wt. of tissue 201 mg. moist | | | |
| No addition | + | 12·3 | 7·5 | 0·97±0·02 | +6·7 | 3·9 | Larger wt. of tissue 375 mg. moist | | | |
| No addition | + | 13·8 | 7·4 | 0·99±0·03 | +6·6 | | + 7·0 | | + 0·2 | — |
| No addition | + | 13·7 | 7·5 | 0·95±0·03 | +6·8 | | + 6·7 | | + 0·5 | — |
| Lactate | + | 14·5 | 8·5 | 1·01±0·03 | +3·8 | | + 3·7 | | + 0·6 [+1·5] | — |
| Lactate | + | 14·8 | 8·8 | 0·98±0·02 | +4·3 | | + 3·5 | | + 0·6 [+2·6] | — |
| Pyruvate | + | 13·7 | 7·7 | 1·41±0·03 | +1·9 | | +11·8 [+24] | | − 9·7 | — |
| No addition | + | 12·8 | 7·5 | 1·00±0·03 | +6·4 | | + 6·0 | | + 0·2 | — |
| Lactate | + | 14·5 | 8·9 | 0·98±0·02 | +3·9 | | + 5·6 | | + 0·8 [+1·4] | — |
| Lactate | + | 14·7 | 8·8 | 0·98±0·03 | +4·6 | | + 4·3 | | + 0·6 [+3·0] | — |
| Pyruvate | + | 14·5 | 8·2 | 1·25±0·03 | +3·1 | | +11·5 [+25] | | − 8·2 | — |
| No addition | + | 13·2 | 6·5 | 0·95±0·05 | +8·4 | | + 8·7 | | − 0·4 | — |
| Lactate | + | 15·1 | 8·1 | 0·99±0·04 | +4·3 | | + 7·1 | | + 0·8 [+1·3] | — |
| Pyruvate* | + | 12·4 | 6·7 | 1·39±0·05 | +3·4 | | +10·4 [+27] | | − 4·6 | — |
| Acetate | + | 14·0 | 8·0 | 0·94±0·05 | +6·0 | | + 7·5 | | − 0·4 [+1·9] | — |
| No addition | + | 14·3 | 8·0 | 1·06±0·04?† | +7·0?† | | + 7·5 | | + 0·9 | +0·4 |
| Lactate | + | 15·8 | 8·8 | 1·00±0·04 | +4·5 | | + 7·4 | | + 0·7 [+6] | — |
| Pyruvate | + | 16·0 | 8·1 | 1·34±0·04 | +2·1 | | +12·1 [+33] | | −11·8 | 0 |
| Acetate | + | 15·4 | 8·8 | 0·98±0·03 | +6·8 | | + 7·5 | | − 0·1 [+3·5] | — |
| No addition | + | 13·4 | 8·3 | 0·95±0·03 | +7·2 | | + 6·7 [+13] | | + 0·3 | — |
| Lactate | + | 14·3 | 9·4 | 0·88±0·03 | +5·3 | | + 1·0? | | + 0·9 [+1·3] | — |
| Lactate | − | 15·2 | 9·9 | 0·82±0·03 | −1·3 | | − 2·9 | | + 0·5 | — |
| Pyruvate | + | 12·7 | 8·3 | 1·17±0·03 | +3·1 | | + 9·9 [+16] | | − 1·8? | — |
| Acetate | + | 12·7 | 8·1 | 0·98±0·04 | +4·5 | | + 7·1 | | + 0·4 | — |
| No addition | + | 14·3 | 7·6 | 0·98±0·03 | +7·9 | 4·2 | + 6·9 | 3·6 | — | — |
| Acetate | + | 14·5 | 8·4 | 1·00±0·02 | +4·8 | 2·8 | + 6·5 | 3·7 | — | — |
| No addition | − | 8·4 | 4·7 | 0·82±0·04 | +1·0 | | — | | — | — |
| Acetate | − | 7·9 | 4·6 | 0·82±0·04 | +1·2 | | — | | — | — |
| Malate | − | 9·1 | 4·8 | 0·87±0·04 | +0·2 | | — | | — | — |
| No addition | − | 6·1 | 3·8 | 0·69±0·08 | +1·0 | | — | | — | — |
| Acetate | − | 6·2 | 3·5 | 0·74±0·08 | +0·7 | | — | | — | — |
| No addition | − | 6·4 | 3·4 | 0·74±0·08 | +0·6 | | + 0·4 | | + 0·5 | — |
| Lactate | − | 17·0 | 9·4 | 0·88±0·03 | −4·3 | | − 6·2 | | + 1·1 [+3] | — |
| Pyruvate | − | 17·0 | 9·1 | 1·29±0·03 | −4·8 | | + 6·7 [+20] | | −10·2 | — |
| Succinate | − | 10·9 | 6·2 | 0·60±0·04 | −0·3 | | (+ 1·7) | | + 0·6 [+2·4] | — |
| No addition | − | 4·8 | 2·3 | 0·82±0·12 | +1·2 | | + 0·9 | | + 0·3 | — |
| Lactate | − | 17·4 | 9·3 | 0·87±0·03 | −3·4 | | − 4·8 | | + 0·3 [+4] | — |
| Pyruvate | − | 15·6 | 8·4 | 1·39±0·03 | −6·1 | | + 4·5 [+32] | | −13·9 | — |
| Succinate | − | 10·4 | 5·4 | 0·61±0·05 | 0·0 | | (+ 2·6) | | − 0·9 [+6] | — |
| No addition | + | 14·3 | 7·3 | 0·98±0·03 | +7·3 | 3·6 | + 7·6 | | + 0·7 | — |
| Succinate | + | 16·0 | 8·8 | 0·91±0·03 | +5·5 | 3·0 | (+ 8·4) | | 0 [+1·3] | — |
| Fumarate | + | 13·1 | 7·2 | 1·05±0·03 | +6·2 | 3·4 | (+11·2) | | 0 [+1·2] | — |
| Malate | + | 13·6 | 7·8 | 1·03±0·03 | +5·9 | 3·3 | (+ 5·8) | | − 0·1 [+2·4] | — |
| No addition | − | 6·3 | 3·1 | 0·75±0·08 | +1·8 | | + 0·6 | | − 0·4 | — |
| Fumarate | − | 7·1 | 3·7 | 0·94±0·06 | +0·4 | | (+ 3·1) | | − 0·2 [+2·5] | — |
| Malate | − | 8·4 | 3·9 | 0·84±0·06 | +0·8 | | (+ 0·2) | | − 0·3 [+2·1] | — |
| Acetate | − | 5·8 | 2·7 | 0·76±0·08 | +1·9 | | + 0·5 | | − 0·1 [+0·6] | — |

Table VI (*cont.*)

Anaerobic

| Substrate | Glucose | $Q_{CO_2}$ (5) | $Q_A$ (5) | $Q_{LA}$ (3) | $Q_{Keto}$ | $Q_{Ald.}$ |
|---|---|---|---|---|---|---|
| No addition | + | 0·4 | +15·3 | +13·5 | 0 | 0 |
| Pyruvate | + | 6·4 (173 μl.) | +13·5 | +19·3 [+21] | −12·5 | 0 |
| Oxaloacetate | + | 15·6 (415 μl.) | + 3·3 (88 μl.) | +20·2 [+16] | — | 0 |
| Oxaloacetate, no tissue | + | — (312 μl.) | — (−310 μl.) | — | — | — |
| No addition | + | 0·6 | +14·5 | +14·0 | − 0·4 | 0 |
| Pyruvate | + | 4·2 | +13·6 | +17·2 [+23] | − 7·6 | 0 |
| No addition | − | 0·4 | + 2·4 | + 0·6 | − 0·1 | 0 |
| Pyruvate | − | 7·5 | + 0·8 | + 7·8 [+22] | −12·2 | 0 |
| No addition | − | 0·5 | + 3·4 | + 0·6 | − 0·3 | — |
| Pyruvate | − | 6·9 (177 μl.) | + 1·3 | + 5·5 [+12] | −12·7 | +0·2 |
| Oxaloacetate | − | 13·1 (342 μl.) | − 9·1 (−239 μl.) | + 5·3 [+7] | — | — |
| Oxaloacetate, no tissue | − | — (295 μl.) | — (−325 μl.) | — | — | — |
| No addition | − | 0·0 | + 4·7 | + 0·6 | 0 | — |
| Pyruvate | − | 5·9 (149 μl.) | + 2·6 | + 7·6 [+23] | −11·6 | — |
| Oxaloacetate | − | 11·9 (268 μl.) | − 5·1 (−114 μl.) | + 5·2 [+7] | — | — |
| Oxaloacetate, no tissue | − | — (219 μl.) | — (−194 μl.) | — | — | — |

(1), (2), (3) and (5) see Table III.
* Pyruvate 0·01 *M*, i.e. half usual concentration.
† Probably error in $h_1$ reading giving too high R.Q. and too low $Q_A$.

$O_2/CO_2$ mixture was bubbling. For each manometer vessel roughly equal amounts of tissue were taken from each of the beakers; the tissue was treated in the usual way, i.e. rinsed in Ringer-Locke solution, drained on filter-paper and weighed rapidly (200–300 mg.).

The first experiments in Table VI show the range of variation to be expected in duplicate experiments.

The metabolism of testis is similar to that of brain in that they both show high anaerobic glycolysis [Warburg, 1927] and their respiration is very dependent on the presence of glucose or other substrate [Dickens & Greville, 1933, 2]. But testis differs from brain and the other normal tissues studied in three respects. First, as was observed by Warburg *et al.* [1924] testis shows a fairly high aerobic glycolysis. This aerobic glycolysis occurs steadily though the rate during the initial period often appears higher than during the experimental period. The glycolysis is not inhibited by added lactate. Secondly, testis carries on a considerable metabolism of pyruvate under anaerobic conditions. Thirdly, testis differs from other tissues in forming, in anaerobic experiments without glucose, a considerable amount of acid which is not lactic acid. The nature and source of this acid cannot yet be suggested.

The behaviour of testis with the substrates under consideration is shown in Table VI and described in the following paragraphs.

*Lactate.* Lactate is quite rapidly oxidized by testis. Even in the presence of glucose excess lactate causes some increase in $O_2$ uptake, although the tissue normally, under our experimental conditions, produces lactic acid faster than it can use it, i.e. it has a considerable aerobic glycolysis. In fact accumulation of lactate ($+Q_{LA}$) continues in spite of excess lactate present and the increased rate of oxidation. In the absence of glucose, addition of lactate increases the $O_2$ uptake above that found with glucose alone; there is a rise in the R.Q. and a disappearance of lactate. It is probable that, as in other tissues, the first immediate oxidation product is pyruvate; there is usually a slight increase in

keto-bodies especially during the initial period of treatment with gas and equilibration. Benoy & Elliott [1937] find no synthesis of carbohydrate from lactate and pyruvate by testis so that the acid disappearing in the absence of glucose is probably all oxidized. Since in the absence of glucose more lactate than acid groups disappear ($-Q_{LA} > -Q_A$) it seems that acid intermediate oxidation products accumulate in these experiments. On the other hand, in the presence of glucose the total lactate and acid groups increase faster than they are oxidized, but more increase of lactate is found than of acid groups. It seems as if in the presence of glucose more intermediate acid substances in the tissue are oxidized away, or possibly lactate plus glucose stimulates protein metabolism so that $NH_3$ is produced[1] and neutralizes some of the lactate.

*Pyruvate.* Pyruvate is rapidly metabolized by testis. In the absence of glucose, addition of pyruvate increases the oxygen uptake considerably; in the presence of glucose, the actual $O_2$ uptake remains about the same but the oxidation processes are probably increased since pyruvate acts as a hydrogen acceptor in addition to oxygen. In both cases there is increased lactate formation due to reduction of pyruvate. As with other tissues, a higher R.Q. is found than corresponds to the complete oxidation of pyruvic acid, owing to the pyruvate acting as a hydrogen acceptor. There is usually a large disappearance of pyruvate; part of this appears as lactate and acid disappearance corresponds approximately to the remainder, which is probably oxidized to $CO_2$ and water.

In anaerobic experiments with testis and pyruvate there is a considerable evolution of $CO_2$. In this respect testis differs strikingly from any tissue we have yet studied. This $CO_2$ evolution does not seem to be due to a simple decarboxylation of pyruvic acid to acetaldehyde and $CO_2$ since this would involve a disappearance of acid groups equivalent to the $CO_2$ evolved, and this is not observed. Also no acetaldehyde formation could be detected either manometrically by cooling the vessels below 20° or analytically. (In testing for aldehyde formation no alkali was introduced into the vessels.) It seemed possible that the following reactions might take place.

$$CH_3.CO.CO_2H = CH_3.CHO + CO_2 \quad (1).$$

$$CH_3.CHO + H_2O + CH_3.CO.CO_2H = CH_3.CO_2H + CH_3.CHOH.CO_2H \quad (2).$$

$$2CH_3.CO.CO_2H + H_2O = CH_3.CHOH.CO_2H + CH_3.CO_2H + CO_2 \quad (3).$$

That is to say, pyruvic acid is decarboxylated to yield $CO_2$ and acetaldehyde (1) and the aldehyde then undergoes a dismutation with unchanged pyruvic acid yielding lactic and acetic acids (2). However, experiments failed to support this hypothesis. Testis was incubated anaerobically under the conditions of a manometric experiment with added pyruvate and acetaldehyde ($M/30$). No increase in lactic acid[2] over controls with pyruvate only was observed. It may be that

[1] In general it is unlikely that $NH_3$ production seriously obscures the figure for $Q_A$. Meyerhof *et al.* [1925] found that liver slices without added amino-acid formed $NH_3$ at a rate corresponding to $Q_{NH_3} = 0{\cdot}17$, $0{\cdot}13$, $0{\cdot}46$. Dickens & Greville [1933, 3] obtained similar comparatively low figures with liver and testis, and with brain Loebel [1925] found values equivalent to $Q_{NH_3} = 0{\cdot}42$–$0{\cdot}96$, and since these values were only slightly affected by the addition of oxidizable substrates, comparisons of $Q_A$ values would not be affected.

[2] When mixtures of pyruvate and acetaldehyde are analysed a slight amount of "lactate" is always found. It is not known whether lactate is actually produced or whether pyruvate prevents the aldehyde from being completely removed in the preliminary distillation. Neither pyruvate nor acetaldehyde alone gives the effect. In the above experiments there was thus found a trace more lactate than in the controls, but the extra "lactate" was no more than that found in an estimation of lactate on a mixture of pyruvate and aldehyde without tissue.

"nascent" acetaldehyde reacts with pyruvate, but for the present equation (3) which integrates (1) and (2) may represent events occurring without any assumption as to intermediate steps. According to equation (3) a dismutation between two molecules of pyruvic acid produces one molecule each of lactic and acetic acids and of $CO_2$. For two equivalents of pyruvate disappearing, one equivalent each of lactate and $CO_2$ should be found but no change in the number of acid groups. This is approximately what was observed. There is a little less acid formed in the presence of pyruvate than without and it is hard to tell whether this means that some of the pyruvic acid has disappeared as acid or if the normal acid formation has been partly inhibited. It is thus impossible to interpret the results in detail.

The same reaction, occurring at a much lower rate, possibly accounts for the small $CO_2$ evolutions with liver, brain, kidney and tumour in anaerobic experiments with pyruvate (see tables above and in Elliott *et al.* [1935]).

*Succinate.* As with other normal tissues, succinate is rapidly oxidized; its addition causes a large increase in $O_2$ uptake, especially in the absence of glucose, and a lowering of the R.Q. On comparing succinate experiments with corresponding normals, it is seen that there is a slight removal of acid groups though the effect is very small compared with the acid removal by kidney. Most of the succinate is oxidized no further than fumarate and malate.

*Fumarate and malate.* As with all other tissues tried, fumarate and malate form an equilibrium mixture in the presence of testis. It is possible that they are oxidized slightly, since small increases in $O_2$ uptake and slight decreases in acid formation have been observed.

*Oxaloacetate.* Testis does not catalyse the decarboxylation of oxaloacetate, since in anaerobic experiments the total $CO_2$ evolved does not exceed the sum of the $CO_2$ evolved spontaneously plus that given off when pyruvate is present. The pyruvate formed by the breakdown of oxaloacetate behaves in the usual way.

*Acetate.* In the presence of glucose addition of acetate often caused a small increase in the $O_2$ uptake and always a decrease in acid formation, while in the absence of glucose there was no increase in $O_2$ uptake or disappearance of acid. It seems that acetate oxidation occurs slowly in testis and it is dependent on simultaneous carbohydrate oxidation; the acetate probably interacts with intermediate products of carbohydrate metabolism.

*General remarks.* Elliott & Greig [1937] found that testis produces a slight amount of succinate from pyruvate. This succinate would be oxidized to fumarate and malate, and the further oxidation of these substances might occur slowly.

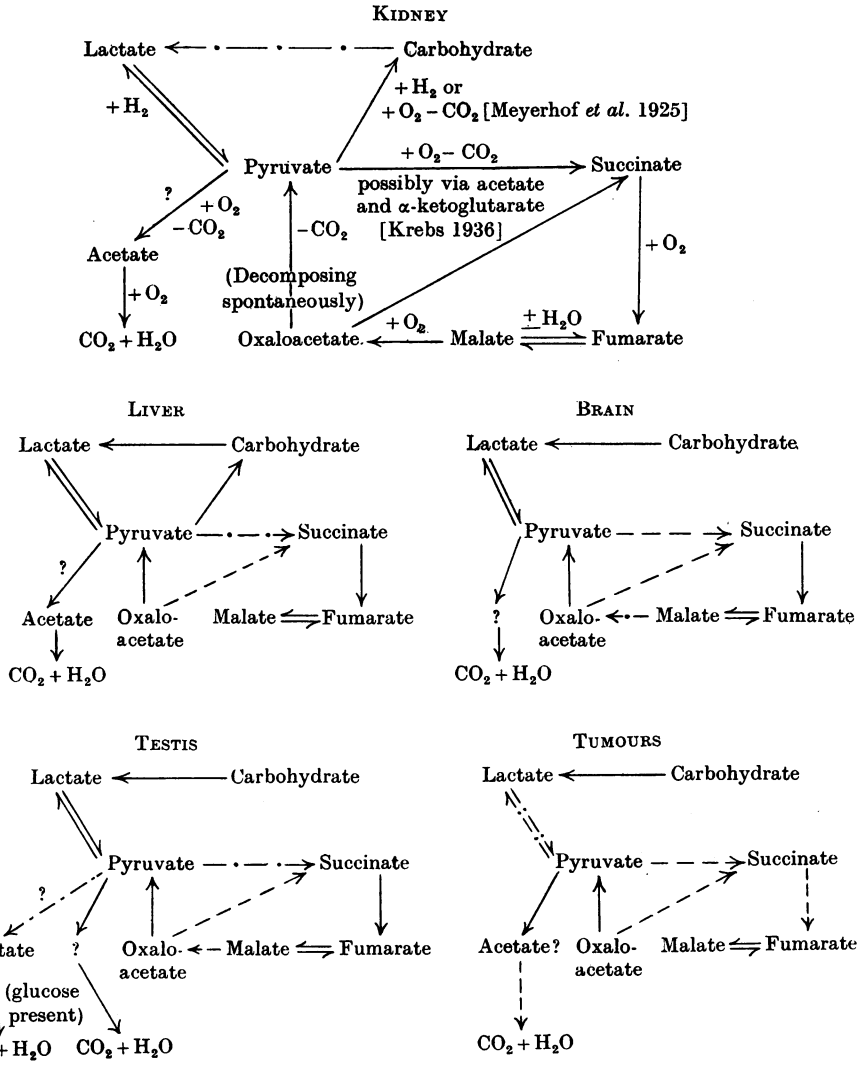
A slight amount of extra keto-body was usually formed in the initial period when succinate, fumarate, malate or acetate was present. Since the amounts were very small, 0·02–0·12 mg., the figures in [brackets] in Table VI are only approximate. Possibly a trace of oxaloacetate or other keto-body is formed by further oxidation of these substances.

No conclusion can be drawn from these experiments as to the main intermediate steps in the oxidation of pyruvate. Acetate is apparently not an essential intermediate since the oxidation of pyruvate, but not of acetate, continues in the absence of glucose.

## Discussion

The chief point that emerges from the work described in this series of papers is that the fate of added lactic and pyruvic acids varies strikingly from tissue to tissue. The results of these papers may be summarized in the following diagrams, which show qualitatively reactions occurring in the various tissues. It is not suggested that the diagrams represent all the events occurring nor is it possible

to estimate strictly the relative rates of the various reactions. However in the diagrams reactions which are known to proceed rapidly are marked →, those which proceed slowly —·—·→, and those which proceed only slightly if at all — — →. In addition, reactions which are presumed but not proved are




*Anaerobically* 2 Pyruvate → Lactate + Acetate + $CO_2$
(This reaction probably occurs to a small extent also in the other tissues.)

marked $\xrightarrow{?}$. No attempt is made to show intermediate steps such as those which must occur between pyruvate and carbohydrate or between pyruvate and succinate.[1] Meyerhof *et al.* [1925] have suggested equations for the synthesis of

[1] In following papers the work on carbohydrate synthesis and succinate production will be reported.

carbohydrate in liver slices, and an interesting scheme for the production of succinate has recently been put forward by Krebs [1936].

The most striking difference between all the normal tissues and the two tumour tissues studied [Elliott *et al.* 1935] is the failure of succinic acid oxidation in the latter. This point is being studied further.

Another point which needs closer study is the observation that during the first few minutes after introducing tissue into the medium a rapid aerobic glycolysis occurs with liver[1] and brain. Testis shows a continued aerobic glycolysis; according to György *et al.* [1928] and Dickens & Weil-Malherbe [1936] the same is true for kidney medulla, and the aerobic glycolysis of retina and tumour tissue is well known. It seems that under certain conditions aerobic glycolysis is a common property of normal and tumour tissues. We have tested kidney cortex and we find that in this tissue there is only a very low initial aerobic glycolysis ($Q_{LA}^{O_2} = +0{\cdot}6,\ +0{\cdot}9$).

Testis is the only tissue tried in which a considerable metabolism of pyruvate occurs under anaerobic conditions. The results did not indicate a simple decarboxylation of pyruvic acid, but rather a dismutation between two molecules of pyruvate yielding a molecule each of lactate, acetate and $CO_2$. In liver, brain, kidney and tumour tissues a slight anaerobic $CO_2$ evolution was observed when pyruvate was added, and possibly a similar dismutation occurs to a small extent in all tissues, or the $CO_2$ may be evolved as a result of other oxidations in which pyruvate replaces oxygen as hydrogen acceptor. In all the tissues, reduction of a fraction of the excess pyruvate to lactate occurs even aerobically; since pyruvate thus acts as a hydrogen acceptor and lactate is readily reoxidized in most tissues, the lactate-pyruvate system may serve a function as a hydrogen transporting agent.

Conclusions drawn from many of the results of these papers are subject to the criticism that the experimental conditions do not exactly reproduce the conditions of the tissues within the living organism: particularly can this criticism be applied to the method of adding excess of the substrate. Nevertheless, the results do show reactions which are likely to occur *in vivo*, and that there are marked differences in the potentialities of the different tissues. The results indicate that fumarate and malate might accumulate in liver, and possibly to a less extent in other tissues, since these substances are formed but apparently are not broken down; further work is necessary to discover whether these substances are normally removed by the circulation or whether under truly physiological conditions they can be broken down by liver. Green [1936] actually finds a considerably larger amount of malic dehydrogenase in extracts from brain and liver than from kidney. In his tests coenzyme I and a carrier (methylene blue) were added and it may be thought that coenzyme or a suitable carrier is lacking in liver, brain and testis, or lost by diffusion from the slices. But this seems unlikely since lactate is readily oxidized by slices of these tissues and lactic dehydrogenase also requires coenzyme I and a carrier. However, experiments to test the possibility will be tried shortly.

## Summary

1. Following the methods of Elliott & Schroeder [1934] and Elliott *et al.* [1935] a study has been made of the metabolism of lactic and pyruvic acids and of various other compounds by rat liver, brain cortex and testis. The previous papers showed that in kidney cortex lactate is oxidized to pyruvate and that the pyruvate is removed largely by a cycle of reactions involving the successive formation of succinate, fumarate, malate, oxaloacetate and pyruvate

[1] With liver this occurs at the expense of glycogen in the tissue and is independent of glucose in the medium; the other tissues mentioned split glucose present in the medium.

in half the original amount. In two types of tumour tissue, it was found that this cycle of reactions does not occur and mechanisms for catalysing the oxidation of lactate, succinate and malate were lacking. In the present paper it is shown that each tissue tried catalyses a different set of reactions, and that only in kidney tissue does the above-mentioned cycle of reactions occur to any extent.

2. In liver tissue lactate, pyruvate and acetate are oxidized. Succinate is oxidized to fumarate, and as in all tissues studied, fumarate is partly converted into malate, but no appreciable further oxidation occurs.

3. In brain grey matter, lactate and pyruvate are oxidized. Succinate is oxidized to fumarate and malate, and these substances are further oxidized to a small extent. Acetate is not appreciably oxidized.

4. In testis lactate and pyruvate are oxidized. Succinate is oxidized to fumarate and malate and these substances are possibly oxidized to a slight extent further. Acetate appeared to be oxidized slowly in the presence of glucose but not in its absence.

As is well known, testis shows a high anaerobic and a considerable aerobic glycolysis. It was noticed that under anaerobic conditions in the absence of glucose, testis produces some acid which is not lactic acid.

5. Under anaerobic conditions, testis is the only tissue tried in which a considerable metabolism of pyruvate occurs. The results did not indicate a simple decarboxylation of pyruvic acid, but rather a dismutation between two molecules of pyruvate yielding a molecule each of lactate, acetate and $CO_2$. In liver and brain and in kidney and tumours [Elliott *et al.* 1935] only a slight $CO_2$ evolution was observed.

6. It was found that liver and brain slices show a rapid aerobic glycolysis during the first few minutes after introduction into fresh medium. It is pointed out that high aerobic glycolysis is a property not only of tumours, but also of many normal tissues under certain conditions.

7. The results of experiments reported in this paper and in papers to appear shortly are summarized in a series of diagrams.

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