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

II. RAT KIDNEY AND TRANSPLANTABLE TUMOURS.

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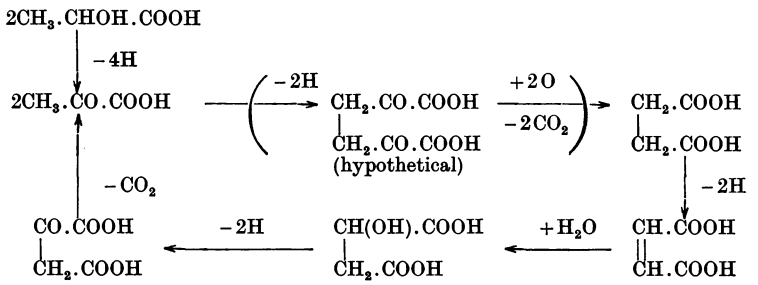
IN the first paper of this series [Elliott and Schroeder, 1934] a study was made by manometric and analytical methods of the oxidative breakdown of lactic and pyruvic acids in rabbit kidney cortex. It appeared that lactate is first oxidised to pyruvate and that the main course of the removal of pyruvate follows a cycle of reactions similar to that put forward by Toenniessen and Brinkman [1930] for muscle tissue. This cycle involves the oxidation of 2 mols. of pyruvic acid, probably by way of an unknown intermediary, to 1 mol. of succinic acid; succinate is then oxidised to fumarate, which, after conversion into malate, is oxidised to oxaloacetate, and oxaloacetate is decarboxylated yielding 1 mol. of pyruvate. The series of reactions is illustrated by the following scheme:



In this paper are presented the results of a corresponding study on transplantable rat cancers, and, for more direct comparison and further discussion, a set of results obtained with rat kidney cortex is given. The methods were similar to those described in the last paper with certain improvements. These results show that tumour tissue is completely unable to remove lactic and pyruvic acids by the above cycle of reactions, mechanisms for the catalysis of several of the steps being absent. In later papers we hope to follow the cycle in other tissues.

Methods.

In general the methods used by Elliott and Schroeder were employed. These consisted in measuring the O_2 uptake, respiratory quotient and acid change of thin slices of tissue in bicarbonate medium in an atmosphere of 95 % O_2 + 5 % CO_2 by means of the Dixon and Keilin apparatus [1933]; at the end of an experiment the contents of the manometer vessels were washed out quantitatively and

chemical estimations carried out on the fluid and tissue. Certain weaknesses in the estimations were discovered and modifications to overcome these are described below.

Pyruvic acid estimation. With the method of Clift and Cook [1932] inconsistent and low recoveries, 80–95 %, as judged by the titration of the acidity of pure pyruvic acid solutions using dilute NaOH and phenolphthalein, were obtained. The fault was found to lie in the fact that, after the addition of sodium bicarbonate suspension to break up the pyruvate-bisulphite compound, the bisulphite rapidly disappeared. If the mixture were allowed to stand for 10 min. at 22–24°, the amount of iodine required was reduced to 50–55 % of the theoretical. However, when the liquid was cooled to well below 10°, this loss was greatly reduced; recoveries of 96 % were then obtained consistently on immediate titration, and on standing 10 min. in the cold the recoveries only went down to 87–91 %. The method is therefore satisfactory provided that the solution is well cooled before adding the bicarbonate suspension and the titration is done immediately. It was found unnecessary to cool before adding the strong iodine to remove excess bisulphite; the loss due to dissociation of the bisulphite compound is slow, being about 5 % if there is a delay of 10 min. at 25° at this stage. The fact that the above source of error was not observed by Clift and Cook was probably due to the lower normal temperature in an English laboratory.

The necessity for cooling is removed if solid disodium hydrogen phosphate is used instead of bicarbonate suspension. After adjusting the initial point in the manner described by Clift and Cook, 2 g. of Na_2HPO_4, $12H_2O$ are added and the iodine titration is carried out at once at room temperature. The titration goes more slowly, but cooling becomes unnecessary, recoveries are consistently good, 97–99 %, and there is no blank value as there is with bicarbonate. This modification has been adopted throughout this work.

Clift and Cook observed that glucose gives a small titration by their method, especially in the presence of amino-acids *etc.*, but that the effect of the sugar is avoided if the solutions are made acid before the bisulphite addition. Larger effects were found when glucose in bicarbonate-Ringer solution had been incubated and kept for some time in dilute acid as in a manometric experiment, and still larger effects when tissue had been present, even though the solutions analysed were about 0·03 *N* acid. Under constant conditions the titre is about proportional to the amount of glucose present, so that an illusory pyruvate disappearance is observed when glucose is being removed by glycolysis. The glucose effects are much reduced and this illusory effect disappears if 0·5 ml. of *N* H_2SO_4 is added to the 5 ml. of solution before bisulphite treatment.

For example, in a normal experiment with tumour tissue in 3 ml. medium containing 7·2 mg. glucose initially, the fluid from the control (L) vessel, after making up to 25 ml. and analysing, apparently contained 0·28 mg. pyruvic acid and the fluid from the experimental (R) vessel, which had lost 2·1 mg. glucose by glycolysis, apparently contained 0·21 mg. pyruvic acid. In a similar experiment with 5 times the amount of glucose initially present the "pyruvate" found in the L and R vessels was respectively 1·40 and 1·24 mg. But when the extra acid was added before estimation in the above experiment with the normal amount of glucose the titres of both L and R vessel fluids were equivalent to only 0·11 mg. pyruvate, in another experiment to 0·06 mg., and in the experiment with five times the amount of glucose, the titres corresponded to 0·35 and 0·37 mg.

Oxaloacetic acid estimation. As Clift and Cook pointed out, the direct estimation of oxaloacetate gives uncertain results since increasing amounts of iodine are taken up owing to the formation of iodoform. Using the above alkaline phosphate method this effect seems even more pronounced, uncertain results as

high as 120–140 % of the theoretical being obtained. If however the solutions are made alkaline and heated on the water-bath for an hour as described by Clift and Cook for the removal of unstable carbonyl compounds, the oxaloacetate is completely changed into pyruvate, and satisfactory consistent titrations of 90–91 % are obtained. (Clift and Cook obtained 93 % for pyruvic acid itself after alkali-heat treatment.) Care must be taken to make the solution properly acid at the end of the alkali treatment by adding about 0·5 ml. N H_2SO_4 more than is required to decolorise the thymolphthalein. All our estimations of oxaloacetate have been made by this method and the results corrected by multiplication by 1·10.

Lactate and malate. The method of Friedemann and Graeser [1933] was followed as before except that the acetaldehyde-bisulphite compound was split by the alkaline phosphate method. Instead of 15 ml. of saturated $NaHCO_3$ solution about 2–3 g. of solid $Na_2HPO_4, 12H_2O$ were added at the beginning of the titration and 1–2 g. more at the appearance of the end-point to ensure a sufficient amount. The recoveries were uniformly good, 97–99 %. A few more experiments were done on the extent to which malate and fumarate affect the estimation. The effect of fumarate is very small. Whereas 1 mg. of lactic acid corresponds to a titre of 11·1 ml. of 0·002 N iodine, 1 mg. of fumaric acid requires about 0·3 ml. Malic acid has a considerable effect, 1 mg. requiring about 3·5 ml. of the 0·002 N iodine. This method however is not suitable for the estimation of malic acid since the recovery varies considerably and also depends on the time (half an hour or overnight) during which the solution has stood with the $Ca(OH)_2$–$CuSO_4$ sugar-removing agent. Nevertheless, when conditions are constant the method is useful as a rough measure of the changes in malate concentration. It should be noted that for the purposes of this work 0·5 mol. malate corresponds to 1 mol. lactate or pyruvate. All the figures under $Q_{L.A}$ in Tables I to X were calculated as if the estimations were on pure lactate. With many of the figures in brackets the estimations were actually mostly of malate and in these cases an idea of the malate change is obtained by multiplication by about $4{\cdot}3\left(\frac{11{\cdot}1}{3{\cdot}5}\times\frac{90}{67}\right)$.

Acetaldehyde. In the previous paper it was mentioned that the presence of acetaldehyde in the manometer vessels could be detected by taking readings of the manometer at 37° and at 10°, since acetaldehyde condenses at 21°. More careful tests showed that, owing to the high solubility of acetaldehyde, vessel constants for this gas are very high, about 200, so that only large amounts would affect the manometer appreciably. In this work therefore we have estimated any acetaldehyde formed, in conjunction with the lactic acid estimation, by carrying out a preliminary distillation for about 10 min. without permanganate. To prevent any permanganate entering the distilling flask, the stem of the permanganate dropping funnel is filled with water. The distillate is collected as usual in a solution of bisulphite and subsequent estimation of bound bisulphite gives the amount of aldehyde. The bisulphite solution is then changed and the lactic acid determination carried out as usual. Tests showed that under ordinary experimental conditions, after the removal of glucose with $Ca(OH)_2$ and $CuSO_4$, recoveries of 85 % of small amounts of added acetaldehyde were obtained. This was close enough as the method was used rather as a qualitative test. The traces of volatile carbonyl compound shown in the experimental part are entered as "acetaldehyde" and corrected for the low recovery by multiplication by 1·18. When the amount is appreciable, the pyruvic acid estimation has been corrected for the acetaldehyde which of course enters additively into that determination.

Glycogen. An appreciable and variable blank value of 0·03–0·045 mg. was found to occur in the method previously described. This was traced to a reducing impurity derived from the rubber stopper which holds the condenser tube into the centrifuge-tube during the HCl hydrolysis of the glycogen. By using a small glass funnel hanging in the centrifuge-tube, instead of the condenser, the blank values became lower and consistent at 0·02 mg.

Solutions. In the experiments with glucose this substance constituted 0·24 % of the medium. All the other substrates were added in the amount necessary to make the concentration in the medium 0·02 *N* (*e.g.* 4·02 mg. malic acid in the 3 ml.), except that with *dl*-lactate twice this concentration was used. The various substrates were made up in neutral solution in Krebs medium as previously described. For pyruvic acid it was found convenient to have a stock 5 % solution of the redistilled acid made up in Krebs medium, with water substituted for the bicarbonate solution. Immediately before use portions of this were neutralised with sodium bicarbonate-saturated Krebs medium and made up to the required volume with the medium.

The oxaloacetic acid used for this work was prepared from 50 g. ethyl sodio-oxaloacetate. The sodium was removed by shaking with cold dilute H_2SO_4 followed by extraction with ether. After removing the ether, the ester was hydrolysed by treatment with 4 vols. of concentrated HCl for 48 hours [Simon, 1903]. Any crystals formed were collected, and a further yield was obtained by repeated extraction of the fluid with ether. After recrystallisation from acetone and benzene 3 g. of the acid were obtained, M.P. 147–148°.

Manometric. With two people working together at the manometric work, it was possible to run four manometers simultaneously with less delay between the death of the animal and the starting of the last manometer than in the previous work with only three manometers at a time. In the majority of the experiments in this paper the Keilin "danglers" were omitted, the substrate solution being pipetted directly into the vessel immediately before placing in the bath.

Terms. The terms defined in the first paper are used for this work and the methods for reducing analytical figures to those terms are shown in the protocol of that paper. However, in this and future work, we shall use the term $Q_{Pyr.}$ only in cases where it is probable that pyruvic acid itself is the main keto-body estimated. In other cases, such as with oxaloacetic acid, we shall use the term Q_{Keto} to include change in all carbonyl compounds (including any traces of acetaldehyde when this substance has not been estimated separately). The term $Q_{Ald.}$ will be used to describe changes in acetaldehyde.

Figures given in brackets under Q_{LA} refer to estimations which were given in whole or in part by malate although the calculation was made as though lactate alone were present. Where chiefly malate was being estimated, these figures, as mentioned above, should be multiplied by 4·3.

Results.

Rat kidney cortex. For these experiments the kidneys of healthy large rats, weighing about 300 g., were used. Enough slices of cortical material could be obtained from the kidneys of one animal to put 60–90 mg. of moist tissue (10–15 mg. dry weight) in each vessel of four manometers. Roughly equal amounts of tissue from the two kidneys were put in each vessel. In Tables I and II results are given of experiments in the presence of glucose and in its absence. It is seen that the results are qualitatively the same in the two cases, and that the events are similar to those observed by Elliott and Schroeder with rabbit kidney.

Table I. *Rat kidney cortex. Glucose present in medium.*

Substrate	$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Keto}	Total glycogen mg. Control vessel	Total glycogen mg. Experimental vessel	Q_{PS}
No addition	24·3	0·85	− 0·6	−0·2	− 0·1	0·101	0·023	−0·9?
dl-Lactate	35·1	0·85	− 9·1	−6·7	+ 1·7*	0·033	0·046	+0·2
Pyruvate	34·9	1·31	−22·6	+3·9	−28·3	0·043	0·041	0·0
Acetate	26·9	0·93	− 6·8	+0·4	− 1·0	—	—	—

* See text.

Table II. *Rat kidney cortex. Glucose absent.*

Substrate	$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Keto}	$Q_{Ald.}$	Total glycogen mg. Control vessel	Total glycogen mg. Experimental vessel	Q_{PS}
No addition	21·5	0·82	+ 0·5	0·0	0·0	—	0·041	0·036	0·0
dl-Lactate	32·1	0·89	− 9·0	−9·3	+ 0·6*	—	0·020	0·151	+0·9?
Pyruvate	33·6	1·28	−20·3	+4·2	−26·0	—	0·062	0·046	−0·1
No addition	19·9	0·75	+ 0·7	+0·1	0·0	—	—	—	—
Succinate (*a*)	32·8	0·68	−14·0	(+3·7)	+ 3·5	—	—	—	—
(*b*)†	30·8	0·88	−15·1	—	—	—	—	—	—
Fumarate	24·5	1·06	−15·0	(+7·1)	+ 4·4	—	—	—	—
l-Malate	24·0	1·19	−14·6	(−8·2)	+ 3·5	—	—	—	—
No addition	17·0	0·77	− 0·4	0·0	0·0	—	—	—	—
Oxaloacetate	27·8	1·81	−32·3	+3·1	−21·7	+0·6	—	—	—
Acetate	20·5	0·88	− 4·1	+0·5	+ 0·3	—	—	—	—
β-Hydroxybutyrate	21·4	0·78	− 3·0	−0·1	0·0	+2·9	—	—	—

* See text. † Done on tissue from a different animal.

Studying Table II we see that lactate has disappeared to the extent of 9·3 m.eq. (manometric equivalents) and this is reflected in the acid disappearance. The oxygen uptake is much increased and there is a rise in the R.Q. In the previous paper it was suggested that pyruvate was the first stage in the removal of lactate and that in the presence of excess lactate a little pyruvate (keto) appeared as a result of a dynamic equilibrium. The above experiment illustrated this clearly since lactate was added to the medium in the vessel immediately after introducing the tissue and not by means of danglers after the equilibration period. It was found that during the 18 min. elapsing before tipping acid into the left-hand vessel, pyruvate had accumulated at the rate of 3·5 m.eq. and had already reached more than half of the maximum amount found in the right-hand vessel after 108 min.

Succinate, fumarate and malate disappear, as acids, at about the same rate. Apparently the oxidation of succinate to fumarate by rat kidney proceeds considerably more rapidly than the further oxidation of the acids. This is indicated by the higher O_2 uptake with succinate and the lowered R.Q. It has been mentioned that malate affects the lactic acid estimation considerably. This estimation thus brings out clearly the position of malate in the cycle. With succinate a considerable amount of malate appeared; with fumarate, which immediately precedes malate in the cycle, still more malate was formed, and with malate itself added, there was a large disappearance which would have been caused partly by oxidation and partly by conversion back into fumarate. The next two steps

in the cycle are oxaloacetate and pyruvate, both keto-bodies, and as would be expected, there is found to be some accumulation of such substances. (The R.Q. with fumarate and malate was raised considerably but did not reach the figure, 1·33, expected if they were being oxidised completely and no other oxidations were occurring.)

With oxaloacetate there is seen to be a very large disappearance of acid, part of which is due to decomposition to pyruvate, thus causing a very high R.Q., and part to the further oxidation of the pyruvic acid. It will be noticed that the keto-body disappearance is a little smaller but of the same order as when pyruvate is added directly, and the same applies to the lactate formed by reduction. As with rabbit kidney, acetate is oxidised to a certain extent as is indicated by increased O_2 uptake, a raised R.Q. and some acid disappearance. The rate however is not sufficiently high to suggest that it is an intermediary in the breakdown of pyruvate, but there is the possibility that acetate may be oxidised *via* the cycle, being first converted into succinate.

For comparison with the above results, Table III gives a set of results obtained with rabbit kidneys using the improved pyruvate estimation. These

Table III. *Rabbit kidney cortex. Glucose absent.*

Substrate	$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Keto}
No addition	11·9	0·75	+ 0·3	−0·1	0
Lactate	14·7	0·84	− 3·3	−3·5	+0·4*
					+0·1
Pyruvate	14·8	1·16	− 5·7	+1·5	−8·0
Fumarate	14·5	1·35	−12·8	(+9·1)*	+0·3
				(−0·1)	

* No danglers were used. Figures marked refer to pyruvate or malate formed during the preliminary 20 mins.

results agree completely with those given in the previous paper. In the experiment with fumarate, Keilin danglers were not used and during the preliminary 20 min. malate was formed in the left-hand vessel, before the acid was tipped in, at the rate of 9·1 m.eq. (as lactate) and, having reached equilibrium with fumarate, increased no further in the right-hand vessel during the experiment.

It is seen that lactate is less rapidly removed by the rabbit tissue than it is by the rat tissue. The same applies, and more strikingly, with added pyruvate. The rabbit tissue appears to remove pyruvate less rapidly than it does fumarate and the other substances of the cycle. Yet, if the cycle of reactions is to proceed, pyruvate should be oxidised at least as rapidly as fumarate; otherwise one would expect pyruvate to accumulate. It is possible that pyruvate added in a concentration of 0·02 *M* has an inhibitory effect on rabbit kidney. With rat kidney, the rate of pyruvate removal is very high, *e.g.* 26 m.eq. of keto-body. Some of this is reduced back to lactate, but the oxygen uptake, though much increased is not sufficient to account for complete oxidation of the acid ($Q_A = 20·3$) disappearing. (1 m.eq. of pyruvate requires 2·5 m.eq. of O_2 for complete oxidation.) It was pointed out in the last paper that the total oxygen uptake of rabbit kidney in the presence of succinate, fumarate *etc.* was not sufficient to account for complete oxidation of the acids disappearing. With the rat kidney the O_2 uptake is sufficient but only if one supposes that oxidation of practically no other materials takes place (1 m.eq. of fumarate requires 1·5 m.eq. of O_2). It is probable therefore that there is another course of metabolism of these substances which involves disappearance of carboxylic acid without complete oxidation. The estimations show that this is not a synthesis to glycogen. It may be

that other carbohydrates are produced, and this possibility will be examined in future work, although Ashford and Holmes [1931], who found a similar unaccountable disappearance of lactate with brain tissue, were not able to find a corresponding synthesis of carbohydrate.

It is necessary here to correct an error in the previous paper where the statements were made that kidney slices catalyse the decarboxylation of oxaloacetate and that the β-carboxylase responsible for the decarboxylation is destroyed by mincing the tissue. Proper controls now show that oxaloacetate, in bicarbonate medium at p_H 7·4 and 38°, decomposes rapidly at about the same rate in the absence of tissue as in its presence. The misleading results with minced tissue are accounted for mainly by the greater dry weight of tissue used, which, divided into the CO_2 evolved, gave a lower Q_{CO_2}. Low CO_2 evolutions were also obtained probably because during the extremely hot weather when the experiments were made the neutralised oxaloacetate had decomposed considerably while standing. Further, the minced tissue seems to inhibit the autodecomposition of oxaloacetate slightly. Table IV shows the results of anaerobic experiments with

Table IV. *Anaerobic experiments with kidney tissue.*

Tissue	Substrate	μl CO_2 evolved in 90 min.	Q_{CO_2}	Q_A	Q_{LA}	Q_{Keto}	$Q_{Ald.}$
Glucose absent:							
Rat kidney cortex slices	None	0	0	0	+1·1	−0·2	+0·45 (9 μl)
„	Pyruvate	22	1·3	− 0·2	+2·1	−4·1	+0·5 (9 μl)
„	Oxaloacetate	322	15·2	−13·8	+1·7	−3·6	+0·4 (9 μl)
Blank	Oxaloacetate	324	—	—	—	—	— (9 μl)
Glucose present:							
Blank	Oxaloacetate	343	—	(363 μl)	—	—	—
Rabbit kidney cortex slices	Oxaloacetate	253	7·0	− 1·0	—	—	—
Rabbit kidney mince	Oxaloacetate	189	1·6	− 0·7	—	—	—
Glucose present:							
Rabbit kidney mince	Oxaloacetate	263	2·6	−1·2	—	—	—
Rabbit kidney cortex slices	Oxaloacetate	321	6·7	+ 0·9	—	—	—
Blank	Oxaloacetate	297	—	(−296 μl)	—	—	—

oxaloacetate and pyruvate. Oxaloacetate apparently gives a high Q_{CO_2} but the blank shows that CO_2 is evolved at the same rate in the absence of tissue. In the two sets of experiments with rabbit tissue it is seen that spontaneous decomposition can account for the whole of the CO_2 evolved by oxaloacetate, in spite of the fact that greater amounts of tissue were used than previously (which explains the lower Q_{CO_2}). In each case with mince (0·35 and 0·30 ml. of tissue) the CO_2 evolved was low, even though in one case the mince experiment was the first to be set up after neutralising the oxaloacetic acid. (The experiments are shown in Table IV in the order in which they were started.) In the presence of glucose, the negative Q_A is lower because the anaerobic glycolysis of the tissue compensates for the loss of acid groups from oxaloacetate. With rat kidney slices the table shows that, anaerobically, pyruvate suffers only a slight decomposition with production of a little CO_2 and some reduction to lactate, the disappearance of

keto-body accounting for both. Estimating acetaldehyde as described in the first section, there was an apparent trace formed but this trace was also found in the absence of tissue. It seems certain that the decarboxylation of pyruvate is negligible and that no acetaldehyde accumulates. In calculating the Q values, the mean of the dry weights of tissue found in the left- and right-hand vessels was used, since kidney tissue in the right-hand vessel after an anaerobic experiment often weighs less than half of that in the left-hand vessel where disintegration has been checked by early acidification. The previous paper showed that β-hydroxybutyrate had practically no effect on the metabolism of rabbit kidney. With rat kidney we find that this acid is oxidised to some extent as is indicated by an increased oxygen uptake and some disappearance of acid. In Table V we

Table V. *Kidney cortex with β-hydroxybutyrate. No glucose.*

Tissue	Substrate	$-Q_{O_2}$	R.Q.	Q_{A^-}	Q_{LA}	Q_{Keto}	$Q_{Ald.}$
Rat kidney cortex	No addition	17·0	0·77	−0·4	0·0	0·0	0·0
	dl-β-Hydroxybutyrate 0·04 *N*	21·4	0·78	−3·0	−0·1	0·0	+2·9
Rabbit kidney cortex	No addition	13·2	0·75	+0·4	+0·1	−0·1	0·0
	dl-β-Hydroxybutyrate 0·04 *N*	13·7	0·74	+0·2	+0·1	0·0	+2·4

show these results together with a new experiment with rabbit kidney confirming the previous results. Another correction to the former paper is necessary here. Realising that small amounts of acetaldehyde formation would not appreciably affect the manometers but would affect the lactate determinations, acetaldehyde was estimated in the experiments with β-hydroxybutyrate according to the method described above. It was found that with rabbit and rat kidneys there was some acetaldehyde (or acetoacetic acid or acetone) formed but no lactate.

Tumour tissue. The experiments described below were done mostly with the Philadelphia No. 1 Rat Sarcoma described by Hueper [Waldschmidt-Leitz *et al.*, 1933]. A set of experiments with the Walker No. 256 Carcinoma is also shown. The tumours were taken 16–28 days after implantation and those used were either free of necrosis or had necrotic speckles fairly evenly distributed. As the slices were cut they were dropped serially into four small beakers containing the Krebs medium with O_2/CO_2 bubbling through, so that using slices from one beaker for each manometer, the manometers each had a reasonably fair sample of tissue. To minimise the amount of lactic acid introduced into the vessels with the tissue, the tumour slices were kept in glucose-free Krebs medium until they were to be used. After rinsing in bicarbonate-free Ringer solution and weighing, they were put back into Ringer solution; the slices for both vessels of one manometer were then drained and introduced into the two vessels simultaneously so that the left- and right-hand vessels started as nearly as possible under the same conditions. The experiments showed that keeping the slices in the absence of glucose did not affect their subsequent behaviour with glucose. It may be mentioned that the ratio wet weight/dry weight of these tumours is high, being between 8 and 9.

Table VI shows the results of duplicate experiments with Phila. No. 1 tumour tissue. It is seen that good agreement in the Q_{O_2} and R.Q. is obtained. The figures for glycolysis do not agree quite so well. The lactate formation does not account completely for the acid formed; this has been found fairly consistently and indicates that aerobically some acid body other than lactate is also formed in small amount, and, as will be seen, this is probably succinate. To make sure that we were losing no lactate by incomplete extraction of the tissues, we have estimated the lactate in the last wash-water and in the undried tissue itself after grinding it up thoroughly with sand and deproteinising the extract. Not more than 0·04 mg.

Table VI. *Duplicate aerobic experiments with tumour (Phila. No. 1) tissue.*

Glucose	Dry wt. mg.	$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	$Q_{Pyr.}$
Present	26·0	10·2	0·88	14·7	13·6	0·0
	22·3	10·0	0·90	16·5	15·4	−0·2
Present	25·6	10·8	0·81	13·8	11·6	+0·1
	22·9	10·0	0·84	13·7	12·9	0·0
Absent	26·4	11·3	0·79	− 0·6	+ 0·1	0·0
	29·9	11·6	0·81	− 0·9	+ 0·1	0·0

lactate, corresponding to $Q_{LA}=0·25$, was found in either. In the anaerobic experiments, of which one example is shown in Table VIII, the lactate found was rather more than enough to account for the acid formation. The experimental period was in all cases 90 min. as with other tissues; the curve of the movement of the manometer fluid was quite linear in the absence of glucose, whilst in the presence of glucose, there was in all experiments a slight steady falling off in the rate of movement throughout the period.

In Tables VII to X are shown the effects of the various substances under consideration on the metabolism of tumour slices in the presence and absence of

Table VII. *Philadelphia No. 1 Sarcoma. Glucose absent.*

Aerobic

Substrate	$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Keto}	$Q_{Ald.}$	Total glycogen mg. Control vessel	Total glycogen mg. Experimental vessel	Q_{PS}
No addition	12·1	0·84	−1·0	0·0	—	—	—	—	—
dl-Lactate	11·2	0·84	−1·7	−1·4	—	—	—	—	—
No addition	13·6	0·74	+0·3	0·0	−0·3	—	0·24	0·24	0·0
dl-Lactate	13·2	0·80	−2·6	−2·3	+0·7	—	0·33	0·33	0·0
Pyruvate	12·8	1·08	−1·3	+3·5	−9·0	—	0·38	0·36	−0·1
Succinate	13·8	0·70	+0·4	(+0·2)	0·0	—	—	—	—
No addition	14·1	0·76	+0·6	−0·6	0·0	—	—	—	—
Fumarate	12·2	0·65?	+1·8?	(+4·2)	0·0	—	—	—	—
l-Malate	13·3	0·73	+0·2	(−1·7)	0·0	—	—	—	—
Oxaloacetate	13·5	1·52	−9·2	+2·7	−7·0	—	—	—	—
No addition	13·0	0·80	−0·5	0·0	0·0	—	—	—	—
Succinate	12·5	0·74	−0·9	(+0·3)	+0·2	—	—	—	—
Fumarate	12·0	0·80	−0·4	(+4·4)	−0·3	—	—	—	—
No addition	11·5	0·78	+0·1	0·0	−0·1	0·0	—	—	—
l-Malate	12·1	0·81	−1·8	(−1·0)	+0·4	0·0	—	—	—
Oxaloacetate	14·2	1·35	−7·2	+1·6	−6·2	0·0	—	—	—
No addition	12·5	0·78	−0·3	−0·4	+0·1	—	—	—	—
Acetate	13·6	0·80	−0·3	0·0	0·0	—	—	—	—
Formate	12·6	0·75	+0·6	+0·2	+0·2	—	—	—	—
β-Hydroxybutyrate	13·6	0·70	0·0	−0·4	−0·3	+0·8	—	—	—
No addition	11·5	0·80	−0·4	+0·4	+0·1	—	—	—	—
Acetate	11·9	0·83	−0·8	+0·1	0·0	—	—	—	—
Formate	10·5	0·81	−0·8	+0·3	0·0	—	—	—	—
β-Hydroxybutyrate	10·7	0·81	−0·9	−0·1	0·0	+0·2	—	—	—

Anaerobic

Substrate	Q_{CO_2}	Q_A	Q_{LA}	Q_{Keto}	$Q_{Ald.}$
No addition	0·2	+1·5	+0·8	+0·2	+0·1
Pyruvate	1·7	+2·0	+3·2	−4·0	+0·1
Oxaloacetate (238 μl)	9·0	−6·1	+3·7	−3·7	+0·1
Blank oxaloacetate (200 μl)	—	—	0·0	0·0	—

Table VIII. *Philadelphia No. 1 Sarcoma. Glucose present.*

	Aerobic						Total glycogen mg.		
Substrate	$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	Q_{Keto}	$Q_{Ald.}$	Control vessel	Experimental vessel	Q_{PS}
No addition	9·9	1·02	+12·5	+11·3	0·0	—	0·47	0·34	−1·0
dl-Lactate	9·8	0·97	+ 9·8	+11·9	+0·4	—	0·51	0·39	−0·8
Pyruvate	10·3	1·28	+ 8·5	+13·4	−7·0	—	0·41	0·52	+0·7
No addition	12·1	0·83	+16·1	+13·0	0·0	—	0·09	0·14	+0·4
dl-Lactate	11·2	0·90	+13·2	+12·3	−0·4	—	0·09	0·09	0·0
Pyruvate	11·0	1·06	+ 7·5	+ 9·4	−6·6	+0·15	0·09	0·14	+0·4
Succinate	11·2	0·84	+14·7	(+14·9)	−0·7	—	—	—	—
No addition	12·4	0·83	+16·7	+14·6	0·0	—	—	—	—
Fumarate	10·0	0·80	+13·0	(+13·8)	0·0	—	—	—	—
l-Malate	9·3	0·84	+12·2	(+11·1)	0·0	—	—	—	—
Oxaloacetate	11·3	1·71	+ 5·2	+17·1	—	—	—	—	—
No addition	11·4	0·81	+17·3	+14·8	0·0	+0·3	—	—	—
Succinate	14·1	0·74	+14·6	(+15·1)	−0·4	+0·3	—	—	—
Fumarate	10·0	0·83	+14·5	(+14·5)	−0·3	+0·2	—	—	—
No addition	12·7	0·76	+15·8	+14·5	0·0	0·0	—	—	—
l-Malate	11·0	0·82	+12·8	(+12·2)	0·0	0·0	—	—	—
Oxaloacetate	10·4	1·63	+ 5·2	+15·9	−5·3	0·0	—	—	—

	Anaerobic				
Substrate	Q_{CO_2}	Q_A	Q_{LA}	Q_{Keto}	$Q_{Ald.}$
No addition	0·0	+23·4	+25·0	0·0	0·0
Pyruvate	0·8	+20·5	+24·3	−4·6	0·0
Oxaloacetate	10·0	+14·7	+27·7	−3·7	0·0

Table IX. *Walker No. 256 Carcinoma. Glucose absent.*

	Aerobic					
Substrate	$-Q_{O_2}$	R.Q.	Q_A	Q_{LA}	$Q_{Keto.}$	$Q_{Ald.}$
No addition	11·5	0·82	− 0·7	0·0	−0·2	0·0
dl-Lactate	11·5	0·82	− 1·5	−0·9	+1·0	—
Pyruvate	13·1	1·04	− 1·2	+3·6	−7·5	+0·?
Succinate	11·0	0·80	− 0·9	+0·1	0·0	—
No addition	11·7	0·78	+ 0·1	0·0	+0·2	—
Fumarate	12·0	0·80	− 0·2	(+2·3)	−0·3	—
l-Malate	10·8	0·82	− 0·8	(−2·4)	+0·2	—
Oxaloacetate	12·3	1·85	−11·7	+3·1	−6·2	—

Table X. *Minced Philadelphia No. 1 Sarcoma.*

	Substrate	$-Q_{O_2}$	Q_A	Q_{LA}	Q_{Keto}
Glucose present	No addition	0·81	+2·3	+1·9	+0·2
Glucose absent	No addition	1·03	+0·8	−0·2	−0·1
Glucose absent	Pyruvate	0·81	+1·1	+0·3	−0·6
Glucose absent	Succinate	1·13	+0·7	(+0·6)	0·0

Note. The metabolism was too small to give a reliable measure of the R.Q.

glucose. The experiments shown are representative and only when similar experiments have differed in some detail has more than one example of an experiment been entered. The effects of the presence of various substrates are more clearly seen in the experiments in the absence of glucose than in its presence since the high glycolysis in the latter case makes variations in Q_A and Q_{LA} more difficult to assess. Studying Tables VII and IX therefore we see that lactate is metabolised to a slight extent only and does not increase the oxygen uptake. With

succinate, fumarate and malate, we find a very striking difference between the behaviours of tumour and kidney tissue. The tables show that tumour is almost completely unable to metabolise these substances. There is no disappearance of acid, no increase in oxygen uptake and no significant change in the R.Q. The enzyme fumarase, which establishes an equilibrium between fumarate and malate, seems to be present however since, as judged by the "lactic acid" estimation, there is an increase in malate when fumarate is added, and a decrease when malate itself is added.

The case of pyruvic acid is interesting. Here there is a definite removal of pyruvate ($Q_{Keto} = -9$). Part of this has been reduced back to lactate as with kidney, but the main amount has been otherwise dealt with, yet there is only a slight disappearance of acid groups. It seems clear that pyruvate has been oxidised to succinate and no further. The raised R.Q. is compatible with this conclusion since the oxidation of pyruvic acid to succinic acid has a CO_2/O_2 quotient of 1·33. There is indeed not always an increase in the O_2 uptake but this is readily explained by the sparing of other oxidations. There is no glycogen formation, and production of other carbohydrate is unlikely since so little acid has disappeared. This behaviour of pyruvic acid with tumour is of importance, since, whilst we have fairly direct evidence for the other steps in the cycle, this is the first clear indication which we have obtained of the transformation of pyruvate into succinate. Dickens and Šimer [1930] observed the effect of pyruvate in raising the R.Q. of tumour tissue and believed that this indicated oxidation of pyruvate in the normal manner. The fact that the acid disappearance does not correspond with the keto-removal shows that this is not so. The results with added oxaloacetate are what would be expected. The very high R.Q. and the acid disappearance are largely due to autodecomposition of oxaloacetate to pyruvate. But, as with added pyruvate, there is also a considerable removal of keto-body and a small reduction to lactate. The results shown in Tables VII and IX are very similar, showing that sarcoma and carcinoma have the same defects in their metabolism. In anaerobic experiments, tumour tissue shows no striking differences from kidney. As with kidney there is possibly a trace of decarboxylation of pyruvic acid but most of the keto-body disappearing is reduced to lactate. No acetaldehyde formation is detectable. Oxaloacetate decomposes to pyruvate at about the same rate in the absence of tumour tissue as in its presence. It may be mentioned that tumour slices do not disintegrate anaerobically as kidney slices do.

Study of Table VIII shows quite clearly that all the points discussed above hold true when glucose is present, if account is taken of the large lactic acid formation.

Meiklejohn *et al.* [1932] have shown that vitamin B_1 is concerned in the oxidation of lactic acid by avian brain tissue. We therefore tried a set of experiments with tumour tissue with 0·5 mg. of a vitamin B_1 concentrate[1] present in the 3 ml. of medium. No effect whatever was observed on respiration, glycolysis or metabolism of succinate or malate. Boyland [1933] showed that the respiration of Jensen rat sarcoma in the presence of lactate is not increased by vitamin B_1.

Included in Table VII are examples of experiments with formate, β-hydroxybutyrate and acetate. The previous paper showed that formate has no effect on the metabolism of rabbit kidney, and it is seen that the same is true for tumour tissue. β-Hydroxybutyrate scarcely affected the metabolism and acetate increased the O_2 uptake only slightly without appreciable acid disappearance. It

[1] Obtained from Dr R. R. Williams of the Bell Telephone Laboratories.

is possible that acetate is normally oxidised first to succinate which is not further oxidised by tumour. This step might constitute a general point of confluence of fat and carbohydrate metabolism in cells.

In Table X are shown some experiments with minced tumour. They illustrate the well-known almost complete destruction of the respiratory and glycolytic mechanisms of tumour on mincing [Barr *et al.*, 1928]. It is also seen that the metabolism of pyruvate, which is shown above to appear in slices, is almost entirely stopped by mincing. In the previous paper it was shown that in minced kidney the succinic dehydrogenase remained very active. The fact that we find only a slight increase in respiration with succinate added to minced tumour is therefore further proof that tumour tissue is almost devoid of the mechanism for the oxidation of succinate.

Discussion.

The results with rat kidney illustrate perhaps more clearly than those obtained with rabbit kidney [Elliott and Schroeder, 1934] that the main course of the metabolism of lactic acid in these normal tissues consists first in an oxidation to pyruvic acid, the pyruvate then being removed by a cyclic series of reactions whereby it is converted successively into succinate, fumarate, malate, oxaloacetate and pyruvate again in half the original amount. With rabbit kidney, it was necessary to assume that part of the succinate, fumarate and malate removed underwent some other change involving loss of acidity but not complete oxidation. The same applies to pyruvate with rat kidney, but with this tissue the oxygen uptake is sufficient to account for complete oxidation of the other compounds if we assume that practically no other substances are being oxidised at the same time. There is no obvious reason why this should not be possible. The dehydrases involved may be present in such high concentrations that, when saturated with their substrates, they are capable of excluding by competition the reduction of the cytochrome, yellow pigment and other intermediaries of tissue respiration, by other enzymes and their substrates. They would thus secure to their substrates nearly the whole of the oxygen made available by these mechanisms. Nevertheless it seems possible that, even with rat kidney, a part of the succinic, fumaric or malic acid removed undergoes an unknown change.

The majority of tissues which form lactic acid in the absence of oxygen show little accumulation of this substance in the presence of oxygen. It is believed that this is explained partly by an oxidative inhibition of the glycolytic mechanism [see *e.g.* Lipmann, 1933] and partly by oxidation of the lactate as it is formed [Krebs, 1931].

The high rate of accumulation of lactic acid in experiments with tumour tissue would be the resultant of the rates at which the acid is formed and removed. There is no doubt that in tumour lactate is formed at an unusually high rate anaerobically and that inhibition of the glycolytic mechanism by oxygen is not complete, but the work described above shows that the accumulation of lactic acid can be explained in part at least by a failure in the oxidation processes. The cycle of reactions we are considering is active in muscle [Needham, 1932] and in kidney, and future papers of this series will show whether it is significant in other tissues. Meanwhile, we see that two, and possibly three, of the oxidative mechanisms are not active in tumours. These are succinic and malic oxidases and possibly lactic oxidase since so little lactate is removed, even though the next step, pyruvate to succinate, seems to occur at a fair rate. The transformation of normal tissue to the malignant habit, then, seems to involve, among other things, the loss of function of these enzymes. It should be pointed out that the mechanisms lost on destroying the tissue structure of kidney by mincing [Elliott and

Schroeder, 1934], namely lactic, pyruvic and malic oxidases, are not the same as those missing from tumour tissue, since tumour slices appear to possess pyruvic oxidase and lack succinic oxidase. According to Barron [1932], slices of various strains of rat tumour, including the Walker No. 256, do possess a succinic dehydrogenase, judging by experiments on the anaerobic reduction of methylene blue. If this is so, it appears that the failure of tumours to oxidise succinate and perhaps also malate and lactate aerobically depends on the absence of some intermediary catalyst, such as cytochrome or the yellow pigment (flavo-protein) of Warburg and Christian [1932]. Cytochrome does not appear to be lacking in tumours [Yaoi *et al.*, 1928; Bierich and Rosenbohm, 1926], but György *et al.* [1934] have shown that the growth-promoting vitamin B_2, which has been identified with flavin [György *et al.*, 1933], is present in very low concentration in tumours whilst it is high in liver and kidney. Euler and Adler [1934] have found the same distribution for flavins themselves. Nevertheless, flavo-protein appears not to catalyse the oxidation of succinate to fumarate [Wagner-Jauregg *et al.*, 1934, 1; Adler and Euler, 1935] and is therefore not necessary for this reaction. But Wagner-Jauregg *et al.* [1934, 2] showed that the yellow pigment is necessary for the anaerobic oxidation of malate and lactate with methylene blue. The lactic and malic dehydrogenases of heart muscle are apparently identical and both need activation by a coenzyme whilst the succinic dehydrogenase does not [Boyland and Boyland, 1934; Andersson, 1934]. Lack of enzyme, coenzyme or flavin might therefore explain the failure of tumours to oxidise lactate or malate. The failure in respect to succinate seems to require some other explanation.

Szent-Györgyi [1935; Gözsy and Szent-Györgyi, 1934] has recently stated that the reversible systems, succinate $\rightleftharpoons$ fumarate, and malate $\rightleftharpoons$ hydroxyfumarate (oxaloacetate), occupy a central position as carrier catalysts for tissue respiration in general. We would agree in the importance of these reactions, but only as stages in the metabolic breakdown of carbohydrate *via* pyruvate, and perhaps in the metabolism of fat *via* acetate and succinate. Apart from the facts that Szent-Györgyi used abnormal material (minced tissue) for his work, and gave no proof of the actual reversion of the reactions during normal activity, we would point out that tumour represents a tissue which respires at a rate corresponding to that of liver, but in which a mechanism responsible for each of his postulated key reactions is missing.

Summary.

1. Following the methods of Elliott and Schroeder [1934] with some improvements, a study has been made of the metabolism of lactic and pyruvic acids and of various other compounds by rat kidney cortex and by two types of tumours.

2. A method is described for avoiding a source of error in the Clift and Cook [1932] method for estimating pyruvic acid. Notes on the interference of glucose in this method are also given and the application of the method to oxaloacetate is described.

3. The previous paper showed that, in rabbit kidney, lactate is reversibly oxidised to pyruvate, which substance is removed mainly by a cycle of reactions involving the successive formation of succinate, fumarate, malate, oxaloacetate and finally pyruvate in half the original amount. This cycle is demonstrated more clearly with rat kidney. It is indicated that some pyruvic acid and possibly some of the other acids are removed by this tissue by a method which does not involve complete oxidation.

4. Oxaloacetic acid decomposes rapidly to pyruvic acid at 37° in neutral solution. Contrary to the statement in the previous paper, this reaction is not catalysed by kidney.

5. Acetate and β-hydroxybutyrate are oxidised to some extent by rat kidney. No lactate is formed from β-hydroxybutyrate; the apparent traces of lactate observed in the previous paper were due to acetoacetic acid, acetone or acetaldehyde affecting the lactate estimation.

6. With two types of tumour it is shown that the cycle of reactions mentioned above does not occur. Mechanisms responsible for two of the reactions are lacking, namely the oxidation of succinate to fumarate and the oxidation of malate to oxaloacetate. The oxidation of lactate to pyruvate also seems defective. On the other hand, the oxidation of pyruvate to succinate can proceed, and the establishment of an equilibrium between fumarate and malate does occur. These results are discussed in relation to the findings of other workers on enzymes and pigments in tumours.

7. Acetate and β-hydroxybutyrate are oxidised by tumours only to a slight extent.

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