CXVII. THE OXIDATIVE DISAPPEARANCE OF LACTIC ACID FROM BRAIN AND THE PASTEUR REACTION.

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(Received March 1st, 1935.)

MEASUREMENTS of the rate of oxidative lactic acid disappearance have been made by Holmes and Ashford [1930] and Ashford and Holmes [1931] in chopped brain, by Meiklejohn [1933] in minced tissue and by Meyerhof and Lohmann in brain slices [1926, 1]. Ashford and Dixon [1935] made some measurements of oxidative lactic acid disappearance and pointed out the small rate of this disappearance in relation to the rate of anaerobic lactic acid production. It seemed desirable to confirm this low value for rate of lactate disappearance in view of the doubt it throws on the generally accepted mechanism of the Pasteur reaction, namely, that oxygen removes the lactic acid which is continually formed as under anaerobic conditions. Further, it was necessary to establish if in reality more lactic acid disappears than can be accounted for by the oxygen uptake, as Ashford and Holmes [1931] have maintained.

In general the lactic acid oxidised is calculated (as Meyerhof did first for muscle) from the extra oxygen uptake caused by the addition of lactate. The justification for this method has been questioned in Ashford and Holmes's paper on the ground that sparing of the oxidation of intracellular substances may result from lactate addition and thus the oxygen uptake is really greater than that calculated. Ashford and Holmes concluded that sparing had not taken place.

Quastel and Wheatley [1932] however showed that lactate spared the oxidation of succinate. They also showed that the excess oxygen uptake caused by small addition of lactate to brain respiring without substrate would account for most of the lactate added if measured over a prolonged period of time. It seems evident however (as indeed they admit) that this only shows that complete oxidation may occur finally. Resynthesis in the Meyerhof sense might well have occurred at first, the resynthesised carbohydrate being later oxidised. Without continuous measurements of lactate disappearance this question could not be answered.

As the rate of respiration without substrate falls off markedly with time and that with added lactate only falls slightly, the difference between these values increases with time (this is seen from O_2 uptake curves in phosphate buffer [Holmes and Ashford, 1930; Ashford and Holmes, 1931] and is demonstrated below for bicarbonate buffer). It thus appeared that data as to the fate of the disappearing lactate could be obtained by studying the lactate disappearance and corresponding O_2 uptake over a period of several hours. If the accepted method of calculating lactate oxidation is correct (that is, that the difference between the lactate and no substrate respiratory values gives the lactate oxidation), then since this difference increases with time, the rate of disappearance of

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lactate should also increase. If on the other hand the oxidation of lactate suppresses the autorespiration, then the lactate will go on disappearing at the same rate. If this latter is really the case the lactate oxidised should be calculated not from the difference or excess O_2 uptake but from the total oxygen uptake.

METHODS.

All experiments were performed with slices of rabbit's brain cortex suspended in bicarbonate Ringer at 37° . Measurements of oxygen uptake and CO_2 production were made by the indirect (two vessel) method of Warburg. Sodium lactate made by neutralising *dl*-lactic acid with NaOH (with heating during the process to ensure hydrolysis of the lactide) was used so as to give a final concentration of 0.2 % lactic acid. Warburg measures aerobic lactic acid production by subtracting the O_2 uptake (Q_{O_2}) from the total CO_2 production in bicarbonate (Q_{SCO_2}). This method assumes a respiratory quotient of 1; if this is the case then that part of the total CO_2 production over and above an amount equal to the oxygen uptake is due to the formation of lactic acid and consequent decomposition of bicarbonate. Conversely, when lactic acid is disappearing the total CO_2 production is less than the oxygen uptake by an amount equal to that bound by the liberated cation of the lactate. This method has been described by Meyerhof and Lohmann [1926, 2]. The disappearance of lactate was thus measured by the value $Q_{O_2} - Q_{SCO_2}$ ¹ which gives the CO_2 absorbed owing to lactic acid disappearance and resulting liberation of base. This assumes R.Q. of 1 for the oxidation of lactic acid². (The R.Q. for the autorespiration of brain slices is 1 [Dickens and Greville, 1933].) The values observed are essentially of the same order as those obtained chemically by Ashford and Holmes. The advantage of this method is that it readily gives the rates of lactic acid disappearance during the whole course of the experiment.

EXPERIMENTAL RESULTS AND DISCUSSION.

Table I shows rates of lactate disappearance ($-Q_L^{O_2}$), O_2 uptake (Q_{O_2}) and also total CO_2 production observed (Q_{SCO_2}) both with and without substrate. In

Table I.

Date	Remarks	Lactate			No substrate		Glucose $Q_L^{N_2}$
		Q_{O_2}	Q_{SCO_2}	$-Q_L^{O_2}$	Q_{O_2}	Q_{SCO_2}	
25. i. 35	2 parallel experiments	7.1	4.9	2.2	—	—	—
		7.9	5.7	2.2	—	—	—
30. i. 35	1st hour	7.4	5.4	2.0	4.25	3.3	—
	3rd hour	7.0	5.3	1.7	2.4	2.6	—
	4th hour	6.9	5.1	1.8	2.0	2.0	—
2. ii. 35	1st hour	7.9	4.7	3.2	3.9	2.1	14.3
	2nd hour	7.4	5.05	2.35	2.8	2.2	10.7
	3rd hour	7.1	5.0	2.1	2.3	2.4	9.7
	4th hour	6.7	4.4	2.3	1.9	2.0	—
15. ii. 35	1st hour	8.0	5.8	2.2	5.6	4.3	—
	2nd hour	6.6	4.7	1.9	3.1	2.6	—
	3rd hour	6.1	3.9	2.2	1.9	1.5	—
	4th and 5th hour	6.1	4.2	1.9	1.1	1.1	—
	6th hour	5.3	3.5	1.8	0.5	0.2	—
	Average	7.0	—	2.1	—	—	—

¹ $Q_{O_2} = \mu l$ O_2 uptake per mg. dry weight per hour. $Q_{SCO_2} = \mu l$ observed CO_2 output per mg. dry weight per hour. $-Q_L^{O_2}$, the rate of disappearance of lactic acid, is equal to $Q_{O_2} - Q_{SCO_2}$.

² If the R.Q. were less than unity, the disappearance of lactic acid would be still less; this would not alter the conclusions reached in this paper.

one experiment the anaerobic glycolysis from glucose was simultaneously measured and values of $Q_L^{N_2}$ are given.

It is first seen that the low values for lactate disappearance are amply confirmed, an average value of $-Q_L^{O_2}$ of 2.1 being obtained.

Secondly, although considerable fall in rate of autorespiration is shown with time, only a slight fall of lactate respiration rate is experienced. This is very strong evidence that lactate addition largely suppresses the autorespiration.

In the third place although the difference between the oxygen uptake with and without lactic acid increases with time, the rate of lactate disappearance maintains either a fairly constant level or even falls slightly, but does not rise as would be the case if the resynthesis theory were correct (see above).

We may conclude then that subtracting the O_2 uptake without substrate from that obtained with added lactate is not a correct method of obtaining a value for the lactic acid oxidised, and that this can be obtained with much more justification from the total oxygen uptake. The existence of resynthesis or unaccounted lactic acid disappearance in brain thus becomes very doubtful. It seems safe to assume that the lactic acid is completely oxidised. The average of the values in Table I ($Q_{O_2}=7.0$ and $-Q_L^{O_2}=2.1$) is quite in harmony with this view.

Ashford and Holmes [1931] described experiments with washed chopped brain and also with chopped brain from animals killed in insulin convulsions. Under these conditions they obtained in some cases more lactate disappearing than could even be accounted for by total oxygen uptake. An experiment has been performed using brain cortex slices from a starved rabbit killed when in hypoglycaemic convulsions caused by an injection of 15 units of insulin $2\frac{1}{2}$ hours previously. The results are shown in Table II.

Table II.

Date	Remarks	Lactate			No substrate	
		Q_{O_2}	Q_{SCO_2}	$-Q_L^{O_2}$	Q_{O_2}	Q_{SCO_2}
21. ii. 35	2nd half hour	7.8	5.3	2.5	4.55	3.7
	2nd hour	6.8	4.6	2.2	(1.7)	(-0.6)
	3rd hour	5.85	3.2	2.65	1.9	2.0
	4th hour	6.8	4.6	2.2	1.1	0.6
	5th and 6th hour	6.35	4.1	2.25	1.0	1.3
	7th hour	6.3	4.3	2.0	0.5	0.5

In this experiment the average Q_{O_2} in lactate (a mean of the values in the table) is 6.65, while the average lactate disappearance is given by $-Q_L^{O_2}=2.3$. This result is nearer the theoretical 3 molecules of oxygen used for every lactic acid molecule oxidised than the values obtained with the normal brain slices. The small excess of the theoretical value of $Q_{O_2}=6.9$ over the observed $Q_{O_2}=6.65$ is almost certainly due to experimental error. In fact the agreement with the theoretical value is better than in the normal brain, where the Q_{O_2} of 7.0 instead of a calculated value of 6.3 was observed. It may well be that a very slight respiration of other substrate occurs in the normal brain and that this is removed by insulin with its attendant hypoglycaemia. The experiment shown in Table II shows the same general course of metabolism as in experiments recorded in Table I, that is, fairly steady disappearance of lactate, while rise in the difference between respiration in lactate and respiration without substrate occurs with increase in time. In this case then also the lactate oxidised should be calculated from the total oxygen uptake and not from the difference between the respiration in lactate and the autorespiration.

It seems clear that the hitherto much favoured theory of mechanism of the Pasteur reaction now requires, at any rate for brain, considerable modification. The rate of lactate disappearance in oxygen is no more than a quarter that obtained for lactic acid production in nitrogen (as is seen in Table I). This precludes the possibility that lactic acid is formed in oxygen as in anaerobic conditions and then removed. It might be said that the metabolism without substrate is still producing lactic acid and this is being re-oxidised to such an extent as to impede the disappearance of added lactate. This view, however, is rendered very unlikely for the following reasons. Firstly, it has been shown by Holmes and Holmes [1925; 1927], Ashford and Holmes [1931] and Holmes and Sherif [1932] that the anaerobic lactate formation without glucose is very small. Secondly, the metabolism without substrate seems to be largely suppressed in the presence of lactate. And, thirdly, the addition of potassium chloride does not cause aerobic glycolysis to appear in brain slices without substrate; this points to the absence of lactic acid formation and lactic acid-forming substance under these conditions.

The use of potassium chloride as an indicator for the presence of lactic acid-forming substances should here be briefly described. It has been shown [Ashford and Dixon, 1935] that addition of this substance causes appearance of marked aerobic glycolysis from glucose as well as increased oxygen uptake. It has since been shown that with fructose only increased oxygen uptake is observed and no aerobic lactic acid formation occurs. This was to be expected since Loebel [1925] and others since have shown that fructose gives hardly any anaerobic glycolysis. Lactate also gives increased oxygen uptake with potassium chloride. If, therefore, in the metabolism without substrate some lactic acid-forming substance were being used we should expect that the addition of potassium chloride would here cause aerobic lactic acid formation as with glucose. The following experiments with added potassium chloride in $M/10$ concentration negative this possibility: it is seen that without substrate there is no aerobic glycolysis, whereas with glucose and mannose which readily glycolyse under anaerobic conditions there is marked aerobic glycolysis.

Table III.

Date		No substrate	Glucose 0.2 %	Fructose 0.2 %	Mannose 0.2 %	Glucose 0.2 % Fructose 0.2 %	Glycogen 0.2 %
22. i. 35	$Q_L^{O_2}$ *	0	14.5	0	—	13.7	—
19. ii. 35	$Q_L^{O_2}$ *	0	10.7	—	11.0	—	0

* Rough direct method of Warburg [see Ashford and Dixon, 1935].

We may conclude then that in oxygen lactic acid is not formed at the same rate as in nitrogen and then reoxidised. Firstly, lactic acid does not disappear fast enough in oxygen for this to be the case. Secondly, if all the lactic acid formed in nitrogen were oxidised completely in oxygen the Q_{O_2} would be of the order of three times the $Q_L^{N_2}$ —it is in fact equal to about half the $Q_L^{N_2}$. For this reason one would have to assume resynthesis of lactic acid or some other form of disappearance unaccounted for by oxygen uptake, to explain a high rate of disappearance. However, the experiments described here disprove the idea of resynthesis, since the lactic acid disappearing is completely oxidised, at any rate at the concentrations employed (0.2 %). It seemed possible that unaccounted disappearance might occur at higher concentrations (0.5 %) employed by Ashford

and Holmes. However, an experiment performed using 0.5 % lactic acid has shown no unaccounted disappearance of lactic acid, as is seen in Table IV.

Table IV.

Date	Remarks	Lactate (0.5 % lactic acid)			No substrate		Glucose $Q_L^{N_2}$
		Q_{O_2}	Q_{SCO_2}	$-Q_L^{O_2}$	Q_{O_2}	Q_{SCO_2}	
13. iii. 35	1st hour	8.3	5.6	2.7	4.9	3.8	19.9
	2nd hour	7.4	4.8	2.6	3.5	2.8	17.5
	3rd hour	7.1	4.5	2.6	1.6	0.8	20.4
	4th hour	6.5	4.1	2.4	1.3	1.0	15.8
	5th hour	6.8	4.9	1.9	0.7	0.5	15.8
	Average	7.2	—	2.4	—	—	—

One must assume that in oxygen lactic acid is either not formed at all from glucose, or, if formed, is produced at a rate slow enough to be removed by complete oxidation. It thus appears that oxygen exerts its sparing effect on glycolysis at some point in the system prior to the formation of lactic acid.

SUMMARY.

1. Low values for rate of disappearance of lactate relative to rate of lactic acid formation in brain slices are confirmed, values averaging $-Q_L^{O_2}=2.1$ for lactate disappearance being obtained.

2. The rate of lactate disappearance is shown to remain fairly constant over prolonged periods of time. Only slight fall in respiration rate in lactate is observed, up to 35 % over 6 hours. Marked fall in respiration without substrate up to 90 % is simultaneously observed.

3. The bearing of these results on the question of the fate of lactic acid disappearing and on the site of action of oxygen in the Pasteur reaction is discussed. It is concluded that the lactic acid oxidised should be calculated from the total oxygen uptake and that complete oxidation occurs.

4. The use of potassium chloride for detection of possible lactic acid-forming substance under aerobic conditions is indicated.

I wish to thank Dr E. G. Holmes and Dr H. A. Krebs for their valuable advice and criticism.

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