LXXXIII. A CONTRIBUTION TO THE STUDY OF THE INTERCONVERSION OF CARBOHYDRATE AND LACTIC ACID IN MUSCLE.

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From the Biochemical Laboratory, Cambridge. Report to the Food Investigation Board.

(*Received October 21st, 1921.*)

I. The Fate of Lactic Acid on Oxidative Recovery.

Some months ago an attempt was made to confirm some recent work by Parnas [1920] on a vexed question of the utmost theoretical interest—that of the fate, during recovery in oxygen, of the lactic acid formed in the muscle during fatigue or survival.

Until the recent series of papers by Meyerhof [1920] there have existed two views as to the explanation of the facts known concerning this recovery. The work of Fletcher and Hopkins [1907] and Parnas [1915] supplied the chemical data, and that of Hill [1911, 1914] and Peters [1913] the thermodynamic data, on which these theories were based.

The facts may briefly be stated thus: The contraction of muscle is a strictly anaerobic process, and is accompanied by the production of lactic acid. The recovery process is dependent on the presence of oxygen, and is accompanied by the removal of lactic acid.

Hill [1914] stated from calculations based on work by himself and also on that of the others mentioned above, that the heat of recovery per grm. of lactic acid removed is 450 calories, while the combustion of the same amount of lactic acid would yield about 3700 calories. From this Hill argued that the lactic acid cannot be oxidised away, but must be rebuilt into the precursor from which it arose. As the breaking down of this precursor was associated with the liberation of energy (the energy of contraction) it is obvious that a supply of energy will be needed for the reverse process. Presumably this energy is supplied by a simultaneous combustion of carbohydrate, which is responsible for the gaseous exchange during recovery.

The views held by the other school will be found in the Croonian lecture by Fletcher and Hopkins [1917]. The lactic acid was believed to be removed by being completely burnt, with the production of carbon dioxide and the consumption of oxygen, the greater part of the energy not being given out as heat, but being retained in the muscle for the restoration of the initial physico-

chemical state. Evidence for this theory was obtained by Parnas [1915], who found that more heat was given out than was found by Hill, and that the amount of oxygen absorbed was equivalent to the lactic acid which disappeared[1].

It should be noted that in the combustion of either glucose or lactic acid, the ratio of carbon dioxide evolved to oxygen absorbed is equal to unity; moreover the combustion of equivalent amounts of glucose and lactic acid yields precisely the same amount of carbon dioxide, and nearly the same quantity of heat. Hence although it has been shown [Meyerhof, 1919] that the respiratory quotient during recovery after fatigue is 1, it is quite impossible to gather from a study of the gaseous exchange and heat production alone, which of these two substances is the "fuel" of the muscle.

Parnas in a communication to the Physiological Congress at Paris [1920] describes what he considers a crucial test for distinguishing between these two hypotheses. Consider an isolated muscle whose reserve is exhausted by prolonged contractions in oxygen. Parnas states that according to the first hypothesis (that of reconstitution) the resulting non-irritable muscle should have a maximum lactic acid content, as there is no fuel present to supply the energy necessary for the rebuilding process. On the other hand, on the alternative hypothesis, the lactic acid formed will be removed by oxidation up to the last, and the muscle in its final state will contain a minimum of lactic acid.

Parnas used sartorii suspended in Ringer's solution, and stimulated at long intervals for two days. When the muscles ceased to contract, he found that the carbohydrate content had been reduced to 0·05 %, and that no lactic acid was present. No lactic acid was formed when the muscles were removed to an atmosphere of hydrogen, and under these conditions *rigor mortis* did not appear.

Believing that this argument was valid, we repeated these experiments on a larger scale and in a modified form, and, though the results have led to no definite conclusions and are in some degree incomplete, we give them in detail below as they show in a very interesting way the equilibrium between lactic acid and carbohydrate, and the extraordinary duration of irritability in amphibian muscle.

It is clear now, however, that such an experiment could not form the crucial test between the two hypotheses. Meyerhof [1920] established that on oxidative recovery one-third of the lactic acid is burnt to carbon dioxide and water, and the remaining two-thirds is rebuilt into glycogen. He showed that the oxygen absorbed is equivalent only to one-third of the lactic acid which disappears, and the heat given out during recovery is approximately equal to the heat of combustion of one-third of the lactic acid disappearing, less the heat of contraction.

It is obvious that the results obtained in Parnas' experiment are explicable just as easily on this view as on the total combustion of the lactic acid. For,

[1] Meyerhof has not been able to confirm these figures. See below.

since there is an abundant supply of oxygen, one-third of the lactic acid will always be burnt away and the energy so obtained will serve to reconstitute the precursor from the remaining two-thirds. Hence when all carbohydrate supplies are exhausted there must be a minimum of lactic acid present.

Experimental Details.

Instead of sartorii we used whole limb pairs, which were suspended in moist oxygen instead of Ringer's solution. The apparatus used was a modification of one used by Parnas [1914] for a different purpose.

The frogs used (in the two experiments described there were 60 in each case) were pithed, the hind limbs severed well above the pelvic girdle and the limb pairs immediately placed in Ringer's solution until all were ready. They were then hung by the anal apertures on aluminium hooks, which were attached to a ring of aluminium about six inches in diameter. Each ring carried thirty hooks and was provided with an aluminium stem, which passed through a hole in the rubber stopper of a large bell jar. The jar stood in a dish containing five litres of Ringer's solution, and the height of the aluminium ring was so adjusted that the limbs dipped into the solution up to the ankles. Two such bell jars were used to support the 60 frogs. An atmosphere of oxygen was maintained inside the bell jar by leading a steady, slow current of the gas from a cylinder through a glass tube which passed through the cork, and opened by means of a four-way piece below the surface of the solution. The oxygen escaped through an opening in the stopper.

The limbs were stimulated with tetanising currents, the induction coil being at 2 cms. The primary circuit contained a battery of 1·4 volts and a key. In the secondary circuit, one copper wire passed from the coil to the aluminium stem, around which it was twisted; the other wire, also of copper, was fastened to a platinum wire, carrying a platinum electrode, which dipped in the Ringer's solution. The limbs themselves completed the circuit.

The limbs were stimulated for ten minutes every hour. During the earlier periods the muscles responded actively the whole time, though signs of the onset of fatigue were visible towards the end of the periods. With the later stimulations response was obtained at the beginning of the period, but failed before the end. In two experiments the muscles remained irritable for 72 hours, *i.e.* after 720 minutes of stimulation; in the third experiment, in which midsummer frogs were used, irritability only lasted for 48 hours under these conditions. The Ringer was changed daily. Some hours before the experiment was stopped a putrefactive smell was noticed, and was believed to arise from the injured muscles of the pelvic girdle, as it is well known that muscle resists infection as long as it is excitable. At the close of the experiment the muscles were almost all non-irritable, but a few responded feebly. The difficulty of deciding the right moment for stopping the experiment is increased by this fact, that the leg muscles lose irritability at different rates.

The limbs, on removal from the apparatus, were placed in ice and shuffled. Groups of ten were selected for the various estimations, set out in detail below. In the case of those brought to heat rigor, they were placed on the bottom of thin-walled beakers, sunk in a thermostat at 45°, and kept at this temperature for 1½ hours. The lactic acid estimations were carried out exactly according to the routine method of Fletcher and Hopkins [1907] and the carbohydrate estimations by the method described by Meyerhof [1920].

Owing to individual variations in the carbohydrate content, comparable estimations were made on the *single* limbs of the same frogs whenever possible; *e.g.* for one estimation 20 left legs were used, and for the estimation to be compared with it, the 20 right legs.

Exp. 17. 13. iv. 21. (See Table I.) 60 frogs used; 72 stimulations made.

The following estimations were made:

1. Initial carbohydrate
2. Heat rigor carbohydrate } 20 half limb pairs.
3. Final resting lactic acid — 10 limb pairs.
4. Final resting carbohydrate
5. Heat rigor carbohydrate } 20 half pairs.
6. Heat rigor lactic acid — 10 limb pairs.

Exp. 18. 20. v. 21. (See Table I.) 60 frogs were used; these were very stale summer frogs, some of which had been kept a month or more, the others a shorter time, but all were very thin. Care was taken that the limbs were well shuffled. 48 stimulations were made.

The following estimations were made:

1. Initial carbohydrate
2. Final resting carbohydrate } 20 half pairs.
3. Rigor carbohydrate — 10 pairs.
4. Rigor lactic acid
5. Final resting carbohydrate } 20 half pairs.
6. Final resting lactic acid — 10 pairs.

Table I. *Exps.* 17 *and* 18.

	Initial carbohydrate (20 half limb pairs)			Final resting carbohydrate (20 half limb pairs)			Difference	Rigor carbohydrate (20 half limb pairs)			Difference	Final resting lactic acid (10 limb pairs)	Heat rigor lactic acid (10 limb pairs)
	Glycogen	Sugar	Total	Glycogen	Sugar	Total		Glycogen	Sugar	Total			
Exp. 17	0·416	0·117	0·533	0·271	0·007	0·278	0·255	0·017	0·009	0·026	0·259	0·036	0·108
								0·016	0·003	0·019			
	(20 half limb pairs)			(20 half limb pairs)				(10 limb pairs)				(10 limb pairs)	(20 half limb pairs)
Exp. 18	0·433	0·108	0·541	0·049	0·013	0·062	0·479	0·014	0·019	0·033	0·029	0·055	
				0·037	0·015	0·052							0·032+

Discussion of Results.

Our results agree with those of Parnas, in that the final lactic acid value was found to be a minimum. In one experiment we succeeded in reducing the total carbohydrate to the value mentioned by Parnas, about 0·05 %. On keeping the muscle at 45° for 1½ hours the carbohydrate content was reduced to 0·03 %; it was intended to estimate the lactic acid formed under the same conditions, but, owing to an accident, the result is only approximate—about 0·03 %.

In the other experiment the carbohydrate was only reduced to 0·277 %. In this case two lots of limbs were put under conditions of heat rigor, and the carbohydrates afterwards estimated; the low values of 0·026 % and 0·019 % were obtained. The rigor lactic acid in this case was 0·108 %—a high value, corresponding to the high final content of carbohydrate.

A Confirmation of some other Recent Work on this Subject.

As mentioned above, Meyerhof has recently published a series of papers in which he brings forward a great mass of evidence in favour of the reconstitution of the greater part (two-thirds to three-quarters) of the lactic acid into its carbohydrate precursor. His arguments are based on a large number of experimental results, which are different from those of Parnas, and are most convincing if these results are to be accepted. A large amount of confirmatory data is given, but all estimations were done by micro-methods, and in a matter of such fundamental importance it seemed worth while to repeat the more crucial of these experiments on a larger scale, and by different methods. Accordingly we selected two problems—the conversion of lactic acid into glycogen on recovery in oxygen and the conversion of glycogen into lactic acid on chopping—and investigated these by Fletcher and Hopkins' method for the lactic acid, Pflüger's method for glycogen and Meyerhof's modification of Parnas' method for the soluble sugars, using ten limb pairs for each estimation. We may say at once that our results were in entire agreement with those of Meyerhof.

Exp. 15. *The recovery process in fatigued muscle.*

Method. 40 limb pairs were, immediately after pithing, fatigued for 45 minutes in an atmosphere of nitrogen. The limbs were suspended from the aluminium rings as in previous experiments. A single cell of the battery was used for stimulation, and the coil was gradually pushed in as the muscle showed signs of fatigue, till finally it was at zero. After the stimulation, 20 limb pairs were removed and placed in a covered beaker sunk in ice; the whole was weighed as quickly as possible. The muscles were then dissected from one leg of each limb pair, with all the usual precautions against injury. During this time nitrogen was passed into the beaker sunk in ice. The lactic acid was extracted in the usual way. The muscles from one leg of the remaining

twenty limb pairs were then similarly treated for carbohydrate estimation. The weight of muscle was in each case determined by difference.

For the recovery, the legs with the remaining muscles were re-suspended in the bell-jars in a current of oxygen, which was maintained for 23 hours. At the end of this time the lactic acid and carbohydrate were extracted again, care being taken that corresponding legs were used, *i.e.* of the muscles of one limb pair those of one leg were used for fatigue carbohydrate and those of the other leg for the recovery value. This is necessary in view of the great individual variations in carbohydrate content.

Table II.

	Glycogen	Soluble sugars	Total carbohydrate	Lactic acid	Total carbohydrate and lactic acid
Fatigue	0·750	0·042	0·792	0·166	0·958
Recovery	0·936	0·073	1·009	0·037	1·046
Difference	...	...	0·217	0·129	

Discussion of Results.

It can be seen from these figures that, contrary to the statement of Parnas and Wagner [1914], an increase in carbohydrate content accompanies recovery in oxygen. The ratio of lactic acid lost to carbohydrate gained is, indeed, not that found by Meyerhof, but the discrepancy in our figures is no doubt to be explained by the individual variations in the limb pairs. Meyerhof, using a micro-method, was able to estimate lactic acid in the gastrocnemii of the same limb pairs used for the carbohydrate estimations.

Exp. 14. *The change of glycogen into lactic acid in chopped muscle.*

Method. The resting carbohydrate and lactic acid were determined on twenty limb pairs—twenty single legs being used for each.

60 more frogs were pithed, cooled in ice, and then skinned; the limbs were placed in beakers sunk in ice. The muscle was dissected off the limbs and chopped with cold knives, on plates resting on ice. The chopped muscle was also cooled as it accumulated. When all the muscle from the 60 frogs had been chopped, it was thoroughly mixed to obtain representative samples, and then divided into six approximately equal portions, which were weighed in tared, cooled beakers.

Table III.

Exp. 14. *The formation of lactic acid in chopped muscle.*

Lactic acid				Carbohydrate				
No.	Time	Total	Difference	No.	Soluble sugar	Glycogen	Total	Difference
5	Initial	0·044		6	0·037	0·902	0·939	
1	One hour	0·134	0·09	2	0·112	0·745	0·857	0·08
3	4 hours	0·326	0·192	4	0·158	0·527	0·685	0·172
A	Resting	0·015		B	0·05	1·18	1·23	

Four of the beakers were then sunk in a thermostat at 20°; the remaining two samples were used for the estimation of initial lactic acid and carbohydrate respectively.

The other samples were removed from the thermostat after one and four hours respectively, being placed in ice at once.

Discussion of Results.

The results obtained are again in agreement with those of Meyerhof, showing absolute parallelism between the loss of carbohydrate and increase in lactic acid, and contrary to those of Parnas, who found that the carbohydrate decrease lagged behind the lactic acid increase.

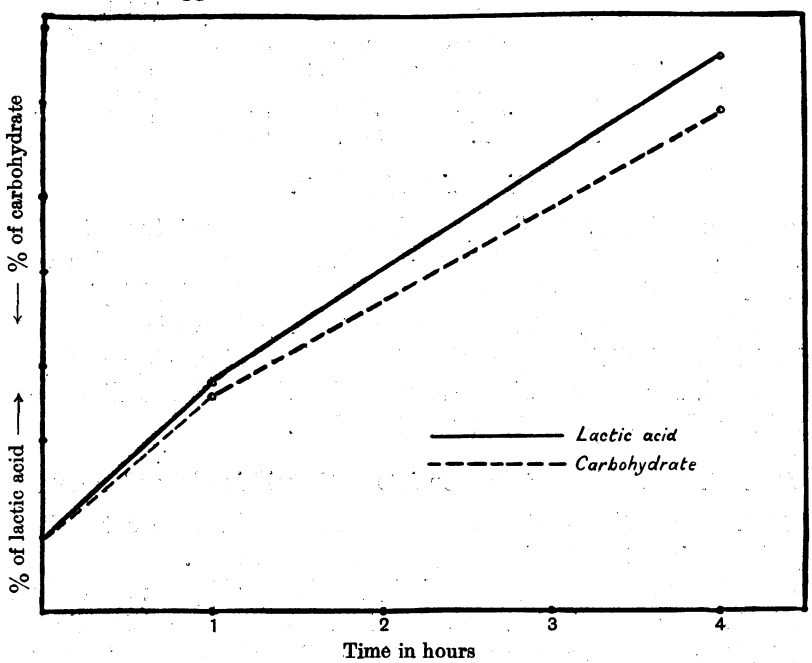

Fig. 1. Experiment 14. The conversion of carbohydrate into lactic acid in chopped muscle.

The values for the resting carbohydrate and lactic acid cannot strictly be compared with the other values, as they were obtained from distinct limb pairs. (See Table III and Fig. 1.)

Further Evidence for Hexosephosphate as the Precursor of Lactic Acid.

In this section we describe an experiment which is of some interest as it indicates that chopped muscle not only possesses the power, as shown by Embden and his co-workers [1915] for muscle juice, of breaking down added hexosephosphate to lactic acid, but that it is also capable of synthesising the hexosephosphate from added glucose and phosphoric acid.

Exp. 16. The muscle from the hind limbs of 80 frogs was dissected off and put through a mincer, being cooled the whole time in a freezing mixture. Approximately equal quantities of the mixed muscle were placed in each of four tared beakers, and weighed. Each portion was then transferred quantitatively to a 500 cc. filtrate jar, covered with a glass plate. Each lot of muscle was treated with 200 cc. of 2 % $NaHCO_3$ solution. The jars were labelled *A*, 1, 2, 3, and their contents were:—

A. Muscle + 200 cc. 2 % $NaHCO_3$ + 35 cc. H_2O.
1. Muscle + 200 cc. 2 % $NaHCO_3$ + 35 cc. hexosephosphate solution.
2. Muscle + 200 cc. 2 % $NaHCO_3$ + 35 cc. glucose solution.
3. Muscle + 200 cc. 2 % $NaHCO_3$ + 35 cc. glucose solution + 2 grms. NaH_2PO_4.

The jars were incubated at 37° for three hours, then removed and placed in a freezing mixture. The liquid in each case was strained through muslin into a beaker which was sunk in boiling water (to coagulate the protein and inactivate any enzyme) and allowed to remain there for an hour. In each case the liquid was neutralised with HCl, and to the control (*A*) 35 cc. of the hexosephosphate solution were added. The residues were extracted with ice-cold alcohol in the usual way. After standing overnight the coagula were filtered from the watery extracts, and united with those to be extracted with alcohol. The watery extracts were taken to dryness on the water bath, and the residues also extracted. The alcoholic extracts were acidified with phosphoric acid to prevent destruction of the lactic acid. From this point the routine method was followed, except that twice the usual quantity of charcoal was added. In the case of 2 and 3 the solutions were coloured, probably owing to the action of the alkali on the sugar; each of these was treated with charcoal a second time with about 1 g. and the lead carbonate treatment was also introduced. The glucose present in the moist lead hexosephosphate was estimated after hydrolysis so that the amount added was known approximately. The moist hexosephosphate was equivalent to 2·5 % glucose. A weighed amount of the Pb hexosephosphate was decomposed by H_2S, the gas removed by a current of air, and the volume made up to 80 cc. 80 cc. correspond with 11·43 g. hexosephosphate, or 0·285 g. glucose, so that 35 cc. correspond with 0·125 g. glucose. The glucose solution was made up so that 35 cc. contained 1·3 g. The results obtained are set forth in Table IV.

Discussion of Results.

These figures show conclusively that not only has the intact muscle the power of breaking down hexosephosphate *in vivo*, but that the chopped muscle apparently possesses the same power. But more interesting still is the evidence for the presence of a mechanism for synthesising this precursor from its ingredients.

Table IV.

Exp. 16. *Hexosephosphate as a precursor of lactic acid.*

No.	Treatment	Weight of muscle	Weight of zinc lactate	Weight of muscle as 100	Treatment	% of lactic acid	Difference
A.	Control	84·00	·542	100	Control	·469	
1	0·125 g. glucose added as hexose-phosphate	84·54	·742	100	0·149 g. glucose as hexose-phosphate	·652	·183
2	1·3 g. glucose added	79·781	·426	100	1·63 g. glucose	·397*	
3	1·3 g. glucose added + equiv. NaH_2PO_4	81·24	·782	100	1·63 g. glucose + equiv. NaH_2PO_4	·715	·246

* This result was unreliable owing to experimental difficulties due to the excess of glucose.

Since this experiment was performed Meyerhof [1921] has published the account of an experiment in which chopped muscle, suspended in a buffer solution containing phosphates, converted added glucose into lactic acid.

Summary.

I. Experiments are described showing the conversion of carbohydrate into lactic acid in the muscles.

II. The conversion of lactic acid into carbohydrate in the intact muscle is demonstrated, as well as the breakdown of carbohydrate in chopped muscle.

III. The breakdown and synthesis of hexosephosphate in chopped muscle are shown.

We are indebted to Professor Harden for the specimen of hexosephosphate used, and to Professor Hopkins for invaluable help and criticism throughout the work.

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