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THE OXYGEN CONSUMPTION AND CARBOHYDRATE METABOLISM OF THE RETRACTOR MUSCLE OF THE FOOT OF *MYTILUS EDULIS*

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THE metabolism of plain muscle has not received the attention which that of striated has, largely owing to experimental difficulties. Evans [1925] has shown that the lactic acid content increases during anaerobiosis and after stimulation, and there is evidence that gut muscle can form lactic acid more readily from glucose than from its own stores of carbohydrate or from added glycogen [Meyerhof and Lohmann, 1926; Rosenthal and Lasnitski, 1928; Haarmann, 1932; Prasad, 1935]. These authors also record values for oxygen consumption.

For the present study the retractor muscle of the foot of *Mytilus edulis* was chosen, partly because it is easier to handle invertebrate than mammalian muscle and partly because it is easy to dissect this muscle free from other tissue, but also because Eggleton [1934] has recently investigated the behaviour of the phosphagen fraction of this muscle during fatigue and recovery, so that something of its metabolism is already known.

MATERIALS AND METHODS

Mussels were obtained from the Marine Biological Laboratory at Plymouth and kept in moist seaweed in the cold room. No differences were observed in muscles from *Mytilus* recently delivered and those kept for up to 4 weeks; longer than this they were not stored. The retractor of the foot was dissected as described by Eggleton [1934] and placed in cooled oxygenated sea water, in most experiments for 2 hours before further treatment.

Oxygen consumption was measured by Warburg's direct method, using Barcroft or Warburg flasks filled with oxygen. The whole of a single muscle was used since the slow rate of oxygen consumption allows thicker tissue to be used, and no differences between large and small muscles were observed. It was placed in 2 or 3 ml. of one of the following solutions: (*a*) artificial sea water (NaCl 0·6 *M* 100 ml., KCl 0·6 *M* 2 ml., $CaCl_2$ 0·4 *M* 4 ml., $MgCl_2$ 0·4 *M* 17 ml.), unbuffered; (*b*) artificial sea water buffered with *M*/150 phosphate; (*c*) buffer alone.

Anaerobic experiments. Muscles were placed in 2–5 ml. of solution in flasks filled with either nitrogen or nitrogen and 5 p.c. CO_2. For short experiments, up to 6 hours, the flasks were shaken in a water bath at constant temperature, but in longer experiments they were incubated without shaking.

Stimulation. Immediately after dissection a muscle was suspended in a chamber similar to that described by Winton [1926] either in natural sea water or in a marine Ringer solution (KCl 0·6 *M* 1·8 ml., $CaCl_2$ 0·4 *M* 2·8 ml., natural sea water to 100 ml.) and attached to a light isotonic lever weighted to give a stretch equal to 15 g. when the muscle was not stimulated. The muscle was allowed to rest with oxygen bubbling through the tube for 2 hours and the sea water was changed before beginning stimulation and nitrogen substituted for oxygen in most experiments. The muscle was stimulated by alternating current pulses from a commutator, 25 per sec., connected to an 8 volt accumulator, using in most cases 5 sec. periods of stimulation once per min.

Chemical. Total carbohydrate and *glycogen* were determined by hydrolysing in hot acid or potash, either the whole muscle or each half separately, after dividing the muscle longitudinally as equally as possible. After hydrolysis the estimation of fermentable carbohydrate was continued as described by Kerly and Ronzoni [1933], and that of glycogen by the method of Good, Kramer and Somogyi [1933]. *Fermentable sugar* was estimated in a mercuric chloride extract [Kerly and Ronzoni, 1933] and *lactic acid* in either a mercuric chloride or a trichloroacetic acid extract [Friedemann, Cotonio and Shaffer, 1927, using Wendel's (1933) receiver]. Estimations on solutions of lithium lactate showed that with quantities of the order estimated in these experiments an accuracy of 3 p.c. could be obtained. Values are given both as mg./100 g. wet weight, and as $Q_G^{N_2}$ (c.mm./mg. dry weight/hour) for comparison with other published figures.

Analysis of resting muscle

The glycogen and total carbohydrate values found during the winter (November and December) were much higher (Table I) than those found in the summer (June and July). Values for opposite halves of the same muscle gave fair agreement, both for total carbohydrate and for glycogen. When total carbohydrate in one half was compared with glycogen in the other on the average the total carbohydrate content was higher, but the differences did not lie outside those found between total carbohydrate or glycogen in two halves of the same muscle. In the few estimations made of fermentable sugar the results were not very constant, mean 47 mg./100 g. The lactic acid content fell the longer the muscles were allowed to recover from dissection in oxygenated sea water. The mean value for muscles left from 10–30 min. was 28 mg./100 g., whilst that for muscles left 1–2 hours was 13 mg./100 g. The water content averaged 83·5 p.c. of the wet weight (11 muscles, spread 81·2–84·5 p.c.).

Table I. Carbohydrate content of resting muscles, mg./100 g.

Total carbohydrate			Glycogen			Excess total carbohydrate over glycogen p.c. of carbohydrate	Date
Half *a*	Half *b*	Diff. p.c.	Half *a*	Half *b*	Diff. p.c.		
2155	2350	8·3	2315	2020	12·6	—	Nov.
2170	2170	0	1970	1850	6·1	—	,,
—	2520	—	2100	—	—	+16·7	,,
—	3280	—	3160	—	—	+ 3·7	,,
2500*	—	—	—	—	—	—	Dec.
3098*	—	—	—	—	—	—	,,
2843*	—	—	—	—	—	—	,,
2533*	—	—	—	—	—	—	,,
Mean 2562	—	—	2236	—	—	—	—
1659	1341	19·2	676	723	6·4	—	June
1139	1144	0·4	1040	1050	1·0	—	,,
—	1020	—	888	—	—	+12·9	,,
—	778	—	823	—	—	− 5·5	,,
1794	1810	0·9	2075	2060	0·7	—	July
885	909	2·5	1728	1782	3·0	—	,,
—	1233	—	1135	—	—	+ 8·0	,,
—	1019	—	1070	—	—	− 4·6	,,
Mean 1228	—	—	1254	—	—	—	—

* Single muscles.

Oxygen consumption

Considerable variations were found for different muscles under the same conditions (Tables II and III). In a few experiments in which measurements were begun immediately after dissection oxygen uptake

was irregular, but not above the subsequent steady level, which continued unchanged throughout the experiment, 6–7 hours. In one experiment after 4 hours' anaerobiosis the oxygen consumption rose from an original value of 40 to 48 c.mm./g./hr. for the first half-hour after readmittance

TABLE II. Influence on oxygen consumption, c.mm. O_2/g. wet weight/hour, of (*a*) temperature, and (*b*) medium.

	No. of exps.	Spread	Mean	Mean Q_{O_2}, calculated from mean dry weight, 16·5 p.c. wet weight
(*a*) Sea water phosphate, *p*H 7·2				
Temp. (° C.)				
7·5	9	12–25	18	0·11
12	31	13–52	34	0·21
15	13	26–52	37	0·22
25	13	27–53	40	0·24
37	3	100–100	100	0·61
(*b*) Temperature 15° C.				
Medium				
Unbuffered sea water, *p*H 8·4	4	27–47	36	0·22
Sea water phosphate, *p*H 6·6	3	22–26	24	0·15
Phosphate alone:				
(*a*) *p*H 7·2	5	14–28	21	0·13
(*b*) *p*H 9·0	6	13–40	26	0·16

of oxygen, but later fell back to 40 c.mm./g./hr. There was little difference between the rates of oxygen uptake at 12, 15 or 25° C., but that at 7·5° was considerably lower. In a few experiments at 37° C. the rate was much greater, but fell off after 3 or 4 hours. The highest rates were recorded from muscles in sea water alone (*p*H 8·4) or sea water buffered with phosphate at *p*H 7·2. (Sea water phosphate mixtures above *p*H 7·2 could not be used owing to precipitation of calcium phosphate.) Sea water phosphate at *p*H 6·6 or phosphate alone at 7·2 or 9·0 gave lower values. The *p*H of the medium did not change significantly during these experiments. No differences were observed between the rates of oxygen uptake of spring (February–April) and summer (June) muscles.

Effect of added substrate and of sodium monoiodoacetate (I.A.A.). The interpretation of the results of these experiments is complicated by the fact that addition of medium alone may cause a decrease in oxygen uptake (Table III). In many of the experiments in which lithium lactate or glucose was added to the medium oxygen uptake was decreased, in some there was no change, but in none was there any increase. In all experiments in which I.A.A. (1/10,000, neutralized NaOH) was added the oxygen uptake was decreased, and this was the case also when glucose

and I.A.A. were added together. On the other hand, when lithium lactate and I.A.A. were added together, in half of the experiments there was no decrease.

TABLE III. Oxygen consumption, c.mm./g. wet weight/hr., (*a*) before, and (*b*) after addition of various substrates. Sea water-phosphate buffer, *p*H 7·2, temperature 12° C.

Sea water phosphate		I.A.A. 1/10,000		Lithium lactate 0·02 *M*		Lithium lactate 0·02 *M* and I.A.A. 1/10,000		Glucose 0·02 *M*		Glucose 0·02 *M* and I.A.A. 1/10,000	
a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
25	24	49	32	13	13	36	22	37	23	46	38
23	24	44	34	44	36	18	19	34	26	37	28
25	18	42	31	39	29	30	27	30	31	21	13
36	27	30	10	33	31	52	25	27	26	38	28
20	13	40	28			40	29				
36	27	36	17			29	28				
		48	38								
		30	20								

I.A.A. = Iodoacetic acid (neutralized with NaOH).

ANAEROBIOSIS

A large number of experiments were carried out in different buffer solutions, both with and without sea water, and at different temperatures, in an attempt to find the optimum conditions for lactic acid formation. The results are summarized in Table IV. Results were very variable, especially during the summer months (May to July), when many muscles treated exactly in the usual manner formed almost no lactic acid. There was some evidence at this season that vigorous bubbling of oxygen through the sea water during the rest period caused lower lactic acid maxima, but the results were not conclusive. Maximum values attained were not influenced by temperature, 8°–20° C., but the rate of formation of lactic acid, calculated from experiments of short duration, Table V, increased with rising temperature. In general the highest amounts of lactic acid were formed in muscles in alkaline media containing phosphate. Some experiments were made in which glucose was added to the fluid surrounding the muscle. Only in those experiments in which both phosphate and bicarbonate were present was the mean value for lactic acid content above the mean for similar experiments without glucose, but even in these the values recorded were not outside the limits of those in experiments without glucose.

No difference was observed in results from intact or minced muscle. All attempts to prepare an active cell-free extract, using the method of

TABLE IV. Lactic acid content of muscles after 16–24 hr. anaerobiosis, mg./100 g.

Buffer	*p*H	Temp. ° C.	Lactic acid (a) No glucose: No. of exps.	Spread	Mean	(b) 0·2 p.c. glucose: No. of exps.	Spread	Mean	Remarks
			(1) Normal						
Phosphate	9·0	8	16	21–126	55	—	—	—	Minced
	9·0	12	26	13–151	70	—	—	—	Minced
	9·0	12	5	17–150	62	2	37, 48	—	Intact
	9·0	20	16	21–178	70	—	—	—	Minced
	9·0	20	5	24–171	81	2	31, 37	—	Intact
	7·2	8	4	18–40	30	—	—	—	Minced
	7·2	20	1	68	—	—	—	—	Minced
Phosphate and bicarbonate	9·0	10	2	20, 37	—	—	—	—	Minced
	9·0	12	5	42–71	53	4	74–165	116	Intact
	9·0	20	2	18, 31	—	—	—	—	Minced
	7·2	10	2	20, 27	—	—	—	—	Minced
	7·2	20	4	18–54	38	—	—	—	Minced
Bicarbonate	9·0	8	6	14–39	28	—	—	—	Minced
	9·0	12	4	36–58	52	—	—	—	Minced
	9·0	12	1	42	—	1	19	—	Intact
	9·0	20	1	35	—	—	—	—	Minced
	9·0	20	2	29, 38	—	2	37, 49	—	Intact
	7·2	8	1	27	—	—	—	—	Minced
Sea water, no buffer	8·4	12	2	28, 38	—	2	29, 45	—	Intact
			(2) Poisoned I.A.A. 1/1000						
Phosphate	7·2	7	1	9	—	—	—	—	Intact
Phosphate and bicarbonate	9·0	12	2	16, 36	—	—	—	—	Intact
	9·0	20	1	20	—	—	—	—	Minced
Bicarbonate	9·0	12	2	16, 17	—	—	—	—	Minced
			(3) Poisoned NaF $M/50$						
Phosphate and bicarbonate	9·0	12	2	34, 42	—	—	—	—	Intact
	9·0	20	1	21	—	—	—	—	Minced
			(4) Poisoned Na_2SO_3 $M/100$						
Phosphate and bicarbonate	9·0	20	2	36, 33	—	—	—	—	Minced

Boyland and Mawson [1934], were unsuccessful. Addition of adenosine triphosphate from rabbit muscle was without effect. Muscles ground with sand before incubation also failed to produce lactic acid.

In muscles containing up to 3000 mg./100 g. glycogen and forming at most 170 mg./100 g. lactic acid, there is no possibility of showing a balance between glycogen lost and lactic acid formed, if such exists. Experiments on winter muscles showed there was no significant change in carbohydrate after 24 hours' incubation. In two experiments on summer muscles the following values were obtained in determinations

TABLE V. Rate of lactic acid production, intact muscles, sea water phosphate buffer, pH 7·2 (Feb.–April).

Temp. °C.	Time hr.	Lactic acid content mg./100 g.	Rate of lactic acid formation mg./100 g./hr.	Mean $Q_G^{N_2}$
7	18·0	25	0·6	
7	17·0	19	0·4 Mean 0·5	0·007
15	6·5	29	2·5	
15	6·5	26	2·0	
15	6·0	30	2·8	
15	6·0	25	2·0 Mean 2·4	0·036
25	4·0	30	4·2	
25	4·0	36	5·7	
25	6·75	40	4·0	
25	6·0	32	3·3 Mean 4·4	0·067
37	5·0	47	6·8	
37	5·0	47	6·8	
37	5·0	57	8·8 Mean 7·5	0·113

Note. Rate calculated taking 13 mg./100 g. (wet weight) as the resting lactic acid value and assuming the rate of formation to be constant. For calculation of $Q_G^{N_2}$ mean dry weight taken to be 16·5 p.c. wet weight.

made on opposite halves of the same muscle before and after 24 hours' anaerobic incubation at 12° C.:

Total carbohydrate, mg./100 g.			Lactic acid, mg./100 g.		
Initial	Final	Decrease	Initial	Final	Increase
1232	1134	98	23	59	36
1257	1191	66	35	93	58

In a few experiments in which fermentable sugar was measured on symmetrical halves of muscles before and after incubation, the values obtained showed great variation in both halves, on the average there was no change.

The results of adding I.A.A., NaF and Na_2SO_3 to the surrounding medium during incubation are shown in Table IV. There was no accumulation of pyruvic acid in the experiments in which Na_2SO_3 was added.

STIMULATION

In the first experiments (April until the middle of May) muscles contracted well and stimulation was continued until no further, or only very small, contractions resulted. From 50 to 80 mg./100 g. lactic acid was found in these muscles. In later experiments, from the middle of May to the end of June, muscles contracted on the whole for shorter

periods and contained, except for one experiment, only 16–36 mg./100 g. lactic acid (Table VI).

TABLE VI. Lactic acid content of muscles after stimulation, mg./100 g.

Date	No. of responses	Lactic acid	Remarks
		(*a*) Aerobic	
Apr. 11	55 + tetanus	47	
16	300	27	
May 21	200	21	
	(*b*) Anaerobic: (1) Normal		
Apr. 3	360	50	
4	200	55	
5	65 + long tetanus	60	
10	Long tetanus	72	
May 8	30	87	Muscle still contracting well
9	30	43	,, ,, ,,
14	60	36	
21	80	16	
22	180	29	
23	60	16	
28	300	35	
29	200	65	
June 17	60	16	
19	60	32	
20	180	23	
	(2) Poisoned I.A.A.		
Apr. 12	28	13	1/1000 I.A.A. soaked 5 min.
30	15	18	1/1000 ,, ,, 5 ,,
May 9	20	7	1/1000 ,, ,, 15 ,,
10	15	11	1/1000 ,, ,, 15 ,,
Apr. 30	30	32	1/5000 ,, ,, 5 ,,
May 7	33	14	1/5000 ,, ,, 15 ,,
8	33	30	1/5000 ,, ,, 15 ,,
	(3) Soaked in 0·6 *M* NaCl		
May 16	30	19	
17	30	19	
20	30	26	
	(4) Soaked in 0·6 *M* NaCl and *M*/10 NaF		
May 16	9	29	
17	10	22	
20	40	23	

Effect of added lactic acid inhibitors. At the end of the rest period either sea water containing I.A.A. or NaCl and NaF replaced the sea water bathing the muscle, stimulation was begun after 5 or 15 min. The experiments in which I.A.A. was added were all made during the earlier period when normal muscles were producing lactic acid. If 1/1000 I.A.A. was used muscles responded to stimulation for 15–28 min., but formed no lactic acid. When 1/5000 I.A.A. was used the response continued for 30 min. and some lactic acid was formed, but less than in

a normal muscle stimulated for an equal length of time. In order to study the effect of added fluoride it is necessary to use a medium containing no calcium during the poisoning period. Normal muscles suspended in 0·6 *M* NaCl for 15 min. before stimulation, but stimulated in natural sea water, gave low values for lactic acid; but as these experiments were made in the latter half of May just when the summer effect was first observed, it was not clear whether this was due to the NaCl or to some change in the muscles. Muscles suspended in NaCl containing *M*/10 NaF for 15 min. before stimulation, and stimulated in natural sea water, failed to respond to stimulation before normal muscles similarly treated, but gave lactic acid value of the same order. From these experiments it cannot be said that fluoride prevents lactic acid formation, but it does cause more rapid fatigue.

Discussion

The mean value of oxygen consumption, $Q_{O_2}-0{\cdot}22$ at 15° C., is nearly the same as that found by Meyerhof and Lohmann [1926] for frog intestine, $Q_{O_2}-0{\cdot}28$ at 20° C., and much lower than the values recorded for mammalian plain muscle [*e.g.* rabbit colon $Q_{O_2}-2{\cdot}64$; Rosenthal and Lasnitski, 1928]. Unlike striated muscle there is no rise on adding lactate, but the decrease due to I.A.A. is in some cases abolished by lactate [cf. Krebs, 1931]. This suggests that unpoisoned muscle has some aerobic glycolysis. No determinations of aerobic glycolysis were made, but Meyerhof and Lohmann [1926] found for frog intestine a value of $Q_G^{O_2}$ 0·14 in the presence of glucose and Rosenthal and Lasnitski [1928] for rabbit colon $Q_G^{O_2}$ 0·39. The decrease in lactic acid content of muscle after rest in oxygenated sea water can be explained either by oxidative removal of lactic acid, or by diffusion into the sea water.

The mean value found for anaerobic glycolysis, $Q_G^{N_2}$ 0·036 at 15° and 0·067 at 25° C., is lower than that found by Meyerhof and Lohmann [1926] for frog intestine, $Q_G^{N_2}$ 0·11 without glucose and 0·50 with glucose, at 20° C. No determinations were made of rate of lactic acid production in the presence of glucose, but it had little effect on the lactic acid maxima. In this respect the behaviour of this muscle is different from that of other samples of plain muscle investigated [Meyerhof and Lohmann, 1926; Rosenthal and Lasnitski, 1928; Prasad, 1935]. It is also in marked contrast to that of striated muscle, where, in a phosphate buffer, almost all the carbohydrate is converted into lactic

acid. In those few experiments in which carbohydrate decrease and lactic acid production were both measured, the two quantities were of the same order. Almost all the carbohydrate present is glycogen, the small and variable amounts of fermentable sugar seeming not to be converted to lactic acid.

The reason for the cessation of lactic acid formation when there are such large amounts of glycogen present is not obvious. Perhaps the supplies of phosphagen, adequate for the work which the muscle performs in life, are rapidly exhausted in anaerobic conditions. Experiment has shown there is an easily hydrolysable phosphorus compound present (in one experiment inorganic P increased from 20 to 25 mg./100 g. after 7 min. hydrolysis in N H_2SO_4); whether or not this compound is a nucleotide similar to the adenosine triphosphate of striated muscle was not determined. Possibly it is this compound which is soon exhausted in anaerobiosis and prevents further glycolysis. The small amount of easily hydrolysable P compound present may also account for the failure to produce active cell-free extracts, as Boyland and Boyland [1935] have suggested in the case of tumour tissue.

No explanation for the failure of summer muscles to produce lactic acid on stimulation, and in many instances during anaerobiosis, is apparent from these experiments. The scanty information available of the breeding period of *Mytilus edulis* suggests it may last from February to June, so that the "alactic acid" season does not coincide with this period. In frogs, where a change in metabolism is observed in summer months, the change occurs after hibernation and breeding have finished.

Summary

1. The carbohydrate of the retractor muscle of the foot of *Mytilus edulis* was found to be almost all glycogen; in winter muscles the mean value was 2562 and in summer muscles 1228 mg./100 g. The lactic acid content of resting muscles was low.

2. The influence of temperature and of pH on the oxygen consumption of the muscle has been studied.

3. Addition of lactate or of glucose did not increase oxygen uptake. 1/10,000 monoiodoacetate depressed oxygen uptake except sometimes in the presence of lactate.

4. Lactic acid production during anaerobiosis was irregular and small. Addition of glucose did not materially increase lactic acid production. Iodoacetate, and to a lesser extent NaF and Na_2SO_3, inhibited lactic acid production.

5. Muscles stimulated to fatigue contained from 50 to 80 mg./100 g. lactic acid. Iodoacetate caused a diminished response to stimulation and inhibited lactic acid production.

6. Summer muscles produced less lactic acid, both during anaerobiosis and after stimulation, than spring or winter muscles.

7. Attempts to produce active cell-free extracts were unsuccessful.

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