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THE DIFFUSION OF LACTATE INTO AND FROM MUSCLE.

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Certain constituents of the voluntary muscles of the frog are able to diffuse out of the muscle into a surrounding saline, and can also diffuse into the muscle if previously dissolved in the saline in a concentration sufficiently high[1]. There exists in such cases a certain concentration of the substance in saline which will be in equilibrium with the tissue. This critical concentration provides a measure of the concentration of the substance in the tissue—or rather in such part of the tissue as is concerned in the diffusion. In the case of creatine this measure of the concentration in the tissue was found by Eggleton [1930] to agree with values obtained by Dulière [1929] by direct analysis. This indicates that the partial osmotic pressure is the factor controlling the direction and extent of diffusion. Similar indirect determination of the concentration of phosphate in muscles [Stella, 1928] gave results rather lower than those obtained by direct analysis; but it must be remembered that direct estimations of the phosphate in resting muscles are liable to yield high results on account of the breakdown of phosphagen at the moment of death.

The present communication presents experiments leading to the conclusion that the concentration of lactate in both resting and fatigued muscles can be measured by the indirect "counter-diffusion" method, since the values obtained agree well with those in the literature obtained by direct analysis.

Technique.

The procedure adopted was that used by Eggleton for determining the concentration of creatine in muscle. One of a pair of resting gastrocnemii was placed in a Ringer's solution containing, for example, no lactate,

[1] Concentrations recorded in this paper were measured in mg. per 100 g. of solution; for calculation purposes these should be expressed in mg. per 100 g. water. For Ringer's solutions, however, the correction is small, and was neglected.

and the other in a solution containing, say, 60 mg. per 100 g. The amount of solution used was the same in both cases, about one and a half to two times the weight of the muscle. Gentle stirring was maintained by bubbling a suitable gas through the solution. Air and nitrogen respectively were used for experiments on resting and fatigued muscles, at a constant rate in all the cases. The tubes containing the muscle and solution were surrounded by ice in a beaker to prevent the formation of lactic acid; all the experiments were carried out at 0° C. After 2½ to 3 hours a known amount of the solution was analysed for lactate. The concentration of lactate in the solutions before diffusion was also determined. As shown by Eggleton, the equilibrium concentration of lactate in the muscle can be calculated from the formula:

$$C = \frac{L_0 l_1 - L_1 l_0}{(L_0 + l_1) - (L_1 + l_0)}, \qquad \text{.....(1)}$$

C = Equilibrium concentration in mg. per 100 g.
L_0 = Initial concentration in "high lactate" Ringer's solution.
L_1 = Final concentration in "high lactate" Ringer's solution.
l_0 = Initial concentration in "low lactate" Ringer's solution.
l_1 = Final concentration in "low lactate" Ringer's solution.

According to A. V. Hill [1930] the concentration of sodium chloride in a Ringer's solution isotonic with resting muscle is 0·71 p.c. This concentration was employed. For fatigued muscle 1·2 p.c. of sodium chloride in Ringer's solution was found to be isotonic with muscle. The concentration of potassium chloride and calcium chloride were 0·014 and 0·0125 p.c. respectively. In order to keep the *p*H of the Ringer's solution at 7·1, a buffer solution of 0·2 molar phosphate containing 65 p.c. of disodium hydrogen phosphate and 35 p.c. of sodium dihydrogen phosphate was prepared; 20 c.c. of this solution were added to 980 c.c. of Ringer's solution, giving a concentration of 12 mg. phosphorus per 100 c.c. For higher or lower concentrations of lactate, an equivalent amount of sodium chloride in the Ringer's solution was replaced by sodium lactate, so that the osmotic pressure of the Ringer's solution in high or low lactate solutions was the same.

Sodium lactate at *p*H 7·1 was prepared by myself. Lactic acid was diluted and boiled for 8 hours to convert all lactic anhydride into lactic acid. The solution was neutralized with caustic soda to *p*H 7·1, the indicator (phenol red) being used externally, lest it should have any toxic effect on the muscle cells. The diffusion was carried out at 0° C. for three hours in all cases.

Lactate in the solution was estimated by Clausen's [1922] method modified by Friedemann, Cotonio and Shaffer [1927], but without aeration [Meyerhof, 1930].

Resting muscle.

In order to obtain maximum quantities of lactate for estimation, muscles of the thigh were chosen. The frog, previously cooled to 0° C., was rendered insensible by a blow on the head or by decerebration, and sectioned immediately above the iliac bones. The abdominal muscles and the viscera in the pelvis were removed, and the skin was carefully dissected off. The legs were separated without injury to the muscles by section of the os pubis with a razor.

Table I. Concentration of lactate in resting frog muscle.

Exp.		Weight of muscles in g. Initial	Weight of muscles in g. Final	Lactate in mg. per 100 g. of Ringer's solution Initial	Lactate in mg. per 100 g. of Ringer's solution Final	Equilibrium calculated (mg. per 100 g.)
1.	*l*	10·35	10·35	0	7·2	30
	L	10·36	10·35	71·6	61·0	
2.	*l*	12·48	12·49	0	6·1	32
	L	12·49	12·50	67·7	61·0	
3.	*l*	13·95	13·96	0	5·0	23
	L	13·87	13·87	67·7	58·4	
4.	*l*	16·40	16·40	0	3·0	27
	L	16·32	16·35	71·0	65·0	
5.	*l*	16·50	16·51	0	7·7	26
	L	15·36	15·35	79·2	63·4	
6.	*l*	15·97	16·02	0	7·2	29
	L	15·94	15·97	37·7	36·0	
7.	*l*	4·71	4·65	0	6·4	24
	L	4·50	4·57	37·7	31·1	
8.	*l*	4·35	4·35	0	9·9	17
	L	4·35	4·35	37·7	26·4	
9.	*l*	1·36	1·37	0	6·2	24
	L	1·39	1·34	44·9	39·7	
10.	*l*	1·83	1·83	0	3·0	13
	L	1·84	1·85	37·0	31·5	
11.	*l*	1·22	1·23	0	4·0	21
	L	1·23	1·24	37·0	34·0	
12.	*l*	1·45	1·45	0	5·3	18
	L	1·25	1·24	37·0	31·6	
13.	*l*	0·98	0·98	0	4·5	16
	L	0·99	0·98	39·4	33·3	
14.	*l*	2·60	2·60	0	3·0	23
	L	2·65	2·64	46·0	43·0	
15.	*l*	2·84	2·83	0	2·5	14
	L	2·90	2·89	47·2	41·1	

l, low lactate Ringer's solution; *L*, high lactate Ringer's solution. Exps. 1–6 were performed on thigh preparations. Exps. 7–15 were performed on gastrocnemii, or calf preparations.

Consistent results of 30 mg. per 100 g. of solution were obtained for the concentration of lactate in equilibrium with frog muscle. To confirm this, muscles were placed in a modified Ringer-lactate solution containing 30 mg. lactate per 100 g. of solution, and it was found that the muscles were in equilibrium with regard to lactate; the concentration of lactate in solution remained constant. This figure, 30 mg. per 100 g., is twice the amount of the accepted figure for resting frog muscle. This was thought to be probably due to partial asphyxia of the muscles, or due to the dissection of the recti muscles which are attached to the skin; even though the injured fibres were cleared away there might be fibrillar contractions taking place, giving rise to lactic acid in the muscle. Therefore they were abandoned and gastrocnemii, preferably with their bony attachments, were selected; in other cases the second segment of the leg with the muscles of the calf were taken. Both these preparations can be made without any injury to the muscles concerned. The diffusion technique was unaltered. The preparations were reweighed at the end of each experiment and found to have neither gained nor lost in weight; therefore the solution was isotonic. For equation (1) to be valid, there should be no transference of water into or from the muscle. The muscles were stimulated and found to be excitable. From each tube 2 g. of the solution were taken and analysed for lactate.

An average of the results recorded for Exps. 7–15 shows a concentration of 20 mg. per 100 g. of solution. From this figure, it is easy to arrive at the concentration of lactate in the muscle. Since the muscle contains 80 p.c. by weight of water the concentration of lactate in the muscle is 16 mg. per 100 g. muscle.

Fatigued Muscle.

In this case the gastrocnemius muscles of the frog were fatigued by causing them to contract isometrically. They were dissected out, weighed and introduced into the tubes containing modified Ringer-lactate solutions. In order to prevent oxidative recovery the solution was covered in the earlier experiments with a layer of liquid paraffin. The steady gentle stirring was maintained by bubbling nitrogen through the solution. The use of liquid paraffin renders it impossible to find if the weight of the muscle had remained constant during the experiment; but the muscles could be tested to see if they were still excitable. Later on the use of liquid paraffin was given up and the tubes were stoppered, after the air had been displaced by nitrogen. The weight of the muscles in these cases

was noted and found to be constant. At the conclusion of the experiments the muscles were found to be still excitable.

TABLE II. Concentration of lactate in fatigued frog muscle.

Exp.		Weight of muscles in g. Initial	Weight of muscles in g. Final	Lactate in mg. per 100 g. of Ringer's solution Initial	Lactate in mg. per 100 g. of Ringer's solution Final	Equilibrium calculated (mg. per 100 g.)
1.	*l*	—	—	0	22	232
	L	—	—	512	487	
2.	*l*	—	—	0	25	220
	L	—	—	500	470	
3.	*l*	—	—	0	23	230
	L	—	—	502	476	
4.	*l*	1·24	1·25	0	16	270
	L	1·25	1·25	502	489	
5.	*l*	1·62	1·62	0	14	214
	L	1·64	1·63	412	399	
6.	*l*	1·36	1·39	0	24	218
	L	1·37	1·36	412	391	
7.	*l*	0·92	0·96	11	34	214
	L	0·90	0·90	418	394	
8.	*l*	1·43	1·44	11	30	205
	L	1·50	1·49	418	396	
9.	*l*	1·09	1·10	11	36	286
	L	1·09	1·09	418	406	

The average equilibrium concentration is 238 mg. per 100 g. of solution. This corresponds to a concentration of 190 mg. of lactate per 100 g. of fatigued frog muscle.

SUMMARY.

Studies of diffusion of lactate into and out of resting and fatigued frog muscles indicate an apparent concentration of lactate in the water of the muscle of 20 and 238 mg. per 100 g. respectively. This is in good agreement with the values obtained from direct analyses of such muscles by other workers. It follows that all the water of the muscle is available to dissolve lactate.

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