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THE DIFFUSION OF CREATINE AND UREA THROUGH MUSCLE.

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If a resting muscle be suspended in well oxygenated Ringer's solution it begins to lose some of its constituents by diffusion. The ability of a substance to diffuse from a muscle and the rate at which it can diffuse cannot be foretold from a knowledge of the behaviour of the substance in aqueous solution. It is probable that a great deal of light will be thrown on the conditions existing in the muscle by a systematic study of these diffusion phenomena, particularly with the aid of the exact mathematical treatment recently published by Hill [1928]. At present the information available is scanty, but very suggestive. For example, lactate diffuses from a dead muscle at exactly the rate that would be expected if the muscle were a lump of jelly containing the same amount of lactate [Eggleton, Eggleton and Hill, 1928]. It is clear that no hindrances to diffusion arise through any structural peculiarities of the dead muscle. This seems to be the case for completely resting muscles, but the rate of diffusion of lactate from fatigued muscles is very greatly reduced by circumstances existing in fatigued, as distinct from dead or resting muscles.

Creatine and phosphate are capable of diffusing from muscle, but the curious circumstance was early observed that phosphagen is quite incapable of diffusion. No explanation has yet been offered for this peculiar behaviour, but it may have considerable significance. In spite of the indiffusibility of phosphagen in a living muscle this substance is perfectly diffusible through collodion when the muscle structure is destroyed. Mr Horton and the writer killed muscles by macerating in dilute acid or alkali, and in alcohol, and in all cases found the phosphagen in the extract to be freely diffusible. More recently a similar result

[1] From a thesis approved for the degree of Doctor of Science in the University of London.

was obtained when muscles were extracted by cutting up finely in saline solution and the extract separated by centrifuging. The phosphagen in the fluid diffused at practically the same rate as orthophosphate. It seems that either the phosphagen of the muscle exists in combination with some colloidal constituent or that the cell walls have a specific impermeability to this substance.

The diffusion of phosphate was studied in a semi-quantitative fashion by Embden in 1922. He observed no diffusion from resting muscles, but an appreciable diffusion from muscles which were being stimulated. Since his estimations of inorganic phosphate in the muscle had shown practically no difference in phosphate content between active and passive muscles, he was forced to conclude that the permeability of active muscle to phosphate was very much greater than that of a muscle in the passive condition. But the increase in orthophosphate now known to occur in fatigue is quite sufficient to render this hypothesis unnecessary, and indeed it is definitely disproved by the recent quantitative studies published by Stella from A. V. Hill's laboratory. Stella [1928] found the diffusion coefficient of phosphate to be the same for resting, fatigued and dead muscles. He showed further that diffusion equilibrium occurred between a dead muscle and the saline solution surrounding it when the latter contained phosphate to the extent of 110 mg. of P per 100 g. This is approximately the figure found for the inorganic phosphate content of a dead muscle by direct analysis when allowance is made for the fact that the muscle contains only 80 p.c. of water. In this respect again therefore the dead muscle is exhibiting the behaviour of a structureless jelly. But in the case of resting and fatigued muscles diffusion equilibrium occurred at concentrations of 8 and 18 mg. per 100 c.c. respectively—figures considerably below those found by direct analysis, more particularly in the case of the fatigued muscle. Meyerhof has expressed the opinion that this discrepancy may be due to the adsorption of part of the phosphate on to some part of the muscle structure.

Regarding creatine no information has been published since the work of Tiegs [1925] who showed that creatine diffuses from fatigued frog muscles into a saline solution, but not from resting, and that the admission of oxygen diminished diffusibility. He rightly supposed that creatine must exist in two forms in the muscle, the diffusible form predominating in the fatigued state. Some recent experiments have indicated that equilibrium occurs at a concentration of about 80 mg. of creatine per 100 c.c. in the surrounding saline solution in the case of resting muscles and at a concentration of about 200 mg. per 100 c.c. in the case of fatigued

muscles. The technique used in these experiments does not appear to have been used before and is accordingly described here.

One of a pair of resting frog sartorii is placed in a Ringer's solution containing, for example, 20 mg. of creatine per 100 c.c. and the other in a similar solution containing, say, 120 mg. per 100 c.c. The amount of solution bears the same ratio to the weight of muscle in both cases; the actual ratio is between 1 and 3. The muscles are kept well supplied with oxygen, and after 2 to 5 hours are removed and a known weight of the solution analysed for creatine. Similar samples of the original solutions are simultaneously analysed. In a typical experiment the creatine concentration in the "low concentration" Ringer's solution is found to have increased slightly (from c_0 to c_f) whilst the other has fallen from its original value (C_0) to a slightly lower (C_F). Since the amount of diffusion in a given time depends only on the first power of the concentration gradient, a simple geometric operation (or its equivalent algebraic calculation) enables one to find that concentration at which no diffusion would have occurred. If the final concentrations are plotted against the initial two points are obtained, and on the straight line joining them is the point corresponding to equal initial and final concentrations. Its position is given by the intersection of this line with the diagonal, for the diagonal includes all points representing equal initial and final concentrations. The equivalent algebraic operation is to find the point on the straight line,

$$\text{(Final concentration)} = a\ \text{(initial concentration)} + b,$$

at which the final concentration equals the initial concentration (= equilibrium concentration, c_e). This is given by

$$c_e = \frac{b}{1-a},$$

or, in terms of the four known values of c,

$$c_e = \frac{C_0 c_f - C_F c_0}{(C_0 + c_f) - (C_F + c_0)}.$$

For this equation to be valid there must be no appreciable exchange of water between the muscle and the surrounding saline: *i.e.* the saline must be isotonic.

Table I gives the details of some of the experiments performed together with the calculated equilibrium concentrations. A few experiments of a similar type were performed with phosphate as the variable, and the results though somewhat irregular gave an average equilibrium value fairly close to that obtained by Stella.

Since a muscle contains about 80 p.c. of water, practically all of which according to the recent work of Hill [1930] is "free" water, with normal

TABLE I. The diffusion of creatine between sartorius muscles (frog) and Ringer's solution containing various concentrations of creatine.

		Muscles		Ringer's solution				Calculated equilibrium concentration (mg./100 gm.)
Exp.	No.	Initial weight (mg.)	Final weight (mg.)	Amount (gm.)	P content (mg./100 gm.)	Initial creatine concentration (mg./100 gm.)	Final creatine concentration (mg./100 gm.)	
1	1	410	392	0·505	9·5	119·5	111·5	7
	1	402	394	0·505	9·5	40	37	
2	1	312	290	0·500	12·5	123·5	114	34
	1	347	319	0·516	12·5	32·4	32·5	
3	1	331	324	0·502	9·5	119·5	98·5	40
	1	308	304	0·509	9·5	40	40	
4	1	372	356	0·509	12·5	125	117	41
	1	395	356	0·505	12·5	32·1	32·9	
5	1	333	335	0·514	9·5	108	98	60
	1	352	356	0·515	9·5	34	39	
6	1	—	291	0·490	9·0	25·7	30·4	70·5
	1	—	297	0·490	9·0	165	155	
7	1	—	326	0·490	9·0	25·7	39·7	72·5
	1	—	324	0·490	9·0	165	137	
8	1	—	270	0·490	9·0	25·7	34·6	75·5
	1	—	243	0·490	9·0	165	149	
9	2	—	455	0·480	6·0	19·1	37·5	80·5
	2	—	448	0·480	6·0	89	86·5	
10	6	1780	1820	2·02	26·7	135	127	87·5
	6	1790	1750	1·98	2·7	23·6	34·4	
11	2	—	382	0·480	6·0	19·1	31·5	89
	2	—	393	0·480	6·0	89	89	
12	4	980	970	1·22	2·7	135	127	104·5
	4	970	930	1·23	26·7	21·7	43·7	
13	1	418	422	0·501	9·5	106	106	106
	1	394	395	0·498	9·5	33	44	
14	1	301	—	0·512	6·0	41·5	43	> 90
	1	285	—	0·518	6·0	88	93	
15	1	418	—	0·502	6·0	21·6	51	98·5
	1	396	—	0·512	6·0	117	110	
16	1	285	—	0·525	6·0	0	12	127·5
	1	293	—	0·505	6·0	170	166	
17	1	404	405	0·567	17·0	306	292	154
	1	435	413	0·552	17·0	131	133·5	
18	1	—	192	0·30	—	93	98·5	170·5
	1	—	194	0·30	—	395	355	
19	1	—	198	0·30	—	93	118·5	183
	1	—	189	0·30	—	395	345	
20	1	559	575	0·554	17·0	299	276	193
	1	534	554	0·557	17·0	131	144	
21	1	327	344	1·01	31·0	258	266	> 270
	1	348	380	1·01	31·0	109	119	
22	1	428	458	1·01	31·0	260	272	> 270
	1	443	464	1·01	31·0	109·5	112	

Of these experiments nos. 17–22 were performed with muscles which had been fatigued to different extents, nos. 14, 15 and 16 with muscles immediately after dissection. In the remaining cases the muscles after dissection had been immersed in well oxygenated Ringer's solution for 30 minutes, then hung in oxygen for a further 30 minutes. This treatment has been found in earlier work to bring the muscles into good condition, and to abolish all tendency to become spontaneously inexcitable.

solvent powers, an "over-all" concentration of creatine in the muscle of 65 mg. per 100 g. is required for equilibrium, on a purely osmotic basis, between the muscle and a surrounding saline solution containing 80 mg. of creatine per 100 c.c. This is the value actually observed by Dulière [1929] in some direct analyses of resting muscles carried out in this laboratory. Dulière used two completely different methods for the direct estimation of free creatine with essentially the same result. It seems, therefore, on the evidence so far available that the creatine of muscle is distributed evenly through all the water of the muscle.

Every ionizable substance so far examined by such diffusion methods has shown some peculiarity in its behaviour. Although the chemist is forced to express his analyses as a percentage of the whole weight of muscle, experience has shown that it may be very misleading to suppose even the diffusible constituents to be distributed evenly through the muscle. That such an indiffusible substance as glycogen is contained only in the muscle fibres and not in the interspaces is a fairly plausible supposition, but there is evidence that chloride, potassium, sodium and phosphagen are by no means equally distributed between the fibres and the fluid in the interspaces. Urea on the other hand was found recently by Horton and the writer to diffuse into a muscle to an extent indicating that 80 p.c. of the weight of the muscle was capable of dissolving urea: that is to say, the apparent water content was 80 p.c.[1] This indicates that urea like creatine distributes itself evenly throughout the muscle and that no hindrance exists to its free diffusion. This simple behaviour may be connected with the very feeble ionization of these substances in water.

The method used was as follows: a sartorius muscle of known weight was immersed in a known volume (about four times the volume of the muscle) of Ringer's solution containing a known concentration (0·10 to 0·15 p.c.) of urea. The urea diffused into the muscle and equilibrium was established (as was shown in some preliminary experiments) in three hours. After 4 to 6 hours the urea still remaining in the solution was estimated (as nitrogen by the Kjeldahl method). The companion sartorius was treated similarly but with a saline solution containing no urea. It enabled the small correction for the diffusion of nitrogenous substances out of the muscle to be made. The calculation then took the following form:

Initial concentration of urea in saline $= x$;
Final ,, ,, $= y$.

[1] Mention was made of the results of these experiments by Prof. A. V. Hill in a recent publication [1930].

Therefore total amount of water in system, assuming even distribution of urea,

$$= \frac{x}{y} \times \text{volume of saline } (v).$$

Hence available water of muscle

$$= \left(\frac{x}{y} - 1\right) \times v.$$

The weight of the muscle being known, the percentage of available water could be calculated.

The results are given in Table II.

TABLE II. Diffusion of urea into frog's sartorii from saline solutions containing urea: the apparent water content of the muscle, calculated from these results.

Weight of muscle		Volume of saline	Titres		Urea in saline			Water in muscle
Initial (mg.)	Final (mg.)	(v) (c.c.)	Initial	Final	Initial (x)	Final (y)	$\left(\frac{x}{y}-1\right) \times v$	(p.c.)
249	251	1·3	0·1	0·20	2·57	2·26	0·18	70·5
268	258	1·3	2·67	2·46				
245	230	1	2·3	2	2·22	1·88	0·18	78
245	230	1	0·08	0·12				
305	280	1	2·3	1·96	2·22	1·81	0·225	80·5
305	280	1	0·08	0·15				
1750	1725	5	0·03	0·57	2·8	2·19	1·39	82
1750	1680	5	2·83	2·76				
Same experiment				0·57	2·8	2·15	1·5	88
"	"			2·67				
323	335	1·3	0·1	0·30	2·57	2·07	0·315	93·5
335	340	1·3	2·67	2·37				
688	637	3	0·05	0·26	3·71	3·16	0·51	79
622	656	3	3·76	3·42				
							Mean	81·5

In this table under the heading "titres" are given the nitrogen concentrations of the urea- and the control-salines in arbitrary units. From these the initial and final urea concentrations (x and y) are obtained also in arbitrary units. The fourth and fifth values were obtained from the same experiment by taking samples at 4 and 5 hours respectively. The 4-hour sample gave slightly the higher result.

SUMMARY.

1. By observing the diffusion of creatine into or out of muscles immersed in salt solutions containing creatine, it has been shown that a concentration of 80 mg. of creatine per 100 c.c. of solution is just sufficient to prevent loss of creatine from a resting muscle (frog's sartorius). If the equilibrium were a simple osmotic one, this would indicate a concentration of 65 mg. of creatine per 100 g. of muscle (80 mg. per 100 c.c. of water in the muscle). This figure of 65 mg. per 100 g. has been previously

obtained by direct chemical analyses. Only one-fifth therefore of the "total creatine" of resting muscles is actually present as creatine.

2. In the case of fatigued muscles equilibrium is obtained at concentrations of 200 to 300 mg. creatine per 100 c.c. of solution. It seems probable, therefore, that stimulation to fatigue trebles the amount of free creatine.

3. Urea distributes itself between resting muscles (frog's sartorii) and surrounding isotonic salt solutions in such a manner as to indicate that the whole of the water in the muscles is capable of dissolving urea.

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