XXXVI. THE PHOSPHATE METABOLISM IN FATIGUED MAMMALIAN MUSCLE.

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THE extreme importance of the changes undergone by the hexosephosphoric esters in the metabolism of muscle has only within recent years been recognised. In a long and important series of papers from Embden and his school the general nature of the changes in these esters (lactacidogen) has been gradually elucidated.

The influence exerted by the various anions on the metabolic changes, anabolic and catabolic, which lactacidogen undergoes, has been the subject of certain recent researches by Embden and Lehnartz [1924], Embden and Haymann [1924], Abraham and Kahn [1924], and Lange and Meyer [1924]. From these investigations it is evident that various anions, such as thiocyanate, chloride, etc., give rise to a disintegration of the lactacidogen molecule, whilst others, especially the fluoride and oxalate, produce a synthesis. From Embden and Haymann's work it is evident that the action of the fluoride is a true synthetic one, because accompanying the disappearance of the free phosphoric acid there is a definite formation of the diphosphoric ester. It is also evident that the process does not require the intact muscle fibre, as it is to be observed in the expressed muscle juice as well as in minced muscle.

It is extremely difficult in many cases, as Embden, Schmitz and Meineke [1921] have proved for frog's muscle, and to a lesser extent for dog's muscle, to show that the lactacidogen diminishes during activity, owing to the rapidity with which a recuperative synthesis of the carbohydrate and the phosphate takes place.

In this communication attention will be directed mainly to the different effects produced by the fluoride anion acting upon resting and stimulated muscle. It was recognised from Embden's investigations and also from many of my own, that this synthetic action might be increased by the addition of glycogen to the fluoride solution; but the effects produced by the fluoride alone are so well marked that such an addition was found to be unnecessary.

Embden, when estimating the amount of phosphoric acid already preformed in the muscle, made use of a method in which he fixed the muscle in hydrochloric acid and then precipitated the protein with mercuric chloride, removing the excess of mercury with sulphuretted hydrogen. The value of free

phosphate which he found in his filtrate he called the A value. Then he warmed the muscle with sodium bicarbonate solution in order to break down the lactacidogen. The total phosphate value thus obtained he called the B value. The difference between B and A was the amount of phosphoric acid bound as ester.

In the experiments which follow I shall make use of these letters when referring to the free and total phosphate values, and the value after the action of fluoride I shall refer to as the C value.

The resting and stimulated muscle of the rabbit, cat and dog were examined. In all the experiments on rabbit muscle the pale variety was used.

TECHNIQUE.

The muscle, immediately it was taken out, was placed in a vessel cooled in ice. Small specimens (about 1 g.) were quickly minced on an ice cold porcelain slab, and placed in 5 cc. quantities of the various solutions. The protein was precipitated by the addition of trichloroacetic acid, and the muscle was finely ground up in a mortar and filtered. The extraction was repeated three times and the washings all passed through the same filter.

The inorganic phosphate was estimated in the filtrate by Embden's gravimetric method [1921], which was found in all the controls to give very accurate results. The preformed inorganic phosphate (Embden's A value) was found by taking the muscle directly into 5 % trichloroacetic acid. As will be seen, this method gives results very comparable with those obtained by the hydrochloric acid extraction method. The lactacidogen values (Embden's B-A value) were obtained by heating the muscle with 2 % sodium bicarbonate for 2 hours at 40° in each case.

Numerous controls were carried out, and duplicates were done with the majority of the specimens. It was found that the duplicates, when the conditions were similar, did not differ by more than 2-3 %.

RABBIT MUSCLE.

In the first three experiments resting rabbit's muscle was examined. The animals had been fed for 2 days previously with oats and carrots. Each was killed by a blow on the back of the head, and bled. Portions of pale muscle were quickly excised from the adductors of the lower limb.

	% H_3PO_4		
	Rabbit I	Rabbit II	Rabbit III
In 5 % trichloroacetic acid	0.356	0.332	0.373
In 2 % sodium bicarbonate, heated 2 hours 40° ...	0.576	0.601	0.623
In 0.9 % sodium chloride, heated 2 hours 40° ...	0.522	0.550	0.546
In N/10 sodium fluoride, 2 hours	0.093	—	—
3 "	—	0.057	0.100
5 "	—	0.057	—
6 "	0.044	—	—
18 "	0.118	—	—
heated 2 "	0.107	—	—
In N/10 sodium fluoride + 0.4 % glycogen, 3 hours 12°	—	0.012	0.057

If we take the trichloroacetic acid value as corresponding to Embden's A value and the heated bicarbonate figure as corresponding to his B value, we obtain in each case the lactacidogen value.

	Rabbit I	Rabbit II	Rabbit III
Lactacidogen	0.220 %	0.269 %	0.250 %

The preformed inorganic phosphate value does not vary much in the three animals.

On heating with bicarbonate there is a marked rise in the inorganic phosphate content, corresponding to increases of I, 61.8 %, II, 81.0 %, III, 67.0 %.

On heating with normal saline, the chloride ion is also found to exercise a disintegrative effect on the lactacidogen, although the increase is never so marked as with bicarbonate. Increases: I, 46.6 %, II, 65.6 %, III, 46.4 %.

Under the action of fluoride there is a marked decrease in the amount of inorganic phosphate; this action seems to reach its maximum in 3-5 hours.

The differences in the trichloroacetic acid value and the fluoride value are practically the same in each of the three rabbits, viz. I, 0.263, II, 0.275, III, 0.273.

When glycogen is added in addition to the fluoride, the inorganic phosphate falls to a very low level, the quantities being too small to permit of accurate determination.

Similar experiments were carried out on strychninised rabbits, and the results may be compared with those obtained in normal resting rabbits.

Rabbit IV. The strychnine was injected in increasing doses at 10-minute intervals for 45 minutes. The animal gradually became more spastic and 5 minutes after the last injection developed strong convulsions and died.

	% H_3PO_4
In N/10 sodium fluoride	0.263
In 5 % trichloroacetic acid	0.354
In 0.9 % sodium chloride, heated 2 hours 40° ...	0.456
In 2 % sodium bicarbonate, heated 2 hours 40° ...	0.531

Rabbit V was injected with strychnine in small doses over a period of 3½ hours. Ten minutes after the last injection it developed strong convulsions and died in 5 minutes.

	% H_3PO_4
In N/10 sodium fluoride, after 6 hours 12°	0.443
In N/10 sodium fluoride, after 7 hours 12°	0.440
In 5 % trichloroacetic acid	0.360
In 0.9 % sodium chloride, heated 2 hours 40° ...	0.466
In 2 % sodium bicarbonate, heated 2 hours 40° ...	0.478

In both animals the preformed or A value is about the normal, but the B value is lower than normal. This gives a lower lactacidogen value than normal, in one case 0.177 %, in the other 0.118 %.

The fluoride values are much higher than those found in resting rabbit's muscle. In the second rabbit the fluoride value was actually higher than the A value. It cannot be the inorganic phosphate that is lacking for synthesis, since this is as high as in the normal animals.

Cohn [1921] found for the lactacidogen values in the pale muscle of the strychninised rabbit figures ranging from 0.1286 % to 0.2057 %, with an average of 0.1635 %.

The values obtained in the above experiments may be summarised as follows:

	% H_3PO_4			
	Synthesis.		Hydrolysis.	
	C	A	B	
Resting muscle	0.044	0.356	0.576	
" "	0.057	0.332	0.601	
" "	0.100	0.373	0.623	
Fatigued "	0.263	0.354	0.531	
" "	0.440	0.360	0.478	

It is of interest to note that Embden and Adler [1921], using 10 g. of muscle, obtained for pale resting rabbit's muscle A values from 0.2008 % to 0.2755 % (average 0.2442 %) and B values from 0.5295 % to 0.6101 % (average 0.5712 %); and for lactacidogen values varying between 0.2800 % and 0.4093 % (average 0.3179 %). Using red muscle they obtained somewhat lower values.

DOG MUSCLE.

Effect of Stimulation.

Exp. 1. The dog was anaesthetised with ether. The sciatic nerve on one side was exposed, severed and the distal portion stimulated for 40 minutes with single induced shocks and then for 10 minutes with interrupted tetanising stimuli. The limb acted against a weight, and at the end the muscles were almost completely fatigued. The muscles in the resting and in the fatigued limbs were then exposed, sprayed with ethyl chloride and corresponding portions excised. They were placed in an ice-cold vessel and rapidly minced. Similar portions were at once placed in the three solutions and weighed.

	% H_3PO_4	
	Resting	Fatigued
In 5 % trichloroacetic acid	0.259	0.225
In 2 % sodium bicarbonate, heated 1 hour 40°	0.457	0.396
In N/10 sodium fluoride, 1 hour 12°	0.067	0.277

Both the A and B values of the muscle on the resting side are higher than those on the fatigued. On the resting side the B value is 76.4 % higher than the A. This corresponds to a store of lactacidogen which furnishes on complete breaking down this increment in free H_3PO_4 . On the fatigued side the increment in free H_3PO_4 is practically the same although the absolute values (A and B) are down.

On the resting side the fluoride has caused a decrement in free H_3PO_4 amounting to 73.3 %. On the fatigued side the fluoride has, instead of producing a decrement in free H_3PO_4 from the A value, given rise to a 23.1 % increment. The stimulated muscle, which contains only 0.171 % of lactacidogen, is unable to synthesise in the presence of fluoride, whereas the resting muscle (0.198 % of lactacidogen) readily produces this effect.

Exp. 2. The dog was anaesthetised with ether, and one sciatic nerve was exposed and cut. A large proportion of the animal's blood was removed by perfusion with normal saline.

When the nerve was stimulated the muscles appeared to become fatigued very quickly—in about a minute. A specimen was taken into fluoride and the stimulation was continued for 5 minutes and again for 15 minutes.

				% H_3PO_4	
				Resting	Stimulated
In $N/10$ sodium fluoride	...	...	...	0.069	—
"	"	"	stimulated 1 minute	—	0.121
"	"	"	" 5 minutes	—	0.120
"	"	"	" 15 "	—	0.126
In 2 % sodium bicarbonate, heated 2 hours	40°			0.400	0.310

The action of the fluoride was again more marked on the resting than on the fatigued side. The total phosphate (preformed and lactacidogen) showed a marked diminution (22.5 %) compared with that obtained on the unstimulated side.

In this animal some experiments were devised to find out how treatment with sodium chloride affected the fluoride synthesis in the muscles on the resting side.

Resting muscle.	% H_3PO_4
In $N/10$ sodium fluoride, 1 hour 11°	0.069
In fluoride 1 hour, then washed and placed in saline 2 hours 40°	0.124
In saline 2 hours 40°	0.603
In saline 2 hours 40°, then left overnight in fluoride	0.361
In saline 20 minutes 40°	0.238
In saline 20 minutes 40°, then in fluoride for 1 hour	0.116

The maximal disintegration of the lactacidogen occurred in the specimen kept in 0.9 % sodium chloride at 40° for 2 hours.

After a preliminary 1 hour action of fluoride followed by 2 hours' action of chloride at 40°, where the fluoride building-up action is counteracted by the chloride breaking-down action, the free H_3PO_4 is brought approximately to the same level as by the fluoride alone acting upon stimulated muscle. When, on the other hand, the breaking-down action of chloride is first allowed to take place and then the muscle is treated with fluoride, the former effect is dominant, so that the fluoride is not able to synthesise to the same extent.

Exp. 3. The dog was anaesthetised with ether. The sciatic nerve on one side was severed and stimulated for 5 minutes with interrupted tetanising stimuli. The muscles showed no evidence of fatigue in this time. Specimens were quickly taken from each side into fluoride. Transfusion was then begun and most of the animal's blood was replaced by saline. At the same time stimulation of the sciatic nerve was continued. The muscles quickly became fatigued—in about 5 minutes fatigue was complete. Specimens of muscle from both sides were then taken.

				% H_3PO_4	
				Resting	Stimulated
Before transfusion:					
In $N/10$ sodium fluoride	...	...	...	0.081	0.077
After transfusion:					
In $N/10$ sodium fluoride	...	...	...	0.071	0.148
In 2 % sodium bicarbonate, heated 2 hours	40°			0.373	0.264

The resting muscle, after heating with bicarbonate, showed a higher H_3PO_4 value (by 41.3 %) than the fatigued.

The fluoride synthetic effect was not changed by 5 minutes' stimulation prior to transfusion, but after the onset of fatigue subsequent to transfusion the synthetic effect of the fluoride was markedly lowered.

A few experiments carried out on the resting muscle alone with regard to the effect of fluoride after the action of chloride at 40° for 2 hours showed no appreciable synthetic effect at 0° .

Exp. 4. A dog was anaesthetised with ether. The sciatic nerve was exposed on one side and stimulated for 20 minutes with interrupted tetanising stimuli. At the end of this period the muscles were still contracting vigorously. Perfusion was commenced and at the same time the animal was bled from the carotid. Stimulation of the sciatic nerve was continued. Unfortunately the heart failed in about two minutes and the animal died. The muscles were quickly excised and examined in the usual way.

	% H_3PO_4	
	Resting	Stimulated
In <i>N</i> /10 sodium fluoride	0.079	0.178
In 5 % trichloroacetic acid	0.244	0.219
In 2 % sodium bicarbonate, heated 2 hours 40° ...	0.375	0.368
In 0.9 % sodium chloride, heated 2 hours 40° ...	0.392	0.374

Here again the H_3PO_4 value in the resting muscle treated with fluoride is much lower than that of the stimulated muscle treated in a similar way.

The A and B values are both lower in the stimulated muscle. The lactacidogen value is slightly higher in the stimulated muscle; but when acted upon by fluoride the free H_3PO_4 is diminished by 67.6 % in the resting muscle and only by 18.7 % in the stimulated.

CAT MUSCLE.

A cat was anaesthetised with ether. The vessels were perfused with saline and while the blood was gradually being washed out the sciatic nerve on one side was exposed and stimulated. After being tetanised for 10 minutes with short interruptions the muscles were completely fatigued.

	% H_3PO_4	
	Resting	Stimulated
In <i>N</i> /10 sodium fluoride	0.124	0.212
In 5 % trichloroacetic acid	0.315	0.278
In 0.9 % sodium chloride, heated 2 hours 40° ...	0.477	0.392
In 2 % sodium bicarbonate, heated 2 hours 40° ...	0.445	0.345

Both the A and B values of the fatigued muscle are lower than those of the resting, and the difference between the A and B values shows in the resting muscle an increment of 41.2 % in free H_3PO_4 and in the fatigued muscle an increment of 24.1 %. The absolute increments are 0.130 and 0.067 % respectively. The lactacidogen value of the stimulated muscle is, therefore, much below that of the resting.

The fluoride effects show a synthesis in both the resting and the fatigued, well marked in the former (0.191 %) and only slight in the latter (0.066 %).

Estimations of glycogen and in many cases of glucose and lactic acid were performed in these muscle specimens, with the object of correlating the carbohydrate changes with those of the phosphoric acid. In this preliminary communication, however, attention has been directed solely to the phosphate changes. It will be of interest to see if the addition of glycogen to the fluoride in the case of fatigued muscle can restore the synthesising power to the level normally found in unfatigued muscle.

SUMMARY.

1. Glycogen when added to the fluoride acting on resting muscle causes practically a disappearance of free phosphate from the mixture.
2. During muscular fatigue there is a slight diminution of the total phosphate (preformed and lactacidogen) and also of free phosphate in the muscle.
3. In fatigued muscle, with practically as much preformed phosphate as in the resting, the synthetic action of fluoride is greatly diminished.

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