LXXV. STUDIES ON PHOSPHATES IN NON-IRRITABLE MUSCLE.

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IN 1921, Foster and Moyle [1921] reported a remarkable condition produced in frog muscles by long exposure to a temperature of 0°. Within a period which varied from a week to a fortnight the muscles had become completely non-irritable, failing to respond even to powerful induction shocks. Their lactic acid content, however, was identical with that of fresh resting muscle; their appearance likewise was normal, showing no trace of the opacity or rigor evident in muscles which have become non-irritable through the action of heat, chloroform and other agents. It could furthermore be shown that in these muscles lactic acid is being slowly and steadily formed and oxidatively removed as in normal muscles; moreover, under the action of heat or chloroform, the non-irritable muscles give a rigor maximum of lactic acid similar to that found in normal muscles.

The behaviour of lactic acid in the muscles studied by Foster and Moyle appears, then, to be almost identical with its behaviour in normal muscles. It would appear, however, from the studies of Embden and his co-workers, that phosphoric acid and some of its esters play a very significant part in muscular contraction [Embden, Griesbach and Schmitz, 1914; Laquer, 1914].

Not only does the precursor of lactic acid in muscle appear to be a hexose-diphosphate, but this compound, as it exists in the muscle, appears to be extraordinarily affected by the ionic character of the fluids with which it is in contact. Certain ions, of which calcium among cations and fluoride among anions are most prominent, bring about a well-marked synthesis of the hexosephosphate from its constituents, and a decided decrease of inorganic phosphate. Sodium, potassium, and other ions, on the other hand, work in the reverse direction, causing an increase in the inorganic phosphate and a breakdown of the hexosephosphate (cf. Embden and Lehnartz [1924], Lange [1924], Embden and Haymann [1924]).

In addition, Embden [1924] has recently advanced the view that the release of phosphoric acid from lactacidogen is the immediate stimulus to muscular contraction, and that the production of lactic acid follows thereafter, serving to maintain a contraction already produced, rather than to initiate that contraction.

It is difficult to reconcile this view with the results obtained by Meyerhof and Hill; on the whole, the weight of the evidence appears to be against it. If Embden's view be correct, however, we might well look for a disturbance in the phosphate metabolism of the non-irritable muscles as a clue to their inability to respond to stimulation. Two possibilities suggest themselves: (1) all, or nearly all, the lactacidogen may have disappeared, so that the available carbohydrate goes over very slowly to lactic acid; this view does not seem very plausible, but deserves consideration; (2) the enzyme which is involved in the ionic effects may have become inactivated, so that the muscle is deprived of the means for producing free phosphate rapidly. From the results to be given below it will be seen that neither of these hypotheses was confirmed.

Method.

The technique employed in the following studies resembled closely that employed by Embden and Lehnartz [1924]. Weighed amounts of frog muscle (about 1 g. per tube), which had been well chopped up with scissors on an ice-cooled glass plate, were placed in stoppered test-tubes containing a known volume of the fluid whose effect on the muscle it was desired to study. The temperature was kept constant at about 15° by immersing the tubes in flowing tap-water. After two hours, trichloroacetic acid was added (in such amount as to make the total acidity 0.25 molar), the whole fluid being made up to a known volume—generally 20 cc. The acid precipitates the proteins present, and inactivates the enzymes involved in the chemical processes studied. One sample of muscle was always treated with acid at the beginning of the experiment, so that the initial phosphate content (Embden's "A" value) might be determined. Another sample was heated for two hours in a water-bath at 40°–45°, in 2 % sodium bicarbonate, to convert all the hexosephosphate present into inorganic phosphate. If the value for inorganic phosphate so obtained ("B" value) be taken, and the "A" value subtracted from it, we obtain the value of the lactacidogen phosphate (Embden's "L" value) on the assumption that all the phosphate broken down by heating with bicarbonate was combined as lactacidogen.

The contents of the test-tubes were well shaken, allowed to stand overnight, and filtered the next morning. The inorganic phosphate content of the solution was determined colorimetrically by Briggs' [1924] modification of the method of Bell and Doisy.

The colorimetric method of estimation, and not the gravimetric method employed by Embden [1921] was used for two reasons. (1) The colorimetric method, although less accurate than the gravimetric, is quite sufficiently accurate for studying the variations found in these researches and has the advantage of greater rapidity. (2) It was felt that confirmation of Embden's studies on ion-effects would have more significance if the results were obtained by a different method.

One difficulty, however, was encountered. Fluorides, whose action on muscle Embden has shown to be so potent, have been found by Rimington [1924] to exert a disturbing influence on the colorimetric determination of phosphate, in concentrations over 0.01 *M*. The samples of fluoride which were obtained for the present investigation were found to disturb the tint and intensity of the colour produced, even in concentrations well below this figure. This may have been due to impurities and not to the fluoride itself. With all the other salts employed perfectly consistent results were obtained. The use of fluoride was therefore abandoned, and calcium salts were employed in studying the synthetic aspect of the ion-effects. Their synthetic action is almost as powerful as that of fluorides, and their functional significance in the animal body much greater.

STUDIES ON NORMAL MUSCLE.

As the colorimetric method has not yet been used in investigating the effects of ions on phosphates in normal muscle it was thought desirable to make studies of this sort before proceeding to the study of non-irritable muscle.

As the synthetic aspect of the phenomena is more striking and distinctive than the breakdown effect, most of the experiments recorded were done with substances which produce the synthetic effect. Below are given the results of two typical experiments. The samples of chopped muscle were allowed to stand two hours with the various solutions tabulated; the figures given state the amount of inorganic phosphate, expressed as H_3PO_4 , found at the end of that time.

Table I.

	Exp. 1	Exp. 2
	%	%
"A" value	0.249	0.281
Molar $CaCl_2$	0.237	0.278
<i>M</i> /10 "	0.194	0.261
<i>M</i> /25 "	0.139	0.230
<i>M</i> /50 "	0.113	0.236
<i>M</i> /100 "	0.108	0.242
<i>M</i> /200 "	0.134	—
Water	0.339	0.409
"B" value	0.388	0.492
"L" "	0.139	0.211

The great diminution in inorganic phosphate under the influence of calcium chloride will be noticed. It is further to be noticed that the effect of the calcium is by no means directly proportional to its concentration. Molar calcium chloride has little or no effect. The effect is a maximum in the neighbourhood of *M*/50, and then diminishes with increasing dilution. In general, these results correspond very closely with those obtained by Lange [1924].

It will also be noticed that the calcium effect is much less in Exp. 2 than in Exp. 1. The cause of this is not altogether clear; Exp. 1, however, is typical of several experiments done in early spring; whilst Exp. 2 was done six weeks

later, when the weather had become much warmer. It is well known that the glycogen content of the frog's muscle is much less in hot than in cold weather [Adler, 1921]. It seems not unlikely, therefore, that the action of calcium is weak in Exp. 2 because of a shortage of glycogen, which is an essential factor in the synthesis of lactacidogen.

The findings of Embden's school on the effect of glycogen in increasing the synthesis of lactacidogen and the influence of sodium and potassium chlorides as contrasted with that of calcium chloride were also confirmed by the colorimetric technique.

STUDIES ON NON-IRRITABLE MUSCLE.

Foster and Moyle's methods [1921] for obtaining non-irritable muscles were followed. The muscles were kept at a constant temperature of 1° in one of the rooms of the Low Temperature Research Station at Cambridge. The appearance of the muscles and their gradual decline to non-irritability was essentially as described by Foster and Moyle. Lactic acid analyses were carried out on some of the muscles by Miss C. E. Lascelles, with the following results:

Resting non-irritable muscle: (a) 0.016 %; (b) 0.017 %.

Non-irritable muscle after heat rigor: 0.448 %.

It will be seen that these results agree with those of Foster and Moyle, and suggest no difference between normal and non-irritable muscles.

As previously indicated, we might look for a disturbance in the phosphate metabolism of these muscles either in a breakdown of the lactacidogen, or in an inactivation of the enzyme concerned in its activity. The following table, based on analyses made on several samples of muscle at various times, show the first hypothesis to be untenable.

Table II. *Non-irritable muscles.*

"A" value	"B" value	"L" value
%	%	%
0.273	0.390	0.117
0.240	0.351	0.111
0.196	0.301	0.105
0.282	0.384	0.102

The values for the lactacidogen phosphate in these muscles are a trifle lower on the average than those for normal muscles, but it is obvious that contraction has not ceased from any lack of the precursor of lactic acid, assuming Embden's "lactacidogen" to be this precursor. We therefore turn our attention to the effects of ions on the phosphates in the non-irritable muscles. If the effects were found to be lost or greatly diminished it might furnish a clue to the nature of the loss of irritability. The following results would appear to give a definite answer to this question.

Table III. *Non-irritable muscles.*

	Exp. 1	Exp. 2	Exp. 3
	%	%	%
"A" value	0.240	0.286	0.282
<i>M</i> CaCl ₂	—	—	0.254
<i>M</i> /5 "	0.241	—	—
<i>M</i> /25 "	0.185	—	—
<i>M</i> /50 "	0.172	0.126	0.149
<i>M</i> /50 "	—	0.076	—
0.5 % glycogen	—	—	—
<i>M</i> /100 CaCl ₂	0.186	—	—
Water	0.286	0.315	0.312
"B" value	0.351	0.255*	0.384
"L" "	0.111	?	0.102

* The "B" value here is obviously an impossible one; the true "B" value cannot be less than the "water" value. Some experimental error seems almost certainly involved.

From these results it would appear that the synthetic power of the calcium ion is fully as great in the non-irritable as in the fresh muscle. These results, therefore, neither support Embden's theory that the liberation of phosphoric acid is fundamental in contraction nor do they serve in any way to refute it, and they certainly give no clue to the nature of the loss of irritability.

SUMMARY.

1. Some of Embden's studies on the influence of various ions on muscle have been repeated and confirmed, with the use of the colorimetric method of estimation of phosphorus.

2. The work of Foster and Moyle on non-irritability in muscle produced by long exposure to low temperature has been extended. There is no apparent difference in chemical behaviour between these muscles and normal fresh muscles. It is possible that some colloidal change has occurred in the non-irritable muscles which we can only hope to understand through a more intimate knowledge of the physico-chemical structure of the muscle fibril.

My thanks are due to Sir F. G. Hopkins, who suggested this line of investigation and has given me much encouragement. The privilege of using one of the rooms in the Low Temperature Research Station has also been of great value in these studies. I am also greatly obliged to Miss Lascelles, who carried out the lactic acid determinations on the non-irritable muscles in the course of other researches on which she is engaged.

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