THE PHYSIOLOGICAL SIGNIFICANCE OF "PHOSPHAGEN."

By PHILIP EGGLETON
AND GRACE PALMER EGGLETON (*Gilchrist Research Student*).

(*From the Department of Physiology and Biochemistry, University College, London.*)

In a recent publication(1) we have described methods for the detection and estimation in muscle tissue of an organic phosphoric acid derivative hitherto undescribed. This substance is of the nature of a hexosephosphate (unpublished results). It has previously escaped observation by virtue of two remarkable characteristics: it is hydrolysed with great rapidity in acid solution, yielding inorganic phosphate; hence the estimation of inorganic phosphate by the methods of Embden(3) or Briggs(2) (which involve the use of strong mineral acids) leads to its complete destruction, and the estimation of its phosphoric acid radicle as "inorganic"; in the second place, incubation of a chopped muscle in the presence of fluoride leads to the conversion of this substance into some acid-stable form. For this reason Embden failed to detect it in his method for isolating the hexosephosphates of muscle(12), inasmuch as the first stage of his preparation was an incubation with fluoride (to increase the yield of "lactacidogen").

This substance has been designated "phosphagen," since it appears to be the precursor of inorganic phosphate in the muscle. It should not be confused with Embden's "lactacidogen," which is very resistant to hydrolysis by mineral acids in the cold. Phosphagen appears to be of special importance to the functioning of voluntary muscle. The muscular lining of the frog's stomach contains so little that we cannot estimate it. The heart muscle contains a little phosphagen, but is hardly comparable in this respect with skeletal muscle (Table I).

TABLE I.

	Stomach	Heart	Gastrocnemius
Inorganic phosphate	20	20	25
Phosphagen	0	5	55

The results are given as mg. of phosphorus per 100 grm. of tissue. In this, as in other tables, the phosphagen figures refer to the "labile" phosphorus of the molecule.

We have examined the behaviour of phosphagen in muscular contraction and recovery, using for the purpose the gastrocnemius muscle of the frog (*R. temporaria*). Fatigue induced by a 2–4 minute tetanus results in a disappearance of the greater part of the phosphagen of the muscle, with the simultaneous production of inorganic phosphate, lactic acid, and a substance (or group of substances) as yet unidentified, which contains phosphoric acid in an acid-stable combination. Table II gives the results of some experiments of this type.

TABLE II.

Experiment (nature of tetanus)	Phosphagen			Inorganic phosphate			Lactic acid		
	Before	After	Fall	Before	After	Rise	Before	After	Rise
1½ min. in air; direct stimulation; secondary coil at 12–10 cm.	40	17	23	38	48½	10½	38	130	92
2 min. in air; stimulation through nerve; 0 cm.	59½	24	35½	28	49	21	—	—	—
2 min. in N_2; direct; 24–10 cm.	47	17	30	33	51½	18½	87	190	103
3 min. in air; stimulation through nerve; 10 cm.	55	25½	29½	25½	45½	20	96	194	98
3 min. in air; stimulation through nerve; 0 cm.	55½	21½	34	27½	46½	19	—	—	—
4 min. in air; stimulation through nerve; 0 cm.	56	23	33	28	45	17	—	—	—

Effect of rapid fatigue. The primary circuit of the stimulating coil contained one 2-volt accumulator. (Results are given as mg. per 100 grm. of muscle, of phosphorus and lactic acid respectively.)

It will be seen from these results that the disappearance of 30 mg. of phosphagen-phosphorus is accompanied by the appearance of about 20 mg. of inorganic phosphorus and about 100 mg. of lactic acid. These figures are suggestive of the glycolysis of a hexosediphosphate, accompanied by the re-synthesis of part of the inorganic phosphate into some compound other than phosphagen. Even so, the lactic acid production is slightly excessive, but the discrepancy might be attributable to some cycle leading to the re-formation of phosphagen, and the subsequent breakdown of this, with the production of more lactic acid. Some support is given to this view by the results in Table III. In these experiments the tetanus was broken up and spread over a longer time in order to facilitate any such anaerobic cycle. The result was a much greater production of lactic acid, with very little alteration in the amount of phosphagen used up or the amount of phosphate liberated.

TABLE III.

Experiment	Phosphagen			Inorganic phosphate			Lactic acid		
	Before	After	Fall	Before	After	Rise	Before	After	Rise
10 sec. tetanus, 30 sec. rest (each 15 times); 2-volt; 10 cm. indirect	53	21½	31½	23	51	28	60	256	196
5 : 15 : 30; 2-volt; 14 cm. direct	50	10	40	29	60	31	66	256	190
5 : 15 : 36; 2-volt; 10 cm. direct	37	2	35	41	69	28	57	263	206

Effect of discontinuous stimulation (in nitrogen).

It could certainly be argued that the lactic acid production is a process independent of the phosphagen disappearance, but the work of Meyerhof(4), on the fermentative properties of muscle extracts, leads one to suspect a connection. There can, however, be little doubt as to the intimate relationship between the inorganic phosphate and phosphagen. If a fatigued muscle (tetanised for about 3 minutes in nitrogen[1]) is exposed to an atmosphere of oxygen, it rapidly regenerates the phosphagen which had been lost during the tetanus. At the end of an hour most of the phosphagen has been replaced, *and an exactly equivalent amount of inorganic phosphate has disappeared.* During this time only a very small amount of lactic acid is removed. The results of five such experiments are quoted in Table IV.

TABLE IV.

Experiment	Phosphagen			Inorganic phosphate			Lactic acid		
	Before	After	Rise	Before	After	Fall	Before	After	Fall
30 min. at 16°	5	22½	17½	70	52½	17½	—	—	—
60 „ 16°	11	32	21	62	41	21	194	169	25
40 „ 4°	6½	41½	35	65	30	35	—	—	—
50 „ 4°	7	46	39	65	26½	38½	—	—	—
60 „ 4°	15½	36½	21	55	35	20	164	152	12

Effect of recovery in oxygen. The muscles had been tetanised for 2–4 minutes in N_2.

Although a comparison of Table III with Table II leads one to suspect the existence of a cycle involving glycogen, phosphate, and phosphagen in the production of lactic acid, yet we have found no direct evidence of any anaerobic recovery leading to an increase in the phosphagen content of a muscle. Whether resting or fatigued, a muscle placed in nitrogen always tends to lose its phosphagen (Table V).

[1] The nitrogen used in all our experiments contained about 1 p.c. of oxygen. In view of the recent work of Furusawa and Hartree (5) this fact may subsequently assume importance.

TABLE V.

Experiment	Phosphagen			Inorganic phosphate		
	Before	After	Fall	Before	After	Rise
Resting muscle (18 hours 16°)	(55)	10	(45)	(25)	63	(38)
Fatigued muscle (1 hour 16°)	16	7½	8½	64½	70½	6
Fatigued muscle (1 hour 16°)	15½	6	9½	59	77	18

Effect of anaerobiosis on resting and fatigued muscles. The initial condition of the resting muscle was not determined: average values have been inserted. In this experiment the lactic acid rose to 535 mg. p.c.

Experimental.

The gastrocnemius muscle of the frog (*R. temporaria*) was used throughout this work. The muscle was dissected away from the bone, with the sciatic nerve attached if required. We have satisfied ourselves that the slight injury necessitated at the origin of the muscle by this technique has no serious effect on its subsequent behaviour. Where it was desired to obtain a close approximation to the theoretical "resting condition," the frogs were previously cooled for a few hours in a room at 0°. Stimulation (either direct or *via* the nerve) was applied through platinum electrodes from a small induction coil set to give a maximal stimulus (as judged by the response of the muscle, which was always allowed to contract freely). It was found convenient, though not essential, to use liquid air for killing the muscles. Where one gastrocnemius was used as a resting control on its companion, it was maintained under the same conditions of temperature and atmosphere as its stimulated companion, and the two were immersed simultaneously in liquid air.

In cases where lactic acid was estimated, batches of four to six frogs were used in each experiment, but in most other cases the measurements here recorded were made on the muscles of a single frog.

In testing the effect of recovery in oxygen, the muscle pairs were stimulated in parallel on the electrodes. One muscle was immediately killed in liquid air, and the other suspended in moist oxygen at a pressure of 80–100 cm. of mercury.

The removal of proteins was in all cases effected by grinding up the tissue in 4 p.c. trichloracetic acid. The method of estimating phosphagen and inorganic phosphate has been outlined in a different publication (1). It is a modification of the Briggs technique, which in its ordinary form does not discriminate between the phosphagen and inorganic phosphate, owing to the rapid hydrolysis of the former. Lactic acid was estimated by a modification of the Clausen technique (6), suggested to us by Prof. Meyerhof. This modification is based on the fact that no aeration is necessary in the Clausen distillation if certain precautions are taken.

Discussion.

There is present in the skeletal muscles of the frog an organic phosphorus compound which has hitherto been confused with inorganic phosphate, owing to its rapid hydrolysis in acid solution to phosphoric acid. There may be more than one such compound, but the hypothesis of a single compound is sufficient to explain the available facts. We have given the name "phosphagen" to this substance.

The results quoted in this paper establish the fact that muscular contraction is accompanied by the removal of phosphagen, and subsequent recovery in oxygen is characterised by a rapid restitution of the phosphagen—a phase of recovery apparently independent of the relatively slow oxidative removal of lactic acid (7). It is of interest to consider the possible relationship between phosphagen, inorganic phosphate, and lactic acid. But it must be remembered, in the first place, that we can estimate phosphagen only in terms of its exceedingly labile phosphate radicle: the molecule may contain phosphate radicles more resistant to acid hydrolysis. In the second place, we have no knowledge of the nature of the organic part of the molecule[1]. Out of the several possibilities we put forward the following scheme, which has the advantage of simplicity:

(*a*) Phosphagen → Lactic acid + Inorganic phosphate.

(*b*) Inorganic phosphate + Glycogen → *X*.

(*c*) *X* → Phosphagen.

Reference to Table II shows that part of the labile phosphagen-phosphorus which disappears in fatigue is not accounted for by the inorganic phosphate which is liberated. In the scheme above this unidentified product has been labelled *X*, and is represented as being formed at the expense of inorganic phosphate, and not directly from phosphagen. *X* may be identical with Embden's lactacidogen. These three reactions form a cycle of changes, which, if properly balanced, leads simply to the conversion of glycogen into lactic acid. By imagining one of these reactions to be temporarily accelerated, as by electrical stimulation or changes in the oxygen supply, the observed effects can be duplicated.

It is evident that this view of the function of phosphorus in the metabolism of striated muscle affords an independent confirmation of the conclusions of Meyerhof (4), in his recent studies of the fermentation processes in muscle extracts.

[1] Recent unpublished work on the isolation of phosphagen shows that it is a hexosemonophosphoric acid, though some doubt attaches to the nature of the hexose.

It is premature at this stage to review the existing literature in the light of this new discovery, but it is necessary to emphasise the fact that all work based on the methods of Briggs(2) or Embden(3), involving the use of strong mineral acid for the estimation of phosphate, becomes of doubtful validity. We have found phosphagen in the skeletal muscles of the tortoise, rabbit and guinea-pig, and from an examination of the work of Beattie and Milroy(8), Andrews(9), and others, we are strongly inclined to suspect the presence of phosphagen in the muscles of cats and dogs. The sudden increase in the inorganic phosphate content of the blood of athletes during a short severe spell of exercise(10), and the increased excretion of phosphate under similar conditions(11), becomes easily explicable on the assumption of the presence of phosphagen in human muscle.

The "synthetic" effect of fluoride on minced muscle will be the subject of a separate communication, but a passing reference will be of interest. Phosphagen disappears from a bicarbonate suspension of minced frog muscle, whether fluoride is present or not: but in the former case it gives rise to no inorganic phosphate. It is only necessary to suppose that fluoride completely inhibits reaction (*c*) in our scheme in order to explain these facts. In the absence of fluoride the complete cycle can be effected, though reaction (*a*) obviously predominates.

Summary.

1. There is present in the gastrocnemius muscle of the frog an organic derivative of phosphoric acid, hitherto undescribed, which takes a part in the chemical mechanism of contractility. This substance has been designated "phosphagen."

2. The amount of this substance in a resting gastrocnemius, measured in terms of its labile phosphorus, is about 55 mg. p.c. In the cardiac muscle there is only about one-tenth of this amount: in the muscular coat of the stomach none has been detected.

3. A gastrocnemius tetanised for 2–4 minutes electrically (either directly or through the nerve) loses most of its phosphagen. About two-thirds of the labile phosphorus which has disappeared is accounted for by the appearance of inorganic phosphate. The simultaneously produced lactic acid bears a ratio to the phosphagen phosphorus lost of rather more than 1 molecule per atom of phosphorus.

4. In a more slowly fatigued muscle (in nitrogen) the lactic acid production is considerably greater, whilst the phosphagen disappearance and the phosphate formation are about the same.

5. No restitution of phosphagen can be observed under anaerobic conditions, but in the presence of oxygen phosphagen rapidly reappears, and an exactly equivalent amount of inorganic phosphate is lost. During this time there is very little removal of lactic acid.

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