CXXXIV. THE DISTRIBUTION OF PHOSPHORUS AND FAT IN THE RESTING AND FATIGUED MUSCLE OF THE CAT, WITH A NOTE ON THE PARTITION OF PHOSPHORUS IN THE BLOOD.

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COSTANTINO [1912, 1914, 1917] was one of the first to attempt a more thorough study of the partition of the element phosphorus in muscle than had previously been undertaken. He recognised the presence of phosphatide, inorganic phosphate, protein phosphorus and an indefinite group of acid-soluble organic compounds. Further, he found that in the dog exercise produced an increase in the inorganic phosphate, but did not alter the total or extractive phosphorus, *i.e.* there was a change in the distribution but not in the total amount present. In the rabbit, however, no such alteration took place.

The work of Embden [1912-22] and his co-workers on the very labile chemistry of "lactacidogen" has rendered the previous determinations of the inorganic and total acid-soluble fractions of little or no value, owing to the rapidity with which this substance is split up or re-synthesised according to whether the muscle is contracting or returning to its resting phase.

In recent times the chief interest in the rôle of phosphorus in muscle metabolism has centred round the hexosediphosphoric ester found by Embden in muscle and now identified with the hexosediphosphoric ester previously demonstrated by Harden and Young [1911] in yeast juice. Of the significance of the other phosphorus compounds very little is known.

The occurrence in the animal body of organic compounds linked with phosphoric acid has given rise to much surmise. Indeed Meyerhof [1924] has put forward the hypothesis that this linkage renders the organic radicle the more accessible to oxidation. Respiratory quotient determinations during prolonged exercise led Benedict and Cathcart [1913], and Krogh and Linhard [1920], to the conclusion that although the energy for muscle work is derived mainly from carbohydrate, yet it cannot be the sole source. On a normal diet, Furusawa [1925] found that the oxidation of fat could only be detected after the oxidation of about 300 g. carbohydrate. On a fatty diet with a basal respiratory quotient of 0.71 the excess metabolism due to a short period

of exercise was that of carbohydrate only, suggesting that fat was used only after conversion into carbohydrate. In long continued exercise on a fatty diet, fat was more quickly called upon than on a normal diet.

Palazzolo [1914] found that the muscle fat in frogs and hedgehogs was consumed even though the glycogen store was not exhausted. On the other hand, Leathes [1906] found no evidence of fat utilisation in muscles tetanised for several hours by stimulation of the sciatic nerve. Winfield [1915] using surviving muscles found that the fat content did not change after prolonged stimulation under anaerobic conditions nor after recovery in oxygen. Lafon [1913], working with the *levator labii superioris* of the horse and ass, found that more fat was removed from the blood when the muscle was working than when at rest. Bloor [1916, 1, 2; 1917; Bloor and MacPherson 1917], as a result of work on the distribution of fat and phosphatide in blood, came to the conclusion that, in general, wherever variations in the fat of the blood occur, there are found corresponding changes in the phosphatide, and at the same time he put forward the suggestion that phosphatide is an intermediate step in the utilisation of fat.

In view of the fact then that phosphorus may be linked with lipins and of the evidence that fat may be utilised as a source of energy, it was thought of interest to determine the fate of fat as well as of phosphatide in fatigued muscle.

GENERAL PLAN OF THE EXPERIMENTS.

The material used was the muscles of the domestic cat, an essentially carnivorous animal. In preference to prolonged stimulation under a general anaesthetic the animals were anaesthetised and decerebrated. After an interval blood was drawn from the left great saphenous vein, then the gastrocnemius, sometimes the triceps and biceps as well, were removed from the left side (first series). The sciatic nerve of the other limb was picked up in the gluteal region but was not cut. Faradic stimulation through the nerve was then carried out for varied periods using single induced shocks at first, then later tetanising the muscles by the application of more rapid stimuli. At the end of the stimulation blood was drawn from the intact right great saphenous vein. The gastrocnemius and the other muscles corresponding to the first series were then dissected out. By determining the distribution of phosphorus in the fractions to be described an attempt has been made to trace the gross changes of the phosphoric compounds during the different states of the musculature.

(a) The total P_2O_5 in the total acid-soluble extract provided an estimate of the changes of inorganic phosphate, lactacidogen and other unknown compounds.

(b) Determinations of the inorganic P_2O_5 were made on "a."

(c) Determinations of the inorganic P_2O_5 in muscle allowed to autolyse in an alkaline medium at 40° for $2\frac{1}{2}$ hours gave the lactacidogen plus in-

organic P_2O_5 content. The former could then be calculated by the difference "c"—"b."

(d) The P_2O_5 of the unknown group was calculated from the difference "a"—"b."

(e) The P_2O_5 estimated in the fraction soluble in anhydrous ether and chloroform gave a rough method of calculating the changes in the phosphatide moiety.

(f) The nucleoprotein P_2O_5 was determined sometimes directly from the residue after the phosphatide and total acid-soluble extractions, sometimes indirectly by the difference of "a" plus "e" from the total P_2O_5 .

The calculations throughout have been stated as percentages of the dried muscle. Such a precaution is necessary owing to the changes in the water relation which take place as the result of stimulation.

EXPERIMENTAL PROCEDURE.

In order to reduce any effects due to recent ingestion of food, the animal received no food on the day previous to the experiment. When attempting the more prolonged operations of the later experiments, a complication, namely an excessive bronchial secretion, arose so frequently that it was found necessary to give an injection of 1/400 g. atropine sulphate hypodermically half an hour beforehand. Though an undesirable factor this allayed the excessive secretion without interfering with the muscular system [see Cushny, 1918]. The animal was anaesthetised with ether and decerebrated according to the method of Schmidt [1922].

After an interval of about 15 minutes, 5 cc. blood were drawn from the great saphenous vein of one limb, and added to 20 cc. of 5 % redistilled trichloroacetic acid¹ in a 25 cc. measuring flask. The flask was stoppered, shaken, and after 10 minutes the contents were filtered through a dry filter paper previously extracted with 1 % HCl.

The inorganic P_2O_5 was determined by the Briggs [1922] modification of the Bell-Doisy method [1920], 10 cc. of the filtrate being used. The total acid-soluble P_2O_5 was estimated colorimetrically on 5 cc. of the filtrate ashed with 0.3 cc. H_2SO_4 and 0.3 cc. HNO_3 in a micro-combustion tube. For the formation of the ammonium phosphomolybdate 2 cc. of a 5 % ammonium molybdate solution were used. The other reagents were as described in the original papers cited. The possible interference of the trichloroacetic acid and sulphuric acid used was tested. *N*/2 sulphuric acid and *N*/10 trichloroacetic acid using a 5 % ammonium molybdate solution to produce the phosphomolybdate had no effect on the colour production².

¹ Martland and Robison [1924] found in some samples of trichloroacetic acid an impurity which produced a blue colour with the reagents used. Although this factor did not occur in the experiments described, the precaution of redistilling the reagent suggested by these authors was adopted.

² Stanford and Wheatley [1925] have recently found the same limiting acid concentrations. The present writer observed that allowing a period of 8–12 hours for colour production accurate readings were obtained with phosphate solutions even when the percentage difference was 50–100 %.

The residue from the above estimations together with the precipitated protein was oxidised with 7 cc. H_2SO_4 and 5–7 cc. HNO_3 and the total P_2O_5 calculated.

In these and subsequent estimations where it was desired to precipitate the phosphorus and weigh as phosphomolybdate, the precaution had to be taken, prior to precipitation, of adding an excess of HNO_3 (5 cc.) to the neutralised acid mixture. A granular ammonium phosphomolybdate which quickly and cleanly precipitated out was formed.

On the same limb from which the blood was drawn the gastrocnemius was dissected out, the vessels ligated and the muscle quickly cleaned of adherent fat and fascial sheath, then immersed in liquid air till frozen. The frozen muscle was then broken into small pieces in a cold stone mortar and divided into four lots—*A*, *B*, *C* and *D*—which were quickly weighed, while still frozen, in small stoppered bottles. This immersion in liquid air inhibits the changes in the isolated muscle, particularly the breakdown of “lact-acidogen.” Though the density of the muscle is decreased by this process, yet the condition is standard throughout the experiment, and is preferable to the rapid evaporation which results from the exposure of a warm isolated muscle even during rapid weighing.

Lot A of about 2 g. was weighed, teased out with needles, then dried in an electric oven at 98° until constant in weight. If drying is carried beyond this point other changes in the tissue take place. The percentage of water was calculated and a total P_2O_5 estimation was made on this dried extract using Neumann's wet ash method.

In this connection it was found that if the 7 cc. H_2SO_4 and 5 cc. HNO_3 used in these experiments were allowed to remain for several days at room temperature in contact with the dried substance to which has been added about 10 cc. water, oxidation proceeded to a considerable extent, and when heating was finally employed the oxidation was rapidly completed, thus obviating the chance of loss by volatilisation.

Lot B, about 2–3 g., was first weighed, then rapidly ground in a mortar containing 5 % redistilled trichloroacetic acid with broken glass. It is essential that the division of the tissue be fine as otherwise the thorough extraction of the acid-soluble material is inhibited by the precipitated film of protein. After about 2 hours the protein precipitate is filtered off, and washed thoroughly up to a known volume (100–200 cc.) with 0.5 % trichloroacetic acid. The inorganic phosphorus content was determined by the Briggs [1922] method. The total P_2O_5 was estimated gravimetrically.

Lot C, 2–3 g., was weighed, then minced finely with scissors with one-fifth of its weight as volume of saturated NaHCO_3 .

The adherent fluid on the scissors was washed off with 15 to 20 cc. mammalian Locke solution containing neither glucose nor NaHCO_3 . The vessel with its contents was immersed in a water bath at 50° for a few minutes and then placed in an incubator at 38° for $2\frac{1}{2}$ hours. Embden and Laquer [1921]

have demonstrated that this alkaline medium allows the breakdown of the lactacidogen by the specific muscle enzyme to go to completion.

An excess of 5 % trichloroacetic acid was added to precipitate the protein completely and extract the acid-soluble material. After standing for about 2 hours the mixture was filtered and washed to a known volume with 0.5 % trichloroacetic acid. Estimations of the inorganic phosphorus represented the P_2O_5 of the inorganic phosphate originally present, plus the additional phosphate liberated from the lactacidogen.

Lot D, approximately 10 g., after being weighed was finely ground in a mortar with broken glass and 95 % alcohol as fluid. This finely ground suspension, after standing for a few days, was filtered through a paper extraction thimble suspended in the interior of an Erlenmeyer flask. The filtrate was washed with alcohol into an evaporating basin and taken down almost to dryness on a water-bath, then desiccated over H_2SO_4 *in vacuo*. Over the suspended thimble a reflux condenser was now fitted and the tissue extracted with 95 % alcohol for 12 hours on an electric hot plate kept at the lowest possible temperature commensurate with steady extraction. After extraction the alcohol was washed with ether into a second evaporating basin and a further 12 hours extraction with ether carried out.

This extract was added to the alcohol in the second basin, and the products of subsequent 12 hours extractions with 95 % alcohol and ether were also added. The total fluid was then evaporated to a few cc. and the final desiccation completed *in vacuo* over H_2SO_4 . The extracts were exposed to the air as little as possible. After as complete a desiccation as is compatible with the preservation of phosphatides unchanged, at least as regards their solubility, the residues of basins 1 and 2 were extracted several times in the cold with anhydrous ether and chloroform; anhydrous ether alone is probably insufficient as kephalin is insoluble in it. These precautions as regards using anhydrous reagents were necessary, for any water remaining in the solvents permits of a partial solution of other compounds, particularly inorganic salts. The combined ether and chloroform extracts were then evaporated almost to dryness on a water-bath and desiccated *in vacuo* over H_2SO_4 . The weight of this dried material gave the total fat and "lipoid" substance, whilst a colorimetric estimation of the P_2O_5 after wet ashing gave the P_2O_5 as combined in phosphatide. An acid mixture of 0.75 cc. H_2SO_4 and 0.5 cc. HNO_3 is sufficient to oxidise the fat in the material obtained from 2 g. muscle.

It was thought that possibly the procedure adopted did not permit of the extraction of the masked fat of Dormeyer [1897] although MacLean [1918] has stated that the ether-insoluble combination of phosphatide and protein is acted on by alcohol and the phosphatide, which can subsequently be extracted by ether, liberated. In order to test if any fat remains unextracted, the residue in some of the experiments was digested with 1 % pepsin in 0.2 % HCl at 40° for 3–4 days. At the end of that period the fluid was neutralised with NaOH, then washed repeatedly with ether in a separating

cylinder and the combined extracts treated as in the previously described fat estimation. A mere trace of fat was obtained which was found each time to be equivalent to the fat content of the enzyme preparation used. The ether and alcoholic extractions therefore remove all the fat.

Nucleoprotein P_2O_5 .

Costantino [1915] demonstrated that the phosphorus-containing protein compounds of smooth muscle were nucleoprotein. To determine the nature of the protein phosphorus in striated muscle the residue from the fat extractions was first treated with 1 % HCl, then, after standing 2-4 hours, the product was filtered and the residue thoroughly washed with distilled water till free from all acid-soluble material. An extraction of this residue with 1 % NaOH for 48 hours at 37° was made according to the directions of Plimmer and Scott [1908]. A total P_2O_5 estimation was done at this stage. The remainder was then treated with 2 cc. glacial acetic acid for every 100 cc. solution and the precipitate filtered off. Since the phosphoric acid is split off the phosphoprotein molecule by this digestion, an estimation of the inorganic P_2O_5 reveals its presence or absence. No phosphoprotein was ever found so that the total P_2O_5 of the extract was due to nucleoprotein.

DESCRIPTION OF THE EXPERIMENTS.

It may be stated that before these experiments were carried out, many estimations of the phosphorus content of the muscle of the cat were made in duplicate and triplicate as a test of the method and of the constancy of the distribution of that element.

Exp. 1. The two gastrocnemii were removed as soon as the depth of anaesthesia permitted.

Exps. 2, 3 and 4. In these experiments the resting gastrocnemii were removed 15 minutes after decerebration. The sciatic nerve in each case was picked up in the gluteal region of the other limb, and, for the first 10 minutes, stimulated with single induced shocks, then subsequently the muscles supplied by the nerve were tetanised by means of a Neef's hammer for periods of 15 to 50 minutes.

Exp. 5. After decerebration the resting gastrocnemius was allowed to remain *in situ* throughout the stimulation period of its fellow.

In Exps. 1 to 5, no atropine was injected. Nucleoprotein P_2O_5 was estimated directly. In all the experiments described, stimulation was carried on till the last moment prior to the actual dissection of the muscle.

Exp. 7. Blood was drawn from the great saphenous vein 15 minutes after decerebration. The lateral and medial cords of the brachial plexus on the right side were cut. Stimulation was started after the three muscles on the left side were removed and was continued for 45 minutes at which point the breathing stopped. A few minutes later the heart ceased beating. Stimulation

was kept up till the moment of removal of the gastrocnemius. No atropine was used.

Table I. *Exps. 1-5.*

Number of experiment	1		2		3		4		5	
State of the muscle (gastrocnemius)	R.	R.	R.	F.	R.	F.	R.	F.	R.	F.
% dry matter	25.41	25.43	25.01	22.89	26.80	22.14	24.83	22.66	26.79	23.16
% water	74.59	74.57	74.99	77.11	73.20	77.86	75.17	77.34	77.86	76.84
Phosphatide P ₂ O ₅ % of dry matter	0.25	0.24	0.19	0.17	0.29	0.28	0.27	0.28	0.17	0.17
Total acid-soluble P ₂ O ₅ % of dry matter	1.84	1.82	1.92	1.95	1.88	1.91	2.02	2.00	1.83	1.82
Inorganic phosphate P ₂ O ₅ % of dry matter	0.88	0.88	0.97	1.20	1.00	1.19	1.05	1.24	0.79	0.97
Nucleoprotein P ₂ O ₅ % of dry matter	0.17	0.15	0.16	0.10	0.17	0.17	0.09	0.12	0.14	0.11
Total P ₂ O ₅ % of dry matter	2.24	2.22	2.25	2.24	2.33	2.32	2.41	2.43	2.13	2.12
Period of stimulation	Resting		50 mins.		20 mins.		30 mins.		15 mins.	
	R. = resting.				F. = fatigued.					

Table II. *Exp. 7. Female Cat.*

	Left gastroc. removed after decerebration but prior to stimulation of right	Right gastroc. stimulated for $\frac{1}{2}$ hour after decerebration. Animal died	Left triceps removed after de- cerebration but before stimulation of gastroc.	Right triceps removed after stimu- lation of gastroc. for $\frac{1}{2}$ hour	Left cephalo- humeral mus- cle removed after decere- bration but before stimu- lation of gastroc.	Right cephalo- humeral muscle removed after stimu- lation of gastroc. for $\frac{1}{2}$ hr.
% dry matter	24.31	20.74	24.16	25.29	24.76	25.74
% water	75.69	79.26	75.84	74.71	75.24	74.26
Phosphatide P_2O_5 % of dry matter	0.30	0.30	0.34	0.35	—	—
Total acid-soluble P_2O_5 % of dry matter	—	—	—	—	—	—
Inorganic phosphate P_2O_5 % of dry matter	1.03	1.37	1.00	0.97	1.05	0.94
Inorganic phosphate plus lactacidogen P_2O_5 % of dry matter	1.51	1.50	1.45	1.42	—	1.42
Lactacidogen P_2O_5 % of dry matter	0.48	0.13	0.45	0.45	—	0.48
Acid-soluble organic P_2O_5 other than lactacidogen % of dry matter	—	—	—	—	—	—
Nucleoprotein P_2O_5 % of dry matter	—	—	—	—	—	—
Total P_2O_5 % of dry matter	2.39	2.40	2.32	2.30	2.29	2.31

Exp. 8. This experiment was in nature a basal one. Half an hour prior to the operation 1/400 g. atropine sulphate was injected. The animal was anaesthetised with ether and decerebrated. Fifteen minutes later 5 cc. blood were drawn from the great saphenous vein on the left side, then the gastrocnemius, triceps and biceps on the same side were removed. The nerve supply to the biceps on the right side was severed. After a period of $1\frac{3}{4}$ hours rest in this condition, 5 cc. blood were drawn from the inferior vena cava and the three corresponding muscles removed from the living animal.

Table III. *Exp. 8. Female Cat. 2.3 kg.*

	Left gastroc. removed $\frac{1}{2}$ hr. after de- cerebration	Right gastroc. removed $1\frac{1}{2}$ hr. after de- cerebration	Left triceps removed $\frac{1}{2}$ hr. after de- cerebration	Right triceps removed $1\frac{1}{2}$ hr. after de- cerebration	Left biceps removed $\frac{1}{2}$ hr. after de- cerebration	Right biceps removed $1\frac{1}{2}$ hr. after de- cerebration during which period its nerve was cut
% dry matter	24.17	24.77	24.71	25.65	25.26	26.00
% water	75.83	75.23	75.29	74.35	74.74	74.00
Phosphatide P_2O_5 % of dry matter	—	—	—	—	—	—
Total acid-soluble P_2O_5 % of dry matter	1.94	1.92	1.92	1.87	1.92	1.95
Inorganic phosphate P_2O_5 % of dry matter	0.92	0.89	0.95	0.91	0.88	0.82
Inorganic phosphate plus lactacidogen P_2O_5 % of dry matter	1.31	1.28	1.37	1.32	1.26	1.25
Lactacidogen P_2O_5 % of dry matter	0.39	0.39	0.42	0.41	0.38	0.43
Acid-soluble organic P_2O_5 other than lactacidogen % of dry matter	0.63	0.64	0.55	0.55	0.66	0.70
Nucleoprotein P_2O_5 % of dry matter	—	—	—	—	—	—
Total P_2O_5 % of dry matter	2.36	2.35	2.43	2.39	2.32	2.35

Exp. 9. The animal was given a hypodermic injection of 1/400 g. atropine sulphate. Twenty minutes after decerebration the gastrocnemius, triceps and biceps of the left side were removed. On the right side, the nerve to the biceps was cut, then the sciatic nerve on the same side was picked up in the gluteal region and stimulated, first by single induced shocks but later fatigued by a Neef's hammer. The load of 150 g. was fixed to the paw so that the gastrocnemius contracted against it. This faradic stimulation was maintained for $2\frac{1}{4}$ hours when the other three muscles were removed for analysis.

Table IV. *Exp. 9. Male Cat. 3.5 kg.*

	Left gastroc. removed after decerebration but prior to stimulation of right	Right gastroc. removed after $2\frac{1}{2}$ hrs. stimulation with a load of 150 g.	Left triceps removed after decerebration but prior to stimu- lation of the gastroc.	Right triceps removed after fatigue of the gastroc.	Left biceps removed after decere- bration but before stimu- lation of the gastroc.	Right biceps remained <i>in situ</i> with its nerve cut during stimulation period of the gastroc.
% dry matter	25.62	22.47	25.12	25.42	26.36	26.72
% water	74.38	77.53	74.88	74.58	73.64	73.28
Phosphatide P_2O_5 % of dry matter	0.29	0.27	0.30	0.30	—	—
Total acid-soluble P_2O_5 % of dry matter	1.84	1.89	1.94	1.96	1.98	1.97
Inorganic phosphate P_2O_5 % of dry matter	0.87	1.17	0.98	0.87	1.00	0.88
Inorganic phosphate plus lactacidogen P_2O_5 % of dry matter	1.40	1.42	1.45	1.46	1.54	1.51
Lactacidogen P_2O_5 % of dry matter	0.53	0.25	0.47	0.59	0.54	0.63
Acid-soluble organic P_2O_5 other than lactacidogen % of dry matter	0.44	0.47	0.49	0.50	0.44	0.46
Nucleoprotein P_2O_5 % of dry matter	0.11	0.06	0.14	0.15	—	—
Total P_2O_5 % of dry matter	2.24	2.22	2.38	2.41	2.31	2.29

Exp. 10. 1/200 g. atropine sulphate was administered. 5 cc. blood were drawn after decerebration from the great saphenous vein of the left limb, then the gastrocnemius, biceps and triceps were removed. The right gastrocnemius was fatigued by stimulation of the sciatic as in the previous experiments. The load was again 150 g. After stimulation for 50 minutes, 5 cc. blood were drawn from the inferior vena cava; the right gastrocnemius, triceps and biceps were then removed, the latter having had its nerve supply cut during the period of fatigue.

Table V. *Exp. 10. Male Cat. 2.85 kg.*

	Left gastroc. removed after decerebration but prior to stimulation of the right	Right gastroc. removed after 50 mins. stimulation with load of 150 g.	Left triceps removed after decerebration but prior to stimulation of the gastroc.	Right triceps which remained <i>in situ</i> during stimulation of gastroc.	Left biceps removed after decerebration but prior to stimulation of the gastroc.	Right biceps removed after the stimulation of the gastroc. during which its nerve was cut
% dry matter	24.16	20.58	23.29	23.75	24.94	24.91
% water	75.84	79.42	76.71	76.25	75.06	75.09
Phosphatide P_2O_5	0.20	0.19	0.21	0.22	—	—
% of dry matter						
Total acid-soluble P_2O_5	1.94	1.96	2.04	2.08	1.88	1.86
% of dry matter						
Inorganic phosphate P_2O_5	0.89	1.17	0.89	0.84	0.80	0.87
% of dry matter						
Inorganic phosphate plus lactacidogen P_2O_5	1.45	1.48	1.44	1.45	1.28	1.25
% of dry matter						
Lactacidogen P_2O_5	0.56	0.31	0.55	0.61	0.48	0.38
% of dry matter						
Acid-soluble organic P_2O_5 other than lactacidogen	0.49	0.48	0.60	0.63	0.60	0.61
% of dry matter						
Nucleoprotein P_2O_5	0.19	0.14	0.14	0.12	0.11	0.13
% of dry matter						
Total P_2O_5 % of dry matter	2.33	2.29	2.39	2.42	2.23	2.24

Table VI. *Exp. 11. Male Cat. 3.9 kg.*

	Left gastroc. removed after decerebration but prior to stimulation of right	Right gastroc. removed after 1½ hr. stimulation with load of 150 g.	Left triceps removed after decerebration but prior to stimulation of the right gastroc.	Right triceps removed after 1½ hr. stimulation of the right gastroc. during which time its nerve was cut	Left biceps removed after decerebration but prior to stimulation of the right gastroc.	Right biceps removed after the 1½ hr. stimulation of the gastroc. but with its nerve intact
% dry matter	24.48	23.75	23.47	24.37	24.31	25.35
% water	75.52	76.25	76.53	75.63	75.69	74.65
Phosphatide P_2O_5	—	—	—	—	—	—
% of dry matter						
Total acid-soluble P_2O_5	1.78	1.81	1.94	1.92	1.84	1.83
% of dry matter						
Inorganic phosphate P_2O_5	0.91	1.12	0.99	0.83	0.93	0.79
% of dry matter						
Inorganic phosphate plus lactacidogen P_2O_5	1.25	1.25	1.24	1.25	1.25	1.25
% of dry matter						
Lactacidogen P_2O_5	0.34	0.13	0.25	0.42	0.32	0.46
% of dry matter						
Acid-soluble organic P_2O_5 other than lactacidogen	0.53	0.56	0.70	0.67	0.59	0.58
% of dry matter						
Nucleoprotein P_2O_5	—	—	—	—	—	—
% of dry matter						
Total P_2O_5 % of dry matter	2.25	2.23	2.32	2.30	2.29	2.26

Exp. 11. 1/400 g. atropine sulphate was injected in this experiment. The blood was drawn before and after stimulation from the great saphenous veins of the left and right sides respectively. Of the resting muscles the triceps had its nerve supply cut whilst the right sciatic nerve was stimulated for $1\frac{3}{4}$ hours. In other respects the procedure was the same as in the previous experiment.

DISCUSSION OF THE RESULTS.

Constancy of Composition. Exp. 1 demonstrates that corresponding muscles under like conditions have the same distribution of phosphorus and the same water content. Many others of this type were carried out with like result. It may be assumed that equality of distribution is the rule.

The Water Relation of Striated Muscle. From Exp. 8, it was observed that the water content of an unexercised muscle remaining *in situ* under the conditions stated, for a period of $1\frac{3}{4}$ hours, decreased by 1 %, and further, this decrease was independent of the integrity of the nerve supply (motor). Several interpretations were possible. The surgical shock may have caused a slight but immediate rise in the water content, due to a dilatation of the blood system in the muscle, and 1–2 hours later this may have returned to normal. Again the “plastic tonus” of decerebration, developing subsequent to the removal of the first series of muscles, may have caused a decrease in the volume of blood in the remaining muscles.

The possibility that these changes may have been due to the exhibition of atropine was, in part, negatived by the fact that they were found in Exp. 7, where the drug was not administered.

Whatever the cause of this effect may be, it occurs in the resting triceps and biceps independent of any stimulation of the leg muscles. In one experiment alone (10), where 1/200 g. atropine sulphate had been injected, did the integrity of the nerve supply affect the result.

The 4–5 % increase in the water content of the muscle after fatigue agreed with the figure obtained by Barcroft and Kato [1916] although these authors stimulated for a period of $7\frac{3}{4}$ hours. The factor responsible for this swelling of the fatigued muscle is probably, in large part, the filling out of the collapsed capillary system during exercise [Krogh, 1919].

The Total Phosphorus Content of Muscle. Fatigue of muscles to the extent described left the total phosphorus practically unaltered.

Phosphorus in the Total Acid-soluble Fraction. This moiety remained apparently constant even after fatigue. The small changes noted, though within the range of experimental error, yet pointed consistently in the direction of a slight increase on fatigue (1–2 %).

Inorganic and Lactacidogen Phosphorus. This group remained the most constant in amount in the varied muscle states.

Inorganic Phosphate. The outstanding feature was the marked increase in the fatigued muscle due to the breakdown of “lactacidogen.” In some of the

experiments a 34 % increase was noted. From the figures in Table III, it was found that under the conditions described there was a consistent but small decrease of 2-4 % in the inorganic phosphate of resting muscles $1\frac{3}{4}$ hours after decerebration. This seemed to take place even although the motor nerve was severed.

Table VII. *Blood Analyses.*

Experiment	...	...	8		10	
Time drawn	...	...	$\frac{1}{4}$ hour after decerebration	$1\frac{3}{4}$ hour after decerebration	$\frac{1}{4}$ hour after decerebration but before stimulation	After 50 minutes' stimulation
Vessel blood drawn from			Great saphenous vein	Inferior vena cava	Great saphenous vein	Inferior vena cava
Inorganic phosphate P_2O_5 as mg. % whole blood			16.8	15.9	12.7	27.6
Total acid-soluble P_2O_5 in mg. % whole blood			33.4	34.1	35.4	48.6
Total P_2O_5 in mg. % whole blood			52.2	—	—	—
Experiment	...	...	11		7	
Time drawn	...	...	$\frac{1}{4}$ hour after decerebration but before stimulation	After $1\frac{3}{4}$ hours' stimulation	$\frac{1}{4}$ hour after decerebration but before stimulation	After 45 minutes' stimulation heart had just stopped
Vessel blood drawn from			Great saphenous vein	Great saphenous vein	Great saphenous vein	Heart
Inorganic phosphate P_2O_5 as mg. % whole blood			13.2	23.5	26.2	48.6
Total acid-soluble P_2O_5 in mg. % whole blood			31.2	34.9	—	—
Total P_2O_5 in mg. % whole blood			42.4	53.5	—	—

On superimposing stimulation on these conditions, the effect on the resting muscles was still further to decrease the inorganic phosphate in some cases by as much as 11 %. With one exception (Exp. 10), similar results were found where the resting muscle had had its nerve severed. In Exp. 10 a 2 % increase was found in the biceps with its nerve cut. This effect may have been due to the relatively large dose of atropine administered.

The question of assessing to what extent changes in the blood phosphorus might affect the estimations of that element in muscle presented itself. On calculation it was found that only in the case of the oedematous fatigued muscle can the changes in the blood appreciably affect that of muscle, and even in this state of the muscle, the extent of the possible change comes within the range of error. Again the changes in the blood to some extent reflect the changes in the muscles.

"*Lactacidogen*" P_2O_5 . Exp. 8 demonstrated that the content of lactacidogen remains practically constant. In the non-stimulated muscle, an increase of 0.38-0.43 % of the dried tissue was found in the resting biceps with the nerve supply cut. On superimposing stimulation on these experimental conditions, it was found that there was a very marked decrease of lactacidogen

amounting to 60 % in some experiments. On the other hand, the effect of exercise was to cause an increase of 20–50 % in the other resting muscles. With the one exceptional case in Exp. 10, this increase was found to be independent of the integrity of the nerve supply.

This increase of lactacidogen in the resting musculature was interesting as suggesting a possible synthesis of that compound at the expense of the inorganic phosphate already present. Another explanation is possible, namely, that the effect of the surgical shock was immediate, wearing off 1–2 hours later, and that while it lasted, it caused a breakdown of lactacidogen with the liberation of inorganic phosphate.

Acid-soluble P_2O_5 in Organic Form other than "Lactacidogen." It was found that this moiety remained very constant in the resting phase and exercise left it practically unchanged. This fraction representing 25 % of the total P_2O_5 is unexplored.

Table VIII. *Fat Analyses.*

Exp.	Fat expressed as % of dried substance			
	Left gastrocnemius	Right gastrocnemius	Left triceps	Right triceps
	R. 4.58 8.97 8.87 7.75	F. 4.60 8.91 8.75 7.35	R. 4.43 7.78 9.00 —	non-stimulated <i>in situ</i> 4.34 7.89 9.05 —

R. = resting.

F. = fatigued.

Fat and Phosphatide Content of Muscle. From the analyses found in Table VIII it will be seen that in three out of the four experiments described, there has been a slight decrease on fatigue. In Exp. 10 the content remained practically constant. These differences however, though tending in the same direction, come so nearly within the range of experimental error, that a definite conclusion cannot be reached.

With regard to the phosphatide phosphorus, exercise for the periods described has not caused any change in its amount.

The data then show practically no evidence of the utilisation of fat or phosphatide in muscle work under the experimental conditions observed, but this does not negative the possibility that these substances may be utilised after the exhaustion of the available carbohydrate. In none of these experiments was the lactacidogen content of the fatigued muscle completely used up.

The Changes in the Distribution of the Phosphorus in the Blood. Kay [1925] has classified the phosphorus compounds of the blood under the following headings: inorganic phosphate, probably two hexosephosphoric esters, an adenine nucleotide [Jackson, 1924] and lecithin. Practically no change takes place in the blood unless muscular exercise is superimposed on the conditions described (Exp. 8, Table VII).

In assessing the changes that take place on fatigue, a number of factors tend to obscure the issue. Schneider and Havens [1915] found that the immediate effect of exercise was a concentration of the blood in the peripheral capillaries, but, in a few minutes the blood was diluted. The increased content of phosphorus in the blood found in Exp. 11 may be due to this peripheral concentration alone, or to this factor accompanied by an actual increase in the circulating phosphoric compounds. There is a very definite increase in the inorganic phosphate of the blood on exercise (see Exps. 7, 10 and 11). This is probably due to the washing out of that metabolite from the active muscles into the circulation. The total acid-soluble phosphorus increases to a small extent. This may be due either to the concentration of the blood or to an increase in the corpuscles, or to both factors. The authors mentioned above found an increased erythrocyte count, whilst Isaacs and Gorden [1924] found the red corpuscles unchanged after exercise. The increase in Exp. 10 is probably due to the fact that the blood was drawn after exercise from the abdominal veins where there may be some accumulation of cells.

The fact that the total acid-soluble P_2O_5 does not increase proportionally with the rise in inorganic phosphate, suggests either that the organic acid-soluble compounds have been removed in part, or have been split up in the circulation with the liberation of inorganic phosphate. The active muscle, having exhausted its reserve of lactacidogen, may call on the hexosephosphates or other phosphorus compounds of the blood, when no further synthesis from its own substance is taking place commensurate with the demands made.

SUMMARY.

1. An experimental procedure has been developed for the examination of the effect of fatigue on the phosphorus content of muscle.

2. A few suggestions relating to the estimation of phosphorus in different tissues and extracts have been put forward.

3. Muscle fatigue to the extent described appeared to cause:

- (a) Practically no change in the total P_2O_5 .

- (b) An increase in the inorganic P_2O_5 of the stimulated muscle, the maximum increase found being 34 %. A decrease in the inorganic P_2O_5 of resting muscles remaining *in situ* during the stimulation period was found; the maximum decrease noted was 11 %.

- (c) A decrease in the lactacidogen P_2O_5 in the fatigued muscle amounting in some experiments to 60 %. An increase in the lactacidogen P_2O_5 of 20–50 % was found in the muscles resting *in situ*.

- (d) No alteration in the total amount of inorganic plus lactacidogen P_2O_5 .

- (e) Little or no effect on the organic acid-soluble P_2O_5 other than lactacidogen.

- (f) A consistent but small increase (1–2 %) in the total acid-soluble fraction.

- (g) No alteration in the phosphatide P_2O_5 .

4. The effect of fatigue on the phosphorus compounds of the blood was found to cause:

(a) An increase of the inorganic P_2O_5 . A 100 % increase was recorded after 50 minutes' stimulation.

(b) An increase in the total acid-soluble P_2O_5 (12–37 %).

(c) A decrease in the organic acid-soluble P_2O_5 (7–36 %).

5. An increase of 4–5 % in the water content was noted after stimulation of a muscle for 45 minutes.

6. The phosphorus-containing proteins of skeletal muscle belong to the nucleoprotein group.

7. An attempt to settle the question of the utilisation of fat and phosphatide in muscle work was undertaken with essentially a negative result. No "masked fat" was found to remain after a thorough alcoholic and ethereal extraction of the muscle tissue.

In conclusion I wish to express my thanks to Professor E. P. Cathcart not only for suggesting this study, but for his advice, criticism and inspiring enthusiasm throughout. To Professor D. Noël Paton for his kindly interest and advice in the operative technique, to Mr Watson and the others who so willingly gave of their time, and under whose licences the experiments were conducted, I desire to express my thanks.

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