THE OCCURRENCE OF LABILE PHOSPHORUS IN VARIOUS KINDS OF MUSCLES.

BY LAURENCE IRVING AND PHILLIP H. WELLS.

(From the Department of Physiology, Stanford University, California.)

(Received for publication, December 19, 1927.)

Since Engelmann's (1871) demonstration of increased phosphorus excretion in urine secreted during muscular exercise, there have been many attempts to show that the increase originated in the muscles. Muscle phosphorus compounds remain obscure, and it has only been during the last 10 years that the inorganic phosphorus of tissues could be conveniently determined.

During this period, tissue inorganic phosphorus has been determined in the extracts from acid protein precipitation, as for example trichloroacetic acid and the hydrochloric acid-bichloride mixtures. In such acid extracts from muscle, phosphorus was found which reacted with reagents for inorganic phosphorus. The study of this acid-soluble inorganic phosphorus fraction has been made particularly significant by Embden's lactacidogen theory, which assigned to phosphorus and lactic acid in muscle a common mother substance.

Although much effort has been expended to connect the facts of inorganic phosphorus and lactic acid metabolism in muscle, it is not easy to find positive evidence of the relation. The acid-soluble inorganic phosphorus is evidently a characteristic quantity for various types of muscles, and there is variation under some physiological conditions, but not uniformly in those where lactic acid is being rapidly produced.

That the conventional acid-soluble inorganic phosphorus does not represent the amount present in living muscle, was first announced by Eggleton and Eggleton (1927, a). When a modification of the Bell-Doisy method of phosphorus determination was used, the color development in ice-cold trichloroacetic acid...
filtrate from frog muscle was much slower than in the standard prepared simultaneously. By comparison of the rate of color development in standard and muscle extract it appeared that the initial inorganic phosphorus of frog striated muscle was only about 30 per cent of the ordinary value given by acid extraction at room temperature. This minimum, estimated from the curves by extrapolation to zero time, was greatly increased by fatigue so that one could believe that muscle exhaustion caused depletion of the labile phosphorus. A muscle in rigor had likewise lost its labile phosphorus, so that evidently the amount of labile compound is also related to the physiological condition of the muscle.

Recently, and apparently independently, Fiske and Subbarow (1927), presented a preliminary report on the results of similar findings in mammalian muscle. They used a colorimetric method of determination previously developed by them (1925) in which the color development is very rapid. Consequently, the estimation of such a labile compound is facilitated. The preliminary statement designates the labile phosphorus as phosphocreatine. Meyerhof (1927) reports also in a preliminary paper, the isolation of Fiske and Subbarow's phosphocreatine and conveniently incorporates its breakdown with the anaerobic energy-giving reactions in muscle.

If the final conclusions substantiate the indications of Fiske and Subbarow's preliminary statement, a new aspect of the part of inorganic phosphates in muscle metabolism is revealed. While Eggleton and Eggleton (1927, b) associate the appearance of labile phosphorus with decomposition of a phosphate carbohydrate, Fiske and Subbarow attribute its appearance to decomposition of a creatine compound. No reconciliation of these two views appears possible, and the first conflicts with the facts supporting Embden's lactacidogen theory.

The conventional method of acid extraction at room temperature does yield a phosphorus fraction having a normal value and capable of reproducible variation under certain experimental conditions. The entire evidence for the part of phosphorus in intermediary carbohydrate metabolism comes from a number of sources (yeast, blood, etc.) besides muscle, and is derived from data obtained by the old method of acid extraction at room temperature. Consequently, the value of the evidence for the conclu-
sions would be invalidated if, as Eggleton and Eggleton (1927, b) believe, the source of labile phosphorus is a hexose monophosphate.

The acid-soluble inorganic phosphorus, as conventionally determined, is evidently the sum of the amount present in natural muscle and the labile phosphorus set free in acid at room temperature. It would seem strange if this variable system could furnish the basic evidence regarding another system so different as that postulated in the lactacidogen theory, but still possible if the sum of labile phosphorus and inorganic phosphorus is a definite quantity. This point has been reexamined and the results presented show that the probable phosphorus-carbohydrate relations are indicated by a different set of data from that which characterizes the labile phosphorus.

If the labile phosphorus originates in a phosphorus-creatine compound, its absence or deficiency would be expected in creatine-poor muscles. From the analytical data compiled by Hunter (1922) the creatine content of smooth muscle is from one-fifth to one-tenth as much as in the striated muscle of the same animals. These small creatine values have, furthermore, not been confirmed by isolation of creatine itself, so that there is room for skepticism as to their accuracy. But in phosphorus content smooth muscles are also lower than striated muscles in about the same ratio as the creatine values reported. Otherwise the work of Evans (1925) suggests that the essential carbohydrate transformations are similar in smooth and striated muscle, at least in so far as the utilization of glycogen and production of lactic acid serve as indicators.

In view of the interest of the facts observed, and their implications, striated muscle, heart muscle, and smooth muscle from a number of sources were examined. In the muscles examined the labile phosphorus was easily demonstrable in the striated muscle of mammals and turtles only. Bird muscle was not examined, and Eggleton and Eggleton had shown the labile compound in frogs. In seven quite different species of fish labile phosphorus was not demonstrated, nor was it present in insect striated muscle. Heart muscle and smooth muscle of vertebrates and invertebrates likewise gave no positive indication of the labile compound.

For determinations the procedure of Fiske and Subbarow (1925) was followed; the muscle was extracted (previously pow-
dered in liquid air) in ice-cold 8 per cent trichloroacetic acid—
normally for 2 hours. Under the conditions observed the extrac-
tion for only a few minutes, reported satisfactory by Eggleton and
Eggleton (1927, a) was not found adequate. No decrease of the
labile compound occurred even after 5 hours at ice temperature.

In view of the suggestion that neutralization prevented de-
composition of the compound, several neutral or alkaline protein
precipitants were tried, but without success. The alkaline zinc
hydroxide protein precipitant used in the Hagedorn-Jensen blood
sugar method extracted no phosphorus whatsoever, and sodium
tungstate reacted with the reagents for the phosphate determina-
tion.

Samples from the ice-cold filtrate were mixed with the reagents
for Fiske and Subbarow’s colorimetric method of determination

<table>
<thead>
<tr>
<th>Table I. Results of Colorimetric and Gravimetric Phosphorus Determination.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus as mg. per gm.</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Colorimetric</td>
</tr>
<tr>
<td>Gravimetric</td>
</tr>
</tbody>
</table>

* Extraction by Schenck method.

and compared with standards having fully developed color. In
this procedure the difference between the phosphorus concen-
tration of unknown and standard includes a discrepancy due to the
time necessary for color development even with Fiske and Sub-
barow’s highly sensitive reagent. This fact, combined with the
steeply rising character of the curve of phosphorus concentra-
tion against time during the first few minutes, and the rapidity with
which the colorimetric matchings must be made, make the early
position of the curve unreliable. To confirm this view a number
of curves were made from observations on the phosphorus of
muscle extracts which had been subsequently kept for 24 hours
at room temperature. The difference between the curves for
development of color in the ice-cold filtrates and those kept for a
day at room temperature represents the amount of labile phosphorus.

Some colorimetric determinations were made with a Duboscq type colorimeter. The majority and most satisfactory were with

![Graph of Mg, P per gm. muscle over time.](image)

Fig. 1. Guinea pig gastrocnemius muscle extracted 3 hours in ice-cold 8 per cent CCl₄COOH. Curve 1, immediate; Curve 2, 24 hours; Curve 3, 48 hours; Curve 4, 72 hours.

...a Leitz-Buerker colorimeter, in which the light passes through the same depth of standard and unknown solutions for each cylinder.
The results in Table I indicate the general reliability of the methods used. Evidently a maximum or, at least, a constant extract is secured in from 1 to 20 hours, but less than 1 hour is insufficient for the method.

Mammalian Muscle.

The labile phosphorus was easily demonstrated in one experiment on the gracilis muscle of a resting cat and in the hind leg muscles of the rat and of ten resting guinea pigs. In Fig. 1 typical curves are shown for the phosphorus (calculated as mg. of P per gm. of muscle) in guinea pig hind leg muscle. Curve 1 shows the increase in phosphorus concentration of the fresh muscle extract, in the colorimeter, with time. Curve 2 shows the results of similar determinations after the extract had stood for 24 hours at room temperature. Curves 3 and 4 indicate determinations on the same filtrate after 48 and 72 hours respectively.

The difference between the curves for muscles examined while the extract was still ice-cold and the curves for muscle extracts kept at room temperature is plain. It becomes more distinct in terms of per cent of full color reached at each time, as shown in Fig. 2. There is no significant difference between Curves 2, 3, and 4 by these methods of representation, and the type of Curve 1 shows the characteristic slope where labile phosphorus is present.

From the experiments made it would be indicated that the average minimum phosphorus of resting guinea pig muscle was about 40 per cent of the usual acid-soluble fraction, or that the labile part decomposed by acid amounted to 1½ times the amount already present. The absolute minimum is hard to determine, because the slope of the curve is steep and the error introduced by the observer in the first few hasty colorimetric readings may be quite large. All of the indications suggest that the actual minimum for inorganic phosphorus in the resting muscles is probably less than 40 per cent of the acid-soluble fraction.

In the single case of rat muscle examined the minimum value was 0.35 mg. of P per gm. of muscle, with a maximum of 0.80. The single cat gracilis muscle examined had a minimum of 0.25 and the unusually low maximum, for these animals, of 0.75.

Muscles from about the pectoral girdle of the turtle had 0.40 and 0.25 as minima, and 0.78 and 0.64 as maxima, indicating about
50 per cent as labile phosphorus in each case. These values may not represent the minimum attainable because of delay and injury incidental to the removal of the shell. It is significant principally that the proportion of labile phosphorus is quite similar in turtles and mammals. In two of the series of guinea pig muscles (Table II) examined the amount of labile phosphorus extracted in different times was considered. While, on account

![Graph showing extraction of guinea pig gastrocnemius muscle](http://www.jbc.org/)

**FIG. 2.** Guinea pig gastrocnemius muscle extracted 3 hours in ice-cold 8 per cent CCl₄COOH. Per cent of maximum P. Curve 1, immediate; Curve 2, 24 hours; Curve 3, 48 hours; Curve 4, 72 hours.

of the uncertainties of the method mentioned, there is considerable variation in the minimum, still the per cent found as labile phosphorus is not perceptibly different for short as compared with long extraction. Unless this results from decomposition during preparation and determination, it is indicated that the velocity of extraction is the same for both labile phosphorus and preexisting inorganic phosphorus.
Fish muscle was first examined from two salt water fish, the midshipman (*Porichthys notatus*) and the hagfish (*Bdellostoma stouti*). If any labile phosphorus is present, it is only in insignificant amounts, in comparison with that of mammals. These fish had been living apparently normally for some time in aquaria.
but it was possible that they were still abnormal. Fresh goldfish muscle was, therefore, examined, but again without positively showing any labile phosphorus. As there was still a chance that transportation might have injured the goldfish, we secured, by the kind assistance of Dr. Evermann and Mr. Seale, four other kinds of fish from the Steinhart aquarium, San Francisco. The

\[
\begin{array}{c}
\text{FIG. 3. Goldfish side muscle extracted 2 hours, 45 minutes in ice-cold 8 per cent CCl}_2\text{COOH. } \text{Curve 1, immediate; Curve 2, 24 hours later; A, per cent of maximum P.}
\end{array}
\]

fish were quickly removed from their tanks, killed, and pieces were cut from the side muscle and placed in liquid air. In this frozen condition the muscles were then transported to the laboratory for the actual determinations. None showed definitely any labile phosphorus.

These different fish included five fresh water and two salt water
species, quite distinct and representing a wide range of type in structure and habit. The figures in Table III show in each case that the minimum value, compared with the standard, is slightly low initially. Fig. 3 shows how the curve for fresh extract compares with that for old extract. There is a small difference between the two such as might arise from the presence of labile phosphorus. But the possible amount would hardly equal 10 per cent of the acid-soluble inorganic phosphorus, and the maxi-

![Diagram: Fig. 4. Muscles of twenty-five grasshoppers' legs extracted 2 hours, 45 minutes in ice-cold 8 per cent CCl₄COOH. Curve 1, immediate; Curve 2, 48 hours later; A, per cent of maximum P.](http://www.jbc.org/)
mum for the curve is reached in less than 10 minutes, while for mammalian muscle the maximum is not reached before 20 minutes. On account of the uncertainty of results in the steep part of the curve, it is safest to conclude that the labile phosphorus is not proved to exist in fish muscles, and that they are essentially different in this respect from the muscles of the other vertebrates.

The creatine content of fish muscle generally is quite of the same order as that of mammalian skeletal muscle (Hunter, 1922), so that if the phosphocreatine is characteristic of mammalian muscle the constituents, at least, are present in fish. But fish muscle is also different in the slowness of its postmortal glycogen decomposition compared with mammalian muscle (Macleod and Simpson, 1927), so that the similarity of fish to other vertebrate muscle may not be very close. It is unfortunate that no trials of elasmobranch muscle could be made, for Hunter (1922) remarks that the meager data on their creatine-creatinine excretion show that, like mammals, they excrete principally creatinine, while the teleosts are like birds in excreting principally creatine.

Invertebrate Striated Muscle.

If labile phosphorus is generally associated with rapid contraction, it might be expected to be present, particularly in the striated muscles of insects. The leg muscles from twenty-five grasshoppers were, however, like fish muscle in not showing the certain presence of labile phosphorus (see Fig. 4). The labile compound, if present at all, is insignificant in amount compared with that of mammals.

Invertebrate Muscle.

The same condition appears in the muscles of the crab's chela, which showed no positive indication of labile phosphorus.

The pallial muscle of the large clam, Schizothaerus nuttallii, was examined, and in Fig. 5 are shown results of several determinations of phosphorus fractions.

The lowest curve shows a small inorganic phosphorus content immediately in cold trichloroacetic acid extract. There is no perceptible increase with time in the colorimeter so that the absence of truly labile phosphorus is certain.

The next curves show that the Schenck method of extraction
gives results agreeing with those on the day old trichloroacetic acid extract. These colorimetric determinations were confirmed by Embden's (1921) gravimetric method. No labile phosphorus, according to its definition, would be expected in the day old extracts, but the amounts are significantly greater than those derived from cold extract. Consequently there has been some decomposition of organic phosphates in the extract. The decomposition occurred exclusively in the extract because the trichloroacetic extract which had remained only 2 hours in contact with the muscle was used in one case and gave results practically agreeing with the Schenck extract, which had been for 15 hours in contact with the muscle.

![Graph](http://www.jbc.org/)

**Fig. 5.** Pallial muscle of *Schizothaerus nuttallii*. Curves 1, clam muscle extracted 2 hours in ice-cold 8 per cent CCl₄COOH. The lower Curve 1 represents determination by the colorimetric method immediately; the upper Curve 1, determination by the same method 24 hours later. Curves 2, clam muscle extracted 15 hours in acid HgCl₂ solution (Schenck). The lower Curve 2 represents determination by the colorimetric method; the upper Curve 2, determination by the gravimetric method. Curves 3, clam muscle digested 2 hours in 2 per cent NaHCO₃ solution (Schenck). The lower Curve 3 represents determination by the gravimetric method; the upper Curve 3, by the colorimetric method.
Not all of the organic phosphorus was released in the acid extraction, because incubation of the muscle at 40° for 2 hours in 2 per cent sodium bicarbonate caused a considerable increase in inorganic phosphorus. This last method, following Embden's directions, gives by its excess over the acid-soluble inorganic phosphorus the magnitude of the lactacidogen content. Judged from the figures, there is an appreciable lactacidogen content. If not absolutely, at least by comparison with the

<table>
<thead>
<tr>
<th>Source</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Time for reaching maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>min.</td>
</tr>
<tr>
<td>Bladder.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig.</td>
<td>0.21</td>
<td>0.31</td>
<td>9</td>
</tr>
<tr>
<td>Two guinea pigs.</td>
<td>0.135</td>
<td>0.16</td>
<td>12</td>
</tr>
<tr>
<td>Turtle.</td>
<td>0.07</td>
<td>0.082</td>
<td>11</td>
</tr>
<tr>
<td>Intestine.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.23</td>
<td>0.26</td>
<td>8</td>
</tr>
<tr>
<td>Two rats.</td>
<td>0.21</td>
<td>0.22</td>
<td>6</td>
</tr>
<tr>
<td>Turtle.</td>
<td>0.22</td>
<td>0.27</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.17</td>
<td>8</td>
</tr>
<tr>
<td>Uterus, virgin.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig.</td>
<td>0.14</td>
<td>0.15</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>0.23</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Three rats.</td>
<td>0.21</td>
<td>0.22</td>
<td>11</td>
</tr>
</tbody>
</table>

amount of inorganic phosphorus originally present, this is similar to the lactacidogen content of vertebrate striated muscle. There is, however, the difficulty of determining which acid extract to take as the initial value. But in either case, the clam smooth muscle is similar to vertebrate striated muscle in having an appreciable amount of inorganic phosphorus released by incubation with bicarbonate. In lacking the true labile phosphorus it differs. Evidently the labile phosphorus is not the sole source of muscle inorganic phosphorus.
Labile Phosphorus in Muscle

Smooth Muscle of Vertebrates.

The examination of smooth muscle from the organs of several vertebrates did not positively show any labile phosphorus present. (This fact has been announced by Eggleton and Eggleton (1927, b) and was first noticed since our work was completed.) In the results quoted in Table IV are given the minimum and maximum of phosphorus values observed. It is evident that muscle from the intestine of rabbits, rats, and turtles, and from the virgin uterus of the guinea pig and rat possesses no labile phosphorus. Extracts from bladder muscle of the guinea pig and turtle show a change in the colorimeter so that they may contain a small amount of labile phosphorus. Otherwise, the inorganic phosphorus of

| TABLE V. |
| Phosphorus Content of Heart Muscle. |
| Phosphorus as mg. per gm. |
| | | | min. |
| Two guinea pigs | 0.30 | 0.33 | 8 |
| Rabbit | 0.40 | 0.44 | 9 |
| Three rats | 0.41 | 0.43 | 10 |
| Turtle | 0.14 | 0.16 | 15 |
| “ | 0.14 | 0.17 | 8 |

bladder muscle is similar in amount to that found in other smooth muscle.

Smooth muscle generally contains lactacidogen as determined by increased inorganic phosphorus after incubation (Evans, 1925), although in small amounts only. Its acid-soluble inorganic phosphorus as shown in Table IV, is only about \( \frac{1}{2} \) that found in skeletal muscle generally. More significant in the light of Fiske and Subbarow's work is the relation between the lack of labile phosphorus and the lack of creatine. According to Hunter (1922) one may be quite skeptical as to the actual presence of creatine in smooth muscle, although it has been reported often, for the reports are not based upon actual isolation of the substance. Riesser (1922) believed that he could establish a relation between the
creatine content and lactacidogen of muscles, showing that heart muscle contained only small amounts of each, while uterus muscle contained even less. The parallelism appears in his figures, but it does not suggest that the lactacidogen and labile phosphorus are identical, although they may well concern each other.

**Heart Muscle.**

In heart muscle we can hardly agree with Eggleton and Eggleton (1927, b) in finding the labile phosphorus equal to \( \frac{1}{4} \) of that usually present in skeletal muscle. Table V shows a slight gradual increase in color when compared with a fully developed standard. This increase is so small as to be of uncertain significance in comparison with the slight delay in color development even in the standard. A comparison of the curve for a still cold extract and one kept for 24 hours at room temperature (Fig. 6) shows a slight lag for the fresh preparation, but no significant difference. The necessary conclusion appears to be that the labile phosphorus, defined as it is by rapid increase in phosphorus in acid solution, is lacking in heart muscle.
DISCUSSION.

The nature of the origin of labile phosphorus in muscle has not yet been established and we have only preliminary reports to indicate the nature of its precursor. Judged by the definition as a substance yielding acid-soluble inorganic phosphorus in acid solution, it is undoubtedly present in the skeletal muscle of vertebrates except fish. It is distinctly different from Embden's lactacidogen, in that by definition the latter yields acid-soluble inorganic phosphorus in weakly alkaline solutions. Furthermore, in acid solution the labile phosphorus is practically completely decomposed within a half hour, after which time the acid-soluble inorganic phosphorus content of the extract remains constant for about a day before showing further increase.

The name "phosphagen" suggested by Eggleton and Eggleton (1927, a) and adopted by Meyerhof (1927) is, therefore, a contradiction of the facts, because the labile phosphorus indicated is only one source of inorganic phosphorus in muscle.

Whether or not the labile phosphorus exists in the muscle before the process of extraction, it has definite physiological significance because of its regular variation under different physiological conditions.

Although the labile phosphorus as found in mammalian skeletal muscle is lacking in fish and insect striated muscle, in invertebrate muscle, smooth muscle of vertebrates, and in heart muscle, its function may be assumed by similar substances which are not, however, preserved by the methods of study. Consequently, we can only deny its presence in the strict terms of the original definition. Until the definitive announcements of the nature of labile phosphorus present their evidence, the suggested importance of their function should not be clouded by rash speculation.

CONCLUSIONS.

Labile phosphorus is easily demonstrated in skeletal muscle of mammals according to the methods defined by its discoverers.

It is distinct from Embden's lactacidogen both by definition of the conditions for determination and by the facts of its appearance. Lactacidogen occurs in smooth muscles entirely lacking labile phosphorus.
Labile phosphorus could not be demonstrated in fish striated muscle, grasshopper striated muscle, smooth muscle generally of vertebrates and invertebrates, and heart muscle. If possibly present in any of these muscles, its amount is of quite a different order from that in mammalian skeletal muscle.

The occurrence and lack of labile phosphorus follow, in some respects, the occurrence of creatine, a natural consequence if Fiske and Subbarow's announcement of the labile compound as a phosphocreatine is substantiated.

**BIBLIOGRAPHY.**

THE OCCURRENCE OF LABILE PHOSPHORUS IN VARIOUS KINDS OF MUSCLES
Laurence Irving and Phillip H. Wells


Access the most updated version of this article at [http://www.jbc.org/content/77/1/97.citation](http://www.jbc.org/content/77/1/97.citation)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/77/1/97.citation.full.html#ref-list-1](http://www.jbc.org/content/77/1/97.citation.full.html#ref-list-1)