MUSCLE PHOSPHORUS IN NUTRITIONAL MUSCULAR DYSTROPHY IN RABBITS

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Rabbits and guinea pigs upon certain diets develop an extreme degeneration of the skeletal muscles (15, 22). The onset of these muscle lesions may be delayed or prevented by the inclusion of vegetable oils in the diet (21, 26, 31) or by vitamin E concentrates (23). In dystrophic muscle the creatine content is lower than normal and roughly proportional to the amount of pathological degeneration (14, 27); there is an increase in moisture and a decrease in total nitrogen (14), an increase in the rate of oxygen consumption (38, 21), a decrease in glycogen, total acid-soluble phosphorus, and fractions thereof, and an increase in cholesterol content (27), a gain in sodium chloride and a corresponding loss in potassium and magnesium, and an increase in calcium (10, 25).

In order to describe dystrophic muscle more completely, analyses of total phospholipid, total acid-soluble phosphorus, and total inorganic orthophosphate phosphorus were made upon the muscle of about 50 normal rabbits and the same number of rabbits in various stages of nutritional muscular dystrophy. Determinations of phosphocreatine and inorganic orthophosphate phosphorus in resting muscle were carried out in a few animals. The values obtained were compared with the extent of pathological degeneration and creatine content.

EXPERIMENTAL

The muscles for analysis were obtained from young and adult rabbits on Diets 11 or 13 (15) with or without supplements of

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natural foods or vegetable oils, or on a diet of grains (10). Normal controls were kept on a stock diet of grains, alfalfa hay, and greens. The rabbit was usually killed by a blow and immediately sampled, although in some instances material was taken some hours after death. For determinations on resting muscle, the rabbit was anesthetized with sodium amytal (Lilly), and the muscle frozen in situ with a mixture of carbon dioxide snow and ethyl chloride, cut in fine slices or powdered in a cold mortar, and the trichloroacetic acid extract prepared according to the method of Davenport and Davenport (8).

The muscles were analyzed separately. The gluteus was used for most of the determinations, but occasionally the gastrocnemius and triceps brachii (caput longum) were also taken. The muscle was freed as far as possible from connective tissue and each pair sampled for chemical analysis and histological study.

Methods

For moisture, the samples were dried for 20 hours at 100°. Creatine was determined by the method of Rose, Helmer, and Chanutin (35). The phosphorus was measured colorimetrically by the Fiske and Subbarow (13) method. For total phosphorus, the dried muscle was incinerated with magnesium nitrate; for phospholipid phosphorus, an alcohol-ether extract of the fresh tissue cut in small pieces was made; for total acid-soluble and total inorganic orthophosphate phosphorus, the finely cut or frozen and powdered muscle was extracted with 5 per cent trichloroacetic acid, the acid being allowed to remain in contact with the tissue for at least 30 minutes. Phosphocreatine was determined by the indirect method of Fiske and Subbarow (13) on extracts prepared by the method of Davenport and Davenport (8).

There was an average difference of 4 per cent between twenty-four pairs of duplicate determinations of total phosphorus in muscle.

Results

The results of the analyses of the muscle phosphorus fractions in normal and degenerated muscle are shown in Figs. 1 to 3 and
Tables I and II. The muscle was arbitrarily graded from 1 + to 4 +, according to the severity of the pathological lesions.¹

**TABLE I**

<table>
<thead>
<tr>
<th>Extent of Muscle Lesions</th>
<th>Normal</th>
<th>++</th>
<th>+++</th>
<th>++++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Phosphorus</td>
<td>![Graph of total phosphorus]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phospholipid Phosphorus</td>
<td>![Graph of phospholipid phosphorus]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 1.** Concentration of total and phospholipid phosphorus in white muscle of normal rabbits and those with nutritional muscular dystrophy. Circles represent total and phospholipid phosphorus; solid dots, determinations of calcified muscles. M shows the position of the mean value (excluding determinations of calcified muscles).

**Total Phosphorus**—Total phosphorus is reported to remain constant during muscle contraction (7) and atrophy (17). A ¹ We are indebted to Dr. A. M. Pappenheimer of the Department of Pathology, Columbia University, for his interpretation of the pathology of the muscles.
marked reduction has been observed in the muscle of rabbits, in which acidosis was induced by feeding hydrochloric acid (16).

The results of analyses of total phosphorus in normal and degenerated muscle are plotted in Fig. 1. As the phosphorus content of normal muscle appeared to have no relation to age, within the limits of 2 months to 2 years, the records of all rabbits have been tabulated together regardless of age.

The following figures, calculated from the analytical values for total phosphorus, are expressed as milliequivalents per kilo of fresh tissue, on the assumption that the phosphorus is present as a monobasic acid: 59 determinations, mean 75.8 ± 0.6, standard deviation ± 7.0 ± 0.4, coefficient of variation 9.2 ± 0.6. The coefficient of variation is about the same as that for creatine and total nitrogen (14). The mean is slightly lower than the values obtained with the Neumann gravimetric method for rabbit muscle by Katz (19), Goto (16), and Sorg (37), but higher than those of Morgulis and Osheroff (25).

In Fig. 1 it is seen that in dystrophic muscle the total phosphorus fluctuates widely. It may increase to a value higher than that indicated in Fig. 1; five severely degenerated muscles with a total phosphorus concentration of from 137 to 322 milliequivalents were not included. This increase has been shown to be related to the amount of calcification in the fibers (10). In dystrophic muscle without calcification the total phosphorus varies widely and tends to decrease. In muscle with slight or moderate lesions the total phosphorus may remain normal or decrease to some extent, but in severely degenerated muscle the decrease is significant. These results are not in agreement with those of Morgulis and Osheroff (25), who found no alteration in the total phosphorus of severely dystrophic muscles, but their series may not have included muscle with as great pathological degeneration as those classified as 4 + in Fig. 1.

Phospholipid Phosphorus—Phospholipid phosphorus is stated not to change during contraction (7) or atrophy (17). A slight decrease has been observed during fasting (6).

The results of analyses of phospholipid phosphorus in normal and dystrophic muscle are plotted in Fig. 1. The concentration of phospholipid phosphorus in normal muscle is seen to vary greatly. The calculated values for normal muscle are, for twenty-
eight determinations, mean $9.1 \pm 0.3$ milliequivalents per kilo of fresh muscle, standard deviation $\pm 2.1 \pm 0.2$, coefficient of variation 23.6. The method used for extracting phospholipids tends to give high values (36). The values obtained are in general agreement with those given by Katz (19) and Bloor and Snider (1), and somewhat lower than those of Sorg (37).

Although there were relatively few determinations of phospholipid phosphorus, it appears from Fig. 1 that this fraction differs, in degenerated muscle, but little from the normal. This finding, which is contrary to that of Morgulis, Wilder, Spencer, and Eppstein (28) who stated that the phospholipid phosphorus is increased in dystrophic muscle, is of particular interest, because the fat content of such muscle may be great. For example, the muscle of Rabbit 51 presented 4 + lesions; the gluteus contained 73 per cent fat by analysis and the triceps, 25 per cent; the phospholipid phosphorus content was respectively 7.1 and 9.7 milliequivalents per kilo.

The presence of calcified fibers in dystrophic muscle does not affect the phospholipid phosphorus.

*Total Acid-Soluble Phosphorus* Total acid-soluble phosphorus remains constant during contraction (7), autolysis (4), fasting (39), fever induced by the injection of 1,2,4-dinitrophenol (5), and in some types of human muscle disorder, such as myasthenia gravis, Graves’ disease, and periodic muscular weakness (29). Low values have been found in thyroxinized and adrenalectomized cats (4), in rats following denervation (18), in human muscle diseases, such as familial periodic paralysis (2), muscle dystrophy (33), pseudohypertrophic muscular dystrophy and dystrophia myotonica (29), and in nutritional muscular dystrophy of the rabbit (27).

Fig. 2 illustrates the data on total acid-soluble phosphorus content of normal and dystrophic muscle. The concentration of total acid-soluble phosphorus in normal muscle is seen to be more variable than that of total phosphorus, a fact which may be due to the method of extraction. The values calculated for normal tissue are, for forty-four determinations, mean $50.2 \pm 0.9$ milliequivalents per kilo of fresh tissue, standard deviation $\pm 9.8 \pm 0.6$, coefficient of variation 19.4. These values are in agreement with those given in the literature (24, 4, 29).
Phosphorus in Muscular Dystrophy

In degenerated muscle the total acid-soluble phosphorus varies more than in normal ones. Higher concentrations are found in calcified tissue, in which it may increase to 3 times the normal amount. The analytical values for four severely degenerated muscles, in which the concentration of total acid-soluble phosphorus varied from 117 to 153 milliequivalents, were not plotted.

![Figure 2](http://www.jbc.org/)  
**Fig. 2.** Concentration of total acid-soluble (TAS) and total inorganic orthophosphate (TI) phosphorus in white muscle of normal rabbits and those with nutritional muscular dystrophy. Circles represent total acid-soluble and total inorganic orthophosphate phosphorus; solid dots, determinations of calcified muscles. *M* shows the position of the mean value (excluding determinations of calcified muscles).

It is seen in Fig. 2 that lower concentrations are found in uncalcified dystrophic muscle and that the decrease is significant in severely degenerated muscle. This observation confirms that of Morgulis and Spencer (27) and is in agreement with the lowered total acid-soluble phosphorus found by Brand and Harris (2), Nevin (29), and Reinhold et al. (33, 34) in human muscle diseases.

**Total Inorganic Orthophosphate Phosphorus**—Inorganic ortho-
phosphate phosphorus increases rapidly during muscle contraction (20) and autolysis, and is derived chiefly from the hydrolysis of phosphocreatine (12, 9).

The amount of total inorganic orthophosphate phosphorus present in normal and dystrophic muscle is also shown in Fig. 2. The following values were calculated: forty-four determinations, mean 42.4 ± 1.0 milliequivalents per kilo of fresh tissue, standard deviation ±9.4 ± 0.7, coefficient of variation 22.2.

Fig. 3 presents a scatter diagram of the concentration of total acid-soluble phosphorus and total inorganic orthophosphate phosphorus, showing their relationship in all types of muscle. Total inorganic orthophosphate phosphorus is roughly 85 per cent of the total acid-soluble phosphorus in postmortem muscle, irrespective of its pathological state.

Phosphocreatine and Inorganic Orthophosphate Phosphorus in Resting Muscle—Phosphocreatine phosphorus is low in the resting muscles of guinea pigs with scurvy and pigeons with polyneuritis (30), in fasting (39), in rats following denervation (18), in thy-
roxinized and adrenalectomized cats (4), in fever induced by the injection of 1,2,4-dinitrophenol (5), in molecular degeneration of muscle in rabbits, produced by freezing or contusion (11), in human muscle dystrophies (29, 34), and in nutritional muscular dystrophy of the rabbit (27).

The concentrations of phosphocreatine and inorganic orthophosphate in resting muscle were determined in seven rabbits with muscular dystrophy, four controls, and one rabbit with extreme muscular atrophy.

The results are given in Table I, together with analyses of other constituents. Whenever possible the analyses are given in duplicate in order to show the variation between samples, the extent of which is not surprising when the heterogeneous histological picture of the muscle is considered.

The values for phosphocreatine in normal resting muscle are similar to those given in the literature (24, 4, 29). In dystrophic muscle with advanced lesions the phosphocreatine phosphorus of resting muscle decreases to very low values, comparable to those found by Nevin (29) in human muscular dystrophy and lower than those reported by Morgulis and Spencer (27) in the rabbit disease. However, although the amount of phosphocreatine is less in degenerated muscle, there is apparently little decrease in the relative concentration. Thus, it will be seen in Table I that the phosphocreatine content of normal muscle is 35 to 40 per cent of the total acid-soluble fraction. In dystrophic muscle with 3+ and 4+ uncalcified lesions, the proportion of phosphocreatine decreases slightly, but not more than might be accounted for by analytical error. In calcified muscle, Rabbits 351 and 368, the phosphocreatine appears to be slightly higher than in the uncalcified dystrophic muscle.

In normal resting muscle the inorganic orthophosphate phosphorus content is one-third to one-half as great as that of phosphocreatine phosphorus, but in degenerated muscle the proportion is greater. In all cases, regardless of the state of the muscle, the sum of these two fractions is approximately equal to 60 per cent of the total acid-soluble phosphorus.

**Phosphorus Fractions in Muscle Atrophy**—The analytical results of Rabbit 366, which presented extreme muscular atrophy, are included in Table I. It will be seen that the moisture is
increased, creatine is less than normal, and although the fractions of phosphorus are low, they are within the limits of biological variation of normal muscle. The results are in agreement with those of Grund (17).

### Table I

**Phosphorus Fractions in Normal and Pathological Rabbit Gluteus Muscle**

<table>
<thead>
<tr>
<th>Normal diet</th>
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<th></th>
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<tbody>
<tr>
<td>Rabbit No.</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Days on diet</td>
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<td>Severity of lesions</td>
<td>Extent of calcification</td>
<td>Creatine, m. eq. per kilo fresh tissue</td>
<td>Phosphorus, m. eq. per kilo fresh tissue</td>
<td>Phospholipid</td>
<td>Total acid-soluble</td>
<td>Total inorganic orthophosphate</td>
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<tr>
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<tr>
<td>61</td>
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<td>-</td>
<td>76.1</td>
<td>37.4</td>
<td>75.8</td>
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<tr>
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<td>83.4</td>
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<tr>
<td>63</td>
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<td>-</td>
<td>76.9</td>
<td>32.0</td>
<td>78.7</td>
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<tr>
<td>64</td>
<td>-</td>
<td>-</td>
<td>75.4</td>
<td>35.9</td>
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<tr>
<td>366*</td>
<td>87</td>
<td>-</td>
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<td>80.6</td>
<td>27.5</td>
<td>58.1</td>
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<td>319</td>
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<td>44.3</td>
<td>74.6</td>
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<tr>
<td>351</td>
<td>107</td>
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<td>+</td>
<td>75.9</td>
<td>45.8</td>
<td>91.5</td>
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<tr>
<td>363</td>
<td>134</td>
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<td>-</td>
<td>22.9</td>
<td>61.9</td>
<td>12.6</td>
</tr>
<tr>
<td>368</td>
<td>138</td>
<td>+++</td>
<td>-</td>
<td>76.7</td>
<td>32.0</td>
<td>98.8</td>
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<tr>
<td>Diet 11</td>
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<td>++</td>
<td>-</td>
<td>80.0</td>
<td>13.0</td>
<td>55.8</td>
</tr>
<tr>
<td>29</td>
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<td>16.0</td>
<td>50.0</td>
<td>15.5</td>
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<tr>
<td>78‡</td>
<td>161</td>
<td>+++</td>
<td>-</td>
<td>9.1</td>
<td>48.4</td>
<td>20.3</td>
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</tbody>
</table>

The muscles had normal fat content, except as indicated. For additional analytical data on some of these muscles see Goettsch and Brown (14) and Victor (38).

* Muscle presented extreme atrophy.
† Muscle fat, 5.3 per cent.
‡ Muscle fat, 20.6 per cent.

**Acid-Insoluble Non-Lipid Phosphorus**—On comparison of Figs. 1 and 2 it will be seen that the concentration of total acid-soluble phosphorus in normal rabbit muscle is significantly lower than that of total phosphorus. There is a difference of 25.6 milli-
equivalents per kilo of fresh tissue between the two means, with a standard deviation of the difference of ±12.0. This acid-insoluble fraction was determined in several muscles by ashing the residue of the trichloroacetic acid extraction. The analyses of a typical muscle (normal gluteus of Rabbit 267) were as follows: total phosphorus 76.8 milliequivalents per kilo of fresh muscle, total acid-soluble phosphorus 47.8, 51.9, total acid-insoluble phosphorus 27.6, 25.2, sum of acid-soluble and acid-insoluble phosphorus 75.4, 77.1.

When the residue of the alcohol-ether extraction of the same muscle was ashed and analyzed, the phosphorus content was found to be higher than that of total acid-soluble phosphorus. The analyses of the normal gluteus of Rabbit 267 were as follows: phospholipid phosphorus 6.5, 7.7 milliequivalents per kilo of fresh muscle, non-phospholipid phosphorus 66.4, 64.1, sum of phospholipid and non-phospholipid phosphorus 72.9, 71.8. The difference between the non-phospholipid phosphorus and the total acid-soluble phosphorus, about 15 milliequivalents, represents the acid-insoluble non-lipid phosphorus, which is presumably nuclear and protein phosphorus. Similar differences between total and total acid-soluble phosphorus in normal dog muscle were reported by Pollack, Flock, and Bollman (32).

From Figs. 1 and 2 it appears that the total acid-soluble phosphorus decreases at a greater rate than the total phosphorus. Since the phospholipid phosphorus remains constant, this observation leads to the assumption of an increase in acid-insoluble non-lipid phosphorus in dystrophic muscle, which is compatible with the histological finding of increased nuclear elements. The increase may also be accounted for by an error in analysis, due to the extraction of fibrous lipomatous tissue with water-soluble reagents. The latter explanation was found to be the more likely.

Powdered frozen muscle was extracted first with alcohol-ether, and then with several portions of trichloroacetic acid. The residue was ashed and analyzed for phosphorus. The analyses of typical muscles are given in Table II. The residues of the dystrophic muscles contained approximately the same amount of phosphorus as the normal ones.

**Relationship of Phosphorus Fractions and Creatine Nitrogen**—Scatter diagrams indicated little correlation between the phos-
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phorus fractions and creatine nitrogen. The coefficients of correlation with their respective probable errors, calculated from forty-four sets of determinations of normal and dystrophic muscle, are as follows: total phosphorus and creatine nitrogen +0.038, total acid-soluble phosphorus and creatine nitrogen +0.552 ± 0.07, total inorganic orthophosphate phosphorus and creatine nitrogen +0.506 ± 0.07. These results are not in accordance with the statement of Brown and Imrie (3) that there is a tendency for the concentration of total acid-soluble phosphorus to be high in cat muscle when the creatine is high. Their value for total acid-soluble phosphorus represents total inorganic orthophosphate phosphorus, since they measured the amount of phosphate in the trichloroacetic acid extract after 50 minutes hydrolysis. When the coefficient of correlation was calculated from their data, it was found to be +0.440 ± 0.10, a value in general agreement with the one given above.

From Table I it is apparent that no clear correlation exists between creatine and the other fractions of phosphorus.

In nutritional muscular dystrophy of the rabbit the loss in muscle creatine is greater in equivalent amount than the loss in phosphorus. Furthermore, the decrease in creatine content is apparent in muscles with slight or moderate lesions, while the changes in phosphorus fractions are not regularly observed until the muscles are severely degenerated. The phosphorus concentration is more strikingly increased than that of creatine in muscles showing histological evidence of calcification.

### Table II

**Acid-Insoluble Non-Lipid Phosphorus in Normal and Pathological Rabbit Gluteus Muscle**

<table>
<thead>
<tr>
<th>Rabbit No</th>
<th>Pathological examination of muscle</th>
<th>Phosphorus, m. eq. per kilo fresh tissue</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>345</td>
<td>Normal</td>
<td>78.6</td>
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<tr>
<td>368</td>
<td>++</td>
<td>98.8</td>
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<tr>
<td>382</td>
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<td>64.4</td>
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<tr>
<td>384</td>
<td>+++</td>
<td>82.0</td>
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</table>
Phosphorus in Muscular Dystrophy

SUMMARY

In nutritional muscular dystrophy of the rabbit, there are no striking changes in the muscle phosphorus fractions until the muscles are severely degenerated. Dystrophic muscles which present calcification histologically are associated with an increase in total, total acid-soluble, and total inorganic orthophosphate phosphorus; those without calcified fibers, with a decrease in these constituents.

The phosphocreatine content of resting degenerated muscles is distinctly lower than normal, but its relationship to total acid-soluble phosphorus remains the same.

There is no change in phospholipid phosphorus of muscle.

BIBLIOGRAPHY

MUSCLE PHOSPHORUS IN NUTRITIONAL MUSCULAR DYSTROPHY IN RABBITS
Marianne Goettsch, Ida Lonstein and John J. Hutchinson