A DIETARY FACTOR ESSENTIAL FOR GUINEA PIGS*

VI. CHANGES IN THE DISTRIBUTION OF ACID-SOLUBLE PHOSPHORUS IN THE MUSCLE DURING A DEFICIENCY OF THE ANTISTIFFNESS FACTOR

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(Received for publication, January 3, 1945)

In diseases in which muscular activity is impaired because of a dystrophic condition, deviations from the normal concentrations of creatine phosphate and adenosine triphosphate can be expected. Reinhold and Kingsley (1) reported a decrease in the concentration of phosphocreatine and adenosine triphosphate in dystrophic muscles of guinea pigs deficient in vitamin E. The low level of creatine in the muscle of animals deficient in vitamin E was recognized by Goettsch and Brown (2) and has since been repeatedly confirmed (3-5). A correlation exists between muscular dystrophy and urinary excretion of creatine (6). These two phenomena are suggestive of a breakdown of creatine phosphate during the deficiency of vitamin E. Houchin (7) and Weissberger and Harris (8) found an increase in phosphate turnover during vitamin E deficiency. Changes in the concentrations of phosphocreatine and adenosine triphosphate in the brain have been reported in experimental poliomyelitis (9).

Guinea pigs raised on a diet mainly composed of skim milk to which the necessary minerals and known essential vitamins had been added developed a characteristic syndrome (10). The first outward sign of the deficiency was the development of a stiffness at the wrist joint. In the advanced stages of the deficiency the muscles were found to be extremely atrophied. Calcium deposits were found in many body tissues. Vitamin E, supplied either in the form of wheat germ oil or as the synthetic α-tocopherol, did not cure or prevent the deficiency disease. The syndrome could, however, be prevented and cured by a factor present in raw cream (11) and several plant sources.†

It was found previously (12) that no creatinuria developed when guinea pigs were raised on a diet deficient in the antistiffness factor, but sup-

* Supported by grants from the Williams-Waterman Fund of the Research Corporation and from the General Research Council of the Oregon State System of Higher Education.
† From a thesis submitted by one of the authors (H. L.) as partial fulfilment of the requirements for the degree of Master of Arts, Oregon State College.
† van Wagtendonk, W. J., and Wulzen, R., to be published.
implemented with α-tocopherol. However, a more severe creatine excretion resulted when the diet was deficient in both α-tocopherol and the antistiffness factor than when only α-tocopherol was omitted. This indicates that the antistiffness factor has some influence in the creatine turnover. The concentration of the easily hydrolyzable phosphorus in the liver and kidneys of the experimental animals was lowered considerably during the deficiency (13). A high concentration of inorganic phosphate was found in these tissues. The level of inorganic phosphate in the blood of these animals was also significantly above normal (14). It might be concluded from these observations that a deviation from the normal levels of creatine phosphate and adenosine triphosphate in the muscle would result from a deficiency of the antistiffness factor. A significant decrease of the concentration of these compounds was found.

EXPERIMENTAL

Method

Guinea pigs were raised on the diet described by van Wagendonk et al. (14). This diet consisted of skim milk powder and water to which the necessary minerals and known vitamins were added. The animals were bedded on autoclaved straw. The animals on the stock diet received rolled barley, greens, and straw ad libitum. At various intervals the guinea pigs were sacrificed. The animals were anesthesized with nembutal, and the musculus rectus femoris rapidly removed. The tissue was frozen at once in a mixture of dry ice and ethyl ether in order to prevent changes in the phosphate distribution (15, 16). After being weighed in the frozen state, the tissue was dispersed in 10 times its weight of ice-cold 10 per cent trichloroacetic acid by means of a Waring blendor. The mixture was filtered immediately after maceration. The filtrate was made alkaline to phenolphthalein and the resulting solution analyzed for inorganic phosphate, creatine phosphate, and adenosine triphosphate according to the method of Stone (17). In order to determine the changes in the concentrations of inorganic phosphate, creatine phosphate, and adenosine triphosphate due to aging, three different age groups (13, 20, and 72 weeks) of animals on the stock diet were also analyzed. Wherever possible groups of fifteen animals were used.

Results

The results are represented in Table I. The creatine phosphate and adenosine triphosphate concentrations increased slightly with progressive age, thus substantiating the findings of Fainshmidt et al. (18). The concentration of creatine phosphate in the musculus rectus femoris coincides with that found by Palladin and Epelbaum for the musculus biceps femoris (19).
Marked changes in the distribution of the acid-soluble phosphate in the muscle were found during the time limit of the deficiency period. The concentration of the inorganic phosphate increased as compared with that found in animals receiving a stock diet. Some fluctuation in the creatine phosphate and adenosine triphosphate content occurred in the early period of the deficiency. In the more advanced stages a significant lower level became established.

**Table I**

*Distribution of Acid-Soluble P in Muscle of Guinea Pigs Receiving Stock Diet and Skim Milk Diet*

<table>
<thead>
<tr>
<th>Age (wks.)</th>
<th>Stock diet</th>
<th>Deficient diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, mg. per 100 gm.</td>
<td>Time on diet</td>
</tr>
<tr>
<td>13</td>
<td>54.1, 7.0, 12.4</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>48.7, 7.8, 18.7</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>69.1, 7.1, 12.4</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>51.6, 10.0, 14.8</td>
<td>7</td>
</tr>
<tr>
<td>20</td>
<td>71.0, 7.0, 14.5</td>
<td>10</td>
</tr>
<tr>
<td>23</td>
<td>54.0, 7.9, 7.6</td>
<td>19</td>
</tr>
<tr>
<td>29</td>
<td>76.1, 4.5, 4.4</td>
<td>28</td>
</tr>
<tr>
<td>41</td>
<td>65.2, 4.2, 3.8</td>
<td>59</td>
</tr>
<tr>
<td>72</td>
<td>80.1, 3.0, 2.8</td>
<td></td>
</tr>
</tbody>
</table>

* ATP and ADP denote respectively adenosine triphosphate and adenosine diphosphate.

**DISCUSSION**

From the experiments reported here it becomes evident that the impaired function of the muscle during the deficiency of the antistiffness factors is correlated with a lowered content of compounds containing energy-rich phosphate bonds (20). This decrease of creatine phosphate and adenosine triphosphate in the muscle tissue parallels the drop in the easily hydrolyzable phosphate concentration in other tissues, e.g. liver and kidneys (13).

In a previous paper of this series (14) a high concentration of calcium was found in the muscle. The simultaneous presence of a high concentration of inorganic phosphate can account for the deposits of calcium phosphate found in so many tissues.

Although both a deficiency of vitamin E and a deficiency of the antistiffness factor result in a lowered concentration of creatine phosphate...
and adenosine triphosphate in the muscle, a distinct difference is found in the fate of creatine. A deficiency of vitamin E is characterized by a severe urinary excretion of creatine. No creatine excretion was found when the diet deficient in the antistiffness factor was supplemented with α-tocopherol. Animals raised on a diet deficient both in vitamin E and the antistiffness factor developed a creatinuria, more severe than when only α-tocopherol was omitted (12). Patrick and Morgan (21) have reported that an unidentified fat-soluble factor, present in yeast and soy bean phosphatides, was necessary for the proper utilization of α-tocopherol by the chick. No evidence for the need of this factor was found in our experiments.

SUMMARY

A deranged distribution of the acid-soluble phosphorus in the muscle is found during a deficiency of the antistiffness factor. The concentration of inorganic phosphate increases during the deficiency, while the concentrations of creatine phosphate and adenosine triphosphate decrease after some fluctuations at the onset of the deficiency. The antistiffness factor and vitamin E have different functions in the animal organism.

BIBLIOGRAPHY

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