THE RÔLE OF INSULIN IN THE METABOLISM OF AMINO ACIDS

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(Received for publication, January 27, 1949)

It is well known that insulin exerts a profound influence on the metabolism of amino acids and protein. For example, the administration of insulin to the fasting, untreated diabetic has been shown to reduce the excessive excretion of nitrogen (1). Leutscher has recently observed that the diabetic has an unusually high concentration of blood amino acid and that insulin brings this to a normal value (2). Conversely, it has been demonstrated that the injection of insulin into the normal fasted man results in a depression of the concentration of blood amino acid to values below normal (3-5). This observation has been confirmed in the rat (3), the rabbit (3), and the dog (6). Harris and Harris (5) have studied the changes in the plasma levels of a limited number of individual amino acids in mental patients during insulin hypoglycemia. While the blood levels of all were depressed, leucine and lysine fell to the greatest extent. These authors offer no conclusive explanation for their findings.

That the liver is not specifically necessary for the action of insulin on amino acids has been shown by the fact that insulin delays the accumulation of blood non-protein nitrogen in the nephrectomized, eviscerated dog (7) and of amino acids in the blood of the eviscerated rat (8). Thus the effect of insulin on amino acids must be exerted on tissues in general, rather than in the liver or any other organ specifically.

In an attempt to understand more fully the nature of the rôle of insulin in amino acid metabolism, it was decided to study the effect of insulin on a representative number of individual amino acids and to examine the results in the light of the best evidence to date which indicates that insulin promotes the synthesis of protein (9, 7). Because of their particular importance in normal growth and nutrition, and because the methods for their analysis are most reliable, it was decided to study the ten “essential” amino acids.1 The results of this study on the normal fasted dog are reported below, and support the belief that insulin is concerned with the synthesis of protein from free amino acids.

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1 Leucine, lysine, arginine, isoleucine, valine, threonine, phenylalanine, methionine, histidine, tryptophan.
Experiments were performed on two normal adult female mongrel dogs, maintained on a diet of laboratory chow, supplemented by horse meat twice a week. The animals were fasted for 18 to 24 hours before an experiment. After a control sample of blood was taken, insulin was injected intravenously in a dose of 2 units per kilo of body weight. Blood samples, in amounts of 35 ml., were drawn into an oxalated syringe at 30 and 60 minutes thereafter. Neutralized tungstic acid filtrates of these bloods were prepared immediately and used for analysis of amino acids. The amino acid analyses were done by microbiological assay techniques. Each method was shown to give quantitative recovery of amino acid added to whole blood, and was therefore considered reliable for use. Only the natural forms of the amino acids are determined by these methods; therefore all data reported herein are for those forms only. Leucine, arginine, valine, threonine, methionine, histidine, and tryptophan were determined according to the method of Stokes et al. (10) with Streptococcus faecalis R as the test organism. Lysine and isoleucine were determined by a modification of the method of Dunn et al. (11) with Leuconostoc mesenteroides P-60 as the test organism. Phenylalanine was determined by a turbidimetric modification of the method of Henderson and Snell (12) with Lactobacillus arabinosus. Amino acid analyses of dog muscle were performed by these same methods on acid and alkaline hydrolysates of muscle. A biopsy of the thigh muscle was obtained under aseptic conditions. It was immediately minced and homogenized in a Waring blender with distilled water. The homogenate was then put in a vacuum flask and lyophilized. The hydrolysates were made from the lyophilized muscle essentially according to the method of Stokes et al. (10).

Blood glucose was followed in all experiments as a check on the activity of insulin, the glucose being determined according to Nelson's photometric adaptation of Somogyi's method (13).

**Results**

It was found that after the intravenous administration of insulin the blood concentration of all ten amino acids fell, but to different degrees. It was further found that these differences remained characteristic from experiment to experiment. This observation lead to speculation concerning the reasons for these differences. If, as has been suggested (7), insulin plays a rôle in protein synthesis, then it would be logical to assume that under the influence of insulin each amino acid would be removed from the blood in quantities commensurate with the proportion of each in the pro-

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2 Iletin, Lilly. The author is grateful to Eli Lilly and Company for the generous supply of insulin for use in this study.
tein in process of synthesis. It was therefore decided to test this hypothesis by comparing the proportions of each amino acid leaving the blood after insulin injection with the proportions of the same amino acids in a representative body protein. Skeletal muscle protein was chosen for this comparison, since it constitutes about 50 per cent of the body weight.

In preliminary experiments in which only three or four amino acids were measured at a time, it became apparent that such a correlation did exist and that the hypothesis was correct. In order to be sure, however, it was felt that it would be necessary to measure blood changes in all ten amino acids simultaneously. The techniques for microbiological assay were modified in order that all amino acids could be analyzed in the filtrate from 35 ml. of blood; the experiments were done on two dogs. In Table I are presented the data from such an experiment. Blood and muscle amino acid data were both obtained from the same dog. Since the blood leucine concentration was lowered to the greatest extent by insulin, and since leucine was in highest concentration in the muscle protein, leucine in both blood and muscle data was given a value of 10 and the other amino acids compared to it on the basis of relative molecular proportions. It is evident from the figures of Table I that in general there is a good correlation between the proportion of each amino acid in skeletal muscle protein and the proportion of each amino acid removed from the blood after insulin. This correlation is illustrated in graphic form in Fig. 1, in which the proportions of the amino acids removed from blood are plotted above the line, and the

### Table I

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>In blood</th>
<th>In muscle protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>μM per ml</td>
<td>μM per ml</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.1740</td>
<td>0.0855</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.4580</td>
<td>0.3840</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.1167</td>
<td>0.0620</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.2370</td>
<td>0.1860</td>
</tr>
<tr>
<td>Valine</td>
<td>0.1400</td>
<td>0.0962</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.1665</td>
<td>0.1227</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.0532</td>
<td>0.0257</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.0537</td>
<td>0.0295</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.0806</td>
<td>0.0626</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.0304</td>
<td>0.0227</td>
</tr>
</tbody>
</table>
muscle amino acids below it. These data, then, substantiate the hypothesis and show that under the influence of insulin the ten essential amino acids do indeed leave the blood in quantities proportional to their concentrations in a representative body protein.

**Figure 1.** A comparison between the molecular proportions of the ten essential amino acids removed from the blood of a dog after insulin and the proportions of the ten essential amino acids in the same dog's skeletal muscle protein.

**DISCUSSION**

These data suggest three possible explanations for the action of insulin on amino acids. First, insulin might promote increased deamination. This is unlikely in view of the fact that insulin affects amino acids even in the absence of the main deaminative organ, the liver. Furthermore, the
data of Bach and Holmes (14) and Stadie et al. (15) indicate that insulin actually inhibits, rather than accelerates, the oxidative deamination of amino acids by liver slices.

Second, insulin might possibly depress the hydrolysis of protein and thus bring about a decrease in the quantity of amino acid entering the blood. This possibility cannot be absolutely ruled out on the basis of the experiments reported here. However, if insulin simultaneously inhibits both the hydrolysis of tissue protein and deamination of amino acids, it would seem highly improbable that the proportionality pattern observed here would have occurred.

Third, as has been suggested, insulin might play a rôle in the process of protein synthesis. This interpretation would explain the data adequately and furthermore would be in harmony with other evidence implicating insulin in protein synthesis. Grey and Thalhimer (16) in 1924 were able to demonstrate greater growth of chick fibroblasts in tissue culture with insulin and glucose than with glucose alone. Mirsky (17) has shown that anterior pituitary extract requires insulin for its protein synthetic function. Indeed in the diabetic animal this extract actually promotes the breakdown of tissue protein rather than its synthesis. This interrelation between insulin and the pituitary was also shown by Frame and Russell (8) who observed that insulin and anterior pituitary extract together are more effective than insulin alone in decreasing the rate of accumulation of amino acids in the blood of the eviscerated rat.

Wilhelmi, Fishman, and Russell (18) have recently stated that "the activity of the anterior pituitary gland in maintaining normal levels of muscle glycogen in the 24 hour fasted hypophysectomized rat ... appears to be a property of the growth hormone.” Thus insulin is not only involved in the protein anabolic function of the pituitary, but the pituitary is also apparently involved in glycostasis, a function known to be affected by insulin.

These studies, linking insulin and the pituitary in both glycostasis and protein synthesis, emphasize the interrelated nature of their functions and add weight to the feeling that the data presented in this paper are most logically interpreted in the light of a protein anabolic function of insulin. Experiments are now planned in which the effects of insulin and growth hormone on the metabolism of the individual amino acids will be studied in vivo, and with muscle strips in vitro. It is hoped that these studies will add further meaning to the experiments reported in this paper.

SUMMARY

The effect of insulin on the blood concentration of the ten essential amino acids has been studied in the normal fasted dog. It has been shown that there is a correlation between the proportions of each amino acid re-
moved from blood after insulin and the proportions of each amino acid in a representative body protein, skeletal muscle. It has been suggested on the basis of this observation that insulin promotes the synthesis of protein from circulating amino acids. This interpretation of the data is in keeping with recent evidence that points to an intimate relationship between insulin and the anterior pituitary gland in both protein and carbohydrate metabolism.

The author wishes to express his thanks to Dr. Robert Pitts and Dr. Jay Tepperman for their helpful suggestions and to Mr. Raymond Cottet for his assistance in the performance of the experiments.

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