LIVER ARGINASE ACTIVITY AS RELATED TO BLOOD UREA IN ACUTE UREMIA OF NEW-BORN RATS*

By I. E. LINER AND M. O. SCHULTZE

(From the Division of Agricultural Biochemistry, University of Minnesota, St. Paul)

(Received for publication, July 28, 1950)

There is evidence to indicate that the demands imposed upon the mammalian organism to metabolize protein may be reflected by changes in the arginase activity of the liver. Particularly those conditions leading to a stimulation of protein catabolism, with its concomitant increase in the formation of urea, frequently elicit a corresponding increase in the arginase activity of the liver. Thus, elevations in liver arginase activity have been observed under the dietary influence of fasting (1, 2), increased levels of protein intake (3, 4), and thiamine deficiency (5). Hormonal secretions, known to control the rate of gluconeogenesis, effect a rise in the arginase content of the liver when injected into hypophysectomized or adrenalectomized animals (6-9).

Previous reports from this laboratory have described a high incidence of early mortality of rats born to mothers maintained on rations composed mainly of crude or purified plant materials (10-12). A characteristic feature of the syndrome which preceded death, referred to as "acute uremia of the new-born," was the very high level of urea in the blood. The subcutaneous administration of vitamin B₁₂ shortly after birth was effective in preventing the onset of the uremic syndrome. If this uremic condition actually reflects an increased rate of nitrogen catabolism which can be prevented by vitamin B₁₂, it appeared possible that, under such circumstances, the arginase activity of the liver might be increased in order to accommodate the need for this enzyme.

In the present paper data are reported on the relationship between arginase activity of the liver and the concentration of urea in the blood of normal and uremic young rats.

EXPERIMENTAL

Animals and Rations—The care and feeding of the animals, as well as the rations used in these experiments, have been described elsewhere in detail (12). The basal ration designated Ration CS-1 was composed of the following ingredients, gm. per kilo of diet: ground yellow corn, 753.4;

* Paper No. 2576, Scientific Journal Series, Minnesota Agricultural Experiment Station. This is the sixth of a series of papers dealing with the nutritional value of plant materials.
expeller soy bean meal, 175.0; alfalfa meal, 50.0; salt mixture (12), 21.6
In Ration CS-3, the protein content was increased by raising the level of the soy bean meal to 375.0 and lowering the corn to 553.4 gm. per kilo of diet. Vitamins A and D were supplied as 2 drops of halibut liver oil (Abbott) per rat per week. Previous experience has shown that in about 40 per cent of the litters born to females maintained on these rations from one to all of the young incur symptoms of acute, fatal uremia (12). The addition to these basal rations of 3 per cent condensed fish solubles or the early postnatal administration of 0.05 γ of crystalline vitamin B₁₂ could prevent this uremic syndrome.

Arginase Activity—As soon as the external symptoms of the acute uremia became apparent, usually within 24 to 48 hours following birth, the animals were decapitated and the blood was collected for the determination of urea in the manner described below. The livers were quickly excised and weighed to the nearest mg. The livers weighed from 100 to 250 mg. The whole liver was homogenized in 5 ml. of ice-cold redistilled water. In order to activate the enzyme an equal volume of 0.1 M MnCl₂·4H₂O was added and the mixture incubated for 1 hour in a water bath, equipped with a shaking device at 38°. A 1 ml. aliquot of the activated homogenate was diluted to 50 ml. with 0.85 per cent NaCl solution. A 1 ml. aliquot of this diluted homogenate was used for measuring the arginase activity, according to the colorimetric procedure described by Van Slyke and Archibald (13). The arginase activity was expressed in terms of the number of arginase units, as defined by Van Slyke and Archibald (13), per mg. of fresh tissue. Calculation of arginase activity on the basis of the weight of dried tissue gave essentially the same results.

Normal appearing young from females maintained on the basal ration supplemented with 3 per cent fish solubles were sacrificed within 24 to 48 hours after birth and the arginase activity of the livers determined in the manner described.

Blood Urea—Following decapitation, the blood was collected into small oxalated glass vials (16 mm. outside diameter and 1 cm. deep). A volume of the blood measured to the nearest 0.001 ml. (volumes of samples ranging from 0.050 to 0.150 ml.) was removed from the vial in an 0.2 ml. Kahn serological pipette and immediately laked in 2 ml. of distilled water. The subsequent steps involving dilution, deproteinization, and the determination of urea have already been described in detail (14).

Several comparative experiments were conducted on liver homogenates which had not been activated in this manner. Although the arginase activity in such instances was about half that of the activated homogenates, the difference in arginase activity between normal and uremic animals was of the same magnitude as in the activated samples.
Results

Effect of Fish Solubles on Liver Arginase Activity and Blood Urea—The data presented in Table I summarize the influence of the maternal diet on liver arginase activity and the concentration of blood urea of young rats within 24 to 48 hours after birth. The uremic new-born from mothers raised on the unsupplemented rations containing the crude plant materials displayed the marked elevation of urea in the blood which is characteristic of this syndrome. Mothers on similar rations supplemented with 3 percent fish solubles in no instance produced young with acute uremia, as evidenced either by their external appearance or by the concentration of urea in the blood, as shown in Table I. The marked elevation in blood urea in the uremic animals was accompanied by a significant increase in the arginase activity of the liver.

<table>
<thead>
<tr>
<th>Ration</th>
<th>No. of animals</th>
<th>Condition of animals</th>
<th>Liver arginase activity*</th>
<th>Blood urea*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-1, unsupplemented</td>
<td>30</td>
<td>Uremic</td>
<td>840 ± 126</td>
<td>225.8 ± 49.2</td>
</tr>
<tr>
<td>&quot; + 3% fish solubles</td>
<td>30</td>
<td>Normal</td>
<td>472 ± 76</td>
<td>70.8 ± 38.8</td>
</tr>
<tr>
<td>CS-3, unsupplemented</td>
<td>19</td>
<td>Uremic</td>
<td>870 ± 165</td>
<td>234.0 ± 68.4</td>
</tr>
<tr>
<td>&quot; + 3% fish solubles</td>
<td>8</td>
<td>Normal</td>
<td>448 ± 37</td>
<td>75.2 ± 10.7</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation.

Variation of Arginase Activity Following Birth—Since it had been previously reported that there is a transient rise in the concentration of urea in the blood of new-born rats during the first few days after birth (12), it appeared likely that this transitory increase in urea concentration might also be reflected by the arginase activity of the liver. A study of this relationship, as influenced by the presence or absence of fish solubles in the maternal diet, was made by analyzing the blood and liver of young from the same litter killed at various intervals. A graphic presentation of the data, obtained by these analyses extending over a 48 hour period following birth, is shown in Fig. 1. The changes in concentration of blood urea of normal and uremic rats followed a course similar to that reported previously (12). Closely paralleling these changes in concentration of blood urea were those which occurred in the activity of arginase in the liver. An increase in arginase activity was observed within 12 to 24 hours after
birth, coinciding with the marked rise in the blood urea which occurred during the same period. This increase in arginase activity was particularly marked in those rats observed in a state of acute uremia, but appreciably less in normal appearing young from others on the same unsupplemented ration. The presence of fish solubles in the maternal diet permitted a more gradual rise in arginase activity in the livers of the new-born. Whereas the concentration of urea in the blood of normal animals tends to return to a lower level within 24 to 48 hours following birth, there appeared to be no immediate tendency for liver arginase activity to decline once maximum activity had been attained. According to the data of Lightbody (15), who also observed a rise in liver arginase activity in rats during the first few days of life, one might expect a gradual return to the level found at birth by the 9th to the 18th day.

**Effect of Crystalline Vitamin B₁₂ on Liver Arginase Activity and Blood Urea**

In this experiment, 0.1 ml. of a solution containing 0.06 μg of crystalline vitamin B₁₂ (Cobione, Merck) was injected subcutaneously within 2 to 3 hours after birth into about half the number of young in each litter from mothers on Ration CS-1. The other half of the litter served as a control. In the 48 hour period following birth, animals observed in the acute uremic

![Image of graph showing changes in liver arginase activity and blood urea concentration](http://www.jbc.org/)

Fig. 1. Changes in concentration of blood urea and liver arginase activity of the new-born rat. Basal Ration CS-1 was used in this experiment. The figures in parentheses indicate the number of animals used in calculating the mean value represented by each point. The vertical lines through each point indicate the magnitude of the standard deviation. O, solid line, young of normal appearance on basal ration; O, dash line, young with acute uremia on basal ration; ●, young of normal appearance on basal ration + fish solubles.
crisis were sacrificed at once. All animals appearing normal were sacri-
ficed at the end of 48 hours. As shown by the data in Table II, vitamin
B\textsubscript{12}, like condensed fish solubles, prevents a continued rise of arginase
activity, as well as the appearance of the external symptoms of acute ure-
emia.

The close relationship that exists between arginase activity in the liver

### Table II

**Effect of Vitamin B\textsubscript{12} on Liver Arginase Activity and Blood Urea of Young Rats Born to Mothers Fed Ration CS-I**

<table>
<thead>
<tr>
<th>Observed condition</th>
<th>Injected with 0.06 γ of vitamin B\textsubscript{12}</th>
<th>Uninjected controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of animals</td>
<td>Liver arginase activity*</td>
</tr>
<tr>
<td>Total of live young born</td>
<td>30</td>
<td>units per gm. fresh tissue</td>
</tr>
<tr>
<td>Young with acute uremia</td>
<td>0</td>
<td>28502 ± 196</td>
</tr>
<tr>
<td>Normal young at end of 48 hrs.</td>
<td>28</td>
<td>1320 ± 122</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation.

![Figure 2](http://www.jbc.org/)

**Fig. 2.** The effect of crystalline vitamin B\textsubscript{12} on concentration of blood urea and liver arginase activity of the new-born rat. Correlation coefficient (\(r\)) = +0.86 (\(r\) at 1 per cent level of significance = +0.35). The equation expressing the regression of arginase units per gm. of fresh liver (\(y\)) on the concentration of blood urea as mg. per 100 ml. of blood (\(x\)) is \(y = 208 + 2.44x\). △, uninjected young with acute uremia; ○, vitamin B\textsubscript{12}-injected young with normal appearance. **
and the concentration of urea in the blood is evident from Fig. 2, a scatter diagram of individual determinations which constitute the basis for the average values shown in Table II. A noteworthy feature revealed by Fig. 2 is that, in spite of the absence of the external features of acute uremia, several of the animals receiving vitamin B₁₂ had concentrations of blood urea equivalent to those usually found in uremic young. The same was true for some of the normal appearing young which had not been injected with vitamin B₁₂. In the case of young born to mothers on basal rations supplemented with fish solubles, however, blood urea levels equivalent to those found in uremic young were never encountered. It would appear that the quantity of vitamin B₁₂ injected, although sufficient to prevent the onset of the uremic crisis as manifested by external appearances, is not sufficient in all instances to prevent the accumulation of large amounts of urea in the blood. On this basis, therefore, one can account for the fact that the mean values for blood urea and liver arginase activity in new-born rats injected with vitamin B₁₂ (Table II) are somewhat greater than those obtained from animals produced by females on the same basal diet supplemented with fish solubles (Table I).

DISCUSSION

Elevated concentrations of non-protein nitrogenous constituents have been observed in the blood of rats (16) and chicks (17, 18) maintained on rations devoid of animal protein. Hartman, Dryden, and Cary (19) and Bosshardt, Paul, and Barnes (20) found that increasing the protein level of diets deficient in vitamin B₁₂ accentuated the requirements for vitamin B₁₂. Charkey et al. (18) have interpreted these results, as well as their own, to indicate that vitamin B₁₂ may function in metabolism by enhancing the utilization of circulating amino acids for the synthesis of tissue protein.

The present data show that the accumulation of large quantities of urea in the blood, and the increase in arginase activity which accompanies it, occur only after the young have nursed. Since it has been consistently observed in this laboratory that, on rations producing acute uremia of the young, the early lactation of the females is excellent (11, 12), it appears that the elevation in blood urea and liver arginase activity is associated with the sudden influx of nutrients from the alimentary tract. In animals deficient in vitamin B₁₂, the failure to utilize absorbed amino acids

* This contention is supported by preliminary observations which indicate that the blood of uremic new-born rats had concentrations of non-urea non-protein nitrogen and α-amino nitrogen, determined by the method of Frame et al. (21), which were about double those found in the blood of normal appearing young of the same age.
protein anabolism might be expected to bring into play those mechanisms which facilitate their elimination from the body in the form of urea. The close relationship demonstrated to exist between liver arginase activity and the concentration of urea in the blood is strong indication that one of the adjustments that the organism makes in order to cope with this defect in nitrogen metabolism is to increase the arginase activity in its liver.

SUMMARY

The acute uremia observed in new-born rats from mothers maintained on rations containing crude plant materials was accompanied by a significant increase in the activity of liver arginase, compared to rats of normal appearance of the same age in which the maternal diet had been supplemented with fish solubles.

Liver arginase activity closely paralleled the marked rise in the concentration of urea in the blood which occurred 12 to 24 hours after birth.

The early postnatal administration of vitamin B\textsubscript{12} prevented the abnormal rise in arginase activity and prevented acute uremia in the new-born rat. Under these conditions a high degree of correlation was found to exist between the arginase activity of the liver and the concentration of urea in the blood.

The assistance of Mr. Raymond Tarleton in the care and feeding of the animals is gratefully acknowledged.

BIBLIOGRAPHY

LIVER ARGINASE ACTIVITY AS RELATED TO BLOOD UREA IN ACUTE UREMIA OF NEW-BORN RATS
I. E. Liener and M. O. Schultze