PROTEIN METABOLISM IN THE CHOLINE-DEFICIENT RAT

I. EFFECT OF CHOLINE ON SERUM PROTEINS

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Unless the choline content is sufficient to supply all of the required methyl groups, the amount of choline in a diet apparently determines the quantity of methionine used for protein synthesis and the quantity reserved for use as a methyl donor. This concept is supported by Treadwell's demonstration (1) of the growth-stimulating effect of choline and Salmon's observation (2) that the nutritive value of protein can be improved by incorporating choline into a deficient diet. In the present study, electrophoretic patterns of sera from rats fed a choline-deficient diet were compared with those from rats fed choline supplements in addition to the basal diet. The data showed that an 18 per cent casein diet did not provide all of the factors essential for the maintenance of normal serum albumin concentration and that the kidney damage of severely deficient animals caused the concentrations of the α-globulins and one of the β-globulins of the sera to increase.

EXPERIMENTAL

Diet—The choline-deficient diet had the following composition: 18 per cent casein, 20 per cent hydrogenated vegetable oil, 57.7 per cent sucrose, 4.0 per cent salt mixture (U. S. P. XIII), and 0.3 per cent L-cystine. The vitamin supplements added to each kilo of diet were 30 mg. of inositol, 5 mg. of thiamine hydrochloride, 5 mg. of pyridoxine, 5 mg. of riboflavin, 10 mg. of calcium pantothenate, 20 mg. of nicotinic acid, and 10 mg. of α-tocopherol acetate (in 0.25 ml. of corn oil). All of the animals were fed 1 drop of cod liver oil concentrate every other day.

Experimental Animals—Female rats of the Sherman strain were taken from our stock colony. When they were 22 and 23 days old, they were distributed into groups consisting of fifteen to twenty-five animals each. The larger groups were used when a high mortality was anticipated. The animals in the same group were caged together. Diets were fed ad libitum.

1 Vitamin-free, Nutritional Biochemicals Corporation.
2 Crisco.
3 Natola, Parke, Davis and Company.
Four experimental series were studied: Series I, basal diet; Series II, basal diet + 2 mg. of oral supplement of choline chloride on each day of the experiment; Series III, basal diet + 6 mg. of oral supplement of choline chloride on each day of the experiment; Series IV, basal diet + 6 mg. of oral supplement of choline chloride on the 6th and subsequent experimental days; and Series V, laboratory chow.4

In each series, a group of animals was sacrificed by decapitation on almost every day of the 15 day experimental period. The blood was collected into a single beaker and allowed to clot. The serum was decanted, centrifuged, and examined in the electrophoresis apparatus. In some cases (Series I and Series III) the lipid phosphorus content of the serum was determined by the method of Fiske and Subbarow (3).

The livers and kidneys of animals in the same group were removed. The kidneys were examined for gross evidence of hemorrhagic enlargement. The livers were ground together in a mortar and two aliquots of the pool were removed for the determination of total lipid by the method of Shipley, Chudzik, and György (4) and in Series I and III for lipid phosphorus (3).

Electrophoresis—After diluting 4 ml. of serum with 8 ml. of Veronal buffer (pH 8.6, μ = 0.1), the sample was dialyzed against 2 liters of the same buffer for 1 hour (5). A field strength of 8.0 volts per cm. was applied for 180 minutes to separate the protein components of the serum. Because the albumin and α-globulin fractions are more sharply defined on the ascending pattern than they are on the descending side, and because the β-globulin fraction separates into two distinct peaks only in the rising arm, all measurements of the total area and of the areas of the individual components were confined to the pattern of the rising boundaries.

RESULTS AND DISCUSSION

The data in Table I show that the basal diet was deficient in choline because the livers of the animals in Series I became fatty and, on the 6th day, renal lesions were evident. A daily supplement of 2 mg. of choline chloride (Series II) prevented, to a large extent, the damage to the kidneys but had a lesser effect on fat deposition in the liver. When the daily supplement was raised to 6 mg. (Series III), liver fat was only slightly greater than normal (Series V) and the kidney lesions were completely prevented. These results are in agreement with those of Griffith and Mulford (6) and will be used as the basis for correlating the signs of choline deficiency with certain changes in the serum protein fractions.

Serum Albumin—Low levels of serum albumin in choline-deficient animals could result from (1) inadequate protein intake, (2) decreased syn-

4 Ralston Purina Company.
thesis of albumin by the fatty liver, or (3) loss of albumin through the damaged kidneys. Fig. 1 shows that, regardless of whether choline was administered or not, the serum albumin concentrations in the experimental groups were lower than normal during the first 6 days, and that on the 4th day a consistent but short lived rise in the albumin area occurred in each of the experimental series but not in the normal controls. On the 7th day elevated serum albumin levels were again noted. In this case, the rise took place in all of the experimental series and in the normal controls and

**Table I**

*Renal Antihemorrhagic and Lipotropic Activity of Choline Supplements*

<table>
<thead>
<tr>
<th>Experimental day</th>
<th>Per cent fat in liver</th>
<th>Per cent damaged kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Series I</td>
<td>Series II</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>12.9</td>
<td>8.5</td>
</tr>
<tr>
<td>3rd</td>
<td>16.9</td>
<td>13.7</td>
</tr>
<tr>
<td>4th</td>
<td>14.0</td>
<td>11.5</td>
</tr>
<tr>
<td>5th</td>
<td>18.9</td>
<td>17.1</td>
</tr>
<tr>
<td>6th</td>
<td>17.8</td>
<td>11.6</td>
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<tr>
<td>7th</td>
<td>22.2</td>
<td>14.1</td>
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<td>11.7</td>
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<td>15th</td>
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* Repaired.

thus cannot be associated with changes in protein metabolism resulting from the experimental diet.

Since choline intake had an insignificant effect upon serum albumin but liver lipide was dependent upon the amount of choline administered, it is evident that liver fat deposition, *per se*, had no effect on albumin synthesis. Furthermore, it seems unlikely that a dietary imbalance or deficiency (e.g., of protein) completely explains the low serum albumin areas noted in the first 6 days of the experiment, for by the 7th day the albumin values returned to normal without any change in the dietary régime.

Several explanations for this finding can be suggested. For example, the metabolism of albumin in the earlier and later days of the experiment may differ in rate, or synthesis of albumin may be suppressed in the earlier days in deference to the synthesis of other temporarily important proteins or to other needs for amino acids. In either case the diet does not ade-
quately support albumin synthesis or utilization during the 1st week but is able to sustain normal albumin levels after that time.

Fig. 1, a shows that the development of kidney lesions affected the serum albumin area. On the 8th day, i.e. 2 days after renal damage first became evident in the deficient animals, the level of albumin in the serum was considerably lower than it was in the choline-treated controls. By the 12th day the kidneys were in the recovery phase and the albumin concent-

Fig. 1. Albumin areas from the electrophoresis patterns of sera from choline-deficient and supplemented rats. ○, values for normal control animals; ●, values for the experimental series. (a) Basal diet; (b) basal diet + 2 mg. of choline chloride daily; (c) basal diet + 6 mg. of choline chloride daily; (d) basal diet; 6 mg. of the choline chloride supplement were given only on the 6th and subsequent experimental days.

tration returned to normal. The data recorded in Fig. 1, d are evidence that the renal antihemorrhagic action of choline prevented the decrease in albumin concentration. In this experiment, a 6 mg. supplement was given to deficient animals on the day on which the kidney lesions were becoming evident (6th day) and on each subsequent day. The supplement prevented the lesions from becoming severe, and recovery of the kidneys was accelerated. As a result, the albumin area did not decrease on the 8th day as it did in deficient animals which were continued on the basal diet. Since Griffith has reported (7) that proteinuria is a result of the renal damage in choline deficiency, it is likely that loss of protein through the kidney caused the lowered serum albumin concentrations on the 8th to the 10th
days in the severely affected animals of Series I. However, one cannot overlook the fact that inanition in the sick animals may be partly responsible for the decrease.

α-Globulins—The outstanding difference between the electrophoresis patterns of the sera of choline-deficient rats and of protected animals is the increased α-globulin content of the sera of deficient rats. Visual inspection of patterns taken after kidney lesions have appeared (Fig. 2) shows that the concentrations of the α1- and α2-globulins are greater than normal and that the α2 fraction is composed of two peaks. No attempt was made to make a quantitative determination of the areas of the α2 components because the double peak was not observed in the normal or supplemented groups.

![CHOLINE-HCl SUPPLEMENT](http://www.jbc.org/)

**Fig. 2.** Electrophoresis patterns showing the effect of choline supplements on the serum proteins. The analyses were made on the 8th experimental day and were performed in Veronal buffer (pH 8.6; μ = 0.1). The time of migration was 180 minutes at a field strength of 8.0 volts cm⁻¹.

In Fig. 3, additional evidence is submitted that the α1- and α2-globulin concentrations are related to the choline intake of the animals and thus to the amount of kidney damage the animals suffer. The animals of Series I received no choline supplements. As a result 90 per cent of them had kidney lesions and their α2-globulin areas were twice the normal value by the 8th day (Fig. 3, a and b). The kidneys of only a small percentage of the animals which were fed 2 mg. of choline chloride a day were visibly damaged and the α-globulins in their sera were slightly elevated (Fig. 3, c and d). Even though no significance can yet be attached to the finding, it is of interest to note that in border-line deficiency the α2 fraction was increased to a greater extent than was the α1 area. Both α-globulin components, however, were present in nearly normal amounts in the sera of animals to which sufficient choline was administered to prevent kidney lesions completely (Fig. 3, e and f), and both globulins were prevented from reaching the values seen in the severely affected animals when the admin-
istration of the 6 mg. daily supplement of choline chloride was begun on the day on which kidney lesions usually appear (Fig. 3, g and h). All of the data, therefore, seem to point to the conclusion that renal hemorrhages and the subsequent degeneration of kidney tubules (7) are responsible for the presence of increased quantities of α-globulins in the sera of deficient rats.

Increases in the α-globulins of human sera have long been associated with "tissue destruction irrespective of its cause" (8). Similar conclusions have been drawn from studies on the injured dog (9) and injured rat (10) and, in the rat, the concentrations of the lipides associated with the α- and β-globulins were shown to be consistently increased by injury or the administration of toxic agents (11). In the present investigation serum and liver phospholipide concentrations were determined on the tissues of rats of Series I and III. Fig. 4 shows that from the 5th to the 8th day the
liver phospholipide of the choline-deficient animals increased 3-fold and that the serum phospholipide decreased at the time at which liver phospholipide began to increase. This suggests that phospholipide from serum was being withdrawn from the blood into the liver. The sudden rise in serum phospholipide concentration, after signs of kidney lesions appeared,

![Graphs showing electrophoresis patterns of sera from choline-deficient and supplemented rats.](http://www.jbc.org/)

coincided with the abrupt increase in the serum \(\alpha\)-globulins. These simultaneous occurrences lead us to believe that the degeneration of kidney tubules in choline-deficient rats liberates phospholipide-containing globulins into the blood stream and that the "liberated choline permits renal recovery even though the liver remains fatty" (7).

**\(\beta\)-Globulins**—The areas of these fractions are shown in Fig. 5, a to h. There is some indication that the \(\beta_1\) component rises after the appearance of hemorrhagic kidney lesions in the animals of Series I and, in this respect,
this fraction resembles the \( \alpha \)-globulins. It has been found that it becomes elevated as a result of tissue damage (10, 11). On the other hand, the \( \beta_2 \) fraction seems to be independent of the kidney lesions encountered in deficient animals. Rather, it appears that the addition of choline to the diets (Fig. 5, d and f) caused more variation in the concentrations of this protein group than did the omission of choline from the diet (Fig. 5, e). Furthermore, a delayed administration of choline (Fig. 5, h) caused the \( \beta \) fraction to increase. So far it has not been possible to draw any meaningful conclusions from these data.

\( \gamma \)-Globulin—The \( \gamma \)-globulin concentration (Fig. 6) of the serum of the normal controls declined during the first 8 days of the experiment, and there does not seem to be any major difference between the data for the normal controls, for the unsupplemented groups, and for the animals which received a choline supplement throughout the experiment. Fig. 6, d, however, shows that administration of choline to deficient animals on the 6th and the remaining days of the experiment caused an increase in \( \gamma \)-globulin area. Since, at the time when the kidneys of deficient rats (Series I) repair spontaneously, the livers remain fatty and there is no increase in \( \gamma \)-globulin, it does not seem reasonable to ascribe the increased \( \gamma \)-globulin content of the sera of the animals of Series IV to the recovery of the kidneys. Rather, it seems likely that the increase in \( \gamma \)-globulin area, which was caused by the delayed administration of choline, is related to the restoration of the liver to its normal structure and fat content.

**SUMMARY**

1. The albumin concentrations of the sera of choline-deficient rats did not differ significantly from those of the choline-fed controls until kidney lesions appeared. Then the albumin concentration was lower in the deficient animals than it was in the controls.

2. Increases in phospholipide, \( \alpha \)-globulin, and \( \beta \)-globulin were demonstrated in the sera of deficient animals. There seemed to be a relationship between these changes and the degeneration of the kidney tissue.

3. Serum \( \gamma \)-globulin content was not affected by feeding the basal diet nor by feeding the basal diet with supplements of choline throughout the experiment. However, when choline was administered to deficient animals to speed their recovery, the \( \gamma \)-globulin concentration increased.

**BIBLIOGRAPHY**
