The Biosynthesis of Rat Serum Albumin

VI. INTRACELLULAR TRANSPORT OF ALBUMIN AND RATES OF ALBUMIN AND LIVER PROTEIN SYNTHESIS IN VIVO UNDER VARIOUS PHYSIOLOGICAL CONDITIONS*

(Received for publication, December 29, 1971)

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SUMMARY

The incorporation of L-[14C]leucine was measured during a 16-min time interval in rats under various physiological conditions. Rates of albumin and liver protein formation in control rats 5 to 7 weeks of age were 3.13 and 28.8 mg/100 g of body weight per hour, respectively. The half-time of replacement of liver protein was 18 hours, appreciably faster than half-times obtained from measurements of catabolism of labeled liver protein.

When synthesis of protein was inhibited by cycloheximide, loss of albumin from hepatic microsomal particles continued at its previously existing rate until the cells were nearly depleted of albumin. Rates of secretion measured in this manner were 0.34 and 0.74 mg per g of liver per hour in adult rats and young nephrotic rats, respectively; corresponding rates of synthesis determined with [14C]leucine were 0.34 and 0.77 mg per g of liver per hour. The agreement between the results obtained with these independent techniques adds some confidence to the use of the [14C]leucine method.

The rate of albumin synthesis was unchanged when measured at 10 p.m. compared to 10 a.m. in rats on a 12-hour light-dark cycle. Fasting for 18 hours caused a 40% reduction. In adult rats the rate of albumin synthesis was 34% and that of liver protein 52% of the rates in control rats. No significant increase in albumin synthesis was detected in the presence of nephrosis caused by puromycin amino-nucleoside, even when a supplement of protein plus hydrocortisone was given.

The dynamics of the transport of albumin and its release from the cytoplasmic particles were similar in all of the conditions studied. The amount of albumin in hepatic microsomes, however, varied directly with the rate of albumin production over a 4-fold range. It is suggested that the stimulus for increased secretion is a small increase in the quantity of albumin in the microsomal particles.

Measurements of the rates of albumin synthesis over a short period of time have generally been restricted to in vitro systems such as liver slices or the perfused liver. In the present paper of this series (1) a procedure was described to estimate the rate of albumin synthesis during a 16-min time interval in vivo. This technique is based upon observations of the maximum label in albumin prior to its secretion from the liver cell. The precursor specific activity is derived by an adaptation to shorter time intervals of the procedure used by Vandermeers et al. (2) and Haider and Tarver (3) of integrating the specific radioactivity of the tracer leucine in liver over the time of its incorporation.

This method has now been applied to measure albumin and liver protein synthesis rates at different times in the diurnal cycle of the rat, and to determine the effects of age of the rat, of a short period of fasting, of increased dietary protein, and of reduction of the circulating albumin level by experimental nephrosis. Measurements were also made of the amount of albumin bound in cytoplasmic particles and of the kinetics of labeling of intracellular albumin. Significant differences were observed under some conditions in the rates of albumin synthesis and in the amounts of albumin in the secretory channel, but there was no apparent change in the time required for secretion of a newly formed molecule under the conditions studied. Upon inhibition of protein synthesis with cycloheximide, albumin secretion was found to continue at the previously existing rate until the supply of particulate albumin was nearly exhausted.

EXPERIMENTAL PROCEDURE

Animals—Wistar rats, raised locally, were housed in plastic boxes with litter of ground corn cobs at 22° and relative humidity 90%, with 12-hour periods of darkness from 6 p.m. to 6 a.m. Unless otherwise noted, the rats used were males 5 to 7 weeks of age, weighing about 200 g, fed ad libitum a rat diet containing 24% protein and 4% fat, supplemented with minerals and vitamins (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.). Experimental procedures were conducted between 9 and 10 a.m., except for those noted as "night" or "nocturnal," which were conducted between 9 and 10 p.m. "Fasting" animals received no food for 18 hours prior to experiment. "High protein" rats received for 14 to 21 days a complete diet containing 64% casein (Nutritional Biochemicals Co., Chagrin Falls, O.).

* This work was supported by United States Public Health Service Research Grants HE-02751 and FR-05498, and by the Stephen Carlton Clark Research Fund of The Mary Imogene Bassett Hospital.
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“Nephrotic” rats had received 6 mg/100 g of body weight of the aminonucleoside of puromycin (Lederle Laboratories Division, American Cyanamid Corp.) by intraperitoneal injection on each of the 3rd, 5th, and 7th days prior to the experiment. The aminonucleoside dosage schedule followed that of Katz et al. (4), and has been shown to cause marked urinary protein loss, primarily of albumin, beginning about the 5th day after the first injection (5, 6). The nephrotic animals showed marked ascites together with hypoalbuminemia. “Nephrotic-high protein” animals were similarly treated and, in addition, received the 64% casein diet for 14 to 21 days, plus intraperitoneal injections of hydrocortisone (Solu-cortef, Upjohn Co., Kalamazoo, Mich.), 1.25 mg/100 g, on each of the 3 days immediately prior to the experiment. The administration of hydrocortisone followed the schedule of Leites and Nikiforova (7). “Adult” rats were 38 ± 3 weeks of age.

**General Procedure**—The measurements of rates of protein synthesis were made with the technique of a previous study (1) on the effects of protein depletion and repletion. Briefly, L-[14C]leucine (L-[U-14C]leucine, 312 to 344 mCi per mmole, Nuclear-Chicago Corp.), 1.5 µCi/100 g of body weight in about 0.2 ml of 0.15 M NaCl, was injected in a tail vein under light ether anesthesia. The amount of leucine injected was calculated to cause no greater than 1% increment in the level of free leucine in the plasma. After a desired interval, usually 16 min, a weighed portion of the left lateral lobe of the liver was homogenized in 0.25 M sucrose and fractionated into a microsome fraction and a combined nuclear plus mitochondrial fraction. LeBouton and Hoffman (8) have shown that there is little distinction in the incorporation into various lobe of the liver when the tracer amino acid is injected into a peripheral vein. The sedimented fractions were extracted with sodium deoxycholate and the extracts dialyzed, treated with chicken serum albumin and anti-chicken serum albumin, and finally with anti-rat serum albumin. The resulting specific precipitates were utilized to determine the quantity and 14C content of rat albumin in the fraction. For measurements of circulating 14C-albumin, serial blood samples were obtained from the tail veins, and albumin isolated from the serum by a trichloroacetic acid-ethanol technique. The amount and radioactivity of liver protein was determined by paper chromatography; results were similar to those obtained at night. The circulating albumin levels were lowered to about one-third of the control in both groups of nephrotic rats, and were slightly depressed in the adult animals.

**Calculations** Rates of synthesis of albumin and liver protein were calculated with the following slight modifications of the formula used before (1).

\[
F = \frac{60(m_{14C})}{\alpha \int_{t_0}^{t_1} L_{t*} \, dt}
\]

(1)

The formula letters are: \(F\), rate of albumin or liver protein synthesis in milligrams per g of liver per hour; \(m_{14C}\) disintegrations per min in microsomal albumin or total liver protein per g of liver at 16 min after injection of [14C]leucine; \(\alpha\), milligrams of leucine per g of albumin, 0.112 (9), or of liver protein, 0.0675 (1); \(L_{t*}\), disintegrations per min in free leucine per g of liver at time \(t\); \(L_0\), free leucine concentration as milligrams per g of liver; 60, conversion factor from minutes to hours. The interval of 0 to 14 min was selected since it requires about 2 min for synthesis of an albumin molecule (10), and the 14C in albumin was measured at 16 min.

If \(L_0\), the free leucine concentration in liver, remains constant during the 14-min period of the integration, Equation 1 becomes

\[
F = \frac{60(m_{14C})L_0}{\alpha \int_{t_0}^{t_1} L_{t*} \, dt}
\]

(2)

\(L_{t*}\) was obtained by correcting the observed 14C concentrations in the protein-free extracts of liver for the fraction of this 14C found to remain in leucine at the time of observation. The integral was obtained graphically. Precision of the determination of the \(L_{t*}\) integral was estimated at ±5%. The standard errors of the values of \(F\) were calculated as the root mean squares of the standard errors of \(m_{14C}\) and \(L_{t*}\), when the standard errors were expressed on a relative basis.

**Results**

**Characteristics of Groups of Rats Studied**—There was a slight weight loss in the fasting rats and in the rats studied at night, at a time 12 hours opposite to the usual time of the experiments (Table 1). Since rats are inactive in a lighted environment, the animals were essentially fasting for about 12 hours at the time of the nocturnal experiments. The high protein, nephrotic, and nephrotic-high protein groups weighed significantly less than the controls; the adult animals were much heavier.

The relative liver weights of the adult animals were lower than those of the controls, as were those of the 18-hour fasted rats and those measured at night. The circulating albumin levels were lowered to about one-third of the control in both groups of nephrotic rats, and were slightly depressed in the adult animals.

Free leucine concentration in liver and serum increased upon consumption of a high protein diet, and was significantly decreased in experimental nephrosis. In the nephrotic animals glycine, valine, methionine, leucine, tyrosine, and histidine were likewise decreased in the liver, and ornithine and lysine were increased. When the nephrotic animals were fed a high protein diet, the free leucine levels returned toward but did not reach the control values.

**Turnover of Free Leucine**—Curves of the rise and fall of 14C in the free leucine of the livers of the different groups of rats are
Protein, albumin, and free leucine contents in livers and serum of rats in various physiological states

The results are given as the mean ± standard error with the number of different animals in parentheses.

<table>
<thead>
<tr>
<th>Condition of rat</th>
<th>Body weight</th>
<th>Liver</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weight</td>
<td>Protein content</td>
</tr>
<tr>
<td>Control (day)</td>
<td>212 ± 9</td>
<td>4.34 ± 0.07</td>
<td>750 ± 16</td>
</tr>
<tr>
<td>Control (night)</td>
<td>194 ± 10</td>
<td>3.88 ± 0.08</td>
<td>759 ± 18</td>
</tr>
<tr>
<td>Fasting</td>
<td>193 ± 9</td>
<td>3.41 ± 0.05</td>
<td>661 ± 15</td>
</tr>
<tr>
<td>High protein</td>
<td>163 ± 13</td>
<td>4.69 ± 0.59</td>
<td>964 ± 86</td>
</tr>
<tr>
<td>Adult</td>
<td>177 ± 7</td>
<td>4.40 ± 0.06</td>
<td>771 ± 18</td>
</tr>
<tr>
<td>Nephrotic</td>
<td>162 ± 5</td>
<td>4.73 ± 0.80</td>
<td>820 ± 80</td>
</tr>
</tbody>
</table>

* Significantly different from "control" with p < 0.01.
* Significantly different from "control" with p < 0.05.

shown in Fig. 1. The turnover of the intrahepatic pool is seen to be rapid even when the tracer amino acid is injected in the tail vein as in these experiments. Peak radioactivity in liver was reached between 1 and 1.5 min in all groups, and by 15 min the activity had fallen to much lower values. The curve for adult rats (Fig. 1e) was somewhat higher than the others and that of the nephrotic-high protein rats (Fig. 1g) was somewhat lower. The integrated [%]leucine values obtained from these curves are listed in the first column of Table II.

Rates of Synthesis of Liver Protein and Albumin—Table II

<table>
<thead>
<tr>
<th>Condition of rat</th>
<th>Average free [%]leucine in liver, 0-14 min</th>
<th>Rate of synthesis</th>
<th>Ratio, albumin to liver protein synthesis rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver protein mg/100 g rat/hr</td>
<td>Albumin mg/100 g rat/hr</td>
<td>%</td>
</tr>
<tr>
<td>Control (day)</td>
<td>3.81</td>
<td>26.8 ± 2.5</td>
<td>3.13 ± 0.27</td>
</tr>
<tr>
<td>Control (night)</td>
<td>3.80</td>
<td>36.6 ± 4.1</td>
<td>3.12 ± 0.27</td>
</tr>
<tr>
<td>Fasting</td>
<td>3.65</td>
<td>28.3 ± 3.7</td>
<td>1.88 ± 0.30</td>
</tr>
<tr>
<td>High protein</td>
<td>5.17</td>
<td>26.2 ± 1.7</td>
<td>2.40 ± 0.22</td>
</tr>
<tr>
<td>Adult</td>
<td>6.44</td>
<td>19.0 ± 1.1</td>
<td>1.00 ± 0.08</td>
</tr>
<tr>
<td>Nephrotic</td>
<td>3.00</td>
<td>37.8 ± 2.2</td>
<td>3.39 ± 0.25</td>
</tr>
<tr>
<td>Nephrotic-high protein</td>
<td>3.24</td>
<td>35.1 ± 3.1</td>
<td>3.41 ± 0.18</td>
</tr>
</tbody>
</table>

* Significantly different from "control" with 0.1 < p < 0.2.
* Significantly different from "control" with 0.01 < p < 0.02.
* Significantly different from "control" with p < 0.01.

shows the rates of synthesis of total liver protein and serum albumin, calculated by use of Equation 2. The basis of measurement was taken as 100 g of body weight rather than weight of liver, owing to the rapid changes in relative liver weight upon fasting or during the diurnal cycle.

There was a tendency for the synthesis of liver protein to ac-
celerate at night. Liver protein synthesis was also more rapid in both groups of nephrotic animals, and was markedly depressed in the adult animals.

On the basis of body weight, the rate of albumin synthesis was unchanged in the nocturnal experiments. Because of the relative shrinking of the liver at night (Table I), there is a significant increase in albumin synthesis if calculated on the basis of liver weight. An 18-hour fast lowered albumin formation by about 60% of the control value. In the adult rats albumin synthesis was only about one-third as rapid as in the 5- to 7-week control rats. Albumin synthesis was not increased by the 84% protein diet, despite elevated levels of free amino acids. The reason may lie in a decreased caloric intake; food intakes were not recorded but were doubtless decreased since the animals on the high protein diet weighed less than controls.

In aminonucleoside nephrosis only a slight increase in albumin formation in response to the severe hypalbuminemia was seen. The high protein diet was added to the nephrotic condition in an effort to compensate for the lower free amino acid levels observed in the nephrotic rats; although the free leucine levels rose appreciably, body weight and the rate of albumin synthesis did not change.

Amount of Intracellular Albumin and Rate of Transport—The amounts of albumin in the microsomes, measured immunochromically, are given in Table III on the basis of weight of liver. If the values are multiplied by the liver weights the difference between the day- and nighttime measurements is eliminated owing to the smaller liver weight at night, but the other relationships to the control animals remain unchanged. The amount of microsomal albumin varies directly with the rate of transport of albumin in the mitochondria fraction included in the mitochondrial fraction, which is involved in an inconsistent manner with the rate of albumin synthesis. The variability may in part be caused by changes in the amount of microsomes included in the mitochondrial fraction in the various groups of rats.

Little difference was seen in the rate of albumin transport in the four groups of rats in which the time curve of labeling of microsomal albumin was determined in some detail. The curves of Fig. 3, which represent animals with a 3-fold range of albumin synthesis rate, show a pattern of an initial sharp rise to a maximum at about 15 min and then a more gradual fall as labeled albumin is secreted. The maximum level is related both to the rate of albumin synthesis and the specific radioactivity of the precursor leucine pool.

The curves of appearance of labeled albumin in blood, Fig. 4, likewise differ in magnitude but not in their time relations. The minimum time of albumin transit of the cell, taken as the intercept on the abscissa of the steeply rising slope of the serum curve, shows no significant difference among any of the six conditions observed (Table III, last column).

### Table III

Intracellular albumin levels and minimum transit times

The results are given as the mean ± standard error with the number of different animals in parentheses.

<table>
<thead>
<tr>
<th>Condition of rat</th>
<th>Microsomes</th>
<th>Nuclei plus mitochondria</th>
<th>Total particulate</th>
<th>Minimum transit time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (day)</td>
<td>389 ± 7.4</td>
<td>54 ± 4</td>
<td>443</td>
<td>16.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>(56)</td>
<td>(39)</td>
<td></td>
<td>(8)</td>
</tr>
<tr>
<td>Control (night)</td>
<td>436 ± 15</td>
<td>80 ± 21</td>
<td>525</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted</td>
<td>346 ± 8</td>
<td>82 ± 10</td>
<td>428</td>
<td>14.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>(21)</td>
<td>(13)</td>
<td></td>
<td>(6)</td>
</tr>
<tr>
<td>High protein</td>
<td>322 ± 19</td>
<td>48</td>
<td>370</td>
<td>15.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(1)</td>
<td></td>
<td>(3)</td>
</tr>
<tr>
<td>Adult</td>
<td>304 ± 19</td>
<td>52 ± 6</td>
<td>356</td>
<td>16.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>(19)</td>
<td>(12)</td>
<td></td>
<td>(7)</td>
</tr>
<tr>
<td>Nephrotic</td>
<td>341 ± 10</td>
<td>31 ± 3</td>
<td>372</td>
<td>14.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>(23)</td>
<td>(12)</td>
<td></td>
<td>(9)</td>
</tr>
<tr>
<td>Nephrotic-high protein</td>
<td>379 ± 9</td>
<td>33 ± 7</td>
<td>412</td>
<td>14.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(3)</td>
<td></td>
<td>(2)</td>
</tr>
</tbody>
</table>

*Significantly different from "control" with p < 0.05.

*Significantly different from "control" with p < 0.01.

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

**Fig. 2.** Albumin content of rat liver microsomes in relation to the rate of albumin synthesis under different conditions. ●, present paper; ○, from Morgan and Peters (1).

![Fig. 3](http://www.jbc.org/)

**Fig. 3.** 14C in albumin of microsomes as a function of time after intravenous injection. Each point is the mean of two to five specimens. Treatment of animals: ▲, nephrotic; ●, control; ○, adult; □, fasted.
cycloheximide injection the albumin content fell in the nephrotic animals at a rate of 0.12 mg per g of liver per hour until it nearly reached zero. In the adult animals the levels in this fraction were more variable, and fell at a rate of about 0.01 mg per g of liver per hour.

The separation of microsomes and mitochondria by the scheme employed here is not complete, and it seems likely that the fall in albumin level in the nuclear plus mitochondrial fractions reflects loss of albumin from included microsomes. Taking the combined rates of fall in the two fractions of Fig. 5 as the rate of secretion of albumin, these rates are 0.74 and 0.35 mg per g of liver per hour for the nephrotic and adult rats, respectively.

**DISCUSSION**

The rate of liver protein synthesis of 28.8 mg/100 g of body weight per hour in the control animals corresponds to a half-time of replacement of the 750 mg of liver protein in a 100-g rat of 18.1 hours, in agreement with the half-times of 10.7 hours found by Vandermeers et al. (2) and of about 20 hours calculated from the data of Haider and Tarver (3). This is a much shorter half-life than those of 1.9 or 2.8 days found by measuring the loss of labeled arginine (11, 12), of 4.9 days with labeled leucine (12), or of 2.2 days with continuous infusion of labeled amino acids (13). The difference between the apparent rates of synthesis and degradation could be caused by the rapid turnover of enzymes such as tyrosine-α-ketoglutarate transaminase or tryptophan pyrrolase, with half-times of 1 hours and 2.4 hours, respectively (14, 15). The metabolism of these enzymes would be included in short term measurements of synthesis but would be omitted from measurements of catabolism where observations are made only at longer intervals.

No difference was found in the rate of albumin synthesis when measured at 10 a.m. or 10 p.m. in rats kept in darkness between 6 p.m. and 6 a.m., provided the nocturnal shrinkage of the liver was taken into account. These are the first data known to the author on the relationship of albumin synthesis to the diurnal cycle. Liver protein synthesis increased at night, a result that is also seen in the data of LeBouton and Handler (16), assuming that the levels of free leucine were constant in the livers of their rats at different times of observations. A more comprehensive study of albumin synthesis at different times in the diurnal cycle would be of interest.

The drop in albumin synthesis observed following a short period of fasting has also been detected in liver slices (17) and in the perfused rabbit liver (18). Liver protein synthesis possibly was maintained by the adaptive changes in composition of hepatic enzymes commonly seen early in the fasting state.

The decreased activity of albumin (19) and liver protein (20) formation with increasing age is well known. The decrease is primarily the result of slower turnover of proteins rather than merely cessation of growth, since it can readily be calculated that in the control rats less than 5% of protein synthesis is attributable to enlargement of the liver or the plasma albumin pool with growth.

Albumin synthesis did not increase in the presence of a markedly lowered albumin level in the aminonucleoside nephrosis, even with dietary compensation of the lowered amino acid levels and addition of hydrocortisone. The response of albumin synthesis in clinical nephrosis is highly variable among
individuals (21-23). Koertge and Oeff (24) found a compensatory increase in albumin synthesis in rats made nephrotic with antikidney serum, but no such increase in rats given puromycin aminonucleoside.

Following cycloheximide injection, the rates of secretion determined by the linear loss of albumin from cytoplasmic particles were 0.74 and 0.35 mg per g of liver per hour for the nephrotic and adult groups of rats, respectively. The corresponding rates of synthesis determined by the incorporation of [14C]leucine, when converted to the same basis of weight of liver, were 0.77 and 0.35 mg per g of liver per hour. This agreement between the rates determined by the two procedures, one chemical, one isotopic, supports the validity of the results obtained with the [14C]leucine procedure.

The cycloheximide experiments confirm the prompt inhibition of protein synthesis in rat liver caused by cycloheximide (25-27) and the independence of albumin secretion from synthesis (28-30). They further show that, although the process of secretion does not require concurrent synthesis, the rate of secretion is apparently established by the previously existing rate of synthesis. The factor influencing the rate of secretion is possibly a small change in the quantity of albumin in microsomal particles awaiting secretion, since this quantity varies directly with the rate of synthesis (Fig. 2).

Acknowledgments—We are grateful to Mrs. Margaret B. Elkan and Mrs. Dorothy Moakler for technical aid, and to Miss Charlene D. Stevens for secretarial assistance.

REFERENCES
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