The Secretion of Serum Protein and the Synthesis of Albumin and Total Protein in Regenerating Rat Liver

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SUMMARY

The regulation of albumin synthesis and serum protein secretion was studied in regenerating rat liver by measuring incorporation of \(^{14}\)C-leucine. Albumin was isolated to radiochemical purity, utilizing a method which eliminates the influence of precursor pool changes on protein labeling. Changes in the size of the product pool were measured. The following results were obtained.

1. The time period between intracaval injection of \(^{14}\)C-leucine and the appearance of radioactive protein in the blood ("secretion time") decreased from 15 min for normal animals to a minimum of 10 min at 48 hours after removal of 70% of the liver.

2. At 10 min after intracaval injection of \(^{14}\)C-leucine, 3.5% of total protein radioactivity was found in albumin in normal liver, whereas in regenerating liver only 1.4% of the total protein radioactivity was incorporated into albumin.

3. Albumin concentration in the serum decreased from 29.2 mg of albumin per ml of serum for normal rats to a minimum of 17.3 mg of albumin per ml of serum at 4 days after partial hepatectomy.

4. The half-life of albumin was 2.66 days for normal rats and 2.13 days for animals, 48 hours after partial hepatectomy.

5. The intravascular pool of albumin decreased from 100 mg of albumin per 100 g, body wt, in normal rats to 57.8 mg of albumin per 100 g, body wt, in partially hepatectomized animals, 48 hours after operation. The extravascular and the total body pool of albumin also decreased after partial hepatectomy to a minimum at 24 hours after the operation. In contrast to the intravascular pool, the extravascular and the total body pool increased again, reaching a plateau between 2 and 4 days after the operation.

6. During regeneration, the proportion of leucine to other amino acids in total liver protein did not change. Also, this proportion did not differ significantly from that found in serum albumin.

7. The net rate of albumin synthesis changed only slightly from 20.1 mg of albumin per day per g of liver for normal to 23.9 mg albumin per day per g of liver for regenerating liver 48 hours after partial hepatectomy. In contrast, the net rate of synthesis of total liver protein increased from 576 mg protein per day per g of liver in normal rats to 1710 mg of protein per day per g of liver in rats 48 hours after partial hepatectomy.

The synthesis of serum albumin is one of the characteristic functions of normal liver (1). Synthesis or breakdown (or both) of albumin seems to be regulated effectively since albumin concentration in the blood is kept constant within narrow limits.

The concentration of albumin in blood decreases after partial hepatectomy (2, 3). This decrease may possibly cause a change in the rate of albumin synthesis in regenerating liver. Therefore, the incorporation of amino acids into serum albumin has been studied repeatedly in regenerating rat liver. An increase (4-7), a decrease (6, 7), and no change at all (6, 8, 9) of amino acid incorporation into albumin have been reported. However, no appropriate corrections for changes of precursor, intermediate, and product pools were made in the interpretation of these experiments and no attention was given to the radiochemical purity of the incorporation product. However, these considerations are imperative for the correct interpretation of incorporation experiments with specific proteins. Inconsistencies in the experimental results can occur if the incorporation products are not radiochemically pure.

Recently, a method has been described for the isolation of radiochemically pure serum albumin from tissue homogenates (10). This paper describes its application to the regenerating rat liver system. Data on the alterations of the kinetics of serum protein secretion and changes of intravascular, extravascular, and total body pools of albumin are also reported. From the obtained values, net synthesis rates of albumin and total liver protein during regeneration were calculated.

MATERIALS AND METHODS

Animals, Operations, and Injection Procedures—Buffalo rats were bred in our laboratory. They were kept in rooms of 20° with constant humidity, light from 9 a.m. to 9 p.m., and dark from 9 p.m. to 9 a.m. They had free access to water and a diet
containing between 18.1 and 19.6% protein (Altromin R of the Altrumn GmbH, Lage-Lippe, Germany). For all experiments only male rats with a body weight of 220 to 300 g were used. Partial hepatectomies were performed between 9 a.m. and 1 p.m. according to the technique described by Higgins and Anderson (11), removing, on the average, 70% of the liver. Radioactive leucine was injected into the caval vein after opening the abdomen by a median incision. Blood samples were taken from the femoral veins, except for the first sample, which was taken from the caval vein 10 min after the injection of radioactive leucine. The animals were kept under ether anesthesia during the operation. Ether anaesthesia does not influence the incorporation of amino acids into albumin and total liver protein (5, 12).

Chemicals, Isotopes, and Determination of Radioactivity—All chemicals used in counting media, buffers, and standard solutions were of scintillation or analytical grade. The polyacrylamide gels were prepared from chemicals bought from Fluka AG (Chemische Fabrik, Buchs, SG, Switzerland). Bovine serum albumin (highest grade) was purchased from Behringwerke AG (Marburg-Lahn, Germany), Evans blue from E. Merck AG (Darmstadt, Germany), and Whatman DEAE-cellulose (DE 32, microgranular) from H. Reeve Angel and Company (London, E.C. 4, United Kingdom). T-l-14C-leucine (59 mCi per mmole), 98% pure, was purchased from the Radiochemical Centre (Amersham, United Kingdom). Standard 14C-toluene was bought from New England Nuclear Corporation (Boston, Massachusetts).

Radioactivity in protein was determined according to Mans and Novelli (13). The absolute counting efficiency, $\eta$, varied with the amount of protein applied to the filter paper disks. The relationship between $\eta$ and the amount of protein applied onto the disks is shown in Fig. 1 for serum protein. It can be approximated by the regression line, $\eta = 0.75 - 0.004$ (milligrams of protein per disk). Determination of radioactivity was performed in Nuclear Chicago liquid scintillation counting systems, type Mark 1, with 0.3% 2,5-diphenyloxazole and 0.03% p-bis[2-(5-phenyloxazolyl)]benzene in toluene as scintillation medium.

Protein Determination—The biuret method (14) with bovine serum albumin as standard was generally used to measure protein concentration. In the more highly purified albumin preparations isolated from liver, protein concentrations were determined by a modified turbidimetric method (15) with rat serum albumin as standard and 10% trichloroacetic acid as precipitating agent. Turbidity was measured at 366 m$\mu$ after incubating for 15 min at room temperature.

Immunological Procedures—For use as antigen, rat serum albumin was purified as described previously (10). The preparation of antiserum against rat serum albumin is published elsewhere (16, 17). The procedure of Mancini, Carbonara, and Heremans (18) was used for the quantitative immunological determination of albumin.

Purification of Albumin from Tissue Homogenates—The purification of albumin from tissue homogenates to constant specific radioactivity was described previously (10). The homogenization medium contained 0.15 m nonradioactive leucine. In the preparative disc electrophoresis on polyacrylamide gel at pH 10.3, 0.05 m Tris-glycine, pH 8.9, 0.1 m Tris-HCl, pH 8.1, 0.05 m Tris-PO$_4$, pH 7.2, and 0.4 m Tris-HCl, pH 8.1, were used as upper, lower and elution, sample, and membrane holder buffer, respectively. At pH 2.7, upper and sample buffer, lower and elution buffer, and membrane holder buffer were 0.005 m acetate-glycine, pH 4.0, 0.075 m acetate-potassium, pH 4.3, and 0.3 m acetate-potassium, pH 4.3, respectively.

Preparation of l4C-Albumin from Serum for Turnover Studies—Injections of 47 $\mu$Ci of L-14C-leucine, 59 mCi per mmole, per 100 g of body weight, were made into the portal vein of 10 rats. After 90 min, 3- to 5-ml portions of blood were taken from the caval vein and immediately replaced by 5 ml of 0.9% NaCl solution. This procedure was repeated about six to eight times until most of the blood was removed from the animal. Serum was obtained by coagulation. Protein concentration was 17 mg per ml. All of the following steps of the purification were performed at 0° to +2°. Solid ammonium sulfate was then added to the serum to a saturation of 54% using the nomograph of Di Jeso (19). After centrifugation, the precipitate was discarded and the supernatant was brought to 72% saturation with ammonium sulfate. The pH was kept at 7.6 by adding small amounts of 10% NH$_4$OH. The resulting precipitate was collected by centrifugation, dissolved in 14 ml of 0.1 m Tris-HCl, pH 7.6, and applied to two columns (2.5 x 91.5 cm) filled with Sephadex G-100. Elution was performed with 0.1 m Tris-HCl, pH 7.6, at a flow rate of 1 ml per min per column. The albumin-containing fractions were combined (220 ml, 1.3 g of protein) and purified further on a column (2.5 x 90 cm) of DEAE-cellulose, precooled with 0.5 x HCl and 0.5 x NaOH, and equilibrated with 0.01 m Tris-HCl, pH 7.6. After applying the sample to the column and washing with 0.15 m Tris-HCl, pH 7.6, albumin was eluted with a gradient of 0.15 m to 0.3 m Tris-HCl, pH 7.6. Concentration of albumin, radioactivity in protein and optical density at 280 m$\mu$ were determined in the eluate. The albumin-containing fractions were combined. Albumin was found to be radiochemically pure when tested by electrophoresis on polyacrylamide gel as described previously (10). The final yield was 546 mg of albumin from a specific radioactivity of 27,100 dpm per mg of albumin.

Determination of Serum Volumes with Evans Blue—Injections of 0.125 mg of Evans blue in 0.5 ml of 0.15 m NaCl were made into the caval vein. After 5, 10, and 15 min, blood was taken from the caval vein. The serum was removed after clotting, and its optical density at 623 m$\mu$ was measured. Serum volumes were calculated from the dilution of the Evans blue solution in the blood of the animal. Only minor differences were found for the serum volume measured after 5, 10, and 15 min. Hence,
in later experiments, blood was taken from the animals between 5 and 10 min only.

**Amino Acid Analysis**—Total liver protein was precipitated with 0.9 N perchloric acid and washed once with 0.6 N, and twice with 0.45 N perchloric acid. The precipitates were hydrolyzed under vacuum at 110° in 6 N HCl in sealed vials for 24, 48, 72, and 96 hours. After removal of HCl by evaporation to dryness, amino acids were dissolved in 0.3 M lithium citrate buffer of pH 2.2. Analyses were carried out with a Calbiochem amino acid analyzer, model BC 200, at a temperature of 35° using the buffer system of Benson, Gordon, and Patterson (20).

**Statistical Calculations**—Analyses of variance and F tests were performed as described by Haseloff and Hoffmann (21) and by Natrella (22). As far as possible, programs for the Olivetti desk computer Programma 101 were used for calculations.

**RESULTS AND DISCUSSION**

**Alterations of Kinetics of Serum Protein Secretion after Partial Hepatectomy**—After intravenous injection of radioactive amino acids, labeled serum protein appeared in the blood with a lag caused by the transport of the amino acids into the cell and by the synthesis and transport of serum protein through the cell (5, 10, 23–27). A rearrangement and replacement of the endoplasmic reticulum takes place during the regeneration of the liver after partial hepatectomy (for review see Reference 28). Since the endoplasmic reticulum is involved in the transport of serum protein through the liver cell (24, 29) the rearrangement of the membrane system may influence the lag time between injection of labeled amino acid and the secretion of radioactive serum proteins. Therefore, we measured the time between injection of radioactive amino acids and appearance of labeled protein in the blood ("secretion time" = time for synthesis and intracellular transport) at several time points after partial hepatectomy.

Intravenous injections of 5 μCi of L-14C-leucine per 100 g body wt, were given to seven different groups of three rats each, at 2, 4, 8, 16, 32, 48, and 72 hours after partial hepatectomy. Blood samples were withdrawn from the caval veins at various times after injection and the specific radioactivity of protein in the serum was determined. The specific radioactivity of the protein in the serum was plotted against time after injection for each group of partially hepatectomized rats and for a nonhepatectomized control group. The curves for the control group and for the 48-hour group are shown in Figs. 2 and 3. The curves for the other time points are not shown. They had shapes similar to those shown in Figs. 2 and 3 with intermediate slopes and secretion times. From the obtained curves, the slopes at 25 min after injection were averaged and plotted against time after partial hepatectomy (Fig. 4). The rate of increase of the specific radioactivity of serum protein dropped to a minimum at 4 hours and reached a maximum at 48 hours after partial hepatectomy.

The time between injection of the labeled amino acid and the appearance of radioactivity in protein in the serum was also averaged and plotted against the time after partial hepatectomy (Fig. 5). There was a steady decrease of the secretion time, reaching a minimum of about 10 min at 48 hours after hepatectomy.

The minimum of the secretion time at 48 hours after hepatectomy was not due to earlier detectability of radioactive protein in the serum caused by higher amounts of radioactivity in protein. This can be demonstrated by measuring secretion times for conditions with increased labeling of serum protein. Increased rates of serum protein labeling were produced by injection of larger amounts of 14C-leucine or by inducing nephrosis in the animals (Table I). Under these conditions, no alteration of the secretion time could be detected, although the rate of labeling of protein in the serum was increased by a factor of 3.6 or 4.7. Partial hepatectomy led only to a 2.9-fold increase in the rate of protein labeling in the serum. However, the secretion time was markedly reduced to about 10 min.

**Ratio of Albumin Synthesis to Total Protein Synthesis in Regenerating Rat Liver**—As discussed above, the incorporation of amino acids into protein is influenced by alterations of precursor and product pools. To evaluate net rates of synthesis of pro-
Between the arginine pool functional in protein synthesis and that functional in the urea cycle (36). Furthermore, it cannot be

changes in the pools of albumin and urea within the body (35).

perfused liver systems, but it is complicated in vivo by possible

proteins (32-34). It gives satisfactory results for studies with

liver has been questioned recently because of the low arginine

standards for the actual amino acid concentration within the

pools. The chemically determined amount of amino acids in

Rats, 48 hours after partial hepatectomy. Each point is derived from the curves for three animals as described in Figs. 2 and 3.

FIG. 5 (center). Time between injection of 14C-leucine and appearance of radioactive protein in the serum of normal (zero

point) and partially hepatectomized rats. Each point is derived from the curves for three animals as described in Figs. 2 and 3.

FIG. 6 (right). Albumin concentration in the serum of partially hepatectomized rats. The number of animals for each point is
given in parentheses. The horizontal dashes indicate the standard error of the mean.

TABLE I

Time between injection of 14C-leucine and appearance of radioactive protein in blood and rate of increase of specific radioactivity of protein in serum at 25 min after injection

The average value for three animals is presented in each group. The injected amount of 14C-leucine is given in parentheses. Nephrosis was produced by injection of the aminonucleoside of puromycin as described in Reference 17.

Animal group | Time between injection of 14C-leucine and appearance of labeled protein in blood | Rate of increase of specific radioactivity of protein in serum, 25 min after injection of 14C-leucine

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Time between injection of 14C-leucine and appearance of labeled protein in blood</th>
<th>Rate of increase of specific radioactivity of protein in serum, 25 min after injection of 14C-leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats (5 μCi of L-1-14C-leucine per 100 g, body wt.)</td>
<td>min</td>
<td>dpm/mg/min</td>
</tr>
<tr>
<td>Normal rats (20 μCi of L-1-14C-leucine per 100 g, body wt.)</td>
<td>15-16</td>
<td>50</td>
</tr>
<tr>
<td>Nephrotic rats (12.5 μCi of L-1-14C-leucine per 100 g, body wt.)</td>
<td>15</td>
<td>183</td>
</tr>
<tr>
<td>Rats, 48 hours after partial hepatectomy (5 μCi of L-1-14C-leucine per 100 g, body wt.)</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Proteins from incorporation experiments with labeled amino acids, it is necessary to consider changes of the functional precursor pools. The chemically determined amount of amino acids in the blood or in the liver after partial hepatectomy (30) may not be identical with the functional precursor pool. An intracellular standard for the actual amino acid concentration within the cell, and, if possible, for the amino acid pool functional in protein synthesis, is therefore desirable. The 14CO2-14C-arginine method was devised for this purpose (31) and used for plasma proteins (32-34). It gives satisfactory results for studies with perfused liver systems, but it is complicated in vivo by possible changes in the pools of albumin and urea within the body (35).

Furthermore, the applicability of this method to regenerating liver has been questioned recently because of the low arginine level in this organ, which may not permit full equilibration between the arginine pool functional in protein synthesis and that functional in the urea cycle (36). Furthermore, it cannot be

excluded that only some of the liver cells are synthesizing albumin, as suggested by immunochemical detection of albumin in only part of the liver cells (37-40). Finally, direct arginylation of albumin may simulate de novo biosynthesis of albumin. An enzyme transferring arginine quantitatively from arginine-tRNA to albumin without involving ribosomes or other parts of the protein synthesizing system has been isolated from rabbit liver (41, 42).

Recently, the purification of albumin from tissue homogenates to constant specific radioactivity has been described (10). The ratio of albumin synthesis to total protein synthesis in the liver can be calculated from the concentration of albumin and protein in the homogenates and their specific radioactivities, provided that the livers were removed within the secretion time, i.e. that no labeled albumin had left the liver, and that albumin synthesis was completed. This method produces data which are independent of all variations in precursor pools. Table II summarizes the results obtained for the purification of albumin from 8 normal and 16 regenerating rat livers, 48 hours after hepatectomy. Only 0.93% of the protein of regenerating liver homogenate was albumin, whereas in normal liver homogenate, 1.6% of total protein consisted of albumin. For the preparation from regenerating liver the separation was sharper, but was offset by a greater loss of albumin. However, the number of purifications carried out by us on both kinds of material is not yet large enough to decide if this difference still lies within experimental variability. Purity as regards protein was reached for regenerating liver after chromatography on Sephadex G-100, and for normal liver after the first chromatography on DEAE-cellulose. Constant specific radioactivity was obtained after the second chromatography on DEAE-cellulose for regenerating liver, and after preparative electrophoresis on polyacrylamide gel at pH 10.3 for the preparation from normal liver. One has to conclude that the eliminated radioactive material was either a protein of very high specific radioactivity or that radioactive leucine which had been bound very tightly to protein was removed.

The specific radioactivities found for albumin (for regenerating rat liver this is the average of the last three purification steps) were then multiplied by the amount of albumin in the homogenate and divided by the radioactivity incorporated into total protein of the homogenates. Thus, in normal liver, 3.5%
of all leucine incorporated into total protein was found in albumin. In regenerating rat liver the ratio of the incorporation into albumin to that into total protein was only 1.4%. Total radioactivity (protein and nonprotein) was \(2.68 \times 10^6\) dpm per ml for the homogenate from normal, and \(4.7 \times 10^4\) dpm per ml for the homogenate from regenerating liver.

**Albumin Concentration in Serum after Partial Hepatectomy**—As shown in Fig. 6, the concentration of albumin in the serum of partially hepatectomized rats decreased from 29.2 mg per ml for normal animals to a minimum of 17.3 mg albumin per ml of serum 4 days after the operation.

**Changes of Intravascular Albumin Pool after Partial Hepatectomy**—The labeling of protein in the serum after injection of \(^{14}\text{C}-\text{leucine}\) is influenced by the size of the leucine pool in the blood, the permeability of the cell membranes for leucine, the intracellular leucine pool, the activity of leucine-tRNA synthetases, the tRNA pool, and the intravascular serum protein pool. Conclusions on the synthesis of serum protein in the regenerating liver have been made from the rate of incorporation of radioactive leucine into protein in the serum (5), assuming that the size of the leucine pool did not change during the regeneration of the liver (30). The possibility of a change in the protein pool in the serum was not considered either (5). Such a change is, however, very likely to occur, due to the loss of blood during the operation and due to the removal of 70% of the liver. A decrease of the protein pool in the serum would lead to an increase of specific radioactivity of protein in the serum, even if its synthesis in the liver was not altered.

The intravascular pool of albumin was therefore determined before and 1, 2, and 4 days after partial hepatectomy by measuring the dilution of Evans blue or of radiochemically pure \(^{14}\text{C}-\text{albumin}\) injected into the caval vein of normal rats and of partially hepatectomized rats 24, 48, and 96 hours after operation. Blood samples were then withdrawn from the femoral veins at different times after injection. The 4 groups of animals consisted of 10 or 11 rats each. After conglutination, albumin concentration and protein radioactivity were determined in the serum and the specific radioactivity of albumin was calculated. The mean values of the natural logarithms of the specific radioactivity of albumin for each time point were plotted against time after injection of \(^{14}\text{C}-\text{albumin}\). The curves obtained for normal animals and for rats 48 hours after partial hepatectomy are shown in Figs. 8 and 9. The curves obtained for rats 24 hours and 96 hours after partial hepatectomy (not shown here) had a similar shape. In an analysis of variance of the data, the variation within each time class was compared with the variation of the means from a hypothetical regression line calculated for the decline of the natural logarithm of the specific radioactivity of albumin between 2 and 12 days after injection. According to
the performed $F$ test, these regression lines were valid on the
level $p = 0.05$. The mathematical characteristics of the regres-
sion lines obtained for the four animal groups are summarized
in Table III. $t_1$ of albumin could be determined easily by sub-
tracting $\ln 2$ from the intercept of the regression lines with the
ordinate. $t_1$ was slightly shorter for partially hepatectomized
rats than for normal animals. The value obtained for normal
rats was, however, significantly higher than the 1.83 days
reported by Sellers et al. (43) for $t_1$ of $^{131}I$-albumin. The difference
between the value reported in this paper and the value published
by Sellers et al. (43) is even more remarkable if the protein con-
tent of the diet is regarded. It was between 18.1 and 19.6%
for the animals described in this paper and 0% for the rats
studied by Sellers et al. (43). A decrease in protein content of
the diet was reported to lead to an increase of the half-life of
serum protein (44) or of albumin (45, 46). Thus, a shorter half-
life of albumin should be expected in our studies from the re-
ciprocal interrelationship between protein content of the diet
and half-life of serum protein. The opposite was observed.
An explanation may be that, in the experiments discussed in this
paper, albumin was labeled by introduction of $^{14}C$-leucine into
albumin through biosynthesis in vivo, whereas in the experiments
of Sellers et al. (43), albumin was labeled by iodination with
$^{131}I$ according to the method of McFarlane (47). It is likely that
the rate of removal of $^{131}I$ from $^{131}I$-albumin is not identical with
the rate of breakdown of $^{14}C$-albumin. To remove denatured or
overiodinated molecules, $^{131}I$-labeled albumin is usually
"screened" by injection into rats which are bled thereafter (48).
However, it may be possible that screening for 2 days (43) or 3
days (49) is not adequate for experimental periods of 7 days
(43).

**Extravascular and Total Body Pool of Albumin**—The total
body pool of albumin was calculated from the intercept with the
ordinate of the extrapolated linear regions of the curves sum-
marized in Table III. According to Sellers et al. (43), this method,
which was described previously by Sterling (50) and
Berson et al. (51), gives more reliable results than the kinetic
analysis method proposed by Matthews (52) or the equilibrium
method of Campbell et al. (33). The extravascular pool was
calculated by subtracting the intravascular pool from the
total body pool. In Fig. 7, the obtained values are plotted
as a function of time after partial hepatectomy. The total body
pool decreased abruptly after the operation, reaching a minimum
at 24 hours after partial hepatectomy, increasing again at 48
hours, and changing only insignificantly between the 2nd and
the 4th day after operation.

**Absolute Amount of Synthesized Albumin and Total Liver Pro-

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**Table III**

Mathematical characteristics of regression lines describing the de-
cline of specific radioactivity of albumin in serum after intrave-
rous injection of $^{14}C$-albumin into normal rats (Fig. 8),
and into rats partially hepatectomized 3 days (Fig.
9), 1 day, or 4 days (not shown in figures) before
injection.

<table>
<thead>
<tr>
<th></th>
<th>Normal rats</th>
<th>Partially hepatectomized rats at time after operation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>2 days</td>
</tr>
<tr>
<td>Intercept with the ordinate</td>
<td>6.46</td>
<td>6.24</td>
</tr>
<tr>
<td>Slope (day$^{-1}$)</td>
<td>-0.261</td>
<td>-0.338</td>
</tr>
<tr>
<td>$t_1$ (days)</td>
<td>2.66</td>
<td>2.10</td>
</tr>
</tbody>
</table>

**Table IV**

Relative liver weight, albumin synthesis, and synthesis and turnover
of total protein in normal and in partially hepatectomized rats

<table>
<thead>
<tr>
<th></th>
<th>Normal rats</th>
<th>Partially hepatectomized rats at time after operation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 days</td>
</tr>
<tr>
<td>(mg synthesized albumin)/(day.100 g, body wt)</td>
<td>90.7</td>
<td>81.6</td>
</tr>
<tr>
<td>(g liver)/(100 g, body wt)</td>
<td>1.4</td>
<td>3.1</td>
</tr>
<tr>
<td>(mg synthesized albumin)/(day.g liver)</td>
<td>20.1</td>
<td>23.9</td>
</tr>
<tr>
<td>(albumin synthesis)/(total protein synthesis)</td>
<td>0.035</td>
<td>0.014</td>
</tr>
<tr>
<td>(mg synthesized total protein)/(g liver-day)</td>
<td>576</td>
<td>1710</td>
</tr>
<tr>
<td>$t_1$ of total liver protein (hours)</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>
Since albumin turnover and total body pool did not change significantly in the partially hepatectomized rats between the 2nd day and the 4th day after operation (Table III and Fig. 7), we assumed that steady state conditions existed both for normal and regenerating liver, the animal 20.1 mg of albumin per g of liver per day, the difference between the values being within the limits of the variation of our method.

Rat serum albumin contains 59 moles of leucine per mole of albumin or 11.4 g of leucine per 100 g of albumin (54). The average leucine content of 29 animal proteins, taken from the tables of Tristram and Smith (55), is 10.3 ± 0.7 g of leucine per 100 g of protein. In an amino acid analysis of the total protein of normal and regenerating liver, we found the values shown in Table V. Leucine comprised 12% of the amino acids in total protein of normal and 13% of the amino acids in total protein of regenerating liver. The average content of leucine being the same for both tissues, it may be legitimate to compare the rates of synthesis of albumin and total protein on the basis of \(^{14}C\)-leucine incorporation. If 20.1 mg of albumin are synthesized per g of liver per day, the difference between the values being within the limits of the variation of our method.

In regenerating liver, 48 hours after partial hepatectomy, 23.9 mg of albumin and 1710 mg of total protein were synthesized per g of liver per day, the ratio of albumin to total protein synthesis being 1.4% (Table IV). Thus, the synthesis of serum albumin per g of liver had increased only insignificantly, whereas total protein synthesis was increased about 3-fold. The decreased concentration of albumin in the blood of partially hepatectomized rats is therefore not an adequate trigger for an increase of its synthesis in regenerating liver.

### Table V

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Normal liver</th>
<th>Regenerating liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>128.0</td>
<td>114.0</td>
</tr>
<tr>
<td>Glutamate</td>
<td>141.0</td>
<td>158.0</td>
</tr>
<tr>
<td>Proline</td>
<td>87.2</td>
<td>89.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>123.0</td>
<td>124.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>131.0</td>
<td>132.0</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>113.0</td>
<td>110.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>40.2</td>
<td>52.4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>81.0</td>
<td>106.0</td>
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<tr>
<td>Leucine</td>
<td>131.0</td>
<td>134.0</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>52.3</td>
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</tr>
<tr>
<td>Phenylalanine</td>
<td>77.5</td>
<td>76.6</td>
</tr>
</tbody>
</table>

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The Secretion of Serum Protein and the Synthesis of Albumin and Total Protein in Regenerating Rat Liver
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