Synthesis and Secretion of Rat Albumin \textit{in Vivo}, in Perfused Liver, and in Isolated Hepatocytes

EFFECTS OF HYPOPHYSECTOMY AND GROWTH HORMONE TREATMENT

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The effects of hypophysectomy on albumin and total protein synthesis in rat liver were investigated \textit{in vivo}, in perfused liver, and in isolated hepatocytes. In all systems, hypophysectomy resulted in about a 50% decrease in the rate of total protein synthesis and a 30 to 50% decrease in the relative rate of albumin synthesis. Albumin synthesis accounted for 11 to 13% of total protein synthesis in all normal systems, but represented only 5 to 8% of the total in all systems derived from hypophysectomized rats. Growth hormone, administered subcutaneously to hypophysectomized rats for 5 days, restored the relative rate of albumin synthesis to normal \textit{in vivo}; however, only partial restoration was demonstrated in the \textit{in vitro} systems. Perfused livers and isolated hepatocytes exhibited linear rates of total protein and albumin secretion for 3 h. The rate of albumin secretion by normal perfused livers was 3 times that of perfused livers from hypophysectomized animals, being 0.54 and 0.17 mg/g of liver/h, respectively. Isolated hepatocytes synthesized total protein and albumin at nearly the same rate as perfused livers. The amount of albumin secreted by cells derived from normal and hypophysectomized rats was 0.38 and 0.10 mg/ml of packed cells/h, respectively. Ribosome half-transit times for albumin and total protein were 1.6 to 1.7 min in isolated liver cells derived from both normal and hypophysectomized rats. Analysis by polyacrylamide gel electrophoresis showed no difference in the qualitative distribution of the proteins secreted by perfused livers and isolated hepatocytes.

Hypophysectomy causes marked alterations in the delicate endocrine balance of the animal that result in many primary and secondary hormonal effects on protein metabolism in the liver. Attempts to understand the biochemical alterations in the liver following hypophysectomy mainly have involved studies on the whole animal or on disrupted and fractionated liver tissue removed from animals following various hormonal treatments. On the basis of these studies, it is well established that hypophysectomy reduces liver growth and the synthesis of hepatic protein and that these defects are corrected by treatment of animals with growth hormone (1, 2). Early studies also established that hypophysectomy reduces albumin synthesis and degradation, and that growth hormone therapy can restore the rates of albumin turnover to normal (3). More recent studies have confirmed that hypophysectomy reduces hepatic protein synthesis and have suggested that amino acid availability may be involved (4, 5). The net synthesis of several plasma proteins is decreased in the perfused liver of the hypox rat (6), but the reason for this effect has not been established.

In the studies presented here, the perfused rat liver and isolated rat hepatocytes were used to investigate the effects of hypophysectomy and growth hormone treatment on total protein and albumin synthesis. Results obtained in the perfused liver and isolated hepatocytes were compared with those obtained \textit{in vivo} in order to establish the reliability of the \textit{in vitro} systems. Rates of synthesis of total protein and albumin were determined in the presence of 5 or 10 mM \textit{L}-[\textit{U-14C]}leucine, a condition which expands the intracellular pool of leucine and maintains the specific activity of intracellular leucine at the same level as that of the extracellular amino acid. This approach minimized problems associated with compartmentation and reutilization of intracellular amino acids and provided a means of determining the actual rates of synthesis. The results show that both \textit{in vitro} systems accurately reflect the effects of hypophysectomy and growth hormone treatment on total liver protein and albumin synthesis. Each \textit{in vitro} system provides certain advantages for investigations of albumin metabolism. The perfusion technique allows the liver to be isolated from the influences of the whole animal and its circulation precisely controlled. The isolated hepatocytes provide a specific cell type in which detailed molecular events can be thoroughly examined. Together, the two systems are complementary in their usefulness for studies on the mechanisms of hormonal control of albumin synthesis and secretion.

**EXPERIMENTAL PROCEDURES**

\textit{Animals}—Normal and hypophysectomized male Sprague-Dawley rats were purchased from Charles River Breeding Laboratories (Wilmington, Mass.). Animals weighing 225 to 250 g were hypophysectomized by the pharyngeal approach and were used in experiments 3 to 6 weeks postoperatively, at which time they weighed 180 to 220 g. All rats were maintained on a 12 h light-12 h dark cycle and were supplied with water and Purina rat chow \textit{ad libitum}.
Hormone Treatment — Where indicated, normal and hypophysectomized rats received daily subcutaneous injections of bovine growth hormone (NIH-GH-B18) at a dose of 0.5 mg/100 g of body weight for the 5 days preceding an experiment. The hormone was dissolved in 0.9% NaCl at pH 9.

Cell Perfusion — Livers were perfused in situ at 37-38° by the technique described in detail by Erton (7). The basic perfusion medium consisted of Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 10 mM glucose, 3% bovine serum albumin (Miles Laboratories, Elkhart, Ind., Fraction V), and sufficient bovine erythrocytes to give a hematocrit of 24 to 26%. The red blood cells were washed three times with 0.9% NaCl and twice with the bicarbonate buffer. The albumin was dissolved in saline and adjusted to pH 7.4 prior to addition to the buffer. The buffer containing albumin was passed through a Millipore filter (pore size, 3 μm) prior to addition of the red blood cells. Ammonium chloride were added at 1 to 2.5 times their concentration in normal rat plasma (8). Other additions to the perfusate are described in the legends. The perfusate was either recirculated or passed through the liver a single time at a flow rate of 7 ml/min. In order to maintain liver weight parity, normal rats were used at 110 to 135 g and hypophysectomized rats at 180 to 220 g. Other experiments showed that the effects of hypophysectomy on total protein and albumin synthesis were the same regardless of whether age-matched controls or liver weight controls were used. At the end of perfusion, livers were rapidly excised, blotted, and frozen in a Wollenberg clamp at the temperature of liquid nitrogen. Liver samples were homogenized in 3 volumes of 0.25 M sucrose at pH 9. The homogenate contained 7.5 times the normal plasma levels of amino acids. The cell membranes were counted as nonviable even if they excluded the dye. In addition any hepatocytes which appeared to have damaged cell membrane (including erythrocytes) were removed by aspiration along with the top 10% which contained large clumps of cells, with 20 volumes of 0.9% NaCl containing 10 mM unlabeled leucine. Samples were subjected to electrophoresis at pH 8.9 on 7.5% polyacrylamide gels (Eastman). Gels, 9 cm in length, were cut into 2-mm slices and assayed in duplicate for radioactivity. Background nonspecific adsorption was measured by adding hen ovalbumin to separate aliquots of the homogenates followed by immunoprecipitation with rabbit antiovalbumin. The ovalbumin immunoprecipitate contained less than 1% of the total radioactivity in protein. For intracelluar experiments, livers were perfused for 12 min with medium containing [3H]leucine at 2.5 μCi/ml. Livers were quickly excised, frozen, and stored at -70° until analyzed. Hepatocyte suspensions were incubated for 12 min with medium containing [3H]leucine at 10 μCi/ml. At each time point, the media were aspirated, 3 volumes of ice cold 0.25 M sucrose were added, and the samples were homogenized and prepared as above. Intragacellular albumin synthesis was expressed as a percentage of total protein synthesis.

Rate of Albumin Secretion — Perfused livers and isolated hepatocytes were exposed for 1.5 to 2 h to media containing leucine at a concentration of either 5 or 10 mM and [3H]leucine at 7.5 to 50 μCi/ml. At either concentration of leucine, the intracellular and extracellular levels of the amino acid were equal as determined by autonimated amino acid analysis (8). Furthermore, the specific activity of intracellular leucine was equal to that of the extracellular amino acid at either leucine concentration. These conditions minimized problems associated with compartmentation and reutilization of amino acids by expanding the intracellular pool of leucine. Mortality rate of hepatocytes which appeared to have damaged cell membranes was determined using a dye which excluded damaged hepatocytes. In most experiments, all hepatocytes which appeared to have damaged cell membranes were exposed for 1.5 to 3 h to media containing leucine at a concentration of 100 μmol. Two concentrations of valine (5 to 10 mM) in the perfusate, the specific activity of intracellular and extracellular valine are equal and the ratio of the specific activities of valyl-tRNA and extracellular valine is 0.96. Thus, the specific activity of valine in the precursor pool for intracellular synthesis is approximately the same as that of the extracellular valine. Under these conditions, this permits a determination of the actual rate of synthesis. Leucine, another branched chain essential amino acid which is not metabolized to any substantial extent by liver (10), appears to be as suitable as valine for determining the rate of protein synthesis in liver. To determine the rate of synthesis of secreted proteins, aliquots of the perfusate or cell incubation medium were removed at 20- to 30-min intervals. The secreted albumin was determined by immunoprecipitation as described above. Total secreted protein was determined by trichloroacetic acid precipitation. Inappropriate controls were included and sample volume corrections made when necessary.

Gel Electrophoresis of Secreted Proteins — Free [3H]leucine was removed from media and perfusate samples by dialysis at 4° against 0.9% saline containing 10 mM unlabeled leucine. Samples were rinsed at 37° with 8.9 g on 7.5% polyacrylamide gels which had been cross-linked with 1.2% N,N'-diallyltartardimide (Eastman). Gels, 9 cm in length, were cut into 2-mm slices and dissolved in 0.5 ml of 2% periodic acid for 30 min at room temperature. Following dissolution, 10 ml of 1% 2-ml scintillation mixture (Yorktown Research, S. Hackensack, N. J.) were added and radioactivity was measured.

Statistical Analyses — The data are presented as the mean of a given number of observations ± S.E. Differences between averages were tested for statistical significance by the Student’s t test. Differences where p values were greater than 0.05 were not considered to be significant. Data on albumin secretion rates were subjected to linear regression analysis.

RESULTS

Incorporation of [3H]leucine into total protein and albumin following a 10 to 12 min pulse was determined in vivo, in perfused liver, and in isolated hepatocytes (Table 1). For this time interval, albumin synthesis can be expressed as a percentage of total protein synthesis since newly synthesized albumin molecules are not secreted in less than 15 min (14), and the leucine content of albumin is approximately the same as that of total liver protein (15, 16). Albumin synthesis accounted for 11 to 13% of total protein synthesis in all normal systems. In each case, hypophysectomy reduced the relative rate of albumin synthesis to 5 to 8% of total protein synthesis.
Treatment of hypophysectomized rats with five daily injections of growth hormone restored the relative rate of albumin synthesis to normal in vivo, however only partial restoration was demonstrated in the in vitro systems. The perfused liver maintained constant rates of total protein and albumin synthesis during a 3 h period of study (Fig. 1). Hypophysectomy resulted in a decrease of about 40 to 60% in total protein synthesis. Furthermore, there was an additional specific decrease in the percentage of albumin synthesis (Table I). Thus, it can be estimated that on an equal weight basis, livers from hypophysectomized rats would only secrete 20 to 30% as much albumin as a normal liver. This finding was examined in detail in subsequent experiments.

Essentially, the same effects of hypophysectomy on total protein and albumin synthesis were demonstrated in isolated hepatocytes. The isolated hepatocytes, whether derived from normal cells and about 24 to 31% of total protein secretion in normal hepatocytes (Fig. 2). The nature and extent of protein secretion by the cells were investigated by continuous incubation in the presence of [3H]leucine. Immunoprecipitation analysis showed that albumin accounted for 31 to 41% of total protein secretion in normal cells and about 24 to 31% of total protein secretion in cells derived from hypophysectomized rats throughout the incubation period (Fig. 3). The proteins secreted by isolated hepatocytes were compared with liver perfusate by analysis with polyacrylamide gel electrophoresis at pH 8.9 as shown in

### Table I

<table>
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<th>Albumin synthesis as percentage of total protein synthesis</th>
<th>S.E.</th>
<th>%</th>
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<td><strong>In vivo experiments</strong></td>
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<tr>
<td>Normal</td>
<td>12.4 ± 0.6* (6)*</td>
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<tr>
<td>Hypox</td>
<td>8.4 ± 0.6 (7)</td>
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<tr>
<td>Hypox + growth hormone*</td>
<td>11.5 ± 0.6* (7)</td>
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<tr>
<td>Normal + growth hormone*</td>
<td>12.3 ± 0.3 (7)</td>
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<td><strong>Perfused liver experiments</strong></td>
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<tr>
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<tr>
<td>Hypox</td>
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<td>10.9 ± 0.5* (8)</td>
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<td><strong>Isolated hepatocyte experiments</strong></td>
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<td>Hypox + growth hormone*</td>
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* Differences from hypox by p < 0.01.
* Numbers in parentheses represent the number of separate determinations.
* Pretreatment with five daily injections of growth hormone at 0.5 mg/100 g of body weight.

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**Fig. 1.** Synthesis of total protein and albumin in the perfused liver. Livers of male Sprague-Dawley rats were perfused in situ as described under "Experimental Procedures." Each liver was perfused in a recirculating system with 300 ml of perfusion medium containing 3 times the plasma levels of amino acids until 12 min before each time point when the liver was switched to a flow-through system with medium containing normal plasma levels of amino acids and [3H]leucine at 2.5 μCi/ml. Following the 12-min pulse livers were excised and frozen in liquid nitrogen. The postmitochondrial supernatant fluids of detergent-treated homogenates were assayed for incorporation into total secreted protein and albumin as described under "Experimental Procedures." The height of the open bars represents incorporation into total protein and the shaded area incorporation into albumin. N, livers of normal rats; H, livers of hypophysectomized rats.

**Fig. 2.** Albumin synthesis as a percentage of total protein synthesis in isolated hepatocytes. Livers were prepared as described under "Experimental Procedures." The hepatocytes were incubated in medium containing 7.5 times the normal plasma levels of amino acids until 12 min before each time point when they were resuspended in medium containing normal plasma levels of amino acids and [3H]leucine at 10 μCi/ml. Following the 12-min incubation, cells were sedimented and homogenized at 2°C with 9 volumes of 50 m M Tris buffer, pH 7.6, containing 25 m M NaCl, 5 m M MgCl2, 1% Triton X-100 (v/v), and 1% sodium deoxycholate (w/v). The postmitochondrial supernatant fluid was assayed for incorporation into total protein and albumin as described under "Experimental Procedures." ○, hepatocytes from normal rats; ●, hepatocytes from hypophysectomized rats.

**Fig. 3.** Total protein and albumin secretion by isolated hepatocytes. Cells were prepared as described under "Experimental Procedures." and preincubated for 30 min in Krebs-Henseleit bicarbonate buffer containing 7.5 times the normal plasma levels of amino acids. Cells were transferred to 40 volumes of bicarbonate buffer containing normal plasma levels of amino acids and [3H]leucine at 10 μCi/ml. At each time point, aliquots of the suspension were centrifuged and assayed for incorporation into total secreted protein and albumin as described under "Experimental Procedures." ○, hepatocytes from normal rats; ●, hepatocytes from hypophysectomized rats.
Fig. 4. In each case, a typical fast migrating albumin peak, which co-migrates with authentic serum albumin, was found to be the major secretion product (around slices 25 to 29). No significant differences in the qualitative distribution of secreted proteins were observed between the perfused liver or hepatocytes from either normal or hypophysectomized rats.

Initial studies indicated that the amount of albumin secreted by liver tissue derived from hypophysectomized rats was reduced to about one-fourth of the normal amount. This finding was confirmed and quantitated in an additional series of experiments. Livers were perfused with either 5 or 10 mM L-[3H]leucine to expand the intracellular leucine pool and thereby minimize potential problems associated with amino acid uptake, compartmentation, and reutilization. The results presented in Fig. 5 demonstrate that hypophysectomy reduced the rate of albumin secretion by the perfused liver to about 25% of normal, indicating that alterations in specific activity of leucine in the precursor pool for protein synthesis did not account for the difference observed. Similar experiments were undertaken with the isolated hepatocyte suspensions. Again, hypophysectomy reduced the rate of albumin secretion to about 25% of normal (Fig. 6). Since the specific activity of the leucine incorporated into total protein and albumin was known along with the molecular weight and leucine content of rat albumin (17), it was possible to calculate the absolute protein synthetic rates (Table II). These data show that in perfused livers or isolated hepatocytes derived from hypophysectomized rats, the rate of total protein synthesis was approximately 50% of normal. However, the synthetic rate of secretory proteins, including albumin, was further reduced to about 30% of normal. Hepatocytes derived from growth hormone-treated hypophysectomized rats showed only a partial restoration of protein synthetic rates, indicating that additional factors are apparently required to maintain normal protein synthesis. The decrease observed in the production of liver protein in the hypophysectomized rat appeared to be compensated by an equivalent increase in the average half-life (Table II).

Following the addition of L-[3H]leucine to perfused livers or incubated hepatocytes, the newly synthesized radioactively labeled albumin required a delay period before secretion into the medium. Although L-[3H]leucine was detected as early as 15 min, a linear rate of accumulation required a slightly longer time (Table II). An additional 10 to 15 min increase in the albumin secretion time was consistently observed for perfused livers and isolated hepatocytes derived from hypophysectomized rats. This increase in secretion time did not result from a difference in the rate of uptake of labeled leucine, since equilibration of the intracellular leucine pool with extracellular leucine occurred in less than 2 min in hepatocytes derived from both normal and hypophysectomized rats (data not presented).

The isolated hepatocytes provided a means to determine whether or not the decrease in albumin synthesis following hypophysectomy was caused by a defect in peptide chain elongation. Determination of ribosome half-transit times by the method of Fan and Penman (18) for total protein and albumin in cells from normal and hypophysectomized rats revealed no significant differences in peptide elongation rates (Fig. 7).

**DISCUSSION**

This study demonstrates the suitability of the perfused liver and isolated hepatocyte systems for studies on the nature of
the defect in albumin metabolism which results from hypophysectomy. Both systems offer unique advantages for studies of albumin metabolism. The perfused liver permits an evaluation of the direct effects of hormones and other factors on the intact organ maintained under physiological conditions. The isolated hepatocytes provide a uniform population of parenchymal cells which can be incubated in an accurately defined medium. Both systems gave values for albumin synthesis as a percentage of total protein synthesis comparable to those obtained in vitro (Table I). Albumin synthesis in vivo, in perfused liver, and in isolated hepatocytes uniformly accounted for 11 to 13% of total protein synthesis in normal liver tissue. This finding is in good agreement with the value of 11% reported for in vivo measurements by Morgan and Peters (16) and Peters and Peters (19). Hypophysectomy reduced the relative rate of albumin synthesis to 5 to 8% in all systems, a value similar to that reported by Keller and Taylor (20) for in vivo measurements in hypophysectomized animals. There is, therefore, a 30 to 50% depression in the relative rate of albumin synthesis following hypophysectomy which can be demonstrated in both in vitro systems as well as in vivo. Growth hormone treatment of hypophysectomized rats restored the relative rate of albumin synthesis to normal in vivo. However, only partial restoration was demonstrated in perfused liver and isolated hepatocytes. This finding suggested that other factors involved in the regulation of albumin synthesis were modified in the in vitro systems.

In addition to the decreased relative rate of albumin synthesis, there was also a 45 to 50% reduction in total protein synthesis following hypophysectomy (Fig. 1). This observation was originally made by Korner in 1960 (21) who concluded that the "changes in the amount of amino acid incorporation into proteins could not be ascribed to hormonally controlled changes in the specific activity of the intracellular amino acid pool." More recently, Tolman et al. (8) established that there was no difference in the intracellular leucine pool nor in leucine uptake by the perfused livers of hypophysectomized rats compared to normals. Albumin secretion following hypophysectomy must, therefore, be the composite result of a 45 to 50% decrease in total protein synthesis and a 30 to 50% decrease in the relative rate of albumin synthesis.

The albumin secreted by perfused livers and isolated cells from normal and hypophysectomized rats was shown to be similar to normal serum albumin by electrophoresis on polyacrylamide gels at pH 8.9 (Fig. 4). The fact that the percentage of albumin secreted into the media relative to total secreted protein was similar when determined by the gel method (Fig. 4) or by immunoprecipitation (Figs. 3, 5, and 6) suggests that the albumin secreted by all systems was identical with respect to immunological, electrophoretic, and molecular weight characteristics.

Under the conditions of our experiments, perfused livers from normal rats secreted albumin at a rate of 0.54 mg/g of liver/h (Table II). This finding is in excellent agreement with the value of 0.47 mg/g of liver/h determined by John and Miller (22) and the value of 0.56 mg/g of liver/h determined by Marsh (23). In contrast, perfused livers from hypophysecto-

![Fig. 7. Determination of ribosome half-transit times (t₁/₂) in liver cells isolated from normal and hypophysectomized rats. Isolated hepatocytes were prepared as described under "Experimental Procedures." Following a 30-min preincubation, cells were transferred to 10 volumes of Krebs-Henseleit bicarbonate buffer containing normal plasma levels of amino acids and L[^3H]leucine at 25 μCi/ml. At each time point, aliquots of the suspension were homogenized at 2°C in 50 mM Tris buffer, pH 7.6, cycloheximide (10 μg/ml), Triton X-100 (1%, v/v), and sodium deoxycholate (1%, w/v). Aliquots of the postmitochondrial supernatant were centrifuged at 200,000 × g for 2 h to sediment polysomes. Portions of both the postmitochondrial (□ - □) and postribosomal (○ - ○) supernatants were assayed for incorporation into total protein (A, C) and albumin (B, D). The half-transit time is given in each panel with the standard error for four experiments with normal cells (A, B) and three with cells from hypophysectomized rats (C, D).

| Table II |
| Protein synthesis by perfused rat liver and isolated hepatocytes |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Secreted albumin | Total secreted protein | Total protein synthesis | t₁/₂ liver protein | Albumin secretion time |
| Perfused liver  |                 |                 |                 |                 |                  |
| Normal (4)     | 0.54 ± 0.02⁴    | 1.54 ± 0.06     | 4.25 ± 0.16     | 1.9             | 22-26            |
| Hypox (3)      | 0.17 ± 0.09     | 0.47 ± 0.05     | 2.19 ± 0.25     | 3.1             | 32-38            |
| Isolated hepatocytes |             |                 |                 |                 |                  |
| Normal (3)     | 0.38 ± 0.06     | 1.21 ± 0.19     | 3.28 ± 0.49     | 2.5             | 19-21            |
| Hypox (3)      | 0.10 ± 0.01     | 0.48 ± 0.05     | 1.82 ± 0.11     | 3.8             | 33-35            |
| Hypox + growth hormone (8) | 0.17 ± 0.01 | 0.75 ± 0.03     | 2.12 ± 0.08     | 3.8             | 24-30            |

⁴ Expressed as milligrams/g of liver/h for perfused liver and as milligrams/mL of packed cell/h for isolated hepatocytes.

Calculated according to 0.693 × mg of protein/g divided by total protein synthesis – secreted protein synthesis.

Mean ± standard error; the number of experiments are in parentheses.
mized rats secreted 0.17 mg of albumin/g of liver/h. Griffin and Miller (6) also noted a 3-fold reduction in the amount of albumin secreted by the perfused liver following hypophysectomy.

During incubation, the isolated hepatocytes were stable with respect to cell viability, total protein synthesis, and albumin synthesis. Less than a 5% decrease in cell viability was observed during a 3-h incubation. In contrast, others have reported as much as a 50% reduction in cell viability with insignificant albumin synthesis by 24 h of incubation (24). Assuming 1 ml of packed cells to be equivalent to 1 g of liver, the isolated hepatocytes from normal rats secreted albumin and synthesized total protein at rates of 70% and 77%, respectively, of those observed in perfused normal liver (Table II).

The rate of albumin synthesis by the isolated hepatocytes is equal to or greater than values reported by others (24-27). The rate of albumin synthesis by the isolated hepatocytes is equal to or greater than values reported by others (24-27).

There was not a specific depression of albumin synthesis in the cells since the relative rate of albumin synthesis was unchanged as was the percentage of albumin secreted relative to total secreted protein.

In both the perfused liver and isolated hepatocytes, a 10- to 15-min increase in albumin secretion time was observed for liver tissue derived from hypophysectomized rats. The reason for this decrease is unknown, but it may be an indirect result of the 3-fold reduction in the amount of total protein secreted by hepatocytes following hypophysectomy (Table II). It may be necessary for secretory vesicles to accumulate a minimum amount of protein before transport out of the hepatocyte. The processing time for protein precursors may also be altered. The large decrease in total secreted protein suggests a proportional decrease in membrane-bound polysomes. Keller and Taylor (20) have, in fact, recently demonstrated a specific decrease in the membrane-bound polysome population following hypophysectomy.

Values calculated for the half-life of liver protein suggest a 2-fold decrease in the catabolic rate in the hypophysectomized systems (Table II), which is a direct consequence of a 50% reduction in total protein synthesis (Fig. 1 and Table II). A general decrease in protein catabolism appears to accompany hypophysectomy (3, 28).

Utilizing the procedure of Fan and Penman (18) in the isolated hepatocyte system, nascent peptides and intracellular proteins were labeled to a very high specific activity to determine ribosome half-transit times (Fig. 7). The identical elongation times for protein and albumin found in cells from both normal and hypophysectomized rats strongly suggest that the defects in protein and albumin synthesis which are found in the livers of hypophysectomized rats do not result from translational deficiencies. It should be noted, however, that the conditions of in vitro incubation may have optimized the elongation times thereby masking any differences which may have existed in vivo.

Taken together, the data demonstrate a 3- to 4-fold reduction in the amount of albumin secreted by perfused liver or hepatocytes isolated from hypophysectomized rats, but no reduction in peptide elongation rates. Since no differences were found in polysome size following hypophysectomy (20), it would appear that the amount of albumin mRNA found in liver tissue following hypophysectomy would be about one-fourth of that found in normal liver tissue. Studies relating to the quantitation of albumin mRNA in liver tissue derived from normal and hypophysectomized rats are in progress.

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