Role of Changes in Protein Degradation in the Growth of Regenerating Livers*

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The significance of changes in rates of synthesis, export and degradation of proteins during liver regeneration was assessed. (a) Proteins were pulse-labeled by the intravenous injection of radioactive leucine and, 5 min later, pactamycin (an inhibitor of the initiation of protein synthesis). One-half of the protein radioactivity was lost from the normal liver within 3 hours. From the radioactivity of the plasma proteins at that time and a study of the disappearance of these proteins from the circulation, it was calculated that 28% of the newly synthesized proteins were exported. Serum albumin accounted for a third of the exported proteins. Thirty-six hours after partial hepatectomy the proportion of albumin to total protein synthesis remained constant, while that of the other plasma proteins increased by 50%. The fraction of the newly synthesized proteins retained by the liver after 3 hours decreased by 20%. (b) During the first 36 hours of liver regeneration the average rates of protein degradation slowed down to one-half the normal values. This was determined either by the loss of radioactivity from total protein (or the guanidino-C of protein-bound arginine) in livers labeled with $^{14}$C bicarbonate, or calculated as the balance between protein synthesis and net protein gain. (c) From these results, and those of our previous study of the protein synthetic machinery of normal and regenerating livers (Scornik, O. A. (1974) J. Biol. Chem. 249, 3876-3883), we conclude that changes in the rate of protein degradation are the single most important factor determining the increase in protein content during liver compensatory growth.

The mass of tissues in adult animals is maintained constant by mechanisms which are not yet sufficiently understood. Stimulation of liver growth by the surgical removal of a portion of the organ (liver regeneration) has been extensively used as a model for the study of these mechanisms (1, 2). Other than water, protein is the most abundant component of this tissue, almost 20% by weight. Its content in the mouse liver is increasing at a maximum rate of 23% per day 36 hours after surgery, just before the first wave of cell division (3). Because normal liver cells synthesize proteins at high rates even in the absence of growth, the net protein gain during regeneration could be attained either by an increased rate of synthesis, by a retention of a larger fraction of the newly synthesized proteins in the liver (as opposed to short-lived liver proteins or exported plasma proteins), or by lower rates of protein degradation. The relative importance of these three variables is ascertained in this paper.

We have analyzed the protein synthetic machinery of normal and regenerating livers in previous publications (3, 4). The concentration of ribosomes and the rate at which they translate mRNA in vivo were the same in both cases. The only significant difference was an increase in the proportion of ribosomes in polyribosomes during regeneration, which resulted in rates of protein synthesis 15% higher than in the normal situation (3). This increase was too small to account for the net protein gain. The proportion of newly synthesized protein retained by the growing liver has not been well documented before (see "Discussion"). A study of the fate of these proteins in the normal and regenerating situations is presented here. Regarding the rate of protein degradation we have found that when proteins were labeled in vivo with L-[guanidino-$^{14}$C]arginine, the rate at which the label disappeared from the liver dramatically decreased after partial hepatectomy (5). We speculated that this was the most important change in overall protein metabolism in this condition (6-8). The slower disappearance from the regenerating livers of protein labeled with L-[guanidino-$^{14}$C]arginine was confirmed by Hill and Malamud (9) and by Swick and Ip (10). However, the latter authors challenged the use of this amino acid; they postulated a continued incorporation of arginine from extrahepatic sources and recommended labeling liver proteins by the administration of $^{14}$C bicarbonate. Using this precursor, they observed that within a 5-day interval, the label disappeared from the regenerating liver protein at a rate estimated to be only 30% slower than in normal livers. This observation compelled us to re-examine in greater detail the role of protein degradation in the net protein gain during regeneration. The results presented in this paper will show that (a) at the time of maximum net protein gain, the loss of radioactivity from protein in livers labeled with $^{14}$C bicarbonate is half as fast as in normal livers; (b) the same result is obtained when rates of protein degradation are calculated as...
the difference between the synthesis of stable liver proteins and the increase in liver protein content; and (c) it is the change in average rates of protein degradation, and not that of protein synthesis or export, that accounts for the net protein gain during compensatory growth.

**EXPERIMENTAL PROCEDURE**

Animals, Injections, Collection of Livers, and Blood—Male, 6-week-old CD-1 mice (Charles River Breeding Laboratories) weighing 30 to 35 g, were used throughout. Their housing and handling, the surgical removal of two-thirds of their livers, the time course of the liver regeneration, and the procedure for injections and collection of the livers have been previously described (9). When a single sample of plasma was needed, an animal was slightly anaesthetized with ether and decapitated. Blood was then collected in a heparin-treated tube, and sedimented at 2000 × g for 15 min to remove the cells. When repeated samples were required, a small incision was made with a razor blade along a lateral vein near the tip of the tail, and 50 to 70 μl of blood was collected in a heparin-treated 100-μl capillary tube, the bottom of which was then sealed with vinyl plastic putty (Critoseal, Fisher Scientific Co.).

**Protein Radioactivity in Various Fractions—**Livers were homogenized in 3 volumes of water and the radioactivity in their protein was determined by scintillation counting of the material remaining after extraction with trichloroacetic acid and perchloric acid (3) and after dissolution in Protosol (New England Nuclear). For the measurement of radioactivity in plasma proteins, 25- to 50-μl aliquots of plasma were mixed with 5 ml of 10% trichloroacetic acid and heated for 15 min at 90°C. The precipitated proteins were collected by centrifugation at 2900 × g for 10 min and the supernatant was mixed with 8 ml of Protosol. Radioactivity in the protein fraction was determined by the acld-alcohol supernatant was transferred to another tube, mixed with 5 ml of 10% trichloroacetic acid, and heated for 15 min at 90°C. The precipitated proteins were collected by centrifugation at 2900 × g for 10 min and the supernatant was mixed with 8 ml of Protosol. Radioactivity in the protein fraction was determined by the acld-alcohol procedure (11) as follows. Aliquots of 50 μl of plasma were mixed with 0.5 ml of 95% ethanol containing 3% solution (12) and preincubated for 3 hours at 37°C before use. A 0.2 ml aliquot was added to 2 ml of 0.9% saline. The precipitated albumin was then treated by the same procedure as described before (4). To determine the specific radioactivity of the incorporated radioactivity from their liver proteins was studied from 6 hours to 6 days afterwards. The results are summarized in the left panel of Fig. 1. The effect of partial hepatectomy in this process was studied at different times after the injection as indicated in that figure. In three of these groups, the radioactivity in the guanidino-C of arginine in proteins was also determined, and the results are presented in the right panel of Fig. 1. It should be noted that in all cases, the injection of the precursor preceded the partial hepatectomy so that in the regenerating animals one was observing the disappearance of proteins labeled in the normal condition.

The results of these experiments agree with those of Swick and Ip (10) in two respects. First, the radioactivity was lost from the regenerating livers at a substantial rate, while when L-[guanidino-14C]arginine was used as the precursor, it had been previously found not to disappear at all over a period of several days following partial hepatectomy (5, 7, 8). Second, the cumbersome determination of the radioactivity in the guanidino-C does not improve upon the much simpler measurement of that in the total protein. The rates of disappearance and the differences between the normal and regenerating livers were essentially the same with either tracer.

The experiment in Fig. 1 differs, however, in one important aspect from that of Swick and Ip (10). These authors measured the loss of the radioactivity after 5 days, and assuming first order kinetics during that interval, they estimated that the apparent degradation rate for regenerating livers was 30% slower than that for normal ones. We obtained very similar results over the same time interval. Five days, however, is too long a period for an accurate representation of the process. The rate of growth reaches a maximum during the 2nd day after the partial hepatectomy, shortly before the first wave of DNA replication, and thereafter becomes progressively slower (3). For this reason we have limited most of our observations to the first 36 hours of regeneration (3). The results presented in Fig. 1 indicate that during the first 36 hours after partial hepatectomy, the rates at which radioactivity was lost from either total protein or the guanidino-C were one-half of those in the normal controls.

The radioactivity disappears from the normal liver with complex kinetics, as one might expect from a mixture of proteins with different half-lives. In the first 36 hours after surgery (Fig. 1, left) apparent degradation rates differed by a factor of 2 when the partial hepatectomy was performed soon

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**RESULTS**

**Disappearance of Protein Radioactivity from Livers Labeled in Vivo with [14C]Leucine—**Mice were injected intraperitoneally with sodium [14C]bicarbonate—Mice were injected intraperitoneally with sodium [14C]bicarbonate and the disappearance of the incorporated radioactivity from their liver proteins was studied from 6 hours to 6 days afterwards. The results are summarized in the left panel of Fig. 1. The effect of partial hepatectomy in this process was studied at different times after the injection as indicated in that figure. In three of these groups, the radioactivity in the guanidino-C of arginine in proteins was also determined, and the results are presented in the right panel of Fig. 1. It should be noted that in all cases, the injection of the precursor preceded the partial hepatectomy so that in the regenerating animals one was observing the disappearance of proteins labeled in the normal condition.

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The radioactivity disappears from the normal liver with complex kinetics, as one might expect from a mixture of proteins with different half-lives. In the first 36 hours after surgery (Fig. 1, left) apparent degradation rates differed by a factor of 2 when the partial hepatectomy was performed soon
Albumin, fibrinogen, and most of the plasma globulins are synthesized in the liver. Once they enter the circulation, their regeneration represented an increasing proportion of the total; different from those in the normal livers. These difficulties will arise from the fact that we were examining the disappearance of proteins labeled in the normal condition. At times soon after surgery this was convenient because we were studying the rapidly turning-over components (with the normal livers containing a smaller proportion of the mixture of these proteins must have progressively changed as the rapidly turning-over components are progressively depleted, the average rate at which the remaining proteins disappeared became considerably slower, but the rates in normal and regenerating livers still differed by a factor of 2.

At times longer than 36 hours after the partial hepatectomy, the differences were less marked (Fig. 1, left, dashed line). This was in part to be expected since the rate of growth was decreasing. The interpretation of the results at these times is complicated, however, by the fact that we were observing the degradation of a mixture of proteins with different rates of turnover. In time, as the normal livers lost more of their radioactive proteins than the regenerating livers, the relative composition of the proteins labeled in the normal condition. The results are summarized in Fig. 2. As shown in the inset of that figure, almost one-half of the injected homologous plasma proteins disappeared from the circulation within 3 hours. The fate of Evans blue, a dye known to bind to plasma proteins was the same during that period. At later times Evans blue was lost faster than the injected proteins, presumably as it became gradually cleared from the circulation by processes other than those affecting the proteins to which it was bound. Fig. 2 also shows the rate of the albumin fraction of injected homologous plasma over a period of 10 days. As indicated in that figure, albumin was eventually distributed in a volume of 14.5 ml/100 g of body weight, and after 3 days disappeared exponentially with a half-life of 3.2 days ($k = 0.215$ day$^{-1}$).

Proportion of Newly Synthesized Proteins Retained and Exported by the Liver—Newly synthesized plasma proteins are secreted by the liver after a lag of a few minutes (16, 17). The proportion of plasma proteins relative to other proteins synthesized in the liver can be studied by labeling them with a radioactive precursor, and then waiting a suitable time until the secretion is completed. One can then measure the protein radioactivity remaining in the liver and that present in the plasma (16). This was done in the first of the experiments in Table I, in which the results with normal and regenerating animals are compared 3 hours after injection of radioactive leucine.

**Fig. 1.** Disappearance of protein radioactivity from livers labeled in vivo with $[^1]C$bicarbonate. Sodium $[^1]C$bicarbonate (40 $\mu Ci/100$ g body weight) was injected intraperitoneally at time 0 (abscissa). Partial hepatectomy ($H_0$) was performed in some groups 0.25 (---), 1.25 (--), or 4.25 (--.--.) days after the injection of the precursor. Left, at the times indicated the livers were obtained and their total protein radioactivity was determined as described under "Experimental Procedure." Each bar represents the average $\pm$ S.E. of a group of mice, the number of which is indicated. Values for normal animals (full bars) were expressed in disintegrations per min per g of tissue (left ordinate). Since the excised tissue represented two-thirds of the organ, values for regenerating livers (open bars) were expressed as their total protein radioactivity in per cent of one-half the total protein radioactivity in the portion removed from the same animals during surgery (5) (right ordinate). Right, the livers in the groups indicated with asterisks in the left panel were pooled and the fraction of total protein radioactivity contributed by the guanidino-C of arginine was plotted in the same manner as in the left panel.

Fate of Plasma Proteins after They Enter the Circulation—Albumin, fibrinogen, and most of the plasma globulins are synthesized in the liver. Once they enter the circulation, their disappearance is a complex phenomenon which has been studied in different species using radioactive proteins (15). After these proteins are injected, the volume in which they are distributed, while initially restricted to the plasma, later expands into extravascular spaces. Some of these spaces exchange their proteins with the plasma within a few hours, whereas others do so more slowly, but after a few days all spaces seem to be equally labeled. Each class of proteins can then be observed to disappear with first order kinetics, probably reflecting its metabolic turnover. For reasons that will become apparent later in this section we wanted to know the characteristics of this phenomenon in our animals. The results are summarized in Fig. 2. As shown in the inset of that figure, almost one-half of the injected homologous plasma proteins disappeared from the circulation within 3 hours. The fate of Evans blue, a dye known to bind to plasma proteins was the same during that period. At later times Evans blue was lost faster than the injected proteins, presumably as it became gradually cleared from the circulation by processes other than those affecting the proteins to which it was bound. Fig. 2 also shows the rate of the albumin fraction of injected homologous plasma over a period of 10 days. As indicated in that figure, albumin was eventually distributed in a volume of 14.5 ml/100 g of body weight, and after 3 days disappeared exponentially with a half-life of 3.2 days ($k = 0.215$ day$^{-1}$).

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ment in radioactivity accumulated during the first interval was calculated to disappear from the circulation as described by the curve in Fig. 2 (inset). The radioactivity thus calculated to remain at 1 hour was subtracted from the actual measurement of the plasma protein radioactivity at that time. The difference was taken to represent the new increment, which was added to the first. The procedure was repeated for each of the successive intervals; at each point the sum of the remaining radioactivity calculated from the previous increments was subtracted from the new measurement. The secretion of the pulse-labeled plasma proteins seemed to have been completed after 1.5 hours and its value exceeded by 65% the radioactivity actually measured at 3 hours.

We have reproduced the conditions for the 7-min and 3-hour periods in the experiment of Fig. 3, and calculated the proportion of labeled liver and plasma proteins, in normal and regenerating animals on two occasions (Table I, Experiments 2 and 3).

In all three experiments summarized in Table I, and in both forms of expressing the results, the regenerating livers retained less of the newly synthesized proteins and exported more of them to the plasma. The larger proportion of plasma proteins in the regenerating animals was due to the non-albumin fraction, whereas the proportion of albumin was the same in both conditions (Experiment 1). When the results of Experiments 2 and 3 were calculated in the same manner as in Experiment 1 the results were very similar, from which we infer that pactamycin had no effect on the relative distribution of plasma and liver protein radioactivity. In Experiments 2 and 3 the sum of the liver protein radioactivity and the estimated exported radioactivity at 3 hours was 103 to 80% of the initial value. The difference remains unaccounted for and we assume it included rapidly turning over liver proteins.

**Estimation of Rates of Liver Protein Degradation from Those of Synthesis and Net Growth**—The experiment described in Fig. 1 indicated that the rate of disappearance of radioactivity from liver protein during the first 36 hours of regeneration was one-half of that in control animals. It seemed desirable to obtain an estimate of the rates of protein degradation by other means. We have previously found that rates of liver protein synthesis 36 hours after partial hepatectomy were 15% higher than in the normal situation (3). With this information, and that presented above on the proportion of newly synthesized proteins retained by the livers, we calculated the rates of degradation, as shown in Table II.

We estimated the absolute rates of synthesis in two entirely different ways. The first one is derived from previous experiments (3) in which the animals were flooded with massive amounts of radioactive leucine, in an attempt to expand the free leucine pool to the point where endogenous sources become negligible. The specific radioactivity of the precursor was 0.04 μCi/μmol, and extrapolation of the results indicated that 5.2 × 10^4 dpm/mg of RNA were incorporated in a 5-min period by the livers of normal animals. Because the concentration of RNA in these livers was 9.9 mg/g of tissue (3), a rate of 168 μmol of leucine/day/g of liver can be calculated. Taking the molecular weight of leucine, 131, and the average proportion of leucine in liver proteins, 10.5% (13), this would result in a rate of 210 mg/day/g of liver for normal animals, while the value of regenerating ones would be 15% higher, or 242 mg day^{-1} g^{-1} (Table II).

The second, more indirect, estimate is derived from experi-

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**Fig. 2.** Disappearance of labeled plasma proteins from the circulation after their intravenous injection to mice. Evans blue or radioactive plasma was injected intravenously to normal animals, and the concentrations of the protein-bound dye and the radioactive plasma proteins were determined at various times thereafter as described under "Experimental Procedure." The results are expressed as the volume, V₀, in which the injected material appears to be distributed (right ordinate) or as a fraction of the initial concentration, calculated from the amount injected and the average plasma volume, 5.0 ml/100 g of body weight (left ordinate). Each bar represents the average ± S.E. of a group of mice, the number of which is indicated. Full bars, Evans blue, 7 min or 3 hours after the injection in two different groups of mice. Open bars, albumin, determined repeatedly 1 to 10 days after the injection in another group. Inset, Evans blue was injected in two animals and radioactive plasma in another three. Samples were obtained at 7 min and 1.5, 3, 6, and 20 hours after the injection. Average values for Evans blue (Δ), albumin (○), and the other plasma proteins ( ● ) are expressed as a fraction of their concentration at 7 min.

Although this approach was useful for comparative purposes, its results were inaccurate. The measurements ignored labeled proteins which may have disappeared from the liver without appearing in the plasma; short-lived proteins, even if present in the liver in only small concentrations, could constitute a sizable proportion of the observed radioactivity. Also, the radioactive proteins in the plasma are not a direct measure of the amount exported by the liver but, instead, reflect the difference existing between net export from the liver and net disappearance from the plasma at any particular time.

We attempted a more accurate definition of these fractions in the experiment presented in Fig. 3. The incorporation of radioactive leucine was interrupted 5 min after its intravenous administration by an injection of pactamycin, in an amount that inhibits the initiation of new proteins but permits the termination of existing nascent chains (4). The effect of the inhibitor was shown to last for at least 6 hours (Fig. 3, inset). Within 3 hours of the injection of the precursor one-half of the pulse-labeled proteins had disappeared from the liver; part of that protein radioactivity appeared in the plasma. From these values a cumulative estimate of the exported plasma protein radioactivity (Fig. 3, Exported) was obtained with the help of a computer program based on the following operations.1 The values in the curve describing the plasma protein radioactivity (Fig. 3, Plasma) were taken at 0.5-hour intervals. The incre-

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1 The computer program was written in the BASIC language. Copies of it are available upon request.
A trace amount of L-[1-14C]leucine (2 μCi) or L-[4,5-3H]leucine (3 μCi) was injected intravenously to normal and 36-hour regenerating animals at zero time, followed when indicated by pactamycin (0.4 mg) 5 min later. The animals were killed 3 hours after the injection of leucine and the total protein radioactivity in their livers, plasma, and the albumin fraction of the plasma was determined as explained under "Experimental Procedure." The total plasma protein radioactivity was calculated from the determination of protein radioactivity in an aliquot of plasma, and the assumption that the plasma volume was 5.0 columns. The next four columns express the results as per cent of the volume of distribution of Evans blue (see "Experimental Procedure") in seven normal and six regenerating animals which yielded values of 7.00 ± 0.12 and 5.07 ± 0.09 ml/100 g of body weight, respectively. Total albumin radioactivity was calculated in the same way; radioactivity in the remaining plasma proteins ("Other" in table) was estimated by difference. Each value is the average ± S.E. of the indicated number of animals. The first four columns present the results as per cent of the liver protein radioactivity in a control group (not shown) killed in each experiment 7 min after the injection of leucine (as in Fig. 3). The exported fraction was estimated as 1.65 times the plasma radioactivity at 3 hours (Fig. 3). "Total" refers to the sum of the first and third columns. The next four columns express the results as per cent of the sum of total protein radioactivities in liver and plasma. The last column represents the proportion of plasma protein radioactivity found in the albumin fraction.

Table I

Distribution of protein radioactivity in liver and plasma 3 hours after injection of radioactive leucine

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mice per group</th>
<th>Precursor at 0 min</th>
<th>PACTAMYCIN at 5 min</th>
<th>Condition</th>
<th>% of 7-min control</th>
<th>% of (Liver + Plasma)</th>
<th>% of plasma in albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
<td>Plasma</td>
<td>Exported a</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>L-[1-14C]leucine</td>
<td>No</td>
<td>Normal</td>
<td>69.5</td>
<td>30.5</td>
<td>10.4</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>Regenerating (36 hr)</td>
<td>60</td>
<td>40</td>
<td>10.5</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>L-[4,5-3H]leucine</td>
<td>Yes</td>
<td>Normal</td>
<td>47</td>
<td>19</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>Regenerating (36 hr)</td>
<td>39</td>
<td>28</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>L-[1-14C]leucine</td>
<td>Yes</td>
<td>Normal</td>
<td>50</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td>Regenerating (36 hr)</td>
<td>38</td>
<td>21</td>
<td>35</td>
</tr>
</tbody>
</table>

*Estimated values.

DISCUSSION

An inverse relationship between overall rate of protein degradation and rate of growth has been first described in bacteria by Mandelstam (18). At the time of our first observations (5, 6) similar changes had been reported in skeletal muscle (19) and in cultured mammalian cells (20). Additional examples of this kind have been recently added to the literature (9, 21). In all these studies, the basic observation has been that the disappearance of radioactivity from the proteins of cells, previously labeled with a precursor amino acid, has been slower under conditions of rapid growth. The extent to
which the apparent rates of degradation were affected by reincorporation of the radioactive amino acid into newly synthesized proteins was not determined.

We have now accumulated sufficient information on the overall rates of protein synthesis, export, and degradation in the normal and regenerating livers to demonstrate conclusively the predominant role of degradation in the regulation of total liver protein. The determination of the specific radioactivity of amino acids in the predominant role of degradation in the regulation of total liver protein.

The measurement of rates of protein synthesis was possible through the use of procedures which did not depend on the determination of the specific radioactivity of amino acids in the precursor pool (3, 4). Of the newly synthesized protein, the normal livers retained 47 to 50%, and the regenerating ones 38 to 39% (Table II). The rest was exported or turned over within 3 hours (Fig. 2). Twenty-eight per cent of the newly synthesized proteins was exported by the normal livers, with serum albumin accounting for one-third. After partial hepatectomy the proportion in albumin remained unchanged, while that in the other plasma proteins increased (Table I). This increased labeling of the non-albumin fraction relative to albumin is consistent with previous reports (22, 23). The values for synthesis of serum albumin relative to total liver protein synthesis presented in this paper differ from those estimated by Schreiber et al. (17), who purified newly synthesized serum albumin from the liver prior to its secretion. Unfortunately, since their work was done before the existence of the albumin precursor was recognized (24), the original interpretation of their measurements must be revised. The advantages of the procedure used in our experiments were explained under “Results.” In addition to these technical considerations, it is possible that in each experimental condition the response of the liver is determined by two conflicting demands: the stimulation of growth, which would be facilitated by a decrease in the fraction of the protein synthetic capacity used for export, and the need to maintain the levels of plasma proteins with only one-third the normal amount of tissue.

In measuring protein degradation, we were fortunate to be working with the liver of an ureotelic animal, in which the high activity of the urea cycle (a) permits the incorporation, much more for liver than for other tissues, of the radioactive carbon atom of [1-14C]bicarbonate into the guanidino moiety of arginine (10), and (b) decreases the reincorporation of the [14C]guanidino group into protein after the arginine is returned to the amino acid pool by protein degradation (25). Furthermore, in agreement with Swick and Ip (10), we found that after labeling the liver proteins with [14C]bicarbonate the fate of the radioactive in whole protein was essentially the same as that in the guanidino-C of protein-bound arginine (Fig. 1). These authors restricted their observations in regenerating livers to a single 5-day interval, a period which, as explained under “Results,” is too long for an accurate representation of the process. We found that at the time of fastest growth, the average rates of protein degradation had slowed down to one-half of the rates for normal livers. This was true whether we measured these rates from the disappearance of both total radioactivity and that in the guanidino-C of protein labeled with [1-14C]bicarbonate (Fig. 1), or whether we calculated them from the balance between protein synthesis and net growth (Table II). The changes were sufficient to account for the net protein gain observed during regeneration. These were average rates and we do not know yet whether all proteins were affected equally. Some information pertinent to this question can be deduced from the data in Fig. 1; here the same difference was observed to exist between normal and regenerating livers, whether measured shortly after the injection of the precursor, when faster components contributed substantially to the average rates, or at later times, when these rates were threefold slower. The question remains an important one: with rates of synthesis unchanged, halving the rates of degradation of a

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**Table II**

*Estimation of rates of protein degradation from those of synthesis and net growth in normal and 36-hour regenerating livers (explanations in the text)*

<table>
<thead>
<tr>
<th></th>
<th>Condition</th>
<th>Total synthesis</th>
<th>Stable liver components</th>
<th>Net growth</th>
<th>Degradation</th>
<th>k (day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg protein/day/g of liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine incorporation</td>
<td>Normal</td>
<td>210</td>
<td>102</td>
<td>0</td>
<td>102</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Regenerating</td>
<td>242</td>
<td>93</td>
<td>42</td>
<td>51 (50%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Albumin turnover</td>
<td>Normal</td>
<td>180</td>
<td>88</td>
<td>0</td>
<td>88</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Regenerating</td>
<td>207</td>
<td>80</td>
<td>42</td>
<td>38 (43%)</td>
<td>0.21</td>
</tr>
</tbody>
</table>
protein with a normal half-life of 2 hours would result in its accumulation at a rate of over 400% per day. The same decrease for a protein with a half-life of 5 days would result in its accumulation at 7% per day. This example serves to stress the fact that so far, we have studied only average rates of synthesis and degradation, and that the harmonious growth of liver components probably requires a readjustment in the relative rates at which individual classes of proteins are turned over.

The predominance of protein degradation in the control of liver mass may be peculiar to this tissue. Normal liver cells, which have one of the highest known rates of protein synthesis, export 30% of this protein while the rest turns over at varying rates. In cells less active in protein synthesis, or in those which export most of their newly synthesized protein (such as the exocrine pancreas or the lactating mammary gland), a decrease in the rates of degradation may not produce the same dramatic effect.

With such a high rate of turnover, a slower rate of degradation is apparently the most economical way for the regenerating liver to gain protein. It would seem wasteful, however, for the normal tissue to maintain this turnover just to avoid growing. Other possible functions of this process have been discussed in recent reviews (26–28): to provide cells with a flexibility in their composition, to secure for them an endogenous source of amino acids, or to scavenge useless proteins. We have examined these possibilities elsewhere (8).

Variable rates of degradation can determine the concentration of individual proteins (26). We do not yet know whether the observed changes in overall rates of degradation result from the action of a single regulatory mechanism, or whether they represent the sum of effects in the regulation of individual protein components of the liver. Our present understanding of the mechanisms involved in protein degradation, much less their regulation, is still rudimentary. The regenerating liver could serve as a useful model for their further study.

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