

## SYNTHESIS AND DEGRADATION OF RIBOSOMAL RNA IN REGENERATING LIVER\*

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One of the most impressive phenomena of mammalian growth is the capacity of the liver to regenerate after partial hepatectomy. For nearly a century (1, 2), and in particular since the classical quantitative study of Higgins and Anderson in 1931 (3), it has been appreciated that within a very short time this organ can be induced to pass from a "resting" state to one of rapid proliferation. An increased mitotic rate in the hepatic remnant can be observed within 24 h of operation (4), and reconstitution of many hepatic components to their initial (prehepatectomy) values can be complete within a few days. Despite increasingly intensive study over the past 15 years, however, it has proved difficult if not impossible to obtain quantitative information about the precise rates of synthesis of various hepatic components during regeneration, or even information about the relative roles of changes in rates of synthesis and degradation in accounting for their enormous net increase in mass. While in most instances regeneration is known to be accompanied by an increased rate of radioactive precursor incorporation, the actual magnitude of the observed increase in incorporation often depends upon the particular precursor used (5), and such observations are difficult to interpret quantitatively because of possible accompanying changes in both intracellular and extracellular precursor pool sizes. Bucher in particular has emphasized the hazards in the interpretation of such observations and has in addition suggested that changes in degradative rates might themselves be of importance in the process of regeneration (6).

The regeneration of ribosomal RNA serves as a possible illustration. This substance, accounting for the bulk of RNA in liver, normally turns over so rapidly [its half life is approximately 5 days (7)] that even a partial decrease in its rate of degradation might, at least in principle, account for a substantial portion of the net increase in ribosomal RNA which is observed during regeneration. A suppression of ribosomal RNA turnover is already known to occur in a variety of other systems [both bacterial (8, 9) and animal (10, 11)] in which cells are induced to pass from a "resting" to a rapidly proliferative state, and it would be of interest to know whether a similar suppression of degradation accompanies the phenomenon of hepatic regeneration; conversely, however, it might be speculated that during the extensive remodeling of hepatic architecture which accompanies the phenomenon of regeneration degradation would instead be accelerated.

\* This work was supported in part by U.S. Public Health Service grants HD-05506 and AM-05397 and by the Perkin Memorial Fund of The Presbyterian Hospital in the City of New York.

‡ Recipient of an Irma T. Hirschl Career Scientist Award.

Despite its intrinsic interest, the subject of cell component turnover during the course of liver regeneration has not proved readily amenable to experimental investigation. In large part this has been for lack of an obvious way in which to determine with precision the size of the initial hepatic remnant left in a given animal at the time of partial hepatectomy and before the onset of regeneration. It is shown here that by employing animals which have previously received both tritiated thymidine and an appropriate  $^{14}\text{C}$ -labeled precursor this difficulty can be overcome, and that it is possible to obtain precise turnover data in individual animals by comparing the concentration and total isotope content of the  $^{14}\text{C}$ -labeled component in the initially excised and regenerating portions of liver. The presence of a  $^3\text{H}$  marker in the liver DNA makes it possible to calculate the exact size of the initial liver remnant and hence to interpret the observed  $^{14}\text{C}$  turnover data in terms of specific rates of synthesis and degradation. As an illustration of its usefulness, this method has been used to determine the day-to-day rates of synthesis and degradation of ribosomal RNA during the course of hepatic regeneration. The approach described here permits accurate determination of turnover rates over intervals considerably shorter than even one half-life, and should be applicable to the study of the specific rates of synthesis and degradation of other cell components as well.

### Materials and Methods

**Materials.** Studies were performed on normal male Sherman (Columbia strain) rats of the weights indicated below, and animals were provided with food and water ad libitum. [*methyl*- $^3\text{H}$ ]-Thymidine (20.2 Ci/mmol) and [ $6\text{-}^{14}\text{C}$ ]orotic acid hydrate (5.4 mCi/mmol) were obtained from the New England Nuclear Corp., Boston, Mass.

**Administration of Radioisotopes.** Two complete studies were performed, each yielding virtually superimposable regeneration and turnover data. In Study I 17 weanling rats weighing  $44 \pm 5$  g (mean  $\pm 1$  SD) were injected intraperitoneally with 20  $\mu\text{Ci}$  of [*methyl*- $^3\text{H}$ ]thymidine in 0.5 ml 0.15 M NaCl, and 22 days later (weight range than 150–170 g) with 4  $\mu\text{Ci}$  of [ $6\text{-}^{14}\text{C}$ ]orotic acid. In Study II the ratio of  $^3\text{H}$  to  $^{14}\text{C}$  was increased, and instead 27 animals weighing  $57 \pm 4$  g received 50  $\mu\text{Ci}$  of [ $^3\text{H}$ ]thymidine followed 17 days later (weight range than 160–180 g) by only 2  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]orotic acid. Bedding was routinely changed every 2 to 3 days throughout the period of maintenance of radioactively labeled animals.

**Partial Hepatectomies.** Partial hepatectomies (approximately 70%, see below) were performed by the method of Higgins and Anderson with only minor modification, and animals were provided with 20% dextrose to drink for a period of 24 h after operation (3). At the time of closing the abdomen each animal was given 15,000 U of crystalline penicillin by intraperitoneal injection. Ether anesthesia was light, the animals regained consciousness rapidly, and postoperative mortality was zero. The weight range of the animals at the time of hepatectomy was  $201 \pm 27$  g (mean  $\pm 1$  SD) for Study I and  $222 \pm 22$  g for Study II. The time elapsed between the administration of [ $^{14}\text{C}$ ]orotic acid and hepatectomy was 5 days in Study I and 8 days in Study II; previous control studies have shown that after these time intervals virtually all radioactivity present in ribosome-associated RNA is in ribosomal RNA per se (7). Animals were sacrificed in groups of five to seven at either 2, 4, and 7 days after partial hepatectomy (Study I) or 1, 3, 5, and 7 days (Study II). Liver samples obtained both at operation and at the time of eventual sacrifice were frozen and stored at  $-20^\circ\text{C}$  before homogenization and the various assay procedures described below.

**Fractionation of Liver RNA.** All liver samples were initially homogenized in 4 vol of "sucrose-TKM" (0.25 M sucrose, 50 mM Tris at pH 7.4, 25 mM KCl, and 5 mM  $\text{MgCl}_2$ ), and aliquots taken in duplicate for protein, DNA, and total RNA determinations. Additional samples of homogenate were then diluted and treated with sodium desoxycholate as described by Blobel and Potter (12), centrifuged in duplicate at 50,000 rpm for  $2\frac{1}{2}$  h at  $4^\circ\text{C}$  (Beckman L2-65B ultracentrifuge and 50 Ti rotor; Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif.) to separate "sedimentable"

and "nonsedimentable" RNA, and the resulting pellets and supernatant fluids assayed for RNA as described below. The fraction of RNA which was sedimentable [consisting almost entirely of ribosomal RNA (7, 12)] was found to account for over 85% of the total; this fraction did not change appreciably during regeneration (see below).

*RNA, DNA, and Protein Determinations.* RNA (sedimentable, nonsedimentable, and total) was determined by the Schmidt-Thannhauser method with minor modifications (13). After gentle alkaline hydrolysis and subsequent acidification of the hydrolysate an extinction at 260 nm of 1.00  $\text{cm}^{-1}$  was taken to correspond to a concentration of 32  $\mu\text{g}$  of acidified RNA hydrolysis products per milliliter (13). The DNA remaining after alkaline hydrolysis of total homogenate RNA was extracted by two successive hydrolyses in 0.5 M perchloric acid for 15 min at 70°C, and the concentration of hydrolysis products in the pooled extracts determined by the method of Giles and Myers (14). Protein was determined by a modification of the Folin-Ciocalteu method (15).

*Radioactivity Determinations.* Although the above selective extraction methods resulted in extremely low levels of  $^{14}\text{C}$  in the DNA hydrolysis products and undetectable  $^3\text{H}$  in the RNA hydrolysis products, a double-channel counting technique was nevertheless employed for the radioactivity determinations. Under typical conditions for differential counting [0.5 ml of hydrolysis products added to 10 ml of Bray's solution (16)] efficiencies were 30.5% for  $^{14}\text{C}$  and 5.1% for  $^3\text{H}$ .

## Results

*Validity of the Method.* If tritiated thymidine is introduced into liver DNA as a "marker" before hepatectomy, the tritium content of the excised and subsequently regenerated pieces can be used to calculate the initial size of the liver remnant if the following two assumptions are true: (a) the tritium label is homogeneously distributed throughout the liver at the time of partial hepatectomy; and (b) there is no appreciable loss of tritiated DNA from the remnant during the course of regeneration. If this is indeed the case, one can readily calculate the size of the initial remnant ( $w_o'$ ) from the size of the portion excised ( $w_o$ ) and from the [ $^3\text{H}$ ]thymidine content of the DNA in the excised and regenerated pieces. Under these conditions:

$$w_o' = w_o \frac{D'}{D},$$

where  $D$  and  $D'$  represent respectively the total [ $^3\text{H}$ ]thymidine content of the excised and the regenerated pieces. If, furthermore, at the time of hepatectomy there is homogeneity with regard to the distribution between the excised and residual lobes of both the mass and radioactive labeling of a given tissue component prelabeled with  $^{14}\text{C}$ , this formula can be used with equal applicability to calculate, from the corresponding value in the excised portion ( $m_o$ ), either the mass of that component or the total  $^{14}\text{C}$  radioactivity that was present in that component in the initial remnant ( $m_o'$ ):

$$m_o' = m_o \frac{D'}{D}.$$

A similarly simple formula can be used to calculate the corresponding quantities ( $w_o''$  or  $m_o''$ ) for the intact liver before hepatectomy:

$$m_o'' = m_o + m_o' = m_o \left[ \frac{D + D'}{D} \right].$$

If the preceding two formulas can be shown to be valid, then  $m_o'$  and  $m_o''$  — two important and hitherto experimentally inaccessible quantities — can be

calculated from  $m_o$ ,  $D$ , and  $D'$ , all of which are readily accessible to direct experimental determination. The validity of the above assumptions was established in the control experiments given below.

(A) *Homogeneity of the Distribution of Bulk Components and of Radioactive Labeling between Excised and Residual Lobes at Time of Operation.* In an initial control experiment five animals previously injected with both [ $^3\text{H}$ ]thymidine and [ $^{14}\text{C}$ ]orotic acid as described in Methods underwent partial hepatectomy. The animals were then killed and the excised and residual lobes were assayed independently for the components illustrated in Table I. It is seen that despite moderate scatter from animal to animal the values for the excised and residual pieces in individual livers were virtually identical.

(B) *Use of the Tritiated Thymidine Content of Excised and Regenerated Pieces to Calculate Residual Liver Mass; Retention of Initial Thymidine Label during the Process of Regeneration.* While under normal circumstances rat liver DNA is known to be exceedingly stable and to undergo virtually no turnover (17), it remained to be determined that this is also the case during regeneration. That indeed this is so is shown in Table II, which shows the percent hepatectomy calculated, on the basis of  $100 D/(D + D')$ , at different stages of regeneration. It is evident (a) that there is no appreciable loss of prelabeled DNA (and hence of pre-existing liver cells) during the course of

TABLE I  
*Homogeneity of Distribution of Liver Components*

Component and concentration	Range (five animals)		Ratio* (residual/ excised)
	"Excised" lobes	"Residual" lobes	
mg DNA per g liver	2.03 - 2.45	2.00 - 2.36	0.99 ± 0.01
mg ribosomal RNA per g liver	5.23 - 6.50	5.74 - 6.36	1.02 ± 0.04
mg protein per g liver	164 - 191	158 - 180	0.98 ± 0.02
dpm $^3\text{H}$ per $\mu\text{g}$ DNA	28.8 - 48.0	28.5 - 50.1	1.05 ± 0.05
dpm $^{14}\text{C}$ per $\mu\text{g}$ ribosomal RNA	23.9 - 38.0	23.4 - 42.0	1.04 ± 0.04

\* Ratio for individual animals (mean ± 1 SE).

TABLE II  
*Percent Hepatectomy Calculated at Different Stages of Regeneration*

Days of regeneration	Hepatectomy = $100 D/(D + D')$ *	
	Study I	Study II
	(%)	(%)
1	—	68.3 ± 1.1
2	70.4 ± 1.2	—
3	—	69.0 ± 2.5
4	70.5 ± 4.9	—
5	—	66.8 ± 1.8
7	69.0 ± 1.5	70.0 ± 1.1

\* Mean ± SE.

regeneration (viz. the constancy of the calculated percentages); and hence (b) that the percent hepatectomy performed on an individual animal is directly calculable by this method after regeneration. The calculated percent hepatectomy (69% overall for Studies I and II) agrees precisely with values reported by Higgins and Anderson (71%, ref. 3) and by Bucher and Swaffield (68%, ref. 18), who in control studies performed hepatectomies by the present technique and removed the remnant immediately afterwards to compare its weight directly with that of the excised portion.

Although the standard errors for the values in Table II appear small, in practice the portion of liver excised by this technique may vary in a given animal from about 62%–78% of the total. A corresponding uncertainty about the size of the initial remnant (i.e., a nearly twofold variability between 22% and 38% of the initial whole) would lead to considerable difficulty in the interpretation of turnover data and makes obvious the advantage of being able to determine, in an individual animal, the precise size of the initial remnant.

*Time-Course of Regeneration.* Fig. 1 shows an application of this method to follow the time-course of regeneration with respect to liver DNA content. The technique permits one to calculate the desired quantities with great precision, and the results obtained in two entirely separate studies are seen to be virtually superimposable. By the end of 24 h the DNA content of the initial remnant has already increased by approximately 30%, the rate of synthesis continues to accelerate for the next 2 days, and by 3 days the DNA content of the original intact liver has been completely reconstituted. Synthesis then stops abruptly, and the DNA content of the remnant shows little change over the next 4 days.

Fig. 2 shows the time-course of the regeneration of ribosomal RNA. Again, appreciable regeneration is evident by the end of the first day, the rate of regeneration becomes maximal by the second day, and regeneration is essentially complete by day 3. Despite disagreement about whether fluctuations in the relative proportions of free and membrane-bound ribosomes take place in

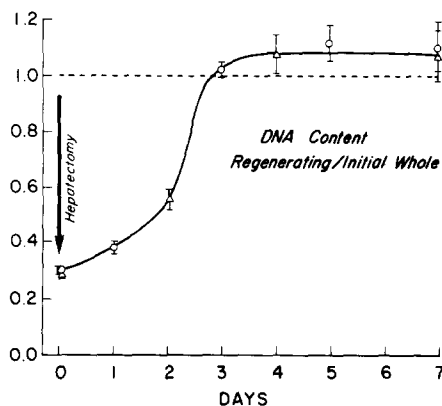


FIG. 1. Time-course of regeneration of liver DNA. Points show the ratio of the DNA content of the regenerating remnant to that of the intact liver just before hepatectomy. Note that regeneration is essentially complete by 3 days. In this and the subsequent figures triangles indicate the results in Study I and circles the results in Study II; all points show means  $\pm$  1 standard error.

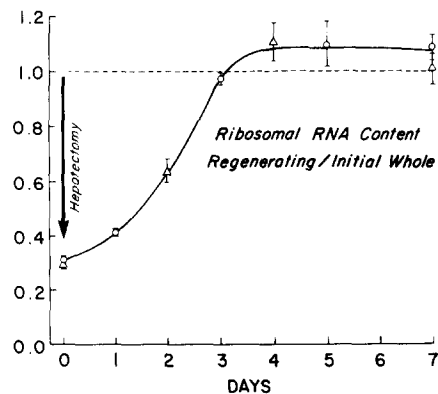


FIG. 2. Time-course of regeneration of ribosomal RNA. Points show the ratio of the ribosomal RNA content of the regenerating remnant to that of the intact liver just before hepatectomy. As in the instance of DNA, regeneration of ribosomal RNA is essentially complete by 3 days.

regenerating liver (19, 20), Table III shows that the fraction of the total RNA that is "nonsedimentable" (12) does not change.

In contrast to the reconstitution of total liver DNA and RNA, both of which are complete within 3 days, the reconstitution of both liver mass and total liver protein takes place considerably more slowly. Fig. 3 shows that even after 7 days these quantities have reached only about 80% of their values in the original intact liver.

Previous isotope incorporation experiments have shown that partial hepatectomy in animals of the weights employed here is followed by a burst of DNA synthesis which reaches a peak at about 24 h (18). The rate of incorporation of radioactive thymidine then falls, and the absence of later well-defined peaks has been attributed to a subsequent loss of synchrony in the dividing cells (21). Fig. 4 presents a semilogarithmic plot of both the DNA and ribosomal RNA content of the remnant as a function of the time after hepatectomy, and shows that despite presumed asynchrony of cell division the growth of the remnant is very nearly exponential during most of the period of regeneration. After an initial lag, shown on the basis of isotope incorporation studies to be about 15 h (22), DNA synthesis becomes exponential and continues until day 3, by which time the remnant has attained the DNA content of the initial intact liver and further synthesis virtually stops. The "doubling time" for DNA synthesis during the period of rapid growth is seen to be only about 36 h, reflecting, in fact, a proliferation rate comparable to what is observed for many rapidly dividing cells in tissue culture. The plateau values for the DNA and RNA ratios (i.e., ratios of the observed final content to the calculated initial content) are both seen to be approximately 3.4 — a value that agrees closely with that expected on the basis of complete regeneration following a 70% (mean) hepatectomy.

*Turnover of Ribosomal RNA during Regeneration; Specific Rates of Synthesis and Degradation during Restoration of Ribosomal Mass.* The method described here lends itself with particular convenience to investigating the rates of synthesis and degradation of various liver cell components in the hepatic remnant during the course of regeneration. If  $m$  denotes the mass of a specific

TABLE III  
*Nonsedimentable RNA as Percent of Total RNA\**

Day	Study I	Study II
	(%)	(%)
0	11.3 ± 0.5	8.9 ± 0.3
1	—	8.6 ± 0.6
2	9.8 ± 0.8	—
3	—	8.0 ± 0.4
4	10.6 ± 0.6	—
5	—	8.7 ± 0.3
7	10.6 ± 0.7	9.0 ± 0.3

\* Means ± 1 SE.

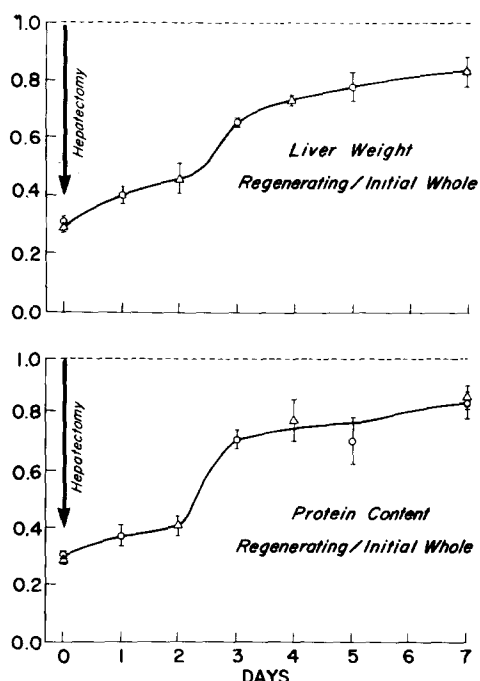


FIG. 3. Time-course of reconstitution of liver mass and of total liver protein. Note that reconstitution of both is considerably slower than regeneration of either DNA or ribosomal RNA.

component prelabeled, for example, by the administration of an appropriate  $^{14}\text{C}$  precursor, and  $s$  its specific activity (e.g., in disintegrations per minute per unit mass), the total radioactivity present in that component at any given time will be given by the product  $sm$ . The net rate of change in this product, reflecting the difference between synthetic and degradative rates, can then be expressed by  $d(sm)/dt = f^* - d^*$ , where  $f^*$  and  $d^*$  represent the actual rates of formation and degradation of radioactively labeled component at any given instant. The specific, or intrinsic, rates of synthesis and degradation, i.e., the rates relative to

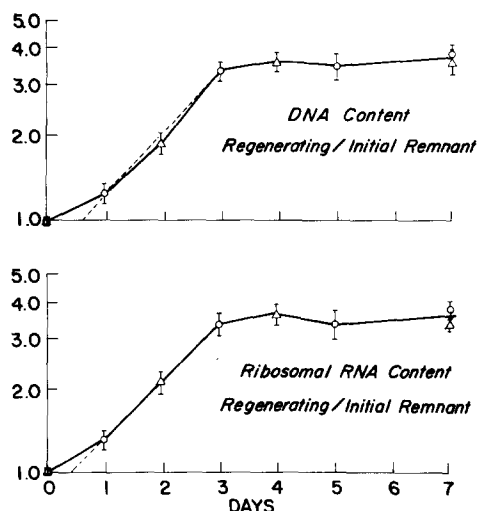


FIG. 4. Semilogarithmic plots of the DNA and ribosomal RNA content of the liver remnant as a function of time. Points show ratios of regenerated to zero-time values calculated in individual animals as described in the text. Note that after an initial lag net synthesis of both DNA and ribosomal RNA is very nearly exponential until regeneration is complete.

the total quantity present at any given time, are in turn given by  $f^*/sm$  and  $d^*/sm$ :

$$\frac{f^*}{sm} - \frac{d^*}{sm} = \frac{1}{sm} \frac{d(sm)}{dt} = \frac{d \ln(sm)}{dt}$$

If the turnover observations are begun sufficiently long after the initial administration of radioisotope and  $f^*$  has in turn become negligible<sup>1</sup>, then the net rate of loss of radioactivity during the period of observation will be given by:  $-d \ln(sm)/dt \cong d^*/sm$ . Similar equations can be written for the net rate of change in the total mass of the component,  $m$ :  $dm/dt = f - d$ , and  $d \ln m/dt = f/m - d/m$ , where  $f$  and  $d$  again correspond to specific formation and degradation rates, only this time for the total component mass.

If the degradation of the component of interest is random and occurs at a rate independent of its age [as has been shown for example to be the case for ribosomal RNA (7, 23–26)], then the specific rate of degradation of the component species previously rendered radioactive will be the same at any given time as that for the component as a whole, and it will follow that  $d/m = d^*/sm$ . By substituting this last relationship in the equations of the preceding paragraph,

<sup>1</sup> The validity of this assumption is, of course, of crucial importance. In the example to follow it is assumed that the "recycling" of radioactive ribosomal RNA degradation products into new radioactive ribosomal RNA has a minimal effect upon the observed turnover rates, as indeed appears to be the case for rat liver: radioactivity in the "acid-soluble" pool is known to fall off rapidly after the administration of radioactive orotic acid (23), and numerous investigators using differently labeled precursors (both with and without a subsequent "chase" of nonradioactive precursor) have obtained virtually identical turnover rates (7, 23–26); Hirsch and Hiatt, examining the turnover of rat liver ribosomes simultaneously labeled with [<sup>3</sup>H]orotic acid and [<sup>14</sup>C]-guanido-labeled arginine, moreover obtained identical values for the half-lives of both ribosomal RNA and ribosomal protein (25)—a finding which would be most unlikely if there were appreciable reutilization of either precursor.



it is seen that the intrinsic rates of degradation and formation,  $d/m$  and  $f/m$ , are simply obtained by plotting  $\ln sm$  and  $\ln s$ , respectively, vs. time:

$$\frac{d}{m} = -\frac{d \ln(sm)}{dt}; \quad \frac{f}{m} = -\frac{d \ln s}{dt}.$$

Plots of these two functions for ribosomal RNA pre-labeled by the administration of [ $^{14}\text{C}$ ]orotic acid are presented in Fig. 5. Beginning within a day of hepatectomy the plot of  $\ln s$  shows a rapid linear decline corresponding to a nearly constant intrinsic formation rate of approximately 53% per day. The linear decline continues until day 3, by which time the ribosomal mass of the original (intact) liver has been restored and the rate of synthesis abruptly falls to only what is necessary to keep pace with degradation. The terminal portion of the plot of  $\ln s$  thereafter becomes parallel to the plot of  $\ln sm$ , indicating that the rates of synthesis and degradation are now about equal, and hence that  $m$  remains approximately constant at its newly regenerated value (see also Figs. 2 and 4). In striking contrast to the changes in specific synthetic rate, the specific degradative rate for ribosomal RNA, illustrated by the plot of  $\ln sm$ , is remarkably constant throughout the course of regeneration, and continues wholly unperturbed at a level of approximately 12% per day — a rate identical, in fact, to that

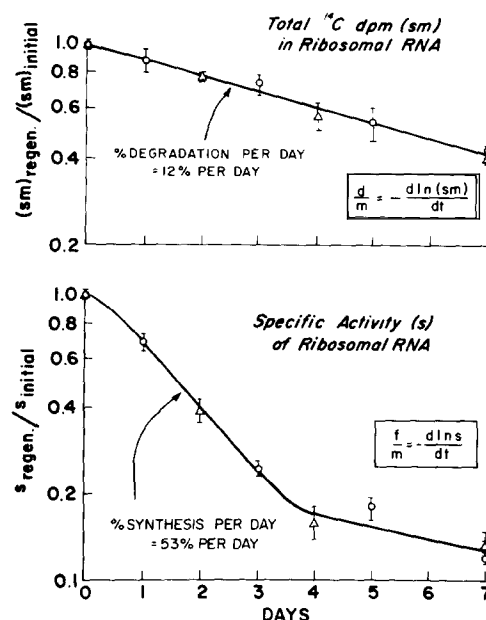


FIG. 5. Semilogarithmic plots of total ribosomal RNA radioactivity and specific activity of ribosomal RNA in the regenerating remnant as functions of time. Ribosomal RNA was rendered radioactive by the administration of [ $^{14}\text{C}$ ]orotic acid approximately a week before hepatectomy as described under Methods. After an initial lag the plot of  $\ln s$  shows a rapid decline corresponding to an intrinsic formation rate ( $f/m$ ) of approximately 53% per day. Exponential decline continues until day 3, by which time the ribosomal mass of the original (intact) liver has been restored and the rate of synthesis falls abruptly. In contrast, the specific rate of degradation of ribosomal RNA, illustrated by the plot of  $\ln sm$ , is remarkably constant throughout the course of regeneration (see text).

observed in normal rat liver (7, 23–26). In normal growing animals of the weights employed for these experiments the intrinsic synthetic rate is about 15% per day, i.e., about 3% greater than the degradative rate and sufficient to account for the slow daily increment in ribosomal mass associated with normal growth. The synthetic rate of 53% per day observed in the present study shows that partial hepatectomy results in an enormous (three- to fourfold) increase in the absolute synthetic rate which begins soon after operation and continues unabated until original ribosomal mass has been restored.

### Discussion

A simple method has been described which permits accurate determination of the size of the initial liver remnant after partial hepatectomy. On the basis of this information it is possible to make a direct comparison of pre- and postregenerative values in individual animals and hence to determine the time-course of regeneration for various hepatic components with a precision heretofore not possible. Application of this method in the present study shows that, after the operative stimulus, the rates of regeneration of both DNA and ribosomal RNA become maximal by the end of 1 day and continue undiminished until regeneration of both of these components is complete 2 days later. Despite the fact that cell division is known to become asynchronous, DNA synthesis is seen to be truly exponential during this period of rapid growth.

We believe that the most useful application of this method, however, is in determining the rates of synthesis and degradation of various liver cell components during the course of regeneration. As shown here one can obtain turnover data of remarkable precision even over very short intervals (considerably less than one half-life) and in relatively small numbers of animals. Application of this method in the present study has made it possible, for example, to determine the intrinsic rates of both synthesis and degradation of ribosomal RNA during the reconstitution of ribosomal mass in the regenerating remnant.

Up to the present time the relative contributions of synthesis and degradation in accounting for the reconstitution of ribosomal mass have remained unclear. Although a twofold increase in nucleolar size is observed within 12 h (27) and is known to be accompanied by a sixfold increase in the rate of incorporation of [ $^{14}\text{C}$ ]orotate into microsomal RNA (22), quantitative interpretation of these findings has not been possible. Bucher in particular has emphasized the hazards of basing conclusions upon such observations, and has in addition suggested that the demands imposed by regeneration might largely be met by a decrease in degradation without the necessity for very large changes in total production (6). Becker and Lane have noted, on the other hand, that partial hepatectomy is followed by a notable increase in the number and size of phagocytic vacuoles, suggesting an increased rate of intracellular autophagy (28).

In view of these earlier observations and conflicting suggestions, one of the striking findings in the present study is that the extraordinarily rapid increase in ribosomal RNA which takes place during regeneration of the liver remnant is in fact virtually entirely accounted for by a nearly fourfold increase in its rate of synthesis. In contrast, its rate of degradation, despite other profound changes occurring in the remnant at the same time, remains wholly unperturbed and

indeed identical to that observed in normal intact rat liver. This "mindless" constancy of degradation is in sharp contrast to the suppression of degradation of ribosomal RNA which is known to take place both in bacteria (8, 9) and in various animal cell culture systems (10, 11) when cells are caused to pass from a "resting" state to one of rapid proliferation. It will be of interest to see whether the same inflexibility of degradation applies to other cell components as well.

### Summary

A simple double-isotope method is described which permits precise determination of both synthetic and degradative rates of liver cell constituents during the course of regeneration after partial hepatectomy. By employing animals which have previously received both tritiated thymidine and an appropriate  $^{14}\text{C}$ -labeled precursor it is possible to obtain precise turnover data in individual animals by comparing the concentration and the total isotope content of the  $^{14}\text{C}$ -labeled component in the initially excised and regenerating portions of liver. The presence of a  $^3\text{H}$  marker in the liver DNA makes it possible in addition to calculate the exact size of the initial liver remnant and hence to interpret the observed  $^{14}\text{C}$  turnover data in terms of specific rates of synthesis and degradation.

As an illustration of its usefulness this method has been employed to study changes in cell proliferation rate after partial hepatectomy, and to determine the day-to-day rates of synthesis and degradation of ribosomal RNA, the major component of rat liver RNA. It is shown that during the first 24 h after a 70% hepatectomy ribosomal RNA synthesis undergoes a nearly fourfold stimulation to a rate of approximately 53% per unit mass per day. This accelerated rate of synthesis is sustained for an additional 2 days and is accompanied by exponential DNA synthesis until the hepatic remnant has more than tripled its initial DNA and ribosomal RNA content to attain values identical to those in the initial intact liver; the rates of DNA and RNA synthesis then fall abruptly. In striking contrast to the marked fluctuations in its rate of synthesis, ribosomal RNA continues to be degraded throughout the course of regeneration at a constant rate of 12% per day, a rate virtually identical to that observed in normal liver. The approach described here permits the accurate determination of turnover rates over intervals considerably shorter than even one half-life, and should be applicable to the study of the specific rates of synthesis and degradation of other cell components as well.

*Received for publication 10 April 1975.*

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