Turnover of Liver Ribosomes in Fed and in Fasted Rats*

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SUMMARY

Evidence from many sources indicates that the ribonucleic acid and protein of normal animal tissues are replaced more rapidly than are the cells themselves. However, the significance of this macromolecular turnover and the mechanisms that regulate synthesis in concert with catabolism to maintain cellular composition have remained obscure. In beginning a study of factors that influence protein and RNA turnover in mammalian cells, we have compared the rates of synthesis and degradation of liver cytoplasmic ribosomes in fed and in fasted animals. Synthesis was measured by labeling ribosomal protein and RNA and by measuring the rate at which the specific activities decreased owing to continuing synthesis from unlabeled precursors. In fed, nearly mature animals, degradation approximates synthesis. In fasting, degradation exceeds synthesis and was measured as the difference between rates of synthesis and of net loss of liver ribosomes. Our results indicate that ribosomal protein and RNA are replaced at the same rate. The normal kinetics are first order, with turnover half-times of 5 days. During starvation, the cellular ribosome content decreases steadily due to an increased degradative and a diminished synthetic rate.

Turnover of proteins and ribonucleic acid is known to occur in dividing and in nondividing cells of normal mammalian tissues (3-8). However, the physiological significance of this continuous replacement of macromolecules has remained obscure, as have the mechanisms that regulate synthesis and catabolism in concert to maintain cellular composition. The catabolic aspect especially has received little attention, relative to the synthetic. Therefore, we have begun a study of macromolecular turnover in mammalian tissues in order to determine, for a given class of macromolecules, the responses of the synthetic and catabolic rates to nutritional changes and to elucidate the mechanisms that mediate the responses observed.

For such a study it seemed both convenient and appropriate to examine the turnover of ribosomes. These particles, which are readily labeled and isolated, permit one to deal relatively easily with a defined structural component of the cell containing both protein and RNA. Also, knowledge of the synthetic and catabolic processes that determine the cellular concentration of these essential components of the protein-synthesizing apparatus should contribute to an understanding of the regulation of protein synthesis.

This report presents measurements of the kinetics of synthesis and degradation of rat liver cytoplasmic ribosomes made under essentially steady state conditions and also under conditions of food deprivation.

METHODS AND MATERIALS

Animals—Male CD rats (Charles River Farms) weighing between 250 and 300 g were used. For each experiment, we selected a group of animals similar in age and weight. Fed animals were maintained on water and Purina Laboratory Chow ad libitum. The gain in liver RNA content due to growth of these animals was about 10% during the intervals of these experiments and was neglected in our calculations. Fasted animals received water only.

Isotopic Labeling—The validity of turnover determinations by the method used requires that the period of labeling be short, relative to the interval of the measurements, and that there be negligible reutilization of isotope. For labeling protein, these conditions were met by the use of arginine containing 14C in the guanido group; isotope in this form either is incorporated or is rapidly eliminated as urea because of the high arginase activity in liver (9). For RNA labeling we employed orotic acid, recognizing that the values obtained would represent maximal estimates of the RNA turnover time because of the persistence of labeled precursor pools (10) and the possibility of nucleotide reutilization (4).

On the day preceding protein labeling, RNA pyrimidines were labeled by treating the animals with intraperitoneal injections of 3H-orotic acid (Calbiochem; approximately 200 C per mole) in 0.9% NaCl; the small amounts given, 25µC or less per animal, did not require neutralization before injection. Food was then

1 Synthesis, in this context, is meant to imply production of entire ribosomal particles that can be isolated from the cytoplasm by centrifugation.
withheld for 12 to 18 hours before intraperitoneal injection of L-arginine-guanido-\(^{14}\)C. With this sequence, both the RNA and the protein components of cytoplasmic ribosomes reached their maximum specific activities at approximately the same time.

The period of fasting before protein labeling was required to obtain comparable specific activities from animal to animal. Such a period of fasting before orotic acid administration did not appear to influence RNA labeling.

**Isolation of Ribosomes**—The rats were decapitated and the livers were immediately removed, weighed, and homogenized in 3 volumes per weight of Medium A (0.05 M Tris-HCl (pH 7.5 at \(0^\circ\)), 1 mM MgCl\(_2\), 0.025 M KCl, and 0.25 M sucrose) at \(0^\circ\). Subsequent operations through the final isolation were performed in a 5°C cold room, except where otherwise specified. The homogenate was centrifuged at 15,000 \(\times g\) for 15 min to yield a supernatant fraction containing microsomes relatively free of debris and of larger cellular components. The upper three-fourths of the supernatant solution was removed and diluted with sufficient Medium A and 10% deoxycholate to double its volume and obtain a final deoxycholate concentration of 1.3%. The ribosomes, released from microsomes by the detergent treatment, were sedimented by centrifuging the mixture for 2 hours at 50,000 rpm in a Spinco No. 50 rotor. After removal of the supernatant solution, the ribosomal pellets were rinsed with Medium A minus sucrose; the tubes were drained; 1 ml of Medium A minus sucrose was added to each tube; and the pellets were allowed to soften overnight. After this treatment, the pellets were readily and almost completely dispersed by gentle shaking. The material from the several tubes was pooled or dispersed in several milliliters of water to yield an opalescent, nearly colorless suspension.

**Isotope Analysis**—Samples of 1 ml of the ribosome suspension were dissolved in 1 ml of Hymine (Packard Instruments), and this solution was washed into vials for simultaneous measurement of \(^3\)H and \(^{14}\)C in a liquid scintillation spectrometer.

**Nucleic Acid Analysis**—Ribosomal RNA was measured by hydrolyzing samples of the ribosome suspension in 5% trichloracetic acid at 95°C for 15 min, centrifuging down the protein, and assayng the purine nucleotide ribose by the orcinol reaction (11). Total liver RNA and DNA were measured by the method of Schneider (11). Adenosine monophosphate served as the orcinol reaction standard, 1.0 mg being equivalent to 1.9 mg of rat liver RNA. Deoxyadenosine served as the diphenylamine reaction standard, 1.0 mg being equivalent to 2.6 mg of rat liver DNA. A deoxyadenosine standard was included in the orcinol assays to permit correction for the DNA contribution to the reaction.

**Protein Analysis**—The method of Lowry et al. (12) was used to measure the protein content of samples from the ribosome suspension dissolved in 1 N NaOH. Bovine serum albumin served as the standard.

**Results**

**Turnover in Fed Animals**

We wished first to determine the kinetics of turnover of liver cytoplasmic ribosomes under conditions approximating a steady state in which synthesis is balanced by catabolism. The method was to label the ribosomal protein and RNA moieties in a series of animals and to measure the specific activities of these components at intervals; the rate of decrease indicates the rate of production of unlabeled ribosomes, which in well-fed, mature animals also approximates the rate of degradation.

Fig. 1 presents the results of such an experiment in which a group of rats was treated with intraperitoneal injections of \(^{3}\)H orotic acid and L-arginine-guanido-\(^{14}\)C and subsequently fed \(ad\) \(libitum\). At daily intervals, a rat was killed, liver cytoplasmic ribosomes were isolated, and the specific activities of ribosomal protein and RNA were determined.

RNA specific activity increased rapidly for about 2 days after injection of label, then decreased more gradually. Protein specific activity reached a maximum between 3 and 24 hours after labeling, and then decreased in parallel with RNA. After peaking, both the RNA and protein specific activity curves decreased exponentially at the same rates, reaching half the initial values in 5 days, equivalent to a turnover time of 7.2 days, a fractional rate of 14% daily, or, for the RNA, an absolute rate of 0.39 mg per mg of DNA per day. The results indicate that the ribosomes are replaced as units and that the entire ribosome population observed is homogeneous with respect to its turnover, which is random, following first order kinetics.

**Turnover in Fasted Animals**

A. Synthesis—Although food deprivation is known to lead to a marked depletion of liver RNA and ribosomes (13, 14), it had not been determined whether this results from decreased synthesis, increased catabolism, or both. To examine these possibilities we measured the rate of ribosome production as in the previous experiment and used two groups of animals, one fed,
Fig. 2. Changes with time in the specific activities of the cytoplasmic ribosomal RNA (disintegrations per min X 10^{-2} per mg) and protein (disintegrations per min X 10^{-2} per mg) in two groups of animals, one fasted, one fed, plotted semilogarithmically. The fed group is represented by points at 0 and 6 days, the fasted by points at 0, 2, 4, and 6 days of fasting (the same zero time points represent both groups). The points for each series on a given day are the results from two animals of a group that received 2 mc of 3H-erotic acid and 7.5 mc of 14C-arginine administered as in the first experiment.

Fig. 3. Ratios of total liver RNA to DNA during fasting. Each point represents the result from one animal.

The fed series is represented by the initial points and a final set at 6 days. If these sets are joined by straight lines, one sees that the RNA- and protein-specific activities decreased in parallel and at the same rate as in the previous experiment. However, in the food-deprived series the decreases were more gradual. More experimental points would be required to define precisely the curves in this food-deprived series, but they appear to be exponential, with a decrease to half in 6 to 8 days, equivalent to a fractional renewal rate of about 9% per day. It is clear that liver cytoplasmic ribosomes are replaced more slowly in the fasted rate, although replacement does continue at an appreciable rate.

B. Catabolism—Catabolism clearly exceeds synthesis during food deprivation, for total liver mass, protein, and RNA-content decrease, but the results of the previous experiment alone do not yield the ribosomal degradative rate under these non-steady state conditions. However, knowing the synthetic rate, one can calculate the catabolic rate from a measurement of the rate of decrease in ribosome content per cell, which represents the sum of synthesis and degradation. The cellular ribosome content is not readily measurable. However, if ribosomal RNA comprises a major and relatively constant fraction of total liver RNA, one may assume that total hepatic RNA content, which is directly determinable, is a measure of hepatic ribosomal content. We have demonstrated that this assumption is valid. Therefore, we can determine the change in cellular ribosome content from the change in the RNA:DNA ratio, taking total liver RNA as a measure of ribosome content and total liver DNA as a measure of the number of hepatic cells.

A series of animals fasted for up to 8 days yielded the RNA:DNA ratios plotted in Fig. 3. The decrease was exponential, the ratio reaching one-half the average initial value in 5 days. The net rate of loss equals the turnover rate in fed animals. However, since synthesis slows but does not cease during fasting, the catabolic rate under this condition must exceed that in the fed state. A fractional catabolic rate of 23% per day may be calculated from the 14% daily net fractional loss and the 9% daily fractional synthetic rate.

The progressively decreasing ribosome content and the constancy of the fractional rates of both synthesis and degradation during fasting indicate that the absolute rates decreased exponentially. Thus, the initial rates for synthesis and for degradation under these conditions were 0.25 and 0.64 mg of RNA per mg of DNA per day, respectively. At 6 days of starvation, the respective rates were 0.12 and 0.29 mg of RNA per mg of DNA per day.

Although intracellular loss of RNA, indicated by the diminished RNA:DNA ratio, accounts for most of the ribosomal depletion in starvation, a portion also may be due to loss of entire cells, for the livers of fasted animals consistently contained less total DNA than did those of the fed group. A reduction in DNA content was seen in all experiments and ranged from 10 to 25% after 5 days of fasting.

DISCUSSION

The half-lives of 5 days for both ribosomal protein and RNA that we have measured in the livers of well fed rats agree with reported values for the RNA component (17) and for total rat liver RNA (8). Identical results for both components were unexpected, for we had assumed that the nucleotide label, but not the amino acid label, would be reutilized to some extent. Thus, an apparently greater half-life could have been obtained for the RNA than for the protein. Recycling of isotopic markers in RNA studies, a potentially serious problem (4, 6), evidently was not a significant source of error in the present experiments. This lack of reincorporation into liver RNA of the RNA catab-

* C. A. Hirsch, manuscript in preparation.

* The assumption that there is a constant proportionality between liver DNA content and hepatic cell number, although not rigorously correct under all conditions (see "Discussion"), serves as a useful approximation (18, 16).
There can be no doubt that the RNA turnover rate that we have measured is the rate for ribosomal RNA itself, for Loeb, Howell, and Tomkins (17) obtained the same value for RNA identified as ribosomal by sucrose gradient analysis; they found that both the 28 S and 18 S components had the same half-lives. However, one may ask whether the protein with which we have dealt is truly ribosomal. The answer depends in part on how one defines ribosomal protein, a definition which must be operational at present since those proteins that are functional components of the ribosome have not yet been individually characterized. But we can exclude the possibility that the labeling observed is attributable to cytoplasmic protein remaining associated with the ribosomes. If this were the case, maximum specific activity should have been attained within the less than 15-min period during which there can be labeling of liver protein by a single injection of guanido-labeled arginine (9), for ribosomes present at the time of injection would carry such protein. In fact, the protein reached maximum specific activity between 3 and 24 hours after injection, a period that must largely represent the time for assembly of the ribosomal components and for transit from nucleus to cytoplasm.

A relevant question not answered by these experiments is whether the observed turnover rates obtain for the entire ribosome population, or only for a fraction that is replaced relatively rapidly; a stable population, of course, would remain unlabeled and unobserved by the technique used. However, such a possibility has been excluded by the results of continuous isotope-feeding experiments (5), which have shown that between 80 and 100% of rat liver RNA is replaced within 10 days, a figure consistent with the ribosomal turnover time of 7.2 days that we have obtained. Nevertheless, we cannot say unequivocally that the population of liver ribosomes is completely homogeneous with respect to its turnover. Although there was no apparent change in the slopes of the steady state curves in our experiments, or in those of Loeb et al. (17) that extended over a longer period, small deviations from linearity might not be detectable. In fact, some heterogeneity would be expected, if different cell types have different ribosomal turnover rates, since nonparenchymal cells comprise 5 or 10% of the liver mass (18, 19).

In any case, it is clear that the normal ribosomal replacement rate far exceeds the turnover time of the liver parenchymal cells, which may exceed 200 days (20, 21). Thus, the fraction of ribosomal turnover normally due to cell replacement is negligibly small. It is possible, however, that during fasting, loss of cells may account for a small portion of the RNA depletion observed, although the change in DNA content apparently need not imply a change in cell number. Lowe and Rand (22) and Lowe et al. (23) have observed a reversible decrease of about 18% in the DNA content per nucleus in rat hepatocytes after cortisone administration. Further, cell number can change without a corresponding change in total DNA content, for Conrad and Bass (24) have reported that, although the liver mass and the number of hepatic nuclei are reduced in rats after a 48-hour fast, the reduction in nuclei is largely offset by an increase in ploidy.

Our results show that the rates of both ribosome synthesis and catabolism vary in response to food deprivation, the latter increasing, the former decreasing. The exponentially decreasing rate for catabolism conforms to a first order kinetic model, one which might be expected. For synthesis, no simple model can explain both the immediate slowing, indicated by the decreased fractional rate, and the subsequent exponentially decreasing absolute rate.

The factors that mediate these responses to nutritional variations remain unknown. Experiments by Munro, Naismith, and Wikramanayake (25) have shown the dietary availability of amino acids (rather than of calories) to be a determinant, for the hepatic RNA content decreased markedly in rats fed a diet deficient in protein but providing a normal caloric intake. But it remains to be established whether or not a decrease in intracellular amino acid concentrations precedes the decreased synthesis and increased catabolism of liver ribosomes that accompany fasting. In fact, it has been reported that tissue amino acid levels are nearly normal in severe malnutrition (28). However, even small changes in the plasma concentrations of amino acids or other metabolites could influence the turnover of cellular components markedly, although indirectly, if, for example, the mechanisms responsible for turnover are modulated by certain hormones, the endocrine system being responsive to relatively slight alterations in the extracellular milieu. Thus, it is plausible that a small, fasting-induced decrease in the serum concentration of one or more essential metabolites may trigger the release of hormonal signals; this release, because of the differential responsiveness of different tissues, would then lead to diminished synthesis and increased catabolism of nonessential components, thereby maintaining nearly normal concentrations of precursors for essential biosynthesis. Clearly, the capacities for protein synthesis among the tissues, and hence the apportionment of metabolites, could be influenced selectively if the ribosome content of the tissues could be differentially altered. Such a differential effect during fasting is observed if the RNA contents of, for example, liver and tumor tissues are compared, the tumor retaining its ribosome complement, the liver becoming depleted (2, 30, 31). That hormones can mediate such differential effects is indicated by the observations that cortisone administration leads in the liver to increased (32-35) and in the thymus to decreased (36) RNA and protein synthesis. Cortisone and growth hormone also markedly influence the turnover of serum albumin (37).

A complete understanding of the regulation of macromolecular turnover clearly requires a detailed knowledge of the reactions involved, as well as of the factors that influence the reactions. Since the composition of mammalian cells is determined by the balance between synthesis and catabolism, both processes require equal attention. The understanding of synthesis of protein and RNA has advanced far more rapidly than has our knowledge of catabolism, although several recent reports have helped to define the conditions under which protein degradation occurs in liver (38) and protein and RNA degradation occur in a microorganism (39, 40). The need for additional work in this area
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is clear, especially in view of reports that turnover of RNA, and presumably of ribosomes, is altered in malignant cells (41-43).

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