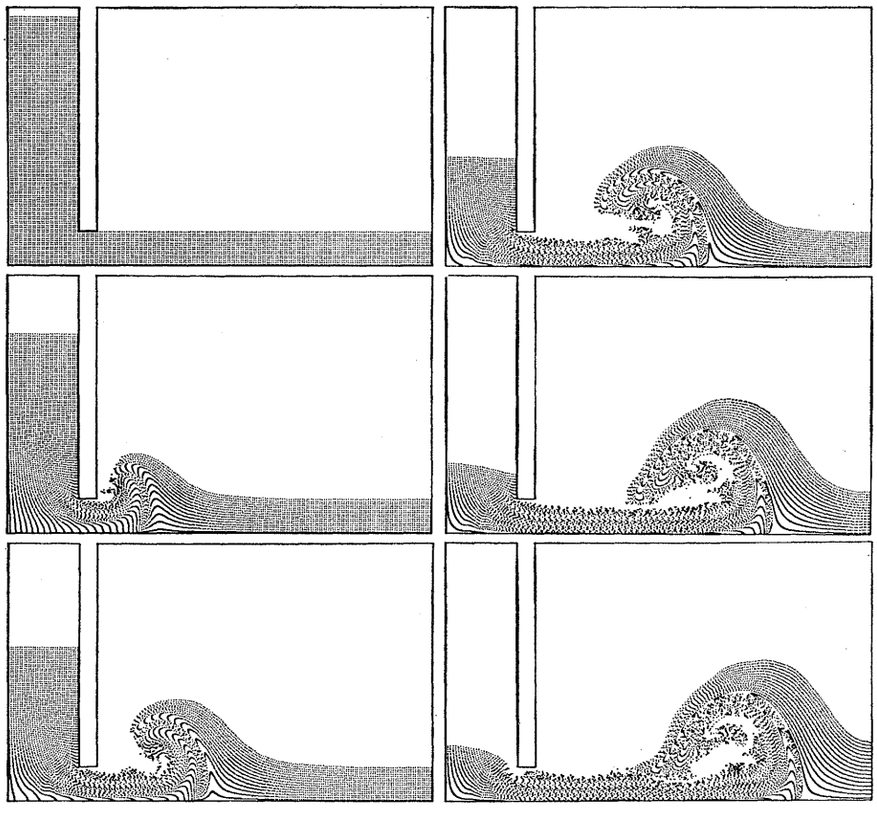

Fig. 1. Sequence of frames, produced by a computer, depicting the escape of water under a newly opened sluice gate into a tranquil pond; frames progress downward and from left to right.

the IBM 7030 (Stretch); a typical problem requires approximately 20 to 60 minutes. Available computer memories limit our studies to two space dimensions, but the method is equally applicable to three-dimensional problems. Configuration plots, such as those in Fig. 1, are obtained directly from the computer through the Stromberg-Carlson microfilm recorder and are not retouched.

The results are generated by the computer through a succession of small time steps or cycles, resembling the frames of a motion picture. The frames shown in Fig. 1 were selected from among hundreds that were obtained in the complete run. The sequence of processes necessary to accomplish the solution for each new frame is as follows:

1) The cycle begins with the full availability of velocity for each cell and of position for each marker particle; velocities and positions either are left over from the previous cycle or have been supplied as initial conditions. The velocities satisfy the conservation equation for the flow of incompressible liquid.

2) Pressures are calculated throughout the fluid in such a way that the resulting accelerations produce a velocity field that, at the end of the cycle, still satisfies the incompressibility equation; this requires the solution of a finite-difference Poisson's equation, accomplished by an iterative procedure.

3) Accelerations and corresponding new velocities are calculated.

4) The marker particles are moved with the velocity of the fluid.

The equations, boundary conditions, and techniques for solution (*1*) are related to those used by Fromm (*2*), but they have required modifications appropriate to a different set of primary dependent variables; Fromm employed the stream function and vorticity, variables that never enter directly into this pressure-velocity technique.

The results of such calculations have been compared with experiments, showing excellent agreement in every case. A study of the water motion from a broken dam, for example, proved that all aspects of the flow can be obtained at least as accurately as experiments could measure them (*1*). An advantage of the calculations is that they give more detailed data than can be obtained from experiments. In some cases, costly or time-consuming experimental studies can even be avoided by the careful use of such computer studies. Even more important, computer studies often provide a valuable basis for analytical studies of physical processes, giving both new ideas for models and bases for comparing the results of analysis of the models.

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3. This work was sponsored by the Atomic Energy Commission.

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## Turnover of Ribosomal RNA in Rat Liver

Abstract. *After a single injection of radioactive orotic acid and a "chase" of nonradioactive precursor, the specific activity of ribosomal RNA in rat liver decreases logarithmically at a rate corresponding to a half-life of about 5 days. The possible significance of this result is discussed with regard to control of protein synthesis.*

In dividing bacteria (*1*) and in L cells growing in tissue culture (*2*), ribosomal RNA (rRNA) turns over slowly, if at all, with respect to the generation time of the cells. This report shows that in normal rat liver, where the generation time of the cells is long, rRNA has a half-life of about 5 days and hence is replenished many times during the lifetime of a cell.

The existence, in the intact animal, of RNA-precursor pools of unknown size and number makes it difficult to estimate the turnover rate of rRNA from the rate of appearance of radioactivity after the administration of a radioactive precursor. For this reason the turnover of rRNA has been esti-

mated in the present studies from the rate of decline of specific activity of rRNA after a single injection of orotic acid-6-$C^{14}$ and a subsequent "chase" of nonradioactive orotic acid. The rate of turnover of rRNA in liver makes this a particularly suitable method (3).

It has previously been shown that, in liver, both 18*S* and 29*S* rRNA become labeled at the same rate after an injection of radioactive precursor (*4*), and for this reason we have not distinguished between the two components of rRNA except in certain control experiments (see below). Moreover, since we have found no difference in the rate at which the rRNA of free and membrane-bound ribosomes becomes labeled with orotic acid (*5*), the following investigation was limited to the study of free ribosomal aggregates—that is, to those ribosomes which can be isolated by sedimentation through 2.0*M* sucrose without the use of a detergent (*6*).

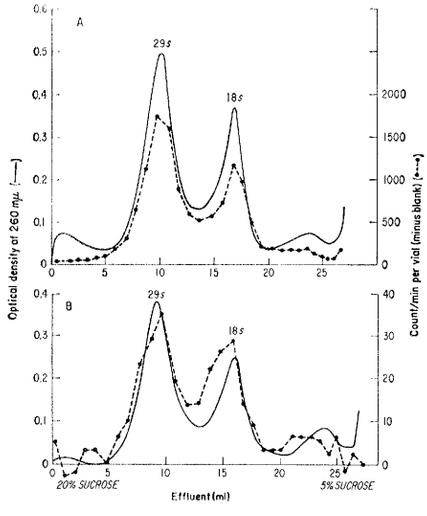


Fig. 1. Distribution of radioactivity in RNA obtained from ribosomes: *A*, from an animal in the first group (killed 70 hours after administration of orotic acid-6-$C^{14}$); and *B*, from an animal in the last group (killed 21 days after administration). Ribosomes were treated with 0.5 percent sodium dodecyl sulfate, and 0.5 ml of each clarified supernatant was layered over a 27-ml linear sucrose density gradient (5 to 20 percent) (*14*) containing 0.005*M* tris, *p*H 7.2; the gradient was then centrifuged at 25,000 rev/min for 19 hours in a Spinco SW 25.1 rotor; the chamber temperature was −5°C. After centrifugation the tube was punctured, and the effluent was passed through a recording spectrophotometer (Gilford Model 2000) equipped with a flow-through cell (a 0.5-cm light path was used; the optical density is that of the effluent corrected to a 1-cm light path). Fractions (45 drops) were collected directly in scintillation vials, and 10 ml of Bray's solution (*15*) was added prior to counting in a Packard Tri-Carb liquid scintillation spectrometer. A blank consisting of 10 ml of Bray's solution and 0.5 ml of 0.5 percent sodium dodecyl sulfate gave 50 count/min. Almost all of the radioactivity is in 18*S* and 29*S* ribosomal RNA. The apparent difference in the specific activities of the 18*S* and 29*S* peaks in *B* was atypical and was not observed at any other times; the radioactivity remaining at 21 days was not great, and the difference observed, which represents only a few counts per minute, is probably not significant.

Thirty male Sprague-Dawley rats (135 to 150 g) were injected intraperitoneally with 33 μc of orotic acid-6-$C^{14}$ (100 μc/ml; specific activity 5 μc/μmole), and 4¼ hours later they were injected with 160 μmoles of nonradioactive sodium orotate in 3.0 ml of isotonic medium. The animals tolerated the injections well and continued their normal Purina diet. They were then killed in 5 groups of 6 each at approximately 4-day intervals from 3 to 21 days after the original injection of radioactive orotic acid. Homogenates of individual livers were prepared and fractionated (*7*). After centrifugation at 20,000*g*, the supernatants were frozen and stored at −20°C. Later these 30 samples were thawed and 6.5 ml of each was layered, in 10-ml centrifuge tubes, over 3.0 ml of a solution containing 2.0*M* sucrose, 0.05*M* tris at *p*H 7.4, 0.025*M* KCl, and 0.005*M* $MgCl_2$. The tubes were centrifuged at 40,000 rev/min for 18 hours in a Spinco Model L preparative ultracentrifuge. Each pellet was then suspended in 2 ml of 0.5-percent sodium dodecyl sulfate and warmed at 37°C for 3 minutes to denature the ribosomes (*4*), and the suspensions were centrifuged at 35,000*g* for 25 minutes at 15°C (Sorvall RC-2) to sediment most of the glycogen. The clear supernatant phases were then decanted, and representative samples were subjected to sucrose density-gradient centrifugation (Fig. 1) to separate the 18*S* and 29*S* components of rRNA from contaminating material. The specific activity of rRNA was calculated from the areas under the 18*S* and 29*S* absorbancy peaks (determined by planimetry) and from the corresponding radioactivity. At 3 and 21 days after the injection of the labeled orotic acid (the extremes encompassed by the experiment) the radioactivity associated with the ribosomes was almost all contributed by rRNA (Fig. 1). This suggested that the radioactivity of rRNA might be sufficiently accurately determined simply by direct measurement of the optical density at 260 mμ and the radioactivity of the supernatants that had been treated with sodium dodecyl sulfate. Indeed the values calculated this way (*8*) generally agreed within 10 to 30 percent with those calculated by the more complicated procedure already described, and this simpler technique was therefore adopted (*9*).

Although it had been shown that several days after an injection of orotic acid-6-$C^{14}$ very little acid-soluble radioactive material remains in rat liver (*10*), an experiment was performed to check the fact that the pools of RNA precursors in liver were suffi-

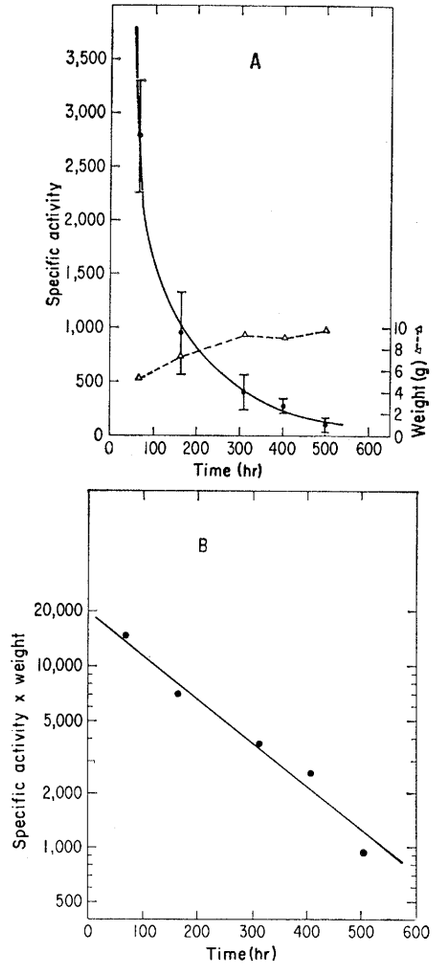


Fig. 2. *A*, Liver weight and specific activity of ribosomal RNA as a function of time (hours) after an injection of orotic acid-6-$C^{14}$. The points show the average values ± 1 SD for each group of six rats. *B*, Product of average specific activity and liver weight as a function of time after an injection of orotic acid-6-$C^{14}$. A very nearly linear logarithmic curve is obtained over a range corresponding to a 25-fold drop in specific activity. The specific activity is expressed as counts per minute per unit of optical density at 260 mμ.

ciently small to justify the plan of our experiment. Twenty-four hours after the administration of radioactive orotic acid the specific activity of rRNA was about the same whether or not a "chase" of nonradioactive orotic acid had been given 4¼ hours after the original injection of labeled acid, showing that even without a "chase" the incorporation of labeled precursor into RNA had become insignificant. The use of a "chase", however, was retained in the experimental procedure as it was well tolerated by the rats and could only serve to decrease still further the specific activity of the precursor pools.

The average specific activity of the RNA isolated from ribosomes from each group of six animals as a function of time after the administration of $C^{14}$-orotic acid is plotted in Fig. 2*A*. An interpretation of the decline of specific activity, however, must take into account liver growth over the course of the experiment, which is also shown in Fig. 2*A*. For this reason the logarithm of the product of the average specific activity and liver weight is plotted as a function of time in Fig. 2*B*. The relation is very nearly linear, and justifies mathematical representation by

$$\frac{\mathrm{d} \ln (s \cdot w)}{\mathrm{d}t} = -k$$

where $s$ is the observed specific activity and $w$ the liver weight at time $t$. Since the product $s \cdot w$ is proportional to the amount of radioactive rRNA in a given liver, $m$, and since the above points were all obtained at times when the rate of *formation* of radioactive rRNA molecules was negligible (as mentioned above), the slope of the line in Fig. 2*B* gives the desired destruction constant directly: $k = -\mathrm{d} (\ln m)/\mathrm{d}t = 121$ hours, or approximately 5 days. The fact that the decrement is logarithmic shows that a molecule of rRNA has no fixed life span, but rather that it is destroyed randomly regardless of its "age."

Estimates of the turnover of messenger RNA in dividing bacteria (*11*) and in HeLa cells growing in tissue culture (*12*) indicate that it is short-lived, and it is thought that, in these cells, the rate of protein synthesis may be controlled exclusively by the rate of synthesis of messenger RNA. In contrast, there is evidence that in liver a significant fraction of messenger RNA has a lifetime of more than 3 days (*13*), and it would therefore seem possible that here the rate of synthesis of specific proteins is dependent upon other factors. In view of the present finding that rRNA in liver, once it is formed, turns over rapidly with respect to the generation time, it would seem possible that protein synthesis in these cells may be influenced at least in part by regulation of ribosomal turnover.

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8. The radioactivity in a 0.5-ml sample of each supernatant treated with sodium dodecyl sulfate was determined in a Packard Tri-Carb liquid scintillation spectrometer after the addition of 10 ml of Bray's solution.
9. An error of 30 percent in the determination of the specific activity of the rRNA from one animal is not important in view of the variation of specific activity seen from one animal to another within a given group (see standard deviations plotted in Fig. 2*A*). More importantly, a discrepancy of this magnitude would not significantly affect the calculation of the turnover rate since, as can also be seen in Fig. 2*A*, there was, over the course of the experiment, more than a *25-fold* change in the observed specific activity.
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## Cystathioninuria: Nature of the Defect

Abstract. *A homogenate of liver obtained by biopsy from two patients with cystathioninuria, an inborn error of metabolism, cleaved radioactive cystathionine only slightly until an excess of pyridoxal phosphate was added. The apoenzyme failed to bind the coenzyme normally. Pyridoxine therapy of familial cystathioninuria thus has a sound basis.*

A grossly increased urinary excretion of the amino acid, cystathionine, has been observed in four patients (*1–4*). Patient A was a female, aged 64 years, imbecile with bilateral talipes calcaneo valgus (*1*). Patient B, now aged 47, is a man with developmental defects about the ears and mental aberrations (*2*). Patient C is a 12-year-old boy with mental deficiency and grand mal convulsions (*3*). Patient D is a 2-year-old boy with thrombocytopenia (*4*). His mental status is probably normal, but difficult to evaluate. The aminoaciduria results from an increase of the amino acid in the blood. Cystathionine is known to be involved in but one biochemical reaction, the transfer of sulfur from methionine to cysteine. The demonstration of probable heterozygotes in the families of three of the individuals suggests an inborn error of metabolism. Cystathioninuria has been attributed to a defect in the cystathionine-cleaving enzyme, cystathionase. The enzyme has been characterized; pyridoxal phosphate is the coenzyme (*5*).

One arresting fact has been the marked reduction in concentrations of cystathionine in urine and blood during administration of high doses of pyridoxine hydrochloride ($B_6$) orally or intramuscularly to three patients (B, C, and D). The $B_6$ appeared to increase the activity of cystathionase, and this effect may be explained by other possibilities. For example, cystathionine may be destroyed by deamination decarboxylation, or a "pre-cystathionine" deviation of sulfur may occur under the influence of $B_6$. In bacterial systems degradation of methionine requires pyridoxal phosphate (*6*). A homocysteine desulfhydrase system in *Proteus morganii* requires pyridoxal phosphate (*7*). A homocysteine desulfurase has been described in liver, kidney, and pancreas (*8*).

Studies in the patients have not