Uptake and Degradation of Cytoplasmic RNA by Hepatic Lysosomes

QUANTITATIVE RELATIONSHIP TO RNA TURNOVER*

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Past evidence has suggested that the lysosomal pathway is an important site of cytoplasmic RNA degradation in the hepatic parenchymal cell (Lardeux, B. R., Heydrick, S. J., and Mortimore, G. E. (1987) J. Biol. Chem. 262, 14507–14519). We now provide additional support for this notion by quantitating degradable RNA in lysosomes and correlating its pool size with hepatic RNA degradation. Rat livers, previously labeled with [6-14C]orotic acid, were perfused with graded levels of amino acids over the full range of induced autophagy; RNA degradation was determined from [14C]cytidine release. Close correspondence between the marker β-acetylglycosaminidase and the breakdown of RNA to cytidine in subcellular fractions indicated that the lysosome was the main site of catabolism, a conclusion supported by the fact that degradation was enhanced when external pH was lowered from 7 to 6. Although [14C]cytidine was also released in homogenates by the action of neutral ribonucleases on cytosolic RNA, this source was eliminated by unlabelled exogenous RNA. The size of the degradable RNA pool in lysosomes, determined from the total release of cytidine in homogenates, correlated directly with rates of hepatic RNA degradation over the full range of basal and induced degradation. A direct correlation was also seen between RNA degradation and cytidine pools within lysosomal particles. Because cytosolic cytidine was not taken up by lysosomes under these conditions, the pool could only have arisen from the breakdown of intralysosomal RNA. As determined by cytidine production, these findings support the view that the lysosomal-vacuolar system is the main, if not sole, site of induced and basal RNA degradation in liver.

Little is presently known of the mechanism of cytoplasmic RNA degradation in the intact cell. Past studies in the rat hepatocyte have revealed three possible pathways (reviewed in Refs. 1 and 2). All involve sequential actions of an endonuclease, an exonuclease, and in most cases, a nucleotidase. One system in mitochondria includes an alkaline endoribonuclease and a polynucleotide phosphorylase; no nucleotidase is known. The second possibility in the cytosol comprises a family of neutral endoribonucleases, a 3′-OH-specific phosphodiesterase, and 3′- and 5′-nucleotidases. The neutral endoribonuclease activity, however, is believed to be suppressed by cytosolic inhibitors under physiological conditions. The third is the lysosomal pathway, involving an acid endoribonuclease, an acid 5′ OH-specific phosphodiesterase, and a nonspecific acid nucleotidase. Acid ribonuclease and phosphodiesterase activity is predominantly lysosomal in distribution (2, 3).

Early views by a number of investigators, including an explicit proposal from Munro’s laboratory (4), have expressed the notion that physiological alterations in polysome aggregation are linked to the breakdown of rRNA by neutral ribonucleases. These views, however, have not found experimental support and have given way to evidence pointing to a possible role of the lysosomal pathway in RNA turnover (5–13). We have shown, for example, that stringent amino acid deprivation in the perfused rat liver induces identical increases in fractional rates of both RNA and protein degradation (5, 6). Together with earlier findings suggesting that ribosomal RNA and associated proteins turn over as a unit (7, 8), these results support the notion that RNA as well as protein is sequestered and catabolized within macroautophagic vacuoles (6). Rough endoplasmic reticulum and free ribosomes are in fact prominent components of these vacuoles in hepatocytes (9–12), and RNA breakdown is inhibitable by lysosomotropic agents (6, 13, 14).

In this study, we have attempted to establish a quantitative correlation between the uptake of RNA by lysosomes and degradation of RNA in the intact hepatocyte. If RNA is in fact catabolized in the same autophagic compartment as long-lived proteins, we should be able to demonstrate a pool of degradable RNA within lysosomal particles having kinetic features similar to those found earlier for protein (16). The results of this study have shown such a pool, and as with protein, its size correlates directly with RNA degradation over the full range of deprivation-induced regulation.

EXPERIMENTAL PROCEDURES

Animals—Male Lewis rats (Harlan Sprague-Dawley, Inc., Indianapolis), weighing 110–150 on the day of perfusion, were maintained on commercial laboratory chow and water ad libitum under controlled temperature and lighting (off 1900 h, on 0700 h). RNA was labeled in vivo by three intraperitoneal injections of [6-14C]orotic acid in 0.5–1.0 ml of 0.85% NaCl at 198, 84, and 60 h before perfusion. The total dose ranged from 10 to 20 μCi. At the time of perfusion, 93% of the total liver radioactivity was recovered in RNA (5).

Liver Perfusion—Livers were perfused in situ at 37 °C in both the cyclic and single-pass modes as described (6, 12, 15). The basic cyclic perfusion medium comprised 4% (w/v) bovine serum albumin (fraction V, Pentex, ICN Biologicals, Costa Mesa, CA) and 27% (v/v) washed bovine erythrocytes in Krebs-Ringer bicarbonate buffer, pH 7.4. The single-pass medium was identical except that 10 mM glucose

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was added and the albumin was reduced to 3%. Single-pass (nonrecirculating) perfusion was employed when it was necessary to maintain constant amino acid concentrations in the inflowing medium. In this situation, the outflow was collected separately and discarded. Amino acid levels equivalent to those in normal plasma (1x), or multiples thereof, were added by the addition of appropriate amounts of a concentrated stock mixture of 20 amino acids in 0.85% NaCl, pH 7.4. The amino acid composition of plasma after addition of the foregoing 1x mixture is given elsewhere (6, 18).

Liver Homogenization and Fractionation—Following perfusion, livers were rapidly excised and homogenized in ice-cold 0.25 M sucrose, 1 mM EDTA, pH 7.7. Percoll-EDTA (Cl- form), one of which was connected immediately below the supernatant. The P + S fraction was separated by spinning the initial homogenate at 16,000 × g for 10 min in the same rotor, leaving the P + S in the supernatant.

Homogenate fractions were incubated in 25-ml glass Erlenmeyer flasks in a shaking water bath at 37 °C for periods up to 180 min. In experiments where sequeserted RNA in lysosomes was quantitated, Torula yeast RNA (80 mg of RNA/ml in the above sucrose-EDTA solution) was added to the homogenate fractions at final concentrations ranging from 8 to 40 mg/ml of incubate. In the experiments of Fig. 3, labeled ribosomes were prepared as described by Nygard and Hultin (20) from rat livers previously labeled with [6-3H]uridine and [14C]cytidine. Samples taken during the course of incubation were treated as follows. RNA, DNA, and protein were precipitated by the method of Lardeux et al. (19). Half of each liver was homogenized in an all-glass tissue grinder in order to minimize damage to lysosomes that can occur when pellets are resuspended. In livers perfused under Ox conditions, the accumulation of fragments in the P + S fraction whose breakdown might not be inhibitable by added RNA.

RNA Degradation—Rates of cytoplasmic RNA breakdown were determined from the release of [14C]cytidine as detailed separately (5, 6). In brief, after 40 min of single-pass perfusion with various amino acid additions, flow was switched to a second, cyclic perfusion reservoir containing 0.5 M sucrose, 10 mM potassium phosphate (K:HPO4, + KH2PO4, pH 7.4) to promote mitochondrial aggregation. The homogenate was added to this fraction and centrifuged. The P + S fraction was separated by spinning the initial homogenate at 16,000 × g for 10 min in the same rotor, leaving the P + S in the supernatant.

Radioactivity was counted in a liquid scintillation spectrometer. All data are expressed as disintegrations/min. All data in figures and tables are given as means ± S.E. Differences between means were evaluated by Student's t test; values of p > 0.05 were considered not significant.

RESULTS AND DISCUSSION

Previous studies have validated the usefulness of cytidine as a marker for RNA degradation in the perfused rat liver (5, 6) and isolated rat hepatocyte (14). Owing to the virtual absence of cytidine deaminase activity in rat liver, cytidine does not enter the uridine degradation pathway and is therefore not oxidized. When the reutilization of [14C]cytidine is prevented by the addition of unlabeled cytidine, the rate of label release has been shown to reflect the direct loss of cytosine nucleotide from RNA (5).

RNA Degradation in Liver Homogenate Fractions—A major obstacle in the evaluation of degradable RNA within lysosomes is interference from the breakdown of RNA by neutral ribonucleases. In homogenates most of these enzymes are bound to cytosolic surfaces of subcellular particles (25, 26) and become active owing to the decreased effectiveness of endogenous inhibitors by dilution. Attempts were made to overcome this activity by adding heparin and a ribonuclease inhibitor from human plasma (Boehringer Mannheim). Unfortunately, both were relatively inactive at levels feasible for routine use. We did discover, though, that Torula yeast RNA was capable of suppressing the release of [14C]cytidine both in the unfractionated homogenate and P + S fraction (Fig. 1B). The effect was not species specific since rat liver RNA proved to be equally effective. Except for a delay in onset, the effect of the added RNA was highly linear over the 180-min incubation period (Fig. 1B). It is important to note that the delay was limited to the P + S fraction since none was seen with the inhibition of exogenous RNA in NML particles (Fig. 2B). The reason for this difference is not known, but it could relate to an unequal distribution of specific neutral ribonucleases among subcellular particles (26, 27) or to the presence of RNA fragments in the P + S fraction whose breakdown might not be inhibitable by added RNA.

Fig. 24 depicts the time course of labeled cytidine release in the NML fraction. In most experiments, release was determined by subtracting values of the P + S from the total homogenate in order to minimize damage to lysosomes that can occur when pellets are resuspended. In livers perfused under Ox conditions, the accumulation of [14C]cytidine in the presence of yeast RNA was rapid during the initial 30 min. Thereafter, labeled cytidine uptake was virtually identical to that previously reported for the breakdown of protein sequeserted in lysosomes (16). The addition of plasma amino acids at concentrations (10x) that maximally inhibit macroautophagy (12) markedly decreased the total quantity of cytidine released.

Degradable RNA Is Sequeserted in Lysosomes—The fact that most of the labeled cytidine fraction released in Figs. 1A and 2A after addition of exogenous RNA was derived from the NML.

2 S. J. Heydrick and G. E. Mortimore, unpublished results.
between these two possibilities, mitochondria were selectively breakdown within lysosomes or mitochondria. To distinguish down in this organelle (28). It should also be mentioned that cytidine pools in lysosomes.

The findings in Tables 40 min they were homogenized and the subcellular fractions incubated with and without 20 mg/ml additions of yeast RNA for the determination of cytidine release. A (upper panel), cytidine release in the total homogenate and P + S fraction. B (lower panel), inhibitory effects of yeast RNA (control minus added RNA) on cytidine release in the total homogenate and P + S fraction. Results are means of five experiments.

fraction with little from the P + S argues for degradable RNA within NML particles. As shown in Table I, the initial release of [14C]cytidine from subcellular particles closely paralleled \( \beta \)-acetylglucosaminidase and citrate synthase activities, but not DNA, suggesting that the label was derived from RNA breakdown within lysosomes or mitochondria. To distinguish between these two possibilities, mitochondria were selectively aggregated with the addition of 10 mM potassium phosphate, a procedure which shifts much of the mitochondria from the M + L to the N fraction. From data in Table II, it is evident that cytidine release and lysosomes were both enhanced 2-fold in the M + L fraction relative to the mitochondrial marker, showing that the lysosome rather than the mitochondrion is the primary intraparticulate source of cytidine. It is known that nucleosides are the major product of RNA breakdown in this organelle (28). It should also be mentioned that the findings in Tables I and II support results from later experiments (see Fig. 7) which demonstrate the presence of cytidine pools in lysosomes.

Fig. 1. Effect of yeast RNA (exogenous or exoRNA) on the release of cytidine in liver homogenate fractions. Livers from nonstarved rats, previously labeled with [6-\(^{14}\)C]orotic acid, were perfused in the single-pass mode without added amino acids (Ox). After 40 min they were homogenized and the subcellular fractions incubated with and without 20 mg/ml additions of yeast RNA for the determination of cytidine release. A (upper panel), cytidine release in the total homogenate and P + S fraction. B (lower panel), inhibitory effects of yeast RNA (control minus added RNA) on cytidine release in the total homogenate and P + S fraction. Results are means of five experiments.

Inhibitory Effectiveness Exogenous RNA on Cytosolic RNA Degradation—Although Torula yeast RNA suppressed the release of labeled cytidine from cytosolic RNA (Fig. 1), its inhibition was less than maximal under the conditions used. To establish its effectiveness, varying quantities were tested against a preparation of radioactive liver ribosomes. The lowest concentration of yeast RNA, 8 mg/ml, decreased the release of [14C]cytidine from the ribosomes by nearly 95% (data not shown). Complete inhibition, though, required much larger quantities. A plot of the reciprocal of RNA concentration versus labeled cytidine release was highly linear, and the slope regressed through both zero intercepts. The mechanism of inhibition is not known, but the findings in Fig. 3 suggest that very high concentrations of exogenous RNA could restrict the access of labeled rRNA to site(s) of catalysis.

A similar test of inhibitory effectiveness was applied to the breakdown of labeled RNA sequestered in NML particles. Since membranes are impermeable to exogenous RNA, the breakdown would not be affected, even at high levels of added
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Livers from nonstarved rats, previously labeled with [6-14C]orotic acid, were perfused without added amino acids as in the experiments of Figs. 1 and 2. Whole homogenates and subcellular fractions, described below, were incubated 10 min for initial cytidine release and prepared for analysis as detailed under "Experimental Procedures." As in Figs. 1 and 2, the heavy particle fractions were analyzed by difference to avoid lysosomal damage from pellet resuspension. Values under N represent differences between results obtained with the total homogenate and the postnuclear supernatant (PNS); M + L, differences between the PNS and P + S fractions. Results are means ± S.E. of five to eight experiments.

### TABLE I

| Subcellular distribution of cytidine release, β-acetylglucosaminidase, citrate synthase, and DNA |

| Livers from nonstarved rats, previously labeled with [6-14C]orotic acid, were perfused without added amino acids as in the experiments of Figs. 1 and 2. Whole homogenates and subcellular fractions, described below, were incubated 10 min for initial cytidine release and prepared for analysis as detailed under "Experimental Procedures." As in Figs. 1 and 2, the heavy particle fractions were analyzed by difference to avoid lysosomal damage from pellet resuspension. Values under N represent differences between results obtained with the total homogenate and the postnuclear supernatant (PNS); M + L, differences between the PNS and P + S fractions. Results are means ± S.E. of five to eight experiments. |

<table>
<thead>
<tr>
<th>% of total homogenate</th>
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<tbody>
<tr>
<td>Cytidine release</td>
</tr>
<tr>
<td>β-Acetylglucosaminidase</td>
</tr>
<tr>
<td>Citrate synthase</td>
</tr>
<tr>
<td>DNA</td>
</tr>
</tbody>
</table>

### TABLE II

Cytidine is released from lysosomes, not mitochondria

Livers from nonstarved rats, previously labeled with [6-14C]orotic acid, were perfused for 40 min in the single-pass mode without amino acids (0x) and homogenized in sucrose-EDTA with and without additions of 10 mM potassium phosphate, pH 7.4, a treatment previously shown to promote mitochondrial aggregation (19). N, M + L, and P + S fractions were prepared as described under "Experimental Procedures." The M + L pellets were resuspended for enzyme assays and measurement of [14C]cytidine release during 10-min incubations at 37°C, pH 7.0. Recoveries of β-acetylglucosaminidase and citrate synthase from all fractions (not shown) averaged 97 and 92% of the total activities. Results are means ± S.E. of six experiments.

<table>
<thead>
<tr>
<th>Information</th>
<th>M + L</th>
<th>P + S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of total activity in M + L fraction</td>
<td>41.1 ± 2.1</td>
<td>33.9 ± 1.2</td>
</tr>
<tr>
<td>Relative separation</td>
<td>0.94 ± 0.07</td>
<td>0.94 ± 0.05</td>
</tr>
<tr>
<td>Normalized to citrate synthase</td>
<td>2.07 ± 0.11</td>
<td>0.46 ± 0.02</td>
</tr>
</tbody>
</table>

RNA, thus allowing the release of [14C]cytidine from the sequestered RNA to be measured. In Fig. 4, yeast RNA strongly suppressed the release of labeled cytidine from the NML fraction at 8 mg/ml both in livers with (0x) and without (10x) induced macroautophagy. Inhibition, though, did not appear to level off until high yeast RNA concentrations were reached. As in the experiment of Fig. 3, plots of the reciprocal of exogenous RNA concentration versus total degradable RNA were linear from 8 to 40 mg/ml in both autophagic states (Fig. 4, inset). However, in contrast to results of Fig. 3, the slopes regressed to positive values at an infinite concentration of exogenous RNA. These intercepts, which indicate the presence of sequestered RNA, were roughly comparable to the [14C]cytidine released from the NML fraction in Fig. 2A under similar conditions. Finally, it should be mentioned that the inhibitory effect of yeast RNA on cytidine release in the P + S fraction did not change between 8 and 40 mg/ml (data not shown), showing that the P + S generated a small amount of cytidine that was not inhibitable by RNA. This could be explained by the breakdown of nucleotides and/or RNA fragments.

Although yeast RNA would not be expected to permeate the lysosomal membrane, Ahlberg et al. (29) have shown that hepatic lysosomes isolated in metrizamide can take up and degrade macromolecular material by membrane invagination. This phenomenon may be an unique feature of metrizamide preparations since we have shown that cytosolic protein is not taken up by lysosomes in lysosome-enriched fractions prepared in 0.25 M sucrose (16). However, we excluded the

![Fig. 3. Effect of exogenous RNA on the breakdown of labeled ribosomes to [14C]cytidine in unlabeled rat liver homogenates. Ribosomes from rat livers previously labeled with [6-14C]orotic acid were isolated as described in (19). Ribosomal samples were then added to unfractionated liver homogenates after which the total release of [14C]cytidine in the presence of graded additions of unlabeled yeast RNA was determined during 120–150 min of incubation. Total release is shown against the reciprocal of yeast RNA concentration. Values are means ± S.E. of four experiments.](image1)

![Fig. 4. Effect of exogenous RNA on cytidine release in the NML fraction of previously labeled rat liver homogenates, prepared as in Figs. 1 and 2. Livers were perfused in the presence (10x) or absence (0x) of a complete mixture of amino acids. The total release of [14C]cytidine in the NML fraction, measured by difference from values in the whole homogenate and P + S fraction (as in Fig. 2A), was determined in the presence of graded additions of yeast RNA. Inset, total release is depicted against the reciprocal of yeast RNA concentration. Results are means ± S.E. of three to eight experiments.](image2)
remote possibility that exogenous RNA might have induced the effect under the present conditions. In four experiments with liver homogenates (prepared as in Fig. 1), only 3.1 ± 0.27% of a trace addition of [14C]methemoglobin was degraded to acid-soluble material in the whole homogenate after 150 min of incubation at 37 °C, pH 7.0, a rate about one-tenth as rapid as that reported in fractions prepared with metrizamide (29). Most (96%) of the degradation occurred in the P + S fraction while only 4% was found in the NML; 10 mg/ml additions of yeast RNA had no effect on NML breakdown.

Effect of pH on Lysosomal RNA Degradation—Isolated lysosomes are relatively stable between pH 5 and 8 (30, 31), and optimal hydrolytic function is obtained within an external pH range of 5 to 6, which maintains a matrix pH of that the increase was similar to that reported for '2sI-albumin point it leveled off in parallel with the control. It is of interest Fig. 5, 6 depicts two correlations between pH range of 5 to 6, which maintains a matrix pH of that the increase was similar to that reported for '2sI-albumin point it leveled off in parallel with the control. It is of interest

Fig. 6. Correlation between hepatic RNA degradation and intralysosomal, degradable RNA. Livers from nonstarved rats, previously labeled with [6-14C]orotic acid, were perfused in parallel in the single-pass mode with graded levels of plasma amino acids (0x, 0.5x, 1x, and 10x); after 40 min, some livers were switched to a second perfusion for the measurement of RNA degradation (see "Experimental Procedures") while others were removed and homogenized for the determination of degradable RNA pools. The latter was determined as in Figs. 1 and 2 by the total release of cytidine from NML particles at pH 7 in the presence of yeast RNA (20 mg/ml). The broken line displays observed pools; the solid line, pool values extrapolated to an infinite concentration of yeast RNA. The latter (0x and 10x) were computed from the slopes of the regressions in Fig. 4 (inset); the slopes at 0.5x and 1x were taken as proportional to the average rates of hepatic RNA degradation at these levels. The points are means of three to five pool and three to five rate measurements.

Table III

<table>
<thead>
<tr>
<th>Plasma AA</th>
<th>Total homogenate</th>
<th>P + S</th>
<th>NML (corr)</th>
<th>RNA degradation (B)</th>
<th>R/A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmoI CYD/liver/100 g rat</td>
<td></td>
<td>nmoI CYD/min/100 g rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0x</td>
<td>159.6</td>
<td>126.0</td>
<td>32.8</td>
<td>19.7</td>
<td>18.7</td>
</tr>
<tr>
<td>±12.5</td>
<td>±10.8</td>
<td>±2.7</td>
<td>±2.1</td>
<td>±1.1</td>
<td>±1.1</td>
</tr>
<tr>
<td>0.5x</td>
<td>110.2</td>
<td>88.0</td>
<td>22.1</td>
<td>13.2</td>
<td>13.2</td>
</tr>
<tr>
<td>±10.2</td>
<td>±8.5</td>
<td>±1.7</td>
<td>±0.9</td>
<td>±1.0</td>
<td>±1.0</td>
</tr>
<tr>
<td>10x</td>
<td>46.0</td>
<td>39.1</td>
<td>6.9</td>
<td>3.2</td>
<td>3.1</td>
</tr>
<tr>
<td>±3.0</td>
<td>±2.6</td>
<td>±1.4</td>
<td>±1.3</td>
<td>±0.2</td>
<td>±0.2</td>
</tr>
</tbody>
</table>

breakdown was maximally suppressed. On the assumption that [14C]cytidine released by the perfused liver was derived from lysosomes, the corrected slope, 0.096 min⁻¹, can be taken
as a first approximation of the rate constant of lysosomal RNA turnover. This estimate, though, is probably too high since pool values, which were determined at an external pH of 7, would be smaller than those obtained at a lower pH. From results in Fig. 5, it is likely that pool sizes at an external pH of 6 (matrix pH of about 5) would be at least 26.6% larger than those at pH 7. A closer approximation cannot be made without evaluating the effects of exogenous RNA at lower pH values. Nevertheless, the present estimates are in accord with reported rate constants for lysosomal protein (16, 34) and volume turnover (11, 12, 35), and any further pH adjustment would not affect the zero intercepts of the regression.

A direct relationship was also found between RNA degradation in the perfused liver and pools of \[^{14}C\]cytidine in the NML fraction determined at the start of incubation (Table III). If it can be shown that the cytidine was generated from the breakdown of previously sequestered RNA, these findings would provide independent confirmation of the correlation in Fig. 6. Uncertainty relating to the use of exogenous RNA would be eliminated since the determinations were made before the RNA was added.

The origin of the cytidine pool was determined by comparing the subcellular distribution of labeled cytidine in livers pulse labeled with \[^{14}C\]cytidine with that in livers previously labeled with \[^{6-14}C\]orotic acid (prelabeled RNA) and perfused 40 min in the single-pass mode without amino acids or in homogenates from unlabeled livers to which \[^{14}C\]cytidine was added immediately after homogenization. Analyses were performed either in homogenates from livers that were perfused in the single-pass mode for 15 min with 0.5 mM \[^{14}C\]cytidine (pulse-label Cyd) or in homogenates to which \[^{14}C\]cytidine was added after homogenization, as above. Values are means ± S.E. of three to four livers; the S.E. of fraction P (shaded) is too small to depict.

Validity of Intralysosomal RNA Measurement—The determination of degradable RNA in the lysosomal matrix rests on three major premises: (i) that CMP, an immediate product of RNA hydrolysis, is quantitatively converted to cytidine; (ii) that interference from the breakdown of labeled RNA outside the lysosome can be eliminated; and (iii) that the sequestered RNA pool is totally degraded during the incubation of lysosomal fractions. The first is well established. Acid ribonuclease and phosphodiesterase activities are primarily lysosomal in location and 3'-CMP is the major if not sole end product (2, 3). Although CMP is neither phosphorylated nor transported, the release can readily escape the lysosomal matrix as cytidine (28) via a nucleoside transporter that has recently been described by Fisoni and Thoene (39).

With regard to the second, we have demonstrated in the present study that the breakdown of labeled ribosomes by neutral ribonucleases is completely inhabitable at an infinite concentration of yeast RNA. Since the addition of yeast RNA failed to alter the low rates of degradation of \[^{14}C\]methemoglobin in homogenate fractions, we may conclude that exogenous RNA had no effect on lysosomal latency or the uptake of macromolecular material into the lysosomal matrix.

The validity of the third premise is based largely on strong parallels between intralysosomal protein (16) and RNA in their kinetics of breakdown. Both exhibit time courses characterized by a rapid initial rate, a slow linear phase, and an abrupt cessation of degradation after 20–120 min. In addition, both respond to a decrease of external pH from 7 to 6 by comparable increases (27–30%) in the amount of substrate degraded (16). Although it has not been possible to measure the sequestered pools of RNA or protein directly, the quantity of degradable protein determined from the total amount of valine released (where the matrix pH was 4 to 5) has agreed closely with values predicted from stereologic measurements of vescular volume multiplied by cytoplasmic protein concentration (34).

Significance of the Correlation Between Pools of Lysosomal RNA and Hepatic RNA Degradation—When considered with the foregoing evidence, the direct relationship in Fig. 6 between cytidine produced from lysosomal pools of degradable RNA and rates of hepatic RNA degradation leaves little doubt that the lysosomal pathway is the major site of cellular RNA breakdown during amino acid deprivation. Independent support for this conclusion is found in Table III from the direct relationship between RNA degradation and intralysosomal cytidine. Because the latter pool is derived solely from the breakdown of sequestered RNA, this direct relationship would not be affected by the degradation of cytosolic RNA or its suppression by exogenous RNA. While the slope of the regression in Fig. 6 of 0.096 min\(^{-1}\) is in reasonable agreement with a previous correlation between intralysosomal protein and proteolysis of 0.087 min\(^{-1}\) (34), there is no a priori reason for expecting the same value since different hydrolytic pathways are involved. The important features of the two correlations are their linearity and regression through zero; closer approximation of the degradable RNA pool based on pH or other

![Graph](image-url)
general factors would very likely affect the slope but not the intercept.

Despite the agreement between these correlations, one cannot fully exclude the possibility that some cytidine is also produced extralysosomally in the intact cell. Sameshima et al. (13) proposed a dual pathway in cultured cells based on the failure of chloroquine to inhibit RNA breakdown completely. This finding, though, must be interpreted with caution since lysosomal function is only partially suppressed by lysosomal agents (reviewed in Ref. 38). In liver, alterations in the aggregate volume of macroautophagic vacuoles mediated by amino acids correlate directly with rates of protein and RNA degradation (6). Thus, if both lysosomal and non-lysosomal mechanisms existed for the production of cytidine, their control would have to be precisely coordinated over the deprivation-induced range in order to account for the regula-

The overall magnitude of the 5' route or, more importantly, its possible role in the selective breakdown of specific classes of RNA has not to our knowledge been evaluated.

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REFERENCES