CONSERVATION OF RIBOSOMAL RNA DURING COMPENSATORY RENAL HYPERTROPHY

A Major Mechanism in RNA Accretion

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ABSTRACT

After removal of one mouse kidney, compensatory hypertrophy in the remaining kidney is marked in 2 days by a 20% average increase in ribosomal RNA (rRNA) per cell. Both 28S and 18S RNA are conserved during the initial stages of compensatory renal hypertrophy to an extent sufficient to account for the rest of the observed accumulation of rRNA. Like some cultured cells, the kidney conserves rRNA during physiological growth.

2 days after unilateral nephrectomy in rats and mice, the amount of RNA per cell in the remaining kidney is increased 20–40% (6, 8, 9, 19, 22, 32). Cell division and DNA synthesis are not pronounced, nor are they necessary for compensatory renal hypertrophy.

Increases in total renal RNA during compensatory hypertrophy are likely to be due to an increase in the quantity of ribosomal RNA (rRNA), since rRNA accounts for at least 85% of cellular RNA (13). However, an increased rate of rRNA synthesis could not actually be demonstrated (11). An alternative explanation for the accumulation of RNA is that rRNA is degraded slower than normal while its rate of synthesis continues unchanged. Although the turnover rate of rRNA in mouse kidneys several days after the onset of renal hypertrophy is the same as that in kidneys of sham nephrectomized mice (3, 22), few measurements of the degradation rate of rRNA have been made during the first stages of growth, when the rate of accretion of RNA is most pronounced (10).

We show that the rate of degradation of both 28S and 18S RNA during the first 4 days of compensatory renal growth is distinctly slower than in nongrowing kidney.

MATERIALS AND METHODS

Animals

40-day old male Charles River mice (Charles River Breeding Laboratories, Wilmington, Mass.) were used for unilateral nephrectomy and controls as described (23). Injections of radiochemicals were intraperitoneal.

Isolation of Ribosmes and RNA

Two kidneys were homogenized in 10 ml of ice-cold RSB buffer with 10 strokes of the tight pestle of a Dounce homogenizer (Kontes Glass Co., Vineland, N. J.). After removal of 1 ml of the homogenate for estimation of RNA and DNA, the remainder was centrifuged at 10,000 *g* for 10 min. $MgCl_2$ was added to the supernate to a final concentration of 70 mM, and the preparation was left for 45 min in ice. After centrifugation at 10,000 *g* for 10 min. the ribosomal pellet was resuspended.

For preparation of polyribosomes the cell homogenate was centrifuged at 10,000 *g* for 12 min. The supernate was layered on a discontinuous sucrose gradient (1.5 ml of 1.5 M sucrose over 2.5 ml of 2.0 M sucrose in 0.25 M NaCl, 0.05 M $MgCl_2$, and 0.01 M Tris, pH 7.4 at 20°C) and centrifuged at 50,000 rpm in a Spinco type 65 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) for 4 h to deposit the polysomes.

Polysome and ribosome preparations were dissolved in

4 ml of NETS buffer and extracted with equal volumes of phenol chloroform:isoamyl alcohol (29). Successive extractions were performed until no interphase was visible, after which three more extractions with chloroform:isoamyl alcohol alone were performed. To the resulting aqueous phase, 2 vol of ethanol was added; RNA was precipitated at −20°C overnight.

The RNA precipitate collected by centrifugation at 15,000 *g* for 15 min was dissolved in 4 ml NEB buffer containing 0.1% Sarkosyl NL-30 (Ciba-Geigy, Ciba Corp. Summit, N.J.) and reprecipitated by addition of 4 ml of 4 M LiCl. The final high-molecular-weight RNA precipitate was concentrated by centrifugation as above and resolved on 15–30% sucrose gradients in NETS buffer.

Estimation of 28S and 18S rRNA by Isotope Dilution

Labeled RNA was prepared from both kidneys of a mouse injected with 100 μCi of [^{14}C]uridine 36 h previously. After the kidneys were homogenized in 10 ml of phenol and 10 ml of NETS buffer at room temperature, total RNA was extracted. 28S and 18S rRNA from LiCl-precipitated high-molecular-weight RNA was resolved on sucrose gradients. To eliminate possible contamination with mRNA, approx. 4 A_{260nm} U of RNA in 2 ml of 10 mM Tris-HCl buffer, pH 7.4, made 0.5 M with respect to NaCl, were applied to a column of 0.2 g of oligo-d(T) cellulose (Type F-2, Collaborative Research, Waltham, Mass.). The column was equilibrated with 0.5 M NaCl, 10 mM Tris-HCl, pH 7.4, and was washed with several column volumes of this same buffer followed by 3 vol of 10 mM Tris-HCl, pH 7.4, at 20°C. Fractions of about 1 ml were collected, from which aliquots were assayed for radioactivity (not shown). The rRNA fraction not bound to oligo-d(T) cellulose was used for further analysis.

To estimate directly the rRNA content of kidneys during compensatory growth, four kidneys were homogenized in the presence of approx. 16,000 cpm of ^{14}C-labeled 28S RNA and 5,000 cpm of ^{14}C-labeled 18S RNA (sp act of 53,000 cpm/A_{260nm}). The rRNA was resolved on sucrose gradients, and the amount of extracted RNA was calculated from the ^{14}C-specific activity of recovered RNA. RNA and DNA were estimated by the method of Munro and Fleck (25) (See also references 7 and 16).

Radiochemicals and Buffers

L-[*methyl*-^{3}H]methionine, [5-^{3}H]orotic acid, [6-^{14}C]-orotic acid, [5,6-^{3}H]uridine and [2-^{14}C]uridine were bought from New England Nuclear Corp., Boston, Mass. NETS buffer: 0.1 M NaCl, 0.01 M ethylenediaminetetraacetic acid (EDTA), 0.01 M Tris, pH 7.4 at 20°C, and 0.2% sodium dodecyl sulfate. NEB buffer: 0.01 M NaCl, 0.01 M EDTA, 0.01 M Tris, pH 7.4 at 20°C. RSB buffer: 0.01 M NaCl, 0.0015 M $MgCl_2$, and 0.01 M Tris, pH 7.4 at 20°C.

RESULTS

Turnover of rRNA in Normal Mouse Kidneys

The half-life of rRNA in normal mouse kidneys after labeling with [^{14}C]orotic acid is about 4 days (3, 22). However, different rates of decay of the two major rRNA species were seen after analysis of 28S and 18S RNA resolved on sucrose density gradients (Fig. 1). As shown in Fig. 2 A, when the two rRNA species extracted from kidney were prelabeled with [^{3}H]methionine, each had a longer apparent half-life than 4 days. Since this finding might have resulted from reutilization of the methionine residue, [^{3}H]uridine was used as precursor in a similar experiment; half-lives of 4.1 days for 28S RNA and 6.1 days for 18S RNA were obtained (Fig. 2 B).

Within the limits of biological variation, the results illustrated in Fig. 2 support the view that decay of rRNA is a first-order process. From the equation of Quincey and Wilson (31) the turnover rate (rate of synthesis and rate of degradation) can be calculated at 0.693 × pool size/half-life. If the half-life of total rRNA is taken to be about 5 days, the turnover rate is approx. 14% per day.

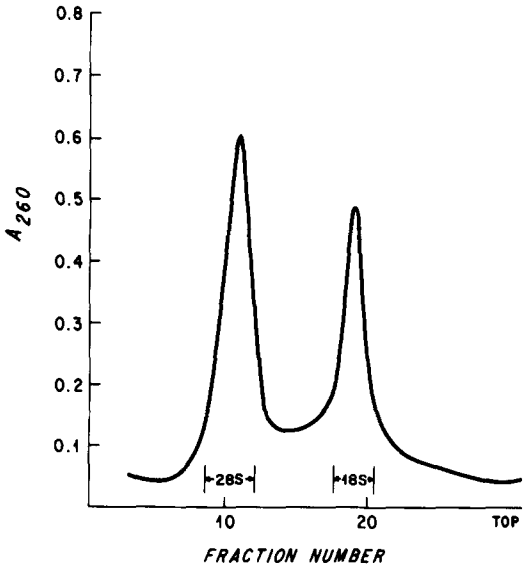

FIGURE 1 Sedimentation pattern of renal rRNA. After centrifugation in a Spinco SW-27 rotor at 23,500 rpm for 15 h at 22°C, the gradient was fractionated with continuous monitoring at 260 nm. RNA in fractions corresponding to the 18S RNA and 28S RNA optical density peaks (brackets) was precipitated with 2 vol of cold ethanol.

Effect of Unilateral Nephrectomy on rRNA Degradation: Prelabeling with [^3H]methionine

During compensatory growth, if increased synthesis of renal rRNA is associated with a normal rate of degradation, the result should be decreased specific activity of prelabeled rRNA. On the other hand, at a stable rate of rRNA synthesis, if rRNA accumulates because ribosomes are degraded less rapidly, the specific activity of rRNA from the growing kidney should be higher. Experiments were designed to test those possibilities.

The specific activity of rRNA in the remaining kidneys of mice subjected to unilateral nephrectomy was followed after prelabeling for 2 days with [^{3}H]methionine. After injection of [^{3}H]methionine, 45S ribosomal precursor RNA is maximally labeled in about 20 min (1). By 90 min, there is comparatively little methyl labeling of 45S RNA, indicating that the precursor pool is rapidly chased. Therefore, if 2 days of prelabeling with methionine precedes nephrectomy, the rRNA from both kidneys should be labeled under similar metabolic conditions.

Although renal mass varies among different animals, the right and left kidneys of any one animal have almost identical masses and are labeled to exactly the same specific activity. Hence an excised left kidney is a control for the growing right kidney. Since the kidneys of normal animals show no increase in rRNA content over the 2-day interval studied, they can be considered in a steady state. Comparison of the specific activity of rRNA from the right kidney undergoing compensatory hypertrophy with the specific activity of rRNA from the excised left kidney of the same animal should provide a valid estimate of the amount of prelabeled rRNA remaining in the growing (right) kidney.

The ratio of the specific activity of rRNA in the growing kidney to that in the excised kidney was higher than would have been expected had degradation been proceeding at the same rate as in the normal kidney (Table I). The number of molecules of rRNA degraded per kidney could be determined. The size of the expanded ribosome pool may be estimated from the content of RNA compared with that of DNA, as will be shown, the proportion of rRNA to total RNA does not change after unilateral nephrectomy, and the DNA content is stable for several days (9, 22). Table I shows a 5% increase of RNA to DNA 1 day after contralateral nephrectomy and an 18% increase at 2 days. When the observed specific activities of 18S and 28S RNA are multiplied by 1.05 (at 1 day) and 1.18 (at 2 days), a measure is obtained of the

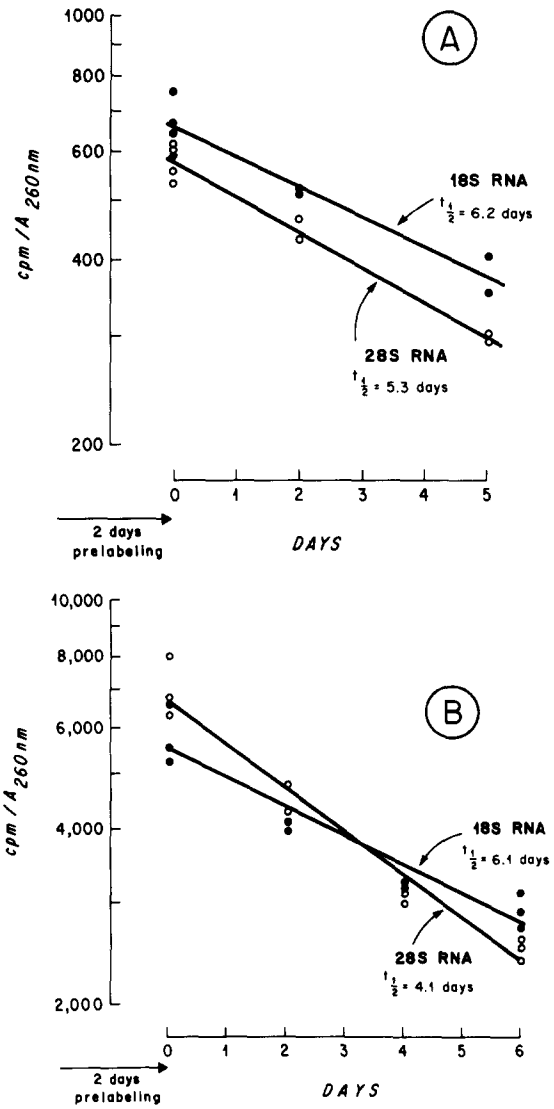

FIGURE 2 Rate of decay in rRNA in normal mouse kidney. (A) Decay in specific activity of rRNA labeled for 2 days with 500 μCi of L[*methyl*-^{3}H]methionine (5 Ci per mmol). rRNA was extracted and purified on sucrose gradients as in Fig. 1. Each point represents two animals. Computer-fitted multiple regression coefficients of the lines are −0.987 for 28S RNA and −0.958 for 18S RNA. (B) Decay in specific activity of rRNA labeled for 2 days with 100 μCi of [5-^{3}H]uridine (4.2 Ci per mmol) per mouse; there were four animals in each group. Computer-fitted regression coefficients of lines are −0.979 for 28S RNA and −0.947 for 18S RNA.

TABLE I

Growing Kidney Compared with Excised Kidney (Ratio)

Time from Nephrectomy	(μg RNA per μg DNA)/ (μg RNA per μg DNA)	(cpm per A_{260nm} U of 28S RNA)/ (cpm per A_{260nm} U of 28S RNA)	(cpm per A_{260nm} U of 18S RNA)/ (cpm per A_{260nm} U of 18S RNA)	(cpm in 28S RNA per kidney)/ (cpm in 28S RNA per kidney)	(cpm in 18S RNA per kidney)/ (cpm in 18S RNA per kidney)
Day 1 Nephrectomy	1.07, 1.03	0.90, 0.96	1.01, 0.98	0.96, 0.99	1.08, 1.01
(Normal)	1.00	0.87	0.89	0.87	0.89
Day 2 Nephrectomy	1.17, 1.20	0.85, 0.86	0.88, 0.84	1.00, 1.03	1.01, 1.01
(Normal)	1.00	0.76	0.80	0.76	0.80

Ratio of RNA:DNA and specific activities of rRNA in kidneys undergoing compensatory hypertrophy compared with those of kidneys removed earlier. There were two mice in each group. Each animal was given 500 μCi of L-[*methyl*-^{3}H]methionine (5 Ci per mmol) 2 days before operation. Normal values are derived from the mice used for Fig. 2 B. See Fig. 2 A for orders of radioactivity present.

number of labeled molecules remaining per kidney. By this estimate, even less degradation of rRNA occurred in the nephrectomized animals than was apparent from the uncorrected specific activities alone (Table I).

Effect of Unilateral Nephrectomy on rRNA Degradation: Prelabeling with [^{3}H]uridine

All the preceding experiments were performed by preparing 18S and 28S RNA from ribosome pellets after homogenizing the kidneys with a Dounce homogenizer. Since this method of homogenization ruptures only 60% of the kidney cells (30), the experiments are open to the criticism that a selected population of kidney cells subject to breakage by reason of their hypertrophy might have higher 28S and 18S RNA specific activities than cells that remain intact.

To examine this possibility, kidneys were homogenized in phenol-NETS mixture to extract all renal rRNA. Parallel isotope dilution analyses were performed to ascertain the recovery of rRNA and thus to permit direct estimation of the accumulation of rRNA. 28S and 18S RNA accounted for 84% of the RNA extracted from normal kidneys. This value is comparable to the 85–90% obtained for liver by Hirsch (13).

The decay of radioactive 18S and 28S RNA from the whole kidney of unilaterally nephrectomized mice prelabeled for 2 days with [^{3}H]uridine was followed for 4 days after the operation and was compared with the rate of decay in control animals (Table II). The UTP pool is labeled maximally by [^{3}H]uridine within 20 min after injection; the specific activity of the UTP pool decays at the rate of approx. 15% per hour thereafter (1, 11). There was no detectable increase in rRNA content of the renoprival kidney on the 1st day, and during this time 28S and 18S RNA labeling declined at the same rate as in normal animals. On the 2nd and 4th days, there was an increase in the amount of 28S and 18S RNA, coincident with higher specific activities of rRNA in the growing kidney.

The change in the 18S:28S ratios of specific activities in liver was used as a control. They were found to increase in time, but the increase was not so marked as in kidney (Fig. 3). In addition, the projection of the ratio to initial labeling time was somewhat higher (0.84 as against 0.75 in the kidney). In other experiments the ratio of specific activities of renal 18S to 28S RNA labeled with [^{3}H]uridine was the same as in normal mouse kidney at all times studied after unilateral nephrectomy (Table III).

Nicks in 28S rRNA

Some of the difference in observed turnover rates of 28S and 18S RNA, but not in conservation of rRNA, might be from progressive nicking of RNA in functional 60S ribosomal subunits (5, 15, 17, 20, 26).[1] If this is so, 60S and 40S ribosomal subunits should turn over at the same rate, but the nicked RNA extracted from the 60S subunits should sediment slower than normal 28S rRNA.

A group of four mice was first labeled with [^{3}H]orotic acid and again labeled 15 days later with [^{14}C]orotic acid. After a further 24 h, kidney polysomes were prepared and dissociated in EDTA; the subunits were centrifuged on a sucrose gradient. Fig. 4 A shows that the labeling patterns of ^{3}H-labeled ribosomal subunits and the ^{14}C-

[1] Scott, J. F. Unpublished observations.

TABLE II A

Ratio of 28S RNA from Growing Kidney Compared with 28S RNA from Excised Kidney

Days after nephrectomy	Sp act				
	Unilateral nephrectomy	Normal	Unilateral nephrectomy	Unilateral nephrectomy	Normal
	cpm per A_{260nm} U/cpm per A_{260nm} U		*μg RNA/μg RNA*	*cpm per kidney/cpm per kidney*	
1	0.83, 0.87	0.85	0.94, 1.04	0.80, 0.86	0.85
2	0.76 ± 0.01 (7)	0.73	1.19 ± 0.09 (4)	0.91 ± 0.06 (4)	0.73
4	0.48, 0.50	0.53	1.38, 1.64	0.69, 0.77	0.53

Table II A and II B. Proportion of prelabeled rRNA remaining in right kidneys after left nephrectomy (ratios). Mice were injected with 100 μCi of 5,6-[^{3}H]uridine (4.22 Ci per mmol) each 2 days before operation. There were four mice in each group. Values are means ± SEM when more than three groups were used; the numbers of groups are given in parentheses. (Table II A) 28S RNA, (Table II B) 18S RNA. See Fig. 2 B for orders of radioactivity present.

TABLE II B

Ratio of 18S RNA from Growing Kidney Compared with 18S RNA from Excised Kinney

Days after nephrectomy	Sp act				
	Unilateral nephrectomy	Normal	Unilateral nephrectomy	Unilateral nephrectomy	Normal
	cpm per A_{260nm} U/cpm per A_{260nm} U		*μg RNA/μg RNA*	*cpm per kidney/cpm per kidney*	
1	0.91	0.89	1.00	0.90	0.89
2	0.85 ± 0.01 (7)	0.80	1.10 ± 0.04 (4)	0.94 ± 0.03 (4)	0.80
4	0.57 ± 0.61 (7)	0.63	1.41, 1.51	0.86, 0.86	0.63

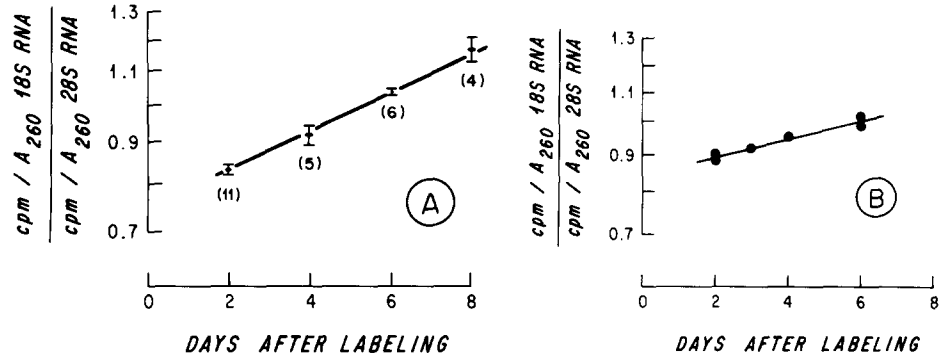

FIGURE 3 Change in ratio of specific activities of kidney (A) and liver (B) 18S RNA and 28S RNA. 100 μCi of [5,6-^{3}H]uridine (42.2 Ci per mmol) was injected per mouse. Values for kidney groups are means ± SEM. Number of groups is shown in parentheses. Single groups were used for liver. Groups contained four mice each.

labeled ribosomal subunits were identical, indicating no difference in the turnover rates of the large and small ribosomal subunits. On the other hand, after extraction, the ^{3}H-28S RNA was partially degraded to smaller pieces, which sedimented in the region between the 18S and 28S peaks (Fig. 4 B). Although most of these degraded species of RNA would have been discarded in earlier experiments when the areas under the A_{260nm} peak were pooled (Fig. 1), some may have cosedimented with the pooled 18S fractions to raise their specific activity factitiously.

TABLE III

Ratio of Specific Activities of 18S:28S RNA in Growing Kidneys (Unilateral Nephrectomy) Compared with Normal Kidneys

Days after operation	Unilateral nephrectomy (cpm per A_{260nm} 18S RNA)/ (cpm per A_{260nm} 28S RNA)		Normal (cpm per A_{260nm} 18S RNA)/ (cpm per A_{260nm} 28S RNA)	
0	0.84 ± 0.01	(11)	0.84 ± 0.01	(11)
2	0.93 ± 0.01	(7)	0.92 ± 0.02	(5)
4	1.03 ± 0.03	(3)	1.04 ± 0.01	(6)

Ratio of specific activities of renal 18S RNA:28S RNA in normal mice and in mice after unilateral nephrectomy. 100 μCi of [5,6-^{3}H]uridine (42.2 Ci per mmol) was given by intraperitoneal injection to each animal 2 days before operation. Values are means ± SEM for number of estimates in parentheses; each estimate represents a pool of four animals.

DISCUSSION

After unilateral nephrectomy, prelabeled rRNA in the remaining kidney is retained to a greater extent than expected. The slower decay of prelabeled rRNA could be due either to decreased degradation of rRNA or to increased reutilization of precursor. Reutilization after the initial period of labeling must have been greater when [^{3}H]methionine was used as precursor than when [^{3}H]uridine was used, since the half-lives obtained with [^{3}H]methionine were longer. Reutilization of methionine from protein breakdown for rRNA synthesis is probably the cause of the longer half-lives observed.

Several arguments suggest no appreciable increase in reutilization of either radiolabeled methionine or uridine directly related to compensatory renal growth. First, during compensatory growth, protein appears to be degraded slower than in normal kidneys (12), probably decreasing the availability of methionine for reutilization. Secondly, there were only 30 cpm in any poly(A)-containing RNA isolated from polysomes 2 days after labeling with uridine compared with 1,500 counts per fraction in poly(A)-lacking RNA. Thirdly, the specific activity of UTP in mouse kidney reaches its peak 20 min after injection of [^{3}H]uridine and then decays at the rate of 15% per hour; only small amounts of radioactivity are present in 45S RNA 24 h after labeling (1). Finally, during compensatory renal hypertrophy, the size and the specific activity of the UTP pool and the kinetics of labeling of RNA with intraperitoneally administered [^{3}H]uridine are unchanged (11).

After unilateral nephrectomy, radioactive label in rRNA was conserved only when rRNA content increased. For example, in Table II, degradation of rRNA occurred at the normal rate during the 1st day, when there was no accumulation of rRNA, but conservation was almost complete on the 2nd day, when there was a marked accumulation of rRNA.

We recognize that the conservation of rRNA may not be specific for compensatory growth, since comparisons were made between growing kidneys and normal kidneys. Although kidneys from sham-nephrectomized mice were also compared, usable data could not be obtained from them. Results were scattered, and there was no way to compare a kidney from the same animal at the time of operation with one later removed at the time of sacrifice. Since kidneys from sham-nephrectomized mice and rats do not grow for more than 1 day as a non-specific effect of an operation (6), one would suspect that over the long term they are more like control kidneys than those undergoing compensatory growth. Fitting alternative curves to data published by Hill (Fig. 1 in reference 10) reveals slower degradation of labeled renal rRNA after contralateral nephrectomy than after sham nephrectomy.

Data from our Table II permit an estimation of the extent to which diminished degradation of preexisting rRNA contributes to the observed accretion of rRNA. The equation of Quincey and Wilson (31) predicts a 14% daily turnover rate of the ribosomal pool. Considering the data for the ratio of 28S RNA radioactivity remaining in the growing kidney compared with that of the normal kidney at 2 days (Table II A), the relative degradation of the rRNA pools during these 2 days is $(1 - 0.91)/(1 - 0.73)$, i.e., 33% of that in normal animals. If synthesis had been normal during this time, the net increase (synthesis less degradation) would have been 28% − 0.33(28%)% = ~ 19%. This figure agrees with the observed increase of 19% in 28S RNA content 2 days after contralateral nephrectomy (Table II A). Thus, at this time the accumulation of rRNA is accounted for by decreased degradation of preexisting RNA coupled with synthesis at a normal rate.

A similar approximation shows that at 4 days after contralateral nephrectomy the proportion of

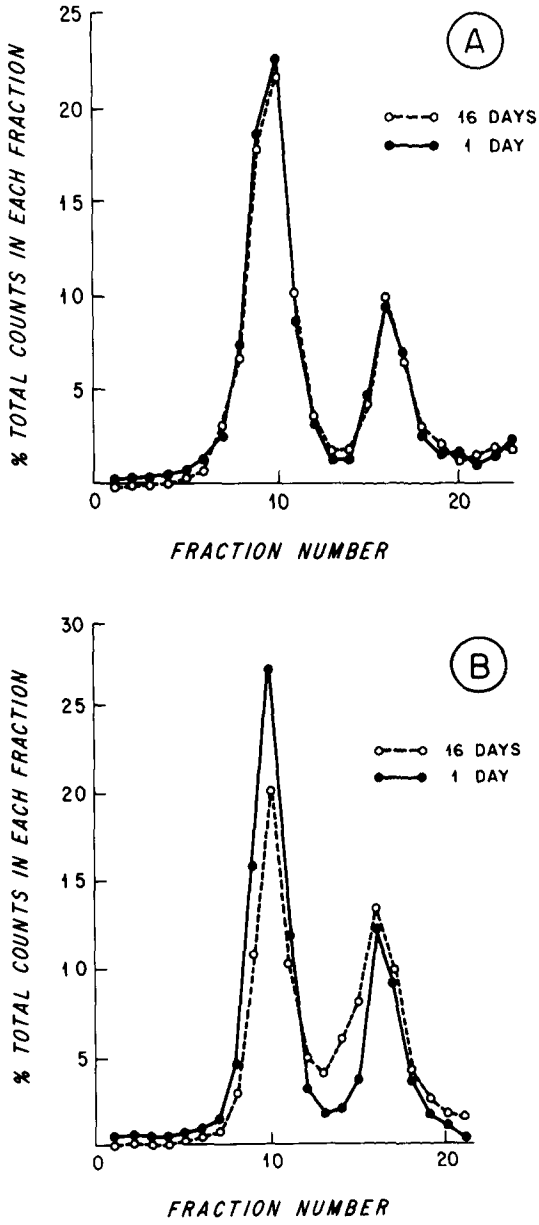

FIGURE 4 Sucrose gradient sedimentation analysis of ribosomal subunits and ribosomal RNA. Each of four mice was labeled with 90 μCi of [^{3}H]orotic acid (11.1 Ci per mmol) 16 days before sacrifice and with 5 μCi of [2-^{14}C]orotic acid (55.2 Ci per mmol) 1 day before sacrifice. Kidney polysomes were prepared by centrifugation at 50,000 rpm for 4 h in a Beckman 65 rotor through a discontinuous gradient of 2.5 ml of 2.0 M sucrose and 1.5 ml of 1.5 M sucrose in 0.25 M NaCl, 0.05 $MgCl_2$, 0.01 M Tris. pH 7.4 at 20°C. The pellet was split. One part was dissolved in NEB buffer and was applied to a 15-30% sucrose gradient in NEB; the other was phenol extracted, and the RNA was applied to a 15-30% sucrose gradient in NETS buffer. Centrifugation was carried out at 23,500 rpm for 17.5 h in a Beckman SW-27 rotor at 4°C (NEB gradient) or 25°C (NETS gradient). (A) Dissociated polysomes; (B) Polysomal RNA.

prelabeled 28S RNA lost compared with controls is (1 – 0.74)/(1 – 0.53), i.e., 55%. With synthesis at a normal rate, the net accumulation would be 56% – 0.55(56%)% = 25%. Since the observed increase was 51% (Table II), half of it would have had to come from increased synthesis of 28S RNA between 2 and 4 days. More exact calculations would require knowledge of the precise rate of rRNA synthesis at each stage of growth.

Similar calculations for kinetics of 18S RNA 4 days after nephrectomy yield a value for conservation of 35% and a requirement of synthesis of 11%. Thus, estimations for the total rRNA pool suggest a requirement for about an 18% (mean of 11% and 26%) increase in synthesis of the original pool between the 2nd and 4th days.

The present paper may be the first report of conservation of rRNA in a mammalian organ during physiological growth. Mature, preexisting rRNA seems to be conserved. A proposal that 45S pre-rRNA is conserved during compensatory renal hypertrophy (10) is derived from a limited number of observations of small changes in radioactive disintegrations in adventitial perinucleolar RNA, which could have contained methylated nucleoplasmic RNA. The kinetics of incorporation of precursors into cytoplasmic rRNA were not reported. Moreover, the assumption that the size of the *S*-adenosylmethionine pool is stable during renal growth is arguable, and equating a 67% in labeling of putative cytoplasmic rRNA with a 67% increase in the amount of cytoplasmic rRNA 36 h after unilateral nephrectomy is inconsistent with much published work (11, and reviewed in reference 6).

Conservation of rRNA during growth has been observed in other systems. In logarithmically growing cultured chick fibroblasts and 3T3 cells, rRNA is not degraded, but turnover commences as the cells become contact-inhibited (2, 18, 33). Decreased degradation of microsomal RNA has been observed during liver growth induced by chronic administration of cortisone (28) and in the kidneys of adrenalectomized rats treated with aldosterone and deoxycortisone (24). Conversely, during liver atrophy as a consequence of protein deprivation, the half-life of liver rRNA is decreased (27). During isoproterenol-induced growth of submandibular glands prelabeled with methio-

nine, the label in rRNA does not decay, but in fact is increased (4); this increase could result from reutilization to the same extent as in normal animals, combined with slower degradation of ribosomes.

Although nicking of 28S RNA may explain the difference in the observed half-lives of the two rRNAs in kidney, it is probably not the mechanism of ribosome degradation and is unrelated to conservation during compensatory renal growth as the rate of nicking is unchanged. Nicking would not have been shown had the species of stable RNA not been extracted from the ribosomal subunits and analyzed separately. Little is known of the mechanism of ribosome degradation except that ribosomal proteins and rRNA turn over with the same half-life (14). Nicking has been observed in rat liver (17. 20), HeLa cells (26), and 3T3 cells (see footnote one). The related observation of differences in turnover rates of 28S RNA and 18S RNA has also been made in parotid glands (4) and 3T3 cells (2, and see footnote one). The proportion of nicked molecules in kidney increases with the age of the molecules by an apparently first-order process. Normal liver, which has the same half-life for total rRNA as kidney (3, 14, 21, 22), has a different rate of nicking of 28S rRNA (Fig. 3).

We have tried to find changes in the composition of ribosomes that could correlate with slower degradation. To date, no changes in buoyant density, sedimentation rate, or electrophoretic migration have been detected in polysomes or in ribosomal subunits from normal and growing kidneys. Both ribosomal subunits have the same turnover rate. Perhaps they are degraded together as an intact ribosome, perhaps as 80S monomers out of the ribosome cycle. However, analysis of polysome profiles in high-salt media (in which monomers dissociate into subunits, but monosomes do not) has shown no change in the proportion of out-of-cycle ribosomes after unilateral nephrectomy.

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