Renal hypertrophy, induced by uninephrectomy (UNI), results in a 40–50% increase in the RNA content per cell 4 days postoperatively (1). Virtually no change in the DNA content occurs over the 4-day period (2). During the first 4 days of renal hypertrophy, incorporation of labeled nucleosides (uridine, orotate, and cytidine) into RNA showed no increase. Rates of synthesis of total kidney RNA from UNI mice, determined by measuring the labeling rates of RNA and UTP, showed no increase when compared to shams (3).

Since the rate of synthesis is unchanged after UNI, the accretion of rRNA could occur by a decrease in degradation. However, the half-life of rRNA labeled before UNI is identical to sham value. Apparently RNA accretion during kidney hypertrophy does not occur by increased rate of transcription or decreased degradation of rRNA. These facts are compatible with the hypothesis that excess production and rapid degradation of newly synthesized rRNA occurs in the unstimulated kidney and that during renal hypertrophy the degradation is reduced. Other investigators have evidence of a similar phenomenon in the estrogen-stimulated uterus (4), phytohemagglutinin-stimulated lymphocytes (5, 6), regenerating liver.
(7, 8), phenobarbital-treated liver (9), hydrocortisone-treated regenerating liver (10), and valine deprivation in human male fibroblasts (11). We present results which show that the kinetics of labeling and processing of kidney pre-rRNA is the same in UNI and sham mice at a postoperative time (16 h) when the appearance of labeled kidney cytoplasmic rRNA shows a 67% increase in specific activity in UNI mice. These facts strongly suggest that accretion of rRNA during renal hypertrophy results from a decrease in degradation of newly synthesized rRNA.

MATERIALS AND METHODS
Male Swiss-Webster outbred mice (35-45 days, 30-34 g) were used in all experiments. Mice were maintained on Wayne chow and housed in quarters with a 12-h light, 12-h dark cycle. UNI and sham nephrectomy (sham) were performed as previously described (2, 3). Isotope was administered by a subcutaneous injection of 1 mCi of L-[methyl-3H]methionine (2.6 Ci/mmol, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N.Y.). At specific times, animals were killed, and kidneys were removed, decapsulated, and homogenized in buffer (0.01 M Tris, pH 7.4, 0.01 M NaCl, 1.5 mM MgCl2, and 0.02% polyvinylsulfate), 10 ml per 250 mg of kidney. DNA was isolated from aliquots of homogenates and determined with the diphenylamine reaction (12) (calf thymus DNA as the standard). The procedures of AB and Malt (13) were used to isolate nuclei, and to extract precursors of rRNA, and cytoplasmic rRNA. Cytoplasmic rRNA was processed as previously described (3). Nuclear RNA was electrophoresed into 7 cm gels of 2.6% (wt/vol) polyacrylamide, 0.11% (wt/vol) bisacrylamide, and 0.5% sodium dodecyl sulfate. Electrophoresis was carried out for 5 h at 5 mA per gel. The gels were scanned at 260 nm, sliced in 1-mm sections, and dissolved in 0.5 ml of 30% H2O2. After evaporation, 10 ml of scintillator (2,5-diphenyloxazole [POPOP], 0.05% wt/vol; dimethyl-1,4-bis[2-(5-phenyloxazo]yl]benzene [PPO], 0.05% wt/vol; Triton 101, 30% toluene, 70%) were added (efficiency 38%).

RESULTS
Degradation Rate of Ribosomal RNA
Mice receive 1 mCi of L-[methyl-3H]methionine subcutaneously 24 h before UNI and sham. The left kidneys are removed from the UNI mice and used to determine the zero time point. At 24-h intervals, up to 5 days, animals are sacrificed, and kidneys removed and homogenized. One aliquot is used to estimate DNA. Another aliquot is used to isolate RNA and to determine radioactivity. Fig. 1 shows the loss of radioactivity in rRNA per milligram DNA. During this 5-day period, the DNA content per kidney is unchanged (2). The degradation rate of rRNA is calculated from the radioactivity in rRNA per milligram DNA. The specific activity of the 32S rRNA is 40% greater than the sham value after 16 h post UNI, and 45% greater after 40 h. The specific activity of the 32S rRNA is 50% greater than the sham value at 16 h post UNI after 60 min of labeling. The specific activity of the 18S rRNA is 40% greater than the sham value. The specific activities of the 45S and 32S pre-rRNA are not different for UNI and sham mice. However, 16 h after UNI, the specific activity of the 18S rRNA is 40% greater than the sham value. The specific activities of pre-rRNAs are not increased in the UNI mice after 60 min of labeling. The specific activity of the 18S rRNA is 50% greater than the sham value at 16 h post UNI after 60 min of labeling. Table I shows the kinetics of labeling of the kidney 45S and 32S pre-rRNA 16 h after UNI and sham. Results obtained from other postoperative times (4, 36, and 96 h) reveal no difference between UNI and sham in the labeling and processing of any kidney pre-rRNAs. Table II gives the specific activity of cytoplasmic rRNA after a 60-min labeling at various times.

![Figure 1](image-url)

**Figure 1**: Mice received a subcutaneous injection of 1 mCi of L-[methyl-3H]methionine 24 h before their operation. The zero point is the mean value from kidneys taken at UNI. All the values are the mean from at least three determinations. The values of UNI and sham mice were not statistically different by the student test at p ≤ 0.05.

**Kinetics of Methylation of rRNA Precursors**

Fig. 2 shows the electropherogram and profiles of radioactivity for renal nuclear RNA after different times (10-60 min) of labeling with L-[methyl-3H]methionine for 4 and 16 h after UNI and sham. The rRNA precursors are extracted from rapidly isolated and partially purified nuclei. After 10 min, a radioactive peak is detectable in the region of the gel coinciding with the 45S pre-rRNA. The specific activities of the 45S pre-rRNA are the same after 10 min labeling for the UNI and sham mice 16 h postoperatively (Table I). After 20 min, two distinctive radioactive peaks are observed in the 45S and 32S regions of the gel (Fig. 2). The maximum specific activity of the 45S pre-rRNA occurs after 20 min of labeling (Table I). The specific activities of the 45S pre-rRNA are 50% greater than the sham value after 16 h post UNI. The specific activities of the 32S pre-rRNA are not different for UNI and sham mice after 60 min of labeling. The specific activity of the 18S rRNA is 40% greater than the sham value. The specific activities of pre-rRNAs are not increased in the UNI mice after 60 min of labeling. The specific activity of the 18S rRNA is 50% greater than the sham value at 16 h post UNI after 60 min of labeling. Table I shows the kinetics of labeling of the kidney 45S and 32S pre-rRNA 16 h after UNI and sham. Results obtained from other postoperative times (4, 36, and 96 h) reveal no difference between UNI and sham in the labeling and processing of any kidney pre-rRNAs. Table II gives the specific activity of cytoplasmic rRNA after a 60-min labeling at various times.
TABLE I

**Methylation and Processing of Pre-rRNA**

| Labeling Time | 45S (mean + SD) | 32S (mean + SD) |
|---------------|-----------------|-----------------|
| UNI Sham     | UNI Sham        |                |
| min 10       | 1320 ± 160      | 160 ± 150       | ND   |
|            | 1160 ± 310      | 160 ± 310       | ND   |
| 20           | 3980 ± 580      | 3840 ± 610      | 1190 ± 250 | 3620 ± 460 |
|            | 610 ± 250       | 1190 ± 250      | 250 ± 1310 | 180 ± 1310 |
| 40           | 1620 ± 190      | 1750 ± 280      | 2810 ± 400 | 460 ± 400   |
|            | 200 ± 3620      | 2010 ± 260      | 460 ± 460   |
| 60           | 820 ± 120       | 710 ± 120       | 2290 ± 270  | 2010 ± 260  |

Methylation and processing of pre-rRNA after subcutaneous injection of 1 mCi of \( \text{L-[methyl-}^{3} \text{H}] \)methionine. The time from operation to sacrifice was 16 h. The specific activities were calculated from electropherograms of nuclear RNA profiles of absorbancy and radioactivity as typified in Fig. 2. The amounts of 45S and 32S RNA were calculated from the area under the peak absorbancy after subtracting the base-line absorbancy. The radioactivity of the peaks was determined by integrating the total amount of radioactivity per slice. The mean and SD are from three experiments. ND, not determined.

TABLE II

**Incorporation of L-[methyl-H]Methionine into Cytoplasmic rRNA (1 h Ending at Sacrifice)**

| Time from operation to sacrifice | rRNA (mean ± SD) |
|---------------------------------|------------------|
| h                               | cpm/mg           |
| 4 UNI                           | 4750 ± 600       |
| 4 sham                          | 5000 ± 900       |
| 16 UNI                          | 8200 ± 800*      |
| 16 sham                         | 4900 ± 800       |
| 36 UNI                          | 8700 ± 1100*     |
| 36 sham                         | 5200 ± 900       |
| 96 UNI                          | 4700 ± 400       |
| 96 sham                         | 4500 ± 700       |

All mice received a subcutaneous injection of 1 mCi of L-[methyl-\(^3\)H]methionine. Kidney cytoplasmic rRNA was isolated and purified, and then centrifuged on linear sucrose gradients. The gradients were fractionated, and RNA was precipitated with 5% TCA, collected on glass filters, and counted. The values for the 18S or 28S rRNA show the same results as do the total (18S + 28S) values. Each value represents the mean ± SD of three determinations.

* Significantly different by student t test at \( p \leq 0.05 \) after UNI and sham. No change is observed at postoperative times of 4 or 96 h, but a 67% increase is observed at 16 and 36 h in the UNI mice. This increase occurs at a time when the kinetic analysis indicates no increase in the specific activities of any pre-rRNA.

DISCUSSION

The results of this study indicate that the synthesis of 45S pre-rRNA is not a rate-limiting factor in the mouse kidney during accretion of RNA induced by UNI. Specifically, the level of methylation of the 45S pre-rRNA with L-[methyl-\(^3\)H]methionine is the same in UNI and sham mice. Since the majority of methylation occurs during or immediately after transcription, we assume that the rate of methylation of the 45 pre-rRNA parallels the rate of synthesis of the 45S pre-rRNA. In other systems, some methylation in the later stages of processing does occur (14), but it does not seem to be quantitatively important. Furthermore, we assume that the pools of S-adenosylmethionine (SAM) are of comparable size in kidneys from UNI and sham mice. The kinetics of labeling and processing of the 45S pre-rRNA presented in Table I suggest that the SAM pools are indeed comparable, since the label amounts to a pulse label and the “chase” kinetics of radioactivity from these pre-rRNAs are comparable. Since a 67% increase in cytoplasmic rRNA occurs 16 h post UNI after a 1-h label, conservation of the newly synthesized rRNA must approach 167%. Therefore, the unstimulated kidney must synthesize at least a 67% excess of 45S pre-rRNA. Non-growing human lymphocytes synthesize excess 45S pre-rRNA and ultimately degrade approximately...
50% of this newly synthesized RNA (5, 6). Stimulation of lymphocytes with phytohemagglutinin results in almost a 100% reduction in the degradation of newly synthesized rRNA (5, 6).

The nuclear RNA displayed in the electrophorograms is extracted from rapidly isolated and partially purified nuclei. This procedure allows the isolation of 45S, 36S, and 32S pre-rRNA species but results in cytoplasmic contamination, i.e. 18S and 28S rRNA. Lengthy isolation procedures which give greater purification do not yield species of RNAs in appreciable quantities larger than 32S pre-rRNA. Ab and Malt (13) have shown that the contribution of 18S and 28S rRNA in the nuclear RNA preparation is 97% of cytoplasmic origin. They further showed that addition of exogenous 18S and 28S rRNA to their nuclear preparations did not alter the observation on the kinetics of processing of the pre-rRNA.

The results of this study and others (4-11) have indicated that in certain systems the controls of rRNA content function so that, in general, an excess of ribosomal RNA precursors is made, compared with the cellular requirement for nascent ribosomes. Apparently the content of pre-rRNA present in the nucleolus at any time is in excess of the needs for nascent ribosomes and normally results in a rapid degradation of the newly synthesized rRNA. In these cells the main control of rRNA and perhaps ribosomes is the efficient utilization (decreased degradation) of the precursor rRNA. Other workers have noted that this type of mechanism (alterations in degradation patterns) may function in the production of some mRNAs from rapidly labeled and rapidly turning over heterodisperse nuclear RNA (15).

Results from the estrogen-stimulated uterus (4), phytohemagglutinin-stimulated lymphocytes (5, 6), regenerating liver (7), phenobarbital-treated liver (9), hydrocortisone-treated regenerating liver (10), and valine deprivation in human male fibroblasts (11) indicate an increase in the utilization of pre-rRNA. In these systems, hypertrophy and hyperplasia play major roles and the rate of transcription as well as the efficiency of utilization of pre-rRNA is increased. UNI in the mouse apparently offers a unique model whereby accretion of rRNA occurs only by a more efficient utilization of pre-rRNA. Renal hypertrophy can be used to investigate changes in the utilization of pre-rRNA without the complication of an increased rate of transcription or increased rate of DNA synthesis.

**SUMMARY**

The degradation rates of kidney rRNA labeled before UNI or sham are unchanged 5 days after the operations (t<sub>1/2</sub> 88 h). Therefore, there is no contribution from pre-existing rRNA to the increased amount of rRNA in the stimulated kidney. After labeling with L-[methyl-<sup>3</sup>H]methionine, the kinetics of incorporation into rRNA precursors (45S and 32S) are the same for the labeling periods 10-60 min and at the postoperative times of 4, 16, 36, and 96 h. The specific activity of cytoplasmic rRNA after 1-h labeling with L-[methyl-<sup>3</sup>H]methionine increased 67% at 16 and 36 h after UNI; no increase occurred at 4 or 96 h. Since (a) the rate of degradation of rRNA, (b) the kinetics of incorporation and processing of rRNA precursors, and (c) the rate of RNA synthesis appear unchanged after UNI, the accretion of rRNA must involve decreased degradation of newly synthesized rRNA.

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