Ribonucleic Acid Labelling and Nucleotide Pools during Compensatory Renal Hypertrophy

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During the first 48 h of compensatory renal hypertrophy induced by unilateral nephrectomy, RNA content per cell increased by 20–40%. During this period, rates of RNA synthesis derived from the rates of labelling of UTP and RNA after a single injection of [5-³H]uridine showed no change in the rate of RNA synthesis (3.1 nmol of UTP incorporated into RNA/min per mg of RNA). ATP and ADP pools were not changed. The rate of RNA synthesis was considerably in excess of the increment of total RNA appearing in the kidneys. With [5-³H]uridine as label, only continuous infusion for 24 h could produce an increase (60%) in the specific radioactivity of renal rRNA in mice with contralateral nephrectomies. With a single injection of [methyl-³H]methionine used to identify methyl groups inserted into newly synthesized rRNA, the specific radioactivity of this rRNA was unchanged 5 h after contralateral nephrectomy, increased by 60% at 9–48 h, and returned to normal values at 120 h. Most RNA synthesized in both nephrectomized and sham-nephrectomized mice has a short half-life. Since total cellular RNA content increases in compensatory hypertrophy despite unchanged rates of rRNA synthesis, the accretion of RNA might involve conservation of ribosomal precursor RNA or a change in rate of degradation of mature rRNA.

Compensatory hypertrophy of the rat or mouse kidney in response to contralateral nephrectomy is marked at the end of 2 days by a 20–40% increase in the amount of RNA, but little change in the amount of DNA (Halliburton & Thomson, 1965; Threlfall *et al.*, 1967; Malt & Lemaitre, 1968; Kurnick & Lindsay, 1968; Bucher & Malt, 1971). This rise in RNA/DNA ratio is probably a result of an increased amount of rRNA, since rRNA constitutes over 80% of cellular RNA (Hirsch, 1967). Because the nominal rate of rRNA turnover appears to be unchanged from 4 to 27 days after nephrectomy and sham-nephrectomy (Malt & Lemaitre, 1968), an increased rate of RNA synthesis has been held responsible for accretion of RNA.

If accretion of RNA is due to an increased rate of transcription, the labelling of RNA with a radioactive precursor should be increased if there is no change in the specific radioactivity of the immediate precursor pool. Preliminary reports (Halliburton, 1969) have suggested a 25–100% increase in labelling of rat kidney rRNA with [³H]orotate within 20–100 min of contralateral nephrectomy. Labelling of mouse kidney rRNA with [5-³H]uridine during compensatory hypertrophy, however, is increased only when compared with labelling in normal mice (Malt & Stoddard, 1966), not with labelling in sham-nephrectomized

mice; rates of labelling of mouse kidney ribosomes are the same as after sham-nephrectomy whether the precursor is orotate, cytidine or uridine (J. M. Hill, G. AB & R. A. Malt, unpublished work). Increased labelling with [³²P]P_i during hypertrophy of the rat kidney has been attributed to an increase in the radioactivity in the acid-soluble pool (Kurnick & Lindsay, 1968), and measurements of radioactivity in AMP in kidney have been related to RNA-labelling data obtained for other purposes (Steinberg & Nichols, 1971).

Knowledge of the specific radioactivity of the immediate nucleoside triphosphate pools is essential to calculate the rate of RNA synthesis from the incorporation of a labelled precursor (Bucher & Swaffield, 1969*a,b*). We have determined the rate of synthesis of RNA in compensatory hypertrophy of the mouse kidney after injection of labelled uridine by measuring the rates of labelling of RNA and the rates of labelling of UTP, an immediate precursor of RNA. We found that under these circumstances nephrectomized, sham-nephrectomized and normal mice have the same rate of renal RNA synthesis despite differences in the rate of accretion of total RNA. Only by continuous infusion of [5-³H]uridine or by a single injection of [methyl-³H]methionine could an increase in the specific radioactivity of renal RNA be found in mice with unilateral nephrectomies.

Experimental

Animals and nephrectomy

Male Charles River mice (45–55 days old, 30–35 g) were caged in groups of eight under constant incandescent lighting and were given free access to Purina rat chow and water. Left nephrectomy, sparing the adrenal, was performed from 09:00 to 11:00 h under light ether anaesthesia. For sham-nephrectomies the left kidney was exposed and touched with forceps.

At intervals after the operation each mouse was given an intraperitoneal injection of 50 μ Ci of [5-³H]uridine in 0.5 ml of water (25–28 Ci/mmol; New England Nuclear Corp., Boston, Mass., U.S.A.). After the labelling period, the mice were anaesthetized with ether-O₂ (Bucher & Swaffield, 1969a), and the right kidneys were exposed and instantly frozen *in situ* with tongs pre-cooled in liquid N₂ (Wollenberger *et al.*, 1960).

Homogenates

The frozen kidneys were weighed, pulverized and homogenized in 7 ml of ice-cold 0.8 M-HClO₄/g. After portions of homogenate were reserved for nucleic acid estimations, the remainder was centrifuged at 9000g for 3 min. A portion of supernatant was neutralized with 0.1 vol. of 7 M-KOH, and after low-speed centrifugation the supernatant was used for analysis of ADP, ATP and UTP, by the methods of Bucher & Swaffield (1969a,b).

Specific radioactivity of total RNA

RNA and DNA were extracted (Bucher & Swaffield, 1969b) and estimated respectively with the orcinol reaction (Mejbaum, 1939) (with yeast RNA as standard) and with the diphenylamine reaction (Burton, 1956) (with salmon spermatozoan DNA as standard).

Since no label appeared in DNA within 60 min, all radioactivity in the supernatant after acid extraction of nucleic acids (0.5 M-HClO₄, 70°C, 15 min) was in RNA. Alternatively, the radioactivity in alkali-solubilized and extracted RNA (0.3 M-KOH, 37°C, 60 min) could be assessed and the DNA estimated in the precipitate. Results from the two methods differed by less than 10%. When labelling times over 1 h were used, radioactivity in RNA could be estimated only in the alkali-extracted RNA.

Radioactivity in UTP per mg of RNA and per mg of DNA

The procedure was that of Bucher & Swaffield (1969b), with modifications. Samples (0.2 ml) of the supernatant from the neutralized homogenate with exogenous UTP (10 μ g) added were spotted

on a polyethyleneimine-impregnated cellulose thin-layer plate (PEI plates; Brinkmann Instruments Inc., Westbury, N.Y., U.S.A.). LiCl (0.2 M) was run to the origin, followed by 1.0 M-LiCl (pH 7.0) saturated with boric acid (Neuhard *et al.*, 1965; Randerath & Randerath, 1967). The UTP spot was located with u.v. light and confirmed by radioautography. Radioactivity of the eluate was determined in Aquafluor (New England Nuclear Corp., Boston, Mass., U.S.A.) at 39% efficiency. Because the volumes of all samples were known, the d.p.m. in UTP/mg of DNA or per mg of RNA could be calculated.

Specific radioactivity of UTP

A sample of the KOH-neutralized supernatant was freeze-dried, the residue dissolved in 0.3 ml of water and a portion (0.28 ml) was streaked and chromatographed on a PEI plate. LiCl (0.2 M) was applied as solvent to the origin and was followed by 0.8 M-LiCl–1 M-acetic acid to 15 cm (Neuhard *et al.*, 1965; Randerath & Randerath, 1967). The UTP streak was eluted with triethylammonium carbonate. After the eluate was diluted with water and freeze-dried, a water solution (0.22 ml) was made and spotted (0.2 ml) on a PEI plate. The solvent for the first dimension was 1 M-LiCl–boric acid (pH 7.2); in the second dimension after desalting it was 0.75 M-(NH₄)₂SO₄. UTP in the eluted spot was estimated at 261 nm (purity was checked by E_{261}/E_{280} ratio at pH 11) and the radioactivity of the samples was measured. The specific radioactivity (d.p.m. in UTP/nmol of UTP) was determined. The size of the UTP pool was calculated from d.p.m. in UTP/mg of DNA divided by d.p.m. in UTP/nmol of UTP.

Determination of amount of ATP and of ADP per mg of DNA

The procedure was that of Bucher & Swaffield (1969a), with modifications. A portion (0.1 ml) of the neutralized supernatant was spotted on a PEI plate. LiCl (0.2 M) was run to the origin, followed by 1.0 M-LiCl (pH 7.0) saturated with boric acid. After the plate was dried, de-salted with methanol and dried again, 0.75 M-(NH₄)₂SO₄ was used as the solvent for the second dimension. The ATP and ADP, identified by marker compounds run parallel in the second dimension, were eluted in 4 M-NH₃ and E_{260} was measured. By using $\epsilon = 15.4 \times 10^3$ litre $\cdot$ mol⁻¹ $\cdot$ cm⁻¹ at alkalinity, amounts (nmol) of ATP and of ADP were determined relative to the DNA content.

Continuous-infusion experiments

Some 2 h after nephrectomy or sham-nephrectomy, a 25-gauge scalp-vein needle placed under the skin

of the back was connected to a screw-driven syringe. Infusion was with $[5\text{-}^3\text{H}]\text{uridine}$ (27.8 Ci/mmol) at 15 $\mu\text{Ci/h}$ (45 $\mu\text{l/h}$), containing 0.1% Evans Blue dye to show possible leakage from the site of injection. Infusion was for 24 h at ambient temperature. Chromatography of the infusate at the beginning and end revealed no change in the specific radioactivity of the uridine and no radioactivity was present in other nucleic acid constituents.

Labelling with $[\text{methyl-}^3\text{H}]\text{methionine}$

To ensure labelling of rRNA in distinction to HnRNA (heterogeneous nuclear RNA) or nRNA, 500 μCi of $[\text{methyl-}^3\text{H}]\text{methionine}$ (4.02 Ci/mmol) was given subcutaneously for a 3 h labelling period.

Extraction of cytoplasmic RNA

Mice were killed by cervical dislocation. The liver and right kidney were rapidly removed, placed in ice-cold Buffer A [0.01 M-Tris (pH 7.4 at 22°C)–0.01 M-NaCl–1.5 mM-MgCl₂] and later weighed. Each kidney and a fragment of liver (0.3 g) were individually homogenized with ten strokes of the loose pestle and ten of the tight pestle in a glass Dounce homogenizer containing cold Buffer A (19 ml/g of tissue) with 20 μg of polyvinyl sulphate/ml (AB & Malt, 1970). Portions were removed from the homogenates for assays of RNA and DNA. Acid-soluble radioactivity was determined from the first 0.5 M-HClO₄ wash.

For extraction of cytoplasmic RNA the top 80% of the supernatant resulting from centrifugation of the homogenate at 1100 g for 3 min was reserved. Sodium dodecyl sulphate (20%, w/v) was added to final concn. 0.5% and EDTA (0.01 M) and the mixture was stirred at 30°C for 1 min (AB & Malt, 1970). An equal volume of a mixture of phenol-*m*-cresol-8-hydroxyquinoline-water (3.0 litres:0.6 litres:3.87 g:0.4 litre) was added immediately thereafter. Chloroform-1% isoamyl alcohol at room temperature was added in equal volume, and extraction was repeatedly carried out on the aqueous phase until it was free of protein (Steele & Busch, 1967). RNA was precipitated with 2 vol. of 95% (w/v) ethanol at 20°C after addition of 0.5 vol. of Buffer B [0.5 M-Tris (pH 7.4 at 22°C)–2.5 M-NaCl–0.25 M-MgCl₂].

Precipitated RNA was layered on 15–30% (w/v) linear sucrose gradients containing 0.5% sodium dodecyl sulphate, 0.01 M-Tris, 0.1 M-NaCl and 0.01 M-EDTA, pH 7.2 at 22°C.

Results

Preliminary experiments showed that at eight different periods beginning 0–48 h after unilateral nephrectomy or sham-nephrectomy the kinetics of labelling of 28S and 18S rRNA were identical in

Table 1. $[^3\text{H}]\text{Uridine}$ incorporation into rRNA

Mice received 50 μCi of $[^3\text{H}]\text{uridine}$ subcutaneously for the times shown, immediately before being killed 24 h after unilateral nephrectomy or sham-operation. Each value is the mean $\pm$ S.E.M. of three determinations on one kidney.

Labelling time (h)	Experimental condition	Radioactivity (c.p.m./ E_{260} unit)	
		18S rRNA	28S rRNA
1	Nephrectomy	370 $\pm$ 20	300 $\pm$ 52
	Sham-nephrectomy	410 $\pm$ 43	380 $\pm$ 49
2	Nephrectomy	1630 $\pm$ 12	1640 $\pm$ 38
	Sham-nephrectomy	1690 $\pm$ 26	1610 $\pm$ 44
4	Nephrectomy	2050 $\pm$ 80	2130 $\pm$ 59
	Sham-nephrectomy	2230 $\pm$ 81	2200 $\pm$ 21

Fig. 1. Labelling of cytoplasmic RNA

RNA was extracted from the right kidney of mice each subcutaneously labelled with 50 μCi of $[5\text{-}^3\text{H}]\text{uridine}$ at 20 h after unilateral nephrectomy (●) or sham-nephrectomy (○). Mice were killed after 4 h and RNA was displayed on sucrose gradients (see the text), SW.41 rotor, 17 h at 24000 rev./min at 23°C. Trichloroacetic acid-precipitated, alcohol-washed RNA on glass-fibre discs was counted for radioactivity in 0.04% Omnifluor in toluene. —, E_{260} ; ○, ●, radioactivity.

mice labelled from 0.5 h to 48 h with $[5\text{-}^3\text{H}]\text{uridine}$. Similar results were obtained with radioactive cytidine and orotate, although labelling with orotate was more intense. Table 1 and Fig. 1 are the results from a typical experiment with $[5\text{-}^3\text{H}]\text{uridine}$.

Since labelling of RNA can be altered by changes in uptake of the precursor (Yu & Feigelson, 1970; Ove *et al.*, 1966) and by changes in the size of the pools (Lucas, 1971; Malamud & Baserga, 1969; Plagemann, 1971a,b), which in turn alter the specific radioactivity of the immediate nucleotide precursors of RNA, measurements of relevant variables

Table 2. *Size of nucleotide pools*

Values are means $\pm$ S.E.M. with numbers of determinations (four kidneys/determination) in parentheses. With only two determinations mean values are given with the duplicate values in parentheses.

Time between operation and death	Experimental condition	Nucleotides (nmol/mg of DNA)			ATP/ADP
		ATP	ADP	UTP	
	Normal control (13)	864 $\pm$ 57	241 $\pm$ 15	105 $\pm$ 9	3.7
20 min	Nephrectomy (6)	915 $\pm$ 154	164 $\pm$ 20	110 $\pm$ 4	5.6
20 min	Sham-nephrectomy (4)	755 $\pm$ 81	180 $\pm$ 41	100 $\pm$ 3	4.2
60 min	Nephrectomy (3)	1046 $\pm$ 37	194 $\pm$ 17	150 $\pm$ 13	5.4
60 min	Sham-nephrectomy (2)	930 (1011; 848)	140 (139; 140)	138 (148; 127)	
24 h	Nephrectomy (2)	996 (943; 1049)	246 (253; 239)	109 (110; 108)	4.0
24 h	Sham-nephrectomy (2)	984 (914; 1054)	198 (209; 187)	110 (105; 115)	5.0
48 h	Nephrectomy (2)	936 (950; 922)	258 (244; 271)	109 (120; 98)	3.6
48 h	Sham-nephrectomy (2)	975 (975; 975)	223 (225; 221)	88 (88; 87)	4.4

Fig. 2. *Rate of entry of UTP into RNA/mg of RNA*

Experiments were begun 60 min after unilateral nephrectomy. Mice received 50 μ Ci of [3 H]uridine in 0.5 ml of water by an intraperitoneal injection. Each time-point for the normal ($\circ$) and unilateral nephrectomized ($\bullet$) mice is an average of four determinations (one kidney/determination).

were made. There was no apparent change in the size of the pools of ADP, ATP and UTP at intervals from 20 min to 48 h after nephrectomy compared with sham-nephrectomy at each time-interval (Table 2).

An estimate of the rate of synthesis of RNA over an initial 20 min period was made by dividing

the specific radioactivity of RNA at the end of the period by one-half the specific radioactivity of UTP (Bucher & Swaffield, 1969b). The factor of one-half was chosen because it was the average value of UTP specific radioactivity, which increased linearly from zero to its final value (Fig. 2); the kinetics of RNA labelling were also linear. No perceptible label appeared in CTP for at least 2 h. Comparative synthetic rates of RNA thus estimated were not appreciably different at each of four times from 20 min to 48 h after operation (Table 3, Expt. A). The 20% decrease in RNA synthesis expressed per mg of RNA in the 48 h-nephrectomized rats is probably a result of the 20% greater content of unlabelled RNA present at that time (Halliburton & Thomson, 1965; Threlfall *et al.*, 1967). Expressed per mg of DNA, rates of synthesis are the same. After 60 min of labelling, no change was observed either (Table 3, Expt. B).

Differences in renal RNA specific radioactivity were seen when the radioactive uridine was administered for 24 h by subcutaneous infusion. Although the specific radioactivity of hepatic RNA was unchanged by unilateral nephrectomy, the specific radioactivity of RNA in the renoprival kidney was increased by 60% (Table 4). Sedimentation analysis revealed that this increase was found in 18S and 28S RNA and that no new species of labelled RNA were detectable. Acid-soluble radioactivity in liver and kidney was similar.

Differences in renal rRNA specific activity were also observed at certain times after unilateral nephrectomy when [*methyl*- 3 H]methionine was given. A 3 h labelling period ending 9, 24, or 48 h after unilateral nephrectomy resulted in a 60% increase in rRNA specific radioactivity (Table 5); no change in rRNA specific radioactivity was observed at 5 or 120 h. The acid-soluble radioactivity was the same in kidneys from sham- and unilaterally-nephrectomized mice.

Table 3. *Rates of RNA synthesis after labelling with a single injection of [5-³H]uridine for 20 min (Expt. A) and 60 min (Expt. B)*

Values are means with numbers of determinations in parentheses, four kidneys per determination.

Time between operation and death	Experimental condition	Sp. radioactivity of RNA (d.p.m./mg of RNA)	Sp. radioactivity of UTP (d.p.m./nmol of UTP)	UTP incorporated into RNA (nmol/mg of RNA)
Expt. A				
	Normal control (5)	101 100	3760	54
20 min	Nephrectomy (6)	94 000	3530	53
20 min	Sham-nephrectomy (4)	86 000	2690	64
60 min	Nephrectomy (2)	81 500	2780	57
60 min	Sham-nephrectomy (2)	88 300	2520	70
24 h	Nephrectomy (2)	106 400	2880	74
24 h	Sham-nephrectomy (2)	97 300	2630	74
48 h	Nephrectomy (2)	86 500	3000	58
48 h	Sham-nephrectomy (2)	110 200	2800	79
Expt. B				
	Normal control (5)	119 000	2350	101
1 h	Nephrectomy (4)	121 500	2580	94
1 h	Sham-nephrectomy (2)	122 800	2340	105
24 h	Nephrectomy (2)	120 600	2500	96
24 h	Sham-nephrectomy (2)	119 900	2320	103
48 h	Nephrectomy (2)	123 700	2460	101
48 h	Sham-nephrectomy (2)	121 600	2380	102

Table 4. *Labelling of RNA for 24 h with [5-³H]uridine by using continuous infusion*Values are means $\pm$ S.E.M. for the numbers of observations in parentheses.

Organ	Sp. radioactivity of cytoplasmic RNA (d.p.m./ μ g of RNA)
Liver	
Sham-nephrectomy	125 $\pm$ 4 (12)
Nephrectomy	126 $\pm$ 7 (14)
Kidney	
Sham-nephrectomy	308 $\pm$ 9 (12)
Nephrectomy	496 $\pm$ 20* (14)

* $P < 0.001$ compared with sham-nephrectomy kidney.Table 5. *Incorporation of [methyl-³H]methionine into rRNA*The radioactivity in rRNA represents the value obtained after combining the 18S and 28S rRNA, which were separated from low-molecular-weight RNA species by centrifugation through sucrose gradients. Labelling was for 3 h ending at death. Values are means $\pm$ S.D., with numbers of determinations (one kidney/determination) in parentheses.

Time from operation to death (h)	Experimental condition	Sp. radioactivity of rRNA (c.p.m./100 μ g of rRNA)	P
5 h	Nephrectomy	610 $\pm$ 190 (3)	
5 h	Sham-nephrectomy	630 $\pm$ 140 (3)	>0.8
	Normal control	580 $\pm$ 30 (3)	>0.8
9 h	Nephrectomy	1080 $\pm$ 150 (4)	
9 h	Sham-nephrectomy	730 $\pm$ 90 (3)	<0.02
	Normal control	570 $\pm$ 130 (3)	<0.01
24 h	Nephrectomy	870 $\pm$ 160 (6)	
24 h	Sham-nephrectomy	550 $\pm$ 50 (7)	<0.001
48 h	Nephrectomy	940 $\pm$ 65 (3)	
48 h	Sham-nephrectomy	650 $\pm$ 130 (4)	<0.02
120 h	Nephrectomy	790 $\pm$ 50 (3)	
120 h	Sham-nephrectomy	800 $\pm$ 90 (4)	>0.9

after contralateral nephrectomy. This calculation is based on the equation of Schimke (1967), whereby:

$$R = \frac{s}{k} + \left(R_0 - \frac{s}{k} \right) e^{-kt}$$

in which s = zero-order synthetic rate, k = first-order degradation constant, and (from $t_{1/2}$ of renal rRNA = 100 h) (Malt & Lemaitre, 1968) $k = 0.17/\text{day}$ at

Discussion

Despite the well-established 20–40% increase in RNA/DNA ratio at the end of 48 h of compensatory renal hypertrophy (Halliburton & Thomson, 1965; Threlfall *et al.*, 1967; Malt & Lemaitre, 1968; Kurnick & Lindsay, 1968; Bucher & Malt, 1971), the present experiments revealed no change in the rate of RNA synthesis assessed in periods of 20 and 60 min labelling. Nonetheless, in principle, one would expect to be able to identify an average 88% increase in renal RNA synthesis to account for a nominal 12.5% increase in RNA/DNA ratios (RNA/cell) during the first day

time t . R_0 is the amount of RNA at steady state; R is the amount of RNA after an increase in RNA content of 12.5% and equals $1.125R_0$ (12.5% increase/day).

The assumptions underlying the calculation of synthetic rate by dividing the specific radioactivity of total renal RNA by one-half the specific radioactivity of the UTP at the end of a labelling period appear to be met (Bucher & Swaffield, 1969b). Most of the label entering RNA seems to remain in RNA, the increase in specific radioactivity of the UTP is linear, RNA labelling is linear and the UMP content of the RNA probably stays constant. Compartmentation of the ribonucleotide pools has not been considered in these calculations. Wu & Soeiro's (1971) evidence was consistent with the existence of a single functional ribonucleotide pool in HeLa cells for the synthesis of nucleoplasmic and nucleolar RNA. Ove *et al.* (1967) identified a rapid equilibrium in ATP between the nucleus and cytoplasm of rat liver, likewise suggesting a single ribonucleotide pool. Plagemann (1971a,b, 1972), however, proposed independent ribonucleotide pools in cultured hepatoma cells.

Support for the validity of the measurement of the specific radioactivity of UTP comes from the measurements of ATP and ADP. The ratios of ATP/ADP were unchanged during the first 2 days of compensatory hypertrophy and were similar to those in rat liver (Bucher & Swaffield, 1969a). An increase in ADP at the expense of ATP would have indicated either unphysiological conditions not supporting the synthesis of ATP or degradation as a result of invalid methods of analysis. The values for ATP and ATP/ADP ratios are greater than or equal to other values reported for kidney (Kirsten *et al.*, 1972; Ross & Weiner, 1972; Miller & Baggett, 1972).

An increase in renal RNA synthesis, if present, should have been detectable. There is no reason to anticipate that peculiarities of RNA metabolism in the renoprival kidney make analytical approaches in other systems (Bucher & Swaffield, 1969b; Malamud & Baserga, 1969; Miller & Baggett, 1972) inapplicable to kidney. For example, in rat liver labelled for 1 h or less, several experiments readily show increased specific radioactivity of hepatic RNA after partial hepatectomy (Blobel & Potter, 1968; Bucher & Swaffield, 1969b; Chaudhuri & Lieberman, 1968; Rizzo & Webb, 1972).

The 20–40% increase of RNA/DNA ratio may have been the result of decreased wastage or of increased stability of rRNA and rRNA precursors. After 1 h labelling in rat liver (Blobel & Potter, 1968) or in HeLa cells (Darnell, 1968) most of the radioactivity is in rRNA or rRNA precursors, and mouse kidney synthesizes rRNA precursors an estimated 2–3 times faster than HeLa cells (AB & Malt, 1970);

therefore most [^3H]uridine incorporated in our experiments after 20–60 min of labelling was probably in precursor rRNA and in rRNA.

After administration of [*methyl*- ^3H]methionine the specific radioactivity of rRNA was increased during compensatory renal hypertrophy (Table 5). Since methylation of rRNA precursor takes place during transcription or immediately after transcription, the increase in rRNA specific radioactivity could have been due to an increase in methylation of rRNA or to decreased degradation of rRNA precursor. In preliminary experiments, no change has been found in specific radioactivity of 45S precursor rRNA after short labelling periods with [*methyl*- ^3H]methionine. It seems possible therefore that the cytoplasmic content of stable rRNA in kidney is regulated in part by the proportion of newly synthesized 45S ribosomal precursor RNA which is processed to mature rRNA (Vaughan, 1972; Busch & Smetana, 1970; Cooper, 1969, 1970; Rubin, 1971; Luck & Hamilton, 1972; Smith *et al.*, 1972; Fantoni *et al.*, 1972; Clissold & Cole, 1973) or perhaps by greater stability of the mature rRNA. Recent experiments (unpublished work) suggest greater stability of rRNA.

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