Ribonucleic Acid Synthesis in the Renal Cortex at the Initiation of Compensatory Growth

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The mechanisms responsible for the increase in RNA per cell during the first 48 h of renal compensatory growth were studied in the renal cortex. Unilaterally nephrectomized, sham-operated or non-operated rats were used. Incorporation into RNA of labelled precursors was studied *in vivo* and *in vitro*. Sham-operation produced significant changes in precursor incorporation, absolute amounts of UTP and RNA, and the rate of RNA synthesis. At 6 h after surgery, the amount of RNA decreased in sham-operated controls, whereas that in growing cortex remained unchanged. Incorporation into RNA *in vivo* was greater in the growing cortex, although the rate of RNA synthesis was not increased. At 24 h, precursor incorporation into RNA and UTP and RNA synthesis were all increased in the growing cortex. In contrast with results obtained *in vivo*, slices of growing cortex incorporated less labelled precursor into RNA than did cortex slices from sham-operated controls, from 3 to 48 h. Maximal differences were found from 6 to 24 h. An attempt was made to equalize endogenous precursor pool sizes by increasing the concentration of unlabelled uridine in the media; incorporation differences were narrowed significantly. Serum from nephrectomized animals did not increase precursor incorporation into RNA *in vitro*. An increase in RNA synthesis is an important factor in RNA accretion in the renal cortex beyond 12 h of compensatory growth. This is accompanied by increased UTP content and preceded by expansion of other pools. The amount of labelled precursor incorporated into RNA is greatly influenced by its delivery rate to the growing kidney *in vivo* and by intracellular dilution of expanded precursor pools *in vitro*.

Unilateral nephrectomy induces a series of metabolic changes in the remaining kidney which lead to its compensatory growth. The metabolism of nucleic acids, proteins and phospholipids are the first to be altered during this process (Malt, 1969; Toback *et al.*, 1974a). Renal cell growth predominates over cell proliferation, and the increase in kidney weight and total content of RNA and protein takes place even when DNA synthesis is blocked (Janicki & Lingis, 1970). Total renal DNA is not significantly increased until the fourth day after nephrectomy (Halliburton & Thomson, 1965; Threlfall *et al.*, 1967), whereas total RNA is increased at 12 h (Halliburton & Thomson, 1965), reaching a maximum of about 20% increase at 48 h (Threlfall *et al.*, 1967; Kurnick & Lindsay, 1968; Malt & Lemaitre, 1968). This change in RNA content is primarily due to rRNA. Although the medulla participates in compensatory renal growth, the

earliest and most prominent mitotic changes take place in the tubular cells of the renal cortex (Ogawa & Sinclair, 1958; Williams, 1961), but it is not known to what extent each fraction contributes to the changes described in total renal RNA concentration.

The study of RNA metabolism during renal growth by the use of labelled precursors has been hindered by the variables that may influence the amount of isotope finally incorporated into RNA. The breakdown rate of RNA during growth has been reported not to differ from controls (Malt & Lemaitre, 1968), although there is no information on the efficiency of nucleotide and nucleoside reutilization, as has been documented in other tissues (Seifert & Vácha, 1974; Vácha & Seifert, 1975). An increase in the rate of RNA synthesis has been inferred from an increase in the incorporation of labelled precursors injected *in vivo* (Halliburton & Thomson, 1966; Johnson & Vera Roman, 1966; Malt & Stoddard, 1966; Sendekci *et al.*, 1972), although it is known that the amount incorporated depends on renal blood flow, plasma

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specific radioactivity of the precursor, membrane transport or changes in intracellular precursor pools. An increase in renal plasma flow has been reported in the remaining kidney shortly after contralateral nephrectomy (Hartman & Bonfilio, 1959; Rous & Wakim, 1967; Potter *et al.*, 1969), and expansion of the intracellular UTP pool has been described in other growing tissues (Bucher & Swaffield, 1969; Koide & Rabinowitz, 1969; Fields & Brox, 1973; Goody & Ellem, 1975). Studies *in vitro* have demonstrated a paradoxically decreased incorporation of labelled precursors into the RNA of renal-cortex slices from growing kidneys, suggesting that RNA-precursor pools may be expanded (Toback & Lowenstein, 1974). Contrary to this, neither the UTP pool nor the rate of RNA synthesis has been demonstrated to be increased in the growing kidney *in vivo* between 20 min and 48 h after unilateral nephrectomy (Hill *et al.*, 1974). The conclusion, reached in recent studies, has been that more efficient processing to mature rRNA of newly synthesized 45S ribosomal precursor RNA and greater stability of mature rRNA account for the increase in RNA (Hill *et al.*, 1974; Hill, 1975), rather than an increase in RNA synthesis.

In this study we have used systems *in vivo* and *in vitro* for the evaluation of RNA synthesis during the initial stages of renal compensatory growth. In both cases the studies were limited to the renal cortex because metabolic differences from the medulla, especially with regard to precursor utilization for RNA synthesis, require separate studies of these tissues (Lee *et al.*, 1962; Bernanke & Epstein, 1965; Liberti & Kline, 1974; Toback *et al.*, 1974b). In addition, a search has been made into factors that could account for the paradoxical results obtained *in vitro*, particularly the possibility that the initiation of compensatory renal growth might depend on the presence of an unidentified serum factor (Lowenstein & Stern, 1963; Preuss *et al.*, 1970; Vichi & Earle, 1970; Lyons *et al.*, 1974). Re-feeding was used to ensure a similar state of nutrition in all animals, since starvation has been known to adversely affect renal hypertrophy (Reiter, 1965; Halliburton & Thomson, 1967; Royce, 1967) and to decrease RNA synthesis (Rickwood & Klemperer, 1970). Further, it is likely that changes in the synthesis of RNA precursors are more evident in the re-fed state, since the activities of enzymes of the pentose phosphate pathway are decreased by starvation, and rapidly restored to normal or above normal values when food is returned (Novello *et al.*, 1969).

Materials and Methods

Animals and infusion technique

Male rats, weighing 140–210 g, were individually caged for 1 week before surgery. During this period

the mean increase in weight was about 4%; animals that increased less than 2% were rejected. Re-feeding of the animals was done by withholding food for 24 h and then returning it 12 h before either nephrectomy, sham-operation or killing of non-operated animals.

Unilateral nephrectomies were done through a lumbar approach under light methoxyflurane (Penthrane; Abbott Laboratories, North Chicago, IL, U.S.A.) and oxygen anaesthesia. A sham-operation was done in all control animals in the same fashion, except in the non-operated control group. Preliminary studies (unpublished work) showed that cortical slices obtained from animals anaesthetized with methoxyflurane or sodium pentobarbital or killed by a blow on the head all showed similar $^{14}\text{CO}_2$ production and ^{14}C incorporation into total lipids when incubated with $[1-^{14}\text{C}]$ palmitic acid or $[\text{U-}^{14}\text{C}]$ glucose.

Animals were stunned by a blow on the head and their kidneys removed and rapidly immersed in 0.9% NaCl at 2°C. The cortex was dissected from the medulla, cut into small pieces, rinsed in 0.9% NaCl and blotted. Two specimens (180–210 mg) were analysed for total RNA and DNA.

Animals used in infusion experiments had an indwelling polyethylene no. 10 catheter with a Silastic (Dow Corning Co., Midland, MI, U.S.A.) tip placed into the inferior vena cava through the left femoral vein. The catheter was brought out subcutaneously through the back and taken out of the cage threaded through a protective coiled wire tubing (Daugharty *et al.*, 1973). Animals were allowed to recover from surgery for 2 weeks before the experiments. At the end of this period there was an increase in weight in all animals.

Unanaesthetized animals were infused at 10 $\mu\text{l}/\text{min}$ for 120 min by using a Sage 220 syringe pump (Orion Research, Cambridge, MA, U.S.A.). The total amount infused per animal was 0.1 μmol of sodium orotate and 75 μCi of $[5-^3\text{H}]$ orotic acid (New England Nuclear Corp., Boston, MA, U.S.A.) dissolved in 0.9% NaCl, the specific radioactivity of the final infusate being 713 mCi/mmol. Orotic acid was chosen as the labelled precursor, since 44% of an injected dose of this compound is taken up by the kidney (Lewan *et al.*, 1975). Without handling of the animals or interrupting the infusion, the animals were lightly anaesthetized (with methoxyflurane) at the end of the infusion period and the left kidneys gently exposed through a lumbar incision. A rim of renal cortex was cut directly into liquid N_2 with ophthalmic enucleation scissors with a strong curve (Weck Eye Instruments, Long Island City, NY, U.S.A.). Any contaminating blood or remaining medulla were later scraped off the cortex under liquid N_2 .

Blood was drawn into heparinized syringes by cardiac puncture, and the plasma immediately separated by centrifugation (1000 g for 10 min at 2°C).

Frozen renal cortex (300–400mg) was pulverized in a liquid-N₂ precooled stainless-steel mortar and immediately transferred to a beaker on ice containing 3ml of 0.1M-HClO₄. Simultaneously, 0.3 μ Ci of [2-¹⁴C]UTP (New England Nuclear Corp.; specific radioactivity 53.3mCi/mmol) was added to the beaker, as internal standard for estimation of UTP recovery. The tissue samples were immediately homogenized at 2°C in an all-glass homogenizer, and the final homogenates brought to 0.2M-HClO₄ with 0.3M-HClO₄ before separation of the precipitate and cold-acid-soluble fraction by centrifugation at 800g for 15min at 2°C. Groups of animals were studied at 0, 6, 12, 24 and 48h after unilateral nephrectomy or sham-operation. A separate group of 14 animals, studied 24h after unilateral nephrectomy or sham-operation, was infused for periods of between 10 and 150min under the same conditions, to follow the amount incorporated as the infusion time was prolonged.

Incubation technique

Kidneys were obtained from stunned animals and rapidly immersed in ice-cold Krebs–Ringer bicarbonate buffer, pH 7.4 (Umbreit *et al.*, 1964). After decapsulation, 180–210mg of cortical-tissue slices was obtained with a Stadie–Riggs microtome, and placed in a vial containing 3ml of cold medium, containing a labelled RNA precursor and either 5mM-glucose or 0.1mM-palmitic acid complexed to albumin (Kessler *et al.*, 1967) as substrate. Slices were incubated for 90min at 37°C in a Dubnoff metabolic incubator at 110 strokes/min after a 5min gassing period with O₂+CO₂ (95:5). Incubations were terminated by placing the vials in ice-cold water. Fatty acids were removed from bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis, MO, U.S.A.) (Chen, 1967). [2-¹⁴C]Uridine (58mCi/mmol) or [6-¹⁴C]orotic acid monohydrate (60mCi/mmol) (Amersham/Searle Co., Arlington Heights, IL, U.S.A.) were used in concentrations of 22.1 and 22.3 μ M respectively. In some experiments unlabelled uridine (Boehringer Mannheim Co., New York, NY, U.S.A.) was added to a final concentration of 2mM. At the termination of all incubations, slices were rinsed in a total of 15ml of ice-cold Krebs–Ringer bicarbonate buffer, pH 7.4, and blotted. The tissue samples were homogenized in cold distilled water.

A separate group of incubations was done with serum added to the incubation media. Blood was obtained on the same day of the experiment, by cardiac puncture by using siliconized syringes, and allowed to clot in plastic tubes. Groups of animals used for providing sera were bled at 12, 24 and 48h after sham-operation or unilateral nephrectomy. By the same techniques, liver slices from non-operated rats were incubated in sera from sham-operated or nephrectomized rats in media containing labelled

uridine. This was done in an attempt to assess non-specific effects produced by these sera (Preuss *et al.*, 1970).

Extraction method and quantification of total ribose phosphate, RNA and DNA

On the same day as the experiment, the cold-acid-soluble fraction, RNA and DNA were extracted as described by Munro & Fleck (1966). The cold-acid-soluble fraction was neutralized with 0.35M-KOH, and a portion taken for freeze-drying. The freeze-dried samples were stored at –40°C for further analysis.

Plasma and neutralized cold-acid-soluble fractions were analysed for glucose by the glucose oxidase reaction (Glucostat; Worthington Biochemical Co., Freehold, NJ, U.S.A.). Ribose was determined by the method of McKay (1964) in the neutralized cold-acid-soluble fraction as well as in the plasma, the orcinol reaction being carried out at 100°C for 8min. Corrections were made for the spectrophotometric interference produced by glucose. The orcinol was purified by recrystallization from benzene, and decolorized with charcoal. Maximal sensitivity of this method was obtained with ribose 5'-phosphate. About half this sensitivity was found with ribose, adenosine and guanosine. Uridine, cytidine and the mono-, di- and tri-phosphates of the four ribonucleosides reacted poorly with orcinol; UMP reacted the most readily, with a sensitivity 1/25 that of the ribose 5'-phosphate sensitivity. Since ribose, adenosine and guanosine are rapidly phosphorylated intracellularly (Hauschka, 1973; Plageman & Richey, 1974), the orcinol method, as used, primarily measures changes in ribose phosphates.

Total RNA was measured as the ribose content (McKay, 1964) by the reaction with orcinol after 30min incubation at 100°C. With this method, 1mg of purified yeast RNA (type XI; Sigma Chemical Co.) gave a colour reaction equivalent to 0.163mg of ribose. Total DNA was measured by the diphenylamine method for deoxyribose (Giles & Myers, 1965); with this method, 1mg of DNA from calf thymus (Sigma Chemical Co.) was equivalent to 0.109mg of deoxyribose. The diphenylamine used (Fisher Scientific Co., Fair Lawn, NJ, U.S.A.) was purified by recrystallization from hexane and decolorized with charcoal. The method of Lowry *et al.* (1951) was used for protein measurement.

Liquid chromatography

The freeze-dried extract obtained from the neutralized cold-acid-soluble fraction of the tissue extract (Brown & Miech, 1972) was resuspended in 0.5ml of distilled water and ultrafiltered at 2°C by using Amicon Centriflo cones (Amicon Corp., Lexington, MA, U.S.A.). The resulting ultrafiltrate was used for analysis.

High-pressure liquid chromatography was carried

out as described by Brown (1970) for nucleotide analysis by using a Varian-4200 system (Varian Instruments, Palo Alto, CA, U.S.A.). Nucleotide separations were done with columns (3m×2mm internal diam.) packed with a strong anion-exchange pellicular resin (AS-Pellionex-SAX; Whatman, Clifton, NJ, U.S.A.). To ensure steady baselines for quantification, an identical column was used on the reference side, and in addition the KH_2PO_4 (Mallinckrodt, St. Louis, MO, U.S.A.) was purified as described by Shmukler (1970) to remove u.v.-absorbing contaminants. Peaks were recorded and integrated simultaneously with a Varian-485 electronic digital integrator. There was a linear correlation between concentrations of UTP from 1.23 to 1.9 nmol and the response in peak area counts. Concave step-wise gradient elutions were obtained by using a low-ionic-strength buffer (which was prepared from approx. 0.006M- KH_2PO_4 adjusted to pH 3.30 at 22°C with 1M- H_3PO_4 and diluted to 0.005M) and a high-ionic-strength buffer (prepared from approx. 0.27M- KH_2PO_4 in 0.25M-KCl adjusted to pH 4.50 at 22°C with 3M-KOH and diluted to 0.25M with 0.25M-KCl). Chromatographic analyses were done at 70°C at a flow rate of 0.5 ml/min with 15 μl of sample. Peaks were detected by simultaneous monitoring of light absorption at 254 and 280 nm. Resolution was determined by the formula $2(T_2 - T_1)/(W_1 + W_2)$ (Grushka, 1975), retention times (T) and peak widths [W] being expressed in minutes. Peaks were identified according to retention times of purified standards, location of the radioactive UTP internal standard, E_{254}/E_{280} ratios and rechromatography. Column effluents were collected in 0.5 ml fractions in an automatic fraction collector (Fractomette-400; Buchler Instruments, Fort Lee, NJ, U.S.A.).

Determination of radioactivity

Plasma samples, cold-acid-soluble fractions, RNA extracts and the collected chromatographic fractions were counted for radioactivity in a three-channel liquid-scintillation counter equipped with an automatic quench corrector (LS-350; Beckman Instruments, Fullerton, CA, U.S.A.). Samples for counting were neutralized with 2M-Tris buffer whenever required. A dioxan-based (Bray, 1960) or a toluene-based scintillator, to which compound BBS-3 (Beckman Instruments) was added (Bray, 1970), was used. Efficiency of counting was from 32 to 40% for ^3H and from 60 to 75% for ^{14}C .

Standards

Ribose and deoxyribose (Sigma Chemical Co.) were used as standards for RNA and DNA quantification respectively. The following purified compounds, obtained from Sigma Chemical Co., were used as standards for chromatography: orotic acid, orotidine 5'-monophosphate, UDP, UTP, UDP-glucose,

UDP-galactose, UDP-*N*-acetylglucosamine, CDP, CTP, ADP, ATP, dTTP and GTP. The UTP standard used for sample quantification was 90.8% pure, as analysed by liquid chromatography. Standards were kept as 4mM solutions in distilled water at -40°C with no evident deterioration (Brown, 1971). The [$2\text{-}^{14}\text{C}$]UTP was analysed for radiochemical purity; this ranged between 76.8 and 98.4% in different batches, depending on the age of the standards when used.

Presentation of data and statistical analysis

The results obtained from the incubations *in vitro* were expressed as a fraction of the initial d.p.m. in the incubation medium incorporated per mg of DNA. Total radioactivity incorporated in infusion experiments was expressed as total d.p.m./mg of DNA or as d.p.m./mg of DNA expressed as a fraction of d.p.m. infused/200g body weight. UTP incorporation was corrected according to the recovery of the internal standard. The rate of RNA synthesis was expressed as the radioactivity incorporated into RNA by using the average specific radioactivity of the UTP during the infusion period, by the method of Bucher & Swaffield (1969). The assumption is made that differences found in the number of labelled molecules entering RNA during the incorporation period are due to molecules which will remain in RNA, and that the rise in UTP specific radioactivity is linear during such a period; nmol of UTP incorporated into RNA/h per mg of DNA will equal the ratio between d.p.m. in RNA/mg of DNA and one-half d.p.m. in UTP/nmol of UTP at the end of the infusion period. All results are presented as means $\pm$ s.e.m. with numbers of observations in parentheses. Student's *t* test was used for statistical analysis, except for the results in incubations *in vitro* with serum, where analysis of variance was applied (Batson, 1956).

Results

RNA and DNA concentrations in the normal rat renal cortex and changes induced by re-feeding

In non-operated controls fed *ad lib.*, RNA and DNA concentrations were 1.020 ± 0.011 (10) and 0.625 ± 0.006 (10) $\mu\text{g}/\text{mg}$ of tissue respectively. As reported by Halliburton & Thomson (1965), who studied the whole kidney, we found no differences between the right and left renal cortices. After 24 h of starvation, RNA concentration was 1.018 ± 0.01 (8) $\mu\text{g}/\text{mg}$ and the RNA/DNA ratio was lower than normal values obtained from controls fed *ad lib.* (Fig. 1). The difference in the RNA/DNA ratio after starvation was still evident after 12 h of re-feeding (Fig. 1).

Changes, induced by unilateral nephrectomy, in the RNA/DNA ratio in the growing renal cortex

The effects of surgery on RNA concentration per cell are depicted by the changes in the RNA/DNA

ratio in the sham-operated control group at different time-intervals after surgery, as compared with the renal cortex of the kidney removed at the time of nephrectomy. At 6h after sham-operation, RNA per cell decreased by about 6% ($P < 0.01$), but had returned to normal at 12h. It was still at this concentration 24h after operation and had increased by about 6% at 48h (Fig. 1). In the growing renal cortex there was no change in RNA concentration per cell until 24h after unilateral nephrectomy. Because of this, RNA concentration was 9% higher than that in the sham-operated animals at 6h after surgery. At 24h, the RNA/DNA ratio increased by 10%, rising to 14% at 48h after nephrectomy (Fig. 1).

Incorporation of [2-¹⁴C]uridine into RNA of growing renal cortex in vitro

The pattern of incorporation of labelled uridine was studied in the growing renal cortex and that from sham-operated controls at 6h after surgery by incubating cortical slices for intervals from 30 to

Fig. 1. Changes in the RNA/DNA ratio in the renal cortex in normal animals after starvation or re-feeding and in operated animals at 0–48h after sham-operation or unilateral nephrectomy

The kidneys were removed from three groups of non-operated Wistar rats; one group had been fed *ad lib*, one had been starved for 24h and one had been starved and re-fed for 12h. Re-fed animals had the left and right kidneys removed with or without ligation of the renal pedicle respectively to determine the effects of this procedure. The results obtained from this group are represented at 0h. Left nephrectomy or sham-operation was done in five additional groups of re-fed rats. At different intervals after surgery the right kidneys were obtained for study. The cortex from the initially removed left kidney at nephrectomy was used as non-operated control. The results obtained from these non-operated controls were pooled and the means $\pm$ S.E.M. values represented as horizontal continuous and broken lines. Each point represents the mean $\pm$ S.E.M. of eight to twelve observations. ■, Non-operated, fed *ad lib*; □, non-operated, 24h starved; △, left cortex with ligation, non-operated, re-fed; ▲, right cortex, without ligation, non-operated, re-fed; ●, growing cortex; ○, cortex from sham-operated controls.

Fig. 2. Changes in [2-¹⁴C]uridine incorporation into cold-acid-soluble fraction and RNA in renal-cortex slices after different periods of incubation

Two groups of re-fed Fisher rats were either unilaterally nephrectomized or sham-operated. Two pools of tissue slices from growing kidneys or from kidneys from sham-operated controls were obtained 6h after surgery and incubated in 3ml of a medium containing 5mm-glucose and 3.7 μ Ci of labelled uridine. Incubations were terminated in the different groups at selected time-intervals. Incorporation of radioactivity was expressed as % of total initial d.p.m. in the medium/ μ g of DNA. The results are means $\pm$ S.E.M. of five or six observations. ●, Growing cortex; ○, cortex from sham-operated controls. (a) Acid-soluble fraction; (b) RNA.

120min. The total amount of radioactivity found in the cold-acid-soluble fraction, containing RNA precursors, reached a maximum value at 60min and remained constant up to 120min. The pattern and total uptake were the same in growing and sham-operated control groups. Incorporation into RNA increased linearly as incubation time was prolonged during the period studied, but the amount incorporated into the growing renal cortex was lower at all intervals of incubation (Fig. 2).

Renal cortical slices obtained from growing kidneys at 6h and 12h after unilateral nephrectomy incorporated into RNA about one-half of the label incorporated by slices from sham-operated controls (Table 1). This difference in incorporation was also obtained when palmitate was substituted for glucose as the substrate. Significant results were demonstrated in two strains of rats, but differences obtained between growing and sham-operated groups were greater in one strain than in the other (Table 1 and Fig. 2a).

Table 1. *Incorporation of [2-¹⁴C]uridine into RNA of renal-cortical slices from growing kidneys at 6–12 h after unilateral nephrectomy*

Renal-cortical slices were prepared from kidneys of unilaterally nephrectomized re-fed Wistar rats. Control slices were obtained from sham-operated animals. Either glucose or palmitate was used as substrate. In one experiment, at 6 h post-nephrectomy the uridine concentration in the medium was increased to 2 mM, thus decreasing the specific radioactivity from 64 mCi/mmol to 0.68 mCi/mmol. Incubations were carried out at 37°C for 90 min, except for the experiment at 12 h post-nephrectomy when the incubation time was 60 min. Values are presented as the means $\pm$ s.e.m., with numbers of determinations indicated in parentheses. Student's *t* test was used for statistical analysis.

Time after nephrectomy (h)	Renal cortex	Substrates (mM)			10 ⁴ × Radioactivity (% of initial d.p.m. in the incubation medium incorporated into the RNA/μg of DNA)	% of control value
		Glucose	Uridine	Palmitate		
6	Control	—	0.022	0.1	90.8 $\pm$ 8.2 (12)	
	Growing				43.1 $\pm$ 1.9 (12)	47.4*
6	Control	5	0.022	—	152.8 $\pm$ 8.2 (12)	
	Growing				82.2 $\pm$ 3.6 (12)	53.7*
6	Control	5	2	—	14.2 $\pm$ 0.8 (12)	
	Growing				10.9 $\pm$ 0.6 (12)	76.9†
12	Control	5	0.01	—	132.2 $\pm$ 4.8 (9)	
	Growing				59.5 $\pm$ 5.9 (9)	57.6*

* *P* < 0.001.† *P* < 0.005.Table 2. *Incorporation of [6-¹⁴C]orotic acid into RNA of renal-cortical slices from growing kidneys at 3–48 h after unilateral nephrectomy*

Renal-cortical slices were prepared from kidneys of unilaterally nephrectomized re-fed Wistar rats. Control slices were obtained from sham-operated animals. Glucose (5 mM) and orotic acid (0.022 mM; specific radioactivity 60 mCi/mmol) were used as substrates in the incubation media. In one experiment at 6 h post-nephrectomy the uridine concentration in the medium was increased to 2 mM. All incubations were carried out at 37°C for 90 min. Values are presented as the means $\pm$ s.e.m., with numbers of determinations indicated in parentheses. Student's *t* test was used for statistical analysis.

Time after nephrectomy (h)	Renal cortex	10 ⁴ × Radioactivity (% of initial d.p.m. in the incubation medium incorporated into the RNA/μg of DNA)	% of control value
3	Control	627.2 $\pm$ 32 (12)	
	Growing	505.7 $\pm$ 25.7 (12)	80.5 (<i>P</i> < 0.01)
6	Control	408.6 $\pm$ 42.1 (11)	
	Growing	251.7 $\pm$ 11.9 (11)	61.6 (<i>P</i> < 0.005)
6	Control	293.6 $\pm$ 54.8 (12)	
	Growing	164.3 $\pm$ 13.1 (12)	55.9 (<i>P</i> < 0.001)*
48	Control	1067.3 $\pm$ 56.1 (11)	
	Growing	910.7 $\pm$ 29.8 (10)	85.2 (<i>P</i> < 0.05)

* 2 mM-Uridine.

At 6 h after surgery, an increase in uridine concentration in the medium from 22.1 μM to 2 mM caused, as expected, both an increase in the total amount of extracellular uridine taken up by the tissue RNA (sham-operated, 0.92 versus 83.9 pmol/μg of DNA; growing, 0.49 versus 65.9 pmol/μg of DNA) and a marked decrease in the incorporation of label (Table 1). The differences previously found between nephrectomized and sham-operated control groups were considerably diminished from 53.7 to 76.9% of

the incorporation in sham-operated rats. This 23.2% decrease was significant (*P* < 0.001).

Incorporation of [6-¹⁴C]orotic acid into RNA of growing renal cortex in vitro

RNA synthesis *de novo* was explored by the use of labelled orotic acid. Renal cortex from sham-operated controls incorporated 1.7 times more and growing cortex 2.1 times more orotic acid than uridine 6 h after surgery (Tables 1 and 2).

The renal cortex from sham-operated controls incorporated variable amounts of label into RNA, depending on the period after surgery when tissue was obtained. There was inhibition from 3 to 6 h after surgery with a subsequent increase in incorporation at 48 h. A parallel change was seen in the growing renal cortex, but, as with the use of uridine, the incorporation of labelled orotic acid was always lower than that observed in the cortex from sham-operated rats at every period studied during the first 48 h after nephrectomy (Table 2).

Fig. 3. Effects produced *in vitro* by the addition of serum from nephrectomized animals on incorporation of $[2-^{14}\text{C}]$ uridine into RNA in growing and control renal-cortical slices

Wistar rats fed *ad lib.* were unilaterally nephrectomized or sham-operated. Both groups were divided into serum donors and experimental animals. Three different experiments were done at 12, 24 and 48 h after surgery. At each time-interval renal-cortical slices were obtained from growing kidneys or kidneys from sham-operated controls in the experimental group and serum obtained and pooled from the nephrectomized or sham-operated serum donors. Two 65–80 mg samples of renal-cortical slices from each individual kidney were incubated separately in vials containing either type of serum. Incubations (60 min) were done in 2 ml of a 5 mM-glucose medium containing 50% (v/v) of the corresponding serum and 1.5–2.0 μCi of $[2-^{14}\text{C}]$ uridine. Radioactivity incorporation was expressed as % of total initial c.p.m. in the medium incorporated into RNA/mg of protein. Results are plotted as % of the mean value ($\pm$ S.E.M.) obtained in the corresponding control group (renal cortex from sham-operated control with control serum) as obtained for each individual experiment. ●, Growing renal cortex with control sera; ▲, growing renal cortex with sera from nephrectomized animals; ○, renal cortex from sham-operated controls with sera from nephrectomized rats.

The addition of unlabelled 2 mM-uridine to the incubation media decreased the incorporation of labelled orotic acid by about 32 % in cortex from both nephrectomized and sham-operated groups. Unlike the effect produced by the addition of unlabelled uridine on the incorporation of labelled uridine, differences between the two groups were not narrowed (Table 2).

*Effects induced by serum from unilaterally nephrectomized or sham-operated animals on incorporation of $[2-^{14}\text{C}]$ uridine into RNA of growing renal cortex *in vitro**

Serum obtained from unilaterally nephrectomized animals did not change the incorporation of labelled uridine in renal-cortex slices from sham-operated control animals when compared with results obtained in incubations with control serum obtained from sham-operated animals. However, in growing renal cortex a further decrease in incorporation, due to the addition of serum from nephrectomized animals, was demonstrated (Fig. 3). The differences produced by the serum were significant at 12 h and 48 h after unilateral nephrectomy, when the decreases were 22% and 26% respectively of the label incorporated by the same growing renal cortex when incubated with control serum. Neither type of serum affected the incorporation of labelled uridine into RNA of liver slices of normal animals under identical incubation conditions.

Chromatographic separation of UTP from renal-cortical tissue

Ion-exchange chromatography with pellicular resins was found to be an excellent method for UTP quantification in the renal cortex. Changes in the pH gradient used were critical in the resolution of UTP. The samples used contained 1.5–1.9 nmol of UTP, the lowest amount quantified with accuracy being approx. 0.75 nmol. No attempt was made to quantify simultaneously other triphosphate nucleotides, since correction for recovery of internal standards was considered necessary. UTP recovery was 82–98% and differences in duplicate determinations done on different days for UTP quantification were less than 11%. Resolution of UTP from ATP and CTP was 1.53 and 3.6 respectively (Fig. 4).

Changes induced by unilateral nephrectomy in UTP concentration per cell in the growing renal cortex

Sham-operation caused an increase in UTP concentration in the renal cortex compared with the normal values in non-operated control animals. This increase was significant at 6 h ($P < 0.025$) and 48 h ($P < 0.025$) and reached the highest value at 24 h ($P < 0.001$) after surgery (Table 3).

UTP concentration was consistently elevated in the growing renal cortex as compared with the

Fig. 4. Separation by high-pressure liquid chromatography of UTP in the acid-soluble fraction obtained from renal-cortical tissue

Continuous infusion with a total of 75.2 μ Ci of [5- 3 H]orotic acid was carried out in a unilaterally nephrectomized rat for 2 h before it was killed. Renal cortex (478 mg) was obtained 12 h after surgery. [2- 14 C]UTP (0.25 μ Ci) was added to the tissue sample before homogenization as internal standard. The cold-0.2 M-HClO₄-soluble fraction (78%) was neutralized, freeze-dried and redissolved in 0.5 ml of distilled water. A 15 μ l sample was used for analysis; UTP recovery was 89%, this sample containing 1.85 nmol of UTP. Gradient limits: concentration of high-ionic-strength buffer 10% initial, 90% final. Retention times of standards are indicated as obtained under identical conditions. These conditions were designed for UTP separation, and other peaks in the sample do not represent pure nucleotides. (a) UMP; (b) UDP; (c) CTP; (d) dTTP; (e) ATP; (f) GTP. —, E_{254} ; ····, E_{280} . ○, 14 C d.p.m.; ●, 3 H d.p.m.; ----, gradient.

Table 3. Changes in UTP and ribose phosphate concentrations in the cold-acid-soluble extract in the growing renal cortex at different intervals after unilateral nephrectomy

Fisher rats, starved for 24 h and re-fed for 12 h, were used. One group of animals was not operated on and four groups were unilaterally nephrectomized or sham-operated. A sample of renal cortex from the right remaining growing kidney or from the right kidney from control animals was obtained at different intervals after surgery. The UTP and ribose phosphate contents were measured in the cold-acid-soluble extract and expressed per unit of DNA measured in the same tissue sample. Values are presented as the means $\pm$ s.e.m., with numbers of observations indicated in parentheses. % changes are based on the value corresponding to the sham-operated control for the same group. Student's *t* test was used for statistical analysis.

Time after nephrectomy (h)	Renal cortex	UTP (nmol/mg of DNA)	% increase of control value	Ribose phosphate (nmol/mg of DNA)	% increase of control value
0	Control (6)	362 $\pm$ 10		8.25 $\pm$ 0.14	
6	Control (3)	425 $\pm$ 18		6.22 $\pm$ 0.24	
	Growing (5)	478 $\pm$ 35	12.5	6.47 $\pm$ 0.31	3.4
12	Control (7)	388 $\pm$ 15		8.33 $\pm$ 0.12	
	Growing (7)	400 $\pm$ 19	3.1	8.71 $\pm$ 0.12	4.6†
24	Control (11)	450 $\pm$ 17		7.14 $\pm$ 0.17	
	Growing (11)	546 $\pm$ 30	21.3†	7.92 $\pm$ 0.23	10.4†
48	Control (7)	444 $\pm$ 25		7.28 $\pm$ 0.23	
	Growing (7)	483 $\pm$ 14	8.8	8.38 $\pm$ 0.20	15.1*

* $P < 0.005$.

† $P < 0.025$.

‡ $P < 0.05$.

corresponding sham-operated control at all periods studied after surgery. The maximal increase, of 21 %, occurred at 24 h (Table 3). UTP concentration in the growing cortex decreased from 6 to 12 h followed by a 36 % rise from 12 to 24 h ($P < 0.001$) (Table 3).

Changes induced by unilateral nephrectomy in the concentration of acid-soluble ribose phosphate in the growing renal cortex

The total ribose phosphate/DNA ratio in the renal

cortex from sham-operated control animals was significantly lower ($P < 0.001$) 6h after surgery than that in non-operated controls. It increased from 6 to 12h after surgery, reaching a maximum value at a time when UTP concentration was at its lowest, as determined in the same acid-soluble extracts (Table 3).

The growing renal cortex showed changes in ribose phosphate/DNA ratio following a pattern similar to that seen in sham-operated controls from 12 to 24h after unilateral nephrectomy (Table 3). The increase in ribose phosphate preceded the increase in UTP in the growing renal cortex. Plasma ribose concentrations did not appreciably change in any of the groups of animals studied, ranging between 3.9 and 5.4 $\mu\text{g/ml}$.

Specific radioactivity of UTP and calculated rate of RNA synthesis at different labelling times in the growing renal cortex and that from sham-operated controls in vivo

Increasing the periods of continuous intravenous infusion of [^3H]orotic acid in sham-operated or unilaterally nephrectomized animals resulted in a linear increase in the radioactivity incorporated into renal-cortex UTP as the infusion was prolonged from 10 to 150min. This resulted in a linear increase in

UTP specific radioactivity in both growing ($r = 0.96$) and sham-operated ($r = 0.98$) groups (Fig. 5). The corresponding rate of RNA synthesis, as calculated from the incorporation at the end of each infusion period, declined rapidly during the first hour, remaining stable from 90 to 150min of infusion. A period of 120min of infusion was chosen for the remainder of the experiments.

The amount of radioactivity found under the CTP peak was less than twice the background counts.

Incorporation of [^3H]orotic acid into renal-cortex UTP and RNA in nephrectomized and sham-operated control rats in vivo

In sham-operated control animals 6h after surgery, less labelled precursor was incorporated into the acid-soluble fraction, UTP and RNA than in non-operated animals (Fig. 6).

The pattern of isotope incorporation into UTP and into the acid-soluble fraction (containing all the metabolic derivatives of orotic acid) at different intervals after surgery was similar in cortex from sham-operated control and nephrectomized animals (Figs. 6a and 6b). Incorporation into UTP, expressed as a fraction of the total amount incorporated into the acid-soluble fraction, varied from 14 to 18%, despite marked changes in the absolute amounts incorporated in different experiments. Incorporation into UTP after nephrectomy or sham-operation varied according to the interval after the operation, maximal values being found at 12h (Fig. 6b). The growing renal cortex incorporated 43% ($P < 0.05$) and 48% ($P < 0.001$) more than that of the sham-operated controls at 6 and 24h respectively, returning to control values at 48h.

The pattern and differences in labelling of RNA closely followed labelling of UTP, the incorporation into the growing renal cortex being greater than in the sham-operated controls at all periods studied up to 48h (Figs. 6b and 6c).

Changes in the rate of RNA synthesis in renal cortex after unilateral nephrectomy or sham-operation in vivo

Changes in the RNA-synthetic rate in renal cortex from nephrectomized or sham-operated rats did not correlate with the total amounts of labelled precursor incorporated into RNA. The RNA-synthetic rate in the renal cortex of sham-operated control animals did not significantly change during the first 24h after surgery, compared with that in non-operated controls (Fig. 7), despite a decrease in RNA per cell at 6h and a subsequent increase at 12h. The decrease in RNA concentration at 6h was evident in different strains of animals and was irrespective of the presence of an indwelling venous catheter (Figs. 1 and 7a). At 48h, there was a significant increase in RNA synthesis ($P < 0.001$), along with a small increase in RNA per cell.

Fig. 5. Changes in the specific radioactivity of UTP in the renal cortex and in the calculated rate of RNA synthesis after different periods of continuous infusion of [^3H]orotic acid

Unanaesthetized re-fed Fisher rats were infused intravenously at 10 $\mu\text{l/min}$ with a solution containing 67 μCi of [^3H]orotic acid/ml and 0.08mM-sodium orotate over different intervals of time before a sample was obtained from the right renal cortex. Animals underwent left nephrectomy or sham-operation 24h before the end of the experiment. The rate of RNA synthesis was calculated on the basis of half the specific radioactivity of UTP at the end of each infusion period. ●, Growing cortex, RNA synthesis; ○, control (sham-operated) cortex, RNA synthesis; ▲, growing cortex, d.p.m. incorporated into UTP/nmol of UTP; △, control (sham-operated) cortex, d.p.m. incorporated into UTP/nmol of UTP.

Fig. 6. Changes in incorporation of $[5\text{-}^3\text{H}]$ orotic acid into the cold-acid-soluble fraction, UTP and RNA of growing renal cortex at 0–48 h after unilateral nephrectomy

Unilaterally nephrectomized, sham-operated or non-operated re-fed Fisher rats were infused for 2 h with $[5\text{-}^3\text{H}]$ orotic acid. A tissue sample (348–585 mg) from the right renal cortex was obtained for study at 6, 12, 24 and 48 h after surgery in the operated animals and at 0 h in the non-operated group. The radioactivity incorporated into the cold-acid-soluble extract, UTP and RNA at the end of the infusion period is expressed as the % of the total d.p.m. infused/mg of DNA. Values for UTP were corrected according to $[2\text{-}^{14}\text{C}]$ UTP internal standard recovery for each individual sample. A total of 52 animals was used; each point represents the mean $\pm$ S.E.M. of three to seven observations. Δ , Cortex from non-operated controls; $\bullet$, growing renal cortex; $\circ$, cortex from sham-operated controls. (a) Acid-soluble fraction; (b) UTP; (c) RNA.

In the growing renal cortex, RNA synthesis at 6 h after unilateral nephrectomy was not different from that observed in non-operated or sham-operated controls. From 6 to 24 h there was a linear increase in the rate of RNA synthesis, reaching a maximal value of 73% higher than that in sham-operated controls. This change in synthesis rate was accompanied by an increase in the differences in RNA per cell between growing cortex and that from sham-operated controls,

Fig. 7. Changes in the rate of RNA synthesis and the RNA/DNA ratio in the growing renal cortex at 0–48 h after unilateral nephrectomy

Unilaterally nephrectomized, sham-operated or non-operated re-fed Fisher rats were infused for 2 h with $[5\text{-}^3\text{H}]$ orotic acid. A sample from the right renal cortex was obtained at different intervals after surgery and at 0 h in the non-operated group. The rate of RNA synthesis was calculated from the average specific radioactivity determined in the UTP pool and the radioactivity incorporated into RNA at the end of the infusion period. RNA/DNA ratio values were determined in the corresponding tissue samples. Each point represents the mean $\pm$ S.E.M. of three to seven observations. Δ , Cortex from non-operated controls; $\bullet$, growing renal cortex; $\circ$, cortex from sham-operated controls. (a) RNA/DNA ratio; (b) rate of RNA synthesis.

from 12% at 6 h to 16% at 24 h (Fig. 7). From 24 to 48 h, the rate of RNA synthesis remained at this value. During this period, there was a small additional increase in RNA concentration.

The increase in RNA per cell in the growing renal cortex compared with that in sham-operated controls 12 h after surgery was not always demonstrated, depending on the strain of animal used and the operative procedure (Figs. 1 and 7a).

Discussion

This study shows that, in the kidney undergoing compensatory growth, an increase in renal-cortex RNA per cell takes place at an earlier stage than has been previously reported for whole kidney (Halliburton & Thomson, 1967; Malt & Lemaitre, 1968; Janicki & Lingis, 1970). Since the early change in RNA concentration was evident only if growing tissue was compared with that from sham-operated controls, rather than with that from non-operated animals, it is obvious that the trauma of surgery produces significant changes in RNA metabolism. Shortly after sham-operation (6h), there is a decrease in RNA per cell, an increase in UTP per cell and a decrease in orotic acid incorporation into UTP and RNA *in vivo*. At this point, RNA synthesis remains unchanged. In addition, there is a marked fluctuation in the ability of the renal cortex to incorporate orotic acid into RNA *in vitro* (3–6h). At 48h after sham-operation, a small but significant increase in RNA is associated with an increase in the rate of RNA synthesis. These results suggest that, shortly after sham-operation, there is increased RNA breakdown which produces an increase in UTP. At the same time, there is a decrease in the utilization of orotic acid for the synthesis of RNA precursors. A reversal of these early changes takes place at 48h after sham-operation. In view of these observations, it is not justifiable to compare, in the same animal, results obtained in the growing kidney with those from the kidney removed at nephrectomy, as has been done in other studies.

Previous studies in the growing kidney *in vivo* and in cultured renal-cortical cells demonstrated that the amount of radioactivity incorporated into the rapidly labelled RNA species reached a plateau at 20–60min after labelling, and that their specific radioactivities at this time were equal in tissue from nephrectomized and sham-operated animals (Lee *et al.*, 1970; Hill, 1975). Longer labelling periods resulted in a progressive increase in incorporation into rRNA (Hill, 1975). The results on incorporation of labelled precursors into RNA obtained in this study reflect changes in rRNA labelling, since long labelling periods were chosen. If shorter periods of continuous infusion (10–30min) were selected, the calculated rates of RNA synthesis were three- to four-fold higher than when periods of 90–120min were used. This difference in the rate of RNA synthesis is probably due to the incorporation of radioactivity into the rapidly labelled RNA species with a rapid turnover, since the specific radioactivity of UTP increases linearly during infusion periods of 10–180min.

It is unlikely that changes in rRNA degradation contribute to the calculated rate of RNA synthesis, since the period of labelling used was a small fraction of the half-life of rRNA, as reported in kidneys from

nephrectomized and sham-operated animals (Malt & Lemaitre, 1968; Hill, 1975). The rate of RNA synthesis could have been overestimated in this study as the result of the conversion of labelled UTP into CTP with a consequent increase in RNA labelling from CTP. Although virtually no radioactivity was found in the CTP peak, as separated by chromatographic analysis of the samples, even small amounts of label could be important, because the CTP pool has such a rapid turnover (Kramer *et al.*, 1973). The validity of the calculation of the rate of RNA synthesis depends primarily on the lack of intracellular compartmentalization of UTP into different pools, each with a different specific radioactivity. Previous studies in HeLa cells have provided results in support of a single ribonucleotide pool (Wu & Soeiro, 1971; Soeiro & Ehrenfeld, 1973), whereas others, using lymphoma cells (Goody & Ellem, 1975) and Novikoff rat hepatoma cells (Plageman, 1971, 1972), have reached the opposite conclusion. The abnormal cells used in those studies differ from the renal cortex in that they are unable to utilize exogenous orotic acid as a precursor for RNA synthesis. It is therefore uncertain if the above findings apply to normal mammalian tissues *in vivo*.

A paradoxical decrease in incorporation of labelled orotic acid and uridine into RNA in the growing renal cortex studied *in vitro* has been demonstrated in this study during the time-interval 3–48h after nephrectomy, despite a progressive increase in RNA per cell during this period. Similar results have also been reported by Toback & Lowenstein (1974) for the period between 60 and 180min after nephrectomy. The decreased incorporation found in this study was obtained during an incubation period when incorporation into RNA was linear with time in both growing and control groups. These results would seem incompatible with the alterations expected to occur in growing tissue, such as an increase in the activity of the enzymes involved in the processing of RNA precursors or in the synthesis of RNA. Enzyme activity is probably increased, since the conversion of orotic acid into UMP in the regenerating liver has been reported to be greater than normal from 12 to 36h after partial hepatectomy (Fausto, 1969).

To investigate further the reasons for the decreased incorporation of labelled precursors into RNA in the growing cortex *in vitro*, an attempt was made to decrease possible differences in the size of the endogenous uridine nucleotide pools between growing and control tissues during their incubation. Under these circumstances, the differences in incorporation should disappear if endogenous dilution of the label incorporated into expanded RNA precursor pools were the cause of the paradoxical results. An increase in uridine concentration in the incubation media by the addition of large amounts of unlabelled uridine

enhances the rate of cellular uptake by diffusion down a concentration gradient and maximally expands the endogenous pool of uridine nucleotides (Plageman, 1972; Plageman & Richey, 1972). This experimental approach, used previously for the same purpose (Forsdyke, 1971; Toback & Lowenstein, 1974), is less direct than measurement of total UTP per cell, but it may reflect more accurately differences in the size of the effective pools of uridine 5'-phosphates involved in RNA synthesis, should intracellular compartmentalization exist. The increase in uridine concentration resulted in a significant decrease in the differences in incorporation between growing and control tissues. Although complete elimination of the differences was not obtained, the results suggest that expansion of the uridine nucleotide pools was an important determinant for the paradoxical results found *in vitro*. The early expansion of the RNA-precursor pools could be due to an increase in their synthesis, as has been described in the regenerating liver (Bresnick, 1965; Fausto, 1969). Additional dilution effects on the incorporated labelled precursor could also be produced by accelerated turnover of the rapidly labelled RNA species, as previously reported in the growing kidney (Willems *et al.*, 1969; Seifert & Vácha, 1974).

In contrast with other studies, where a humoral factor in the nephrectomized animal was demonstrated *in vitro* to stimulate precursor incorporation into RNA (Preuss *et al.*, 1970; Lyons *et al.*, 1974), we found that serum obtained from unilaterally nephrectomized animals consistently decreased the incorporation of labelled uridine into RNA of slices of growing renal cortex. Therefore the decreased incorporation demonstrated in the growing cortex *in vitro* is not due to the absence of a facilitating humoral factor in the incubation media. The presence of serum from nephrectomized animals had little effect on cortex from sham-operated controls or normal liver slices. This is consistent with the hypothesis that a humoral factor in nephrectomized animals would accentuate the changes in the size of endogenous pools in the growing cortex, supporting the theory of a humoral factor as the regulator of renal mass (Preuss & Goldin, 1976). These results, as well as those from other studies where serum was added to the incubation media, must be interpreted with caution, since the effects elicited could be due to non-specific changes in the serum from nephrectomized animals, such as in enzyme activity or substrate composition (Dohi *et al.*, 1959; Simpson, 1963; Royce, 1967; Toback *et al.*, 1973). The lack of effect of the serum on liver slices is not absolute proof for the presence of a factor specific for the kidney facilitating growth, since changes in substrate composition could variably influence different or identical tissues which have dissimilar substrate affinities.

To confirm the early increase in RNA-precursor

pools, UTP concentration per cell was quantified *in vivo* and compared with the values obtained in sham-operated controls. Contrary to previous observations in the growing kidney after unilateral nephrectomy (Hill *et al.*, 1974) and in the regenerating kidney after tubular damage (Oertel & Herken, 1974), an increase in UTP concentration was found in this study during the development of compensatory renal growth. A similar increase in UTP has also been described in the growing liver from 3 to 12 h after partial hepatectomy (Bucher & Swaffield, 1969). Orotic acid incorporation into UTP after its infusion *in vivo* increased in the growing cortex, compared with that in sham-operated controls, between 6 and 24 h after nephrectomy. Two factors probably contribute to this increased incorporation: an early increase in delivery of labelled precursor due to a greater blood flow in the remaining kidney and increased rate of synthesis (as suggested by the greater amount of UTP per cell).

The incorporation of orotic acid into RNA *in vivo* was increased in the growing renal cortex, compared with that in sham-operated controls, in contrast with the results obtained *in vitro*. Shortly after unilateral nephrectomy (6h), precursor incorporation was significantly increased, probably as a consequence of an increased delivery of label overcoming dilution effects, since the rate of RNA synthesis was unchanged at this time. From 12 to 48 h after nephrectomy the rate of RNA synthesis increased in the growing cortex. The greatest difference over the control values was found at 24 h, this coinciding with the peak value in UTP concentration. Although this study does not provide any information on the rate of RNA breakdown, it demonstrates that an increased rate of rRNA synthesis must be an important, if not the exclusive, factor in the RNA increase found in the kidney during the first 2 days of compensatory growth. It is uncertain as to what extent the increased synthesis of RNA depends on an increased supply of UTP, but both seem to change simultaneously as compensatory growth is initiated, and to be preceded by expansion of other precursor pools.

As reported by others (Ross *et al.*, 1975), this study demonstrates that both growing and normal renal cortex preferentially use exogenous orotic acid instead of uridine as a precursor for RNA. This preference was evident when high concentrations of uridine in the incubation media were only partially effective in decreasing orotic acid incorporation into RNA in renal-cortical slices.

The results reported here illustrate the complexity of the process that determines the amount of labelled precursor finally incorporated into RNA *in vivo* and *in vitro*, and the number of variables that should be taken into account in the study of RNA metabolism or its regulation by humoral factors during the development of compensatory renal growth.

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References

- Batson, H. C. (1956) in *An Introduction to Statistics in the Medical Sciences* (Batson, H. C., ed.), pp. 16–20, Chicago Medical Book Co., Chicago
- Bernanke, D. & Epstein, F. G. (1965) *Am. J. Physiol.* **208**, 541–545
- Bray, G. A. (1960) *Anal. Biochem.* **1**, 279–285
- Bray, G. A. (1970) in *The Current Status of Liquid Scintillation Counting* (Bransome, E. D., Jr., ed.), pp. 170–180, Grune and Stratton, New York
- Bresnick, E. (1965) *J. Biol. Chem.* **240**, 2550–2556
- Brown, P. R. (1970) *J. Chromatogr.* **52**, 257–272
- Brown, P. R. (1971) *Anal. Biochem.* **43**, 305–306
- Brown, P. R. & Micch, R. P. (1972) *Anal. Chem.* **44**, 1072–1073
- Bucher, N. L. R. & Swaffield, M. N. (1969) *Biochim. Biophys. Acta* **174**, 491–502
- Chen, R. F. (1967) *J. Biol. Chem.* **242**, 173–181
- Daugharty, T. M., Veki, I. F., Nicholas, D. P. & Brenner, B. M. (1973) *J. Clin. Invest.* **52**, 21–31
- Dohi, S. R., Terzian, J. F., Widman, A., Bretani, R., Fausto, N., Liberman, B. & Rabinovitch, M. (1959) *Am. J. Physiol.* **196**, 924–926
- Fausto, N. (1969) *Biochim. Biophys. Acta* **182**, 66–75
- Fields, T. & Brox, L. (1973) *Can. J. Biochem.* **51**, 954–957
- Forsdyke, D. R. (1971) *Biochem. J.* **125**, 721–732
- Giles, K. W. & Myers, A. (1965) *Nature (London)* **206**, 93
- Goody, H. E. & Ellem, K. A. O. (1975) *Biochim. Biophys. Acta* **383**, 30–38
- Grushka, E. (1975) *J. Chromatogr. Sci.* **13**, 25–37
- Halliburton, I. W. & Thomson, R. Y. (1965) *Cancer Res.* **25**, 1882–1887
- Halliburton, I. W. & Thomson, R. Y. (1966) *Biochem. J.* **99**, 44p
- Halliburton, I. W. & Thomson, R. Y. (1967) *Cancer Res.* **27**, 1632–1638
- Hartman, M. E. & Bonfilio, A. C. (1959) *Am. J. Physiol.* **196**, 1119–1121
- Hauschka, P. V. (1973) *Methods Cell Biol.* **7**, 401–418
- Hill, J. M. (1975) *J. Cell Biol.* **64**, 260–265
- Hill, J. M., Ab, G. & Malt, R. A. (1974) *Biochem. J.* **144**, 447–453
- Janicki, R. H. & Lingis, J. (1970) *Am. J. Physiol.* **219**, 1188–1191
- Johnson, H. A. & Vera Roman, J. M. (1966) *Am. J. Pathol.* **49**, 1–13
- Kessler, J. I., Demeny, M. & Sobotka, H. (1967) *J. Lipid Res.* **8**, 185–190
- Koide, T. & Rabinowitz, M. (1969) *Circ. Res.* **24**, 9–18
- Kramer, G., Wieggers, V. & Hiltz, H. (1973) *Biochem. Biophys. Res. Commun.* **55**, 273–281
- Kurnick, N. B. & Lindsay, P. A. (1968) *Lab. Invest.* **18**, 700–708
- Lee, J. B., Vance, V. K. & Cahill, G. F., Jr. (1962) *Am. J. Physiol.* **203**, 27–36
- Lee, M. J., Vaughan, M. H. & Abrams, R. (1970) *J. Biol. Chem.* **245**, 4525–4533
- Lewan, L. Petersen, I. & Yngner, T. (1975) *Hoppe-Seyler's Z. Physiol. Chem.* **356**, 425–429
- Liberti, J. P. & Kline, E. S. (1974) *Life Sci.* **15**, 1815–1826
- Lowenstein, L. M. & Stern, A. (1963) *Science* **142**, 1479–1480
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Lyons, H. J., Evan, A. P., McLaren, L. C. & Solomon, S. (1974) *Nephron* **13**, 198–211
- Malt, R. A. (1969) *N. Engl. J. Med.* **280**, 1446–1459
- Malt, R. A. & Lemaitre, D. A. (1968) *Am. J. Physiol.* **214**, 1041–1047
- Malt, R. A. & Stoddard, S. K. (1966) *Biochim. Biophys. Acta* **119**, 207–210
- McKay, E. (1964) *Clin. Chim. Acta* **10**, 320–329
- Munro, H. N. & Fleck, A. (1966) *Methods Biochem. Anal.* **14**, 113–176
- Novello, F., Gumaa, J. A. & McLean, P. (1969) *Biochem. J.* **111**, 713–725
- Oertel, J. & Herken, H. (1974) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **284**, 383–393
- Ogawa, K. & Sinclair, J. G. (1958) *Tex. Rep. Biol. Med.* **16**, 215–218
- Plageman, P. G. W. (1971) *J. Cell. Physiol.* **77**, 241–258
- Plageman, P. G. W. (1972) *J. Cell Biol.* **52**, 131–146
- Plageman, P. G. W. & Richey, D. P. (1974) *Biochim. Biophys. Acta* **344**, 263–305
- Potter, D., Sakai, T., Harrah, J. & Holliday, M. A. (1969) *Clin. Res.* **17**, 444
- Preuss, H. G. & Goldin, H. (1976) *J. Clin. Invest.* **57**, 94–101
- Preuss, H. G., Terryi, E. F. & Keller, A. I. (1970) *Nephron* **7**, 459–470
- Reiter, R. J. (1965) *Lab. Invest.* **14**, 1636–1643
- Rickwood, D. & Klempner, H. G. (1970) *Biochem. J.* **120**, 381–384
- Ross, J. S., Malamud, D., Caufield, J. B. & Malt, R. A. (1975) *Am. J. Physiol.* **229**, 952–954
- Rous, S. N. & Wakim, K. G. (1967) *J. Urol.* **98**, 30–35
- Royce, P. C. (1967) *Am. J. Physiol.* **212**, 924–930
- Seifert, J. & Vácha, J. (1974) *Arch. Biochem. Biophys.* **160**, 285–288
- Sendecki, W., Ombach, M. & Kwiatkowska-Patzer, B. (1972) *Bull. Acad. Pol. Sci.* **20**, 273–278
- Shmukler, H. W. (1970) *J. Chromatogr. Sci.* **8**, 581–583
- Simpson, D. P. (1963) *Am. J. Physiol.* **205**, 1049–1052
- Soeiro, R. & Ehrenfeld, E. (1973) *J. Mol. Biol.* **77**, 177–187
- Threlfall, G., Taylor, D. M. & Buck, A. T. (1967) *Am. J. Pathol.* **50**, 1–14
- Toback, F. G. & Lowenstein, L. M. (1974) *Growth* **38**, 17–34
- Toback, F. G., Mayers, A. M. & Lowenstein, L. M. (1973) *Am. J. Physiol.* **225**, 1247–1251
- Toback, F. G., Smith, P. D. & Lowenstein, L. M. (1974a) *J. Clin. Invest.* **54**, 91–97

- Toback, F. G., Smith, P. D. & Lowenstein, L. M. (1974b) *Am. J. Physiol.* **226**, 1474–1479
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1964) in *Manometric Techniques*, pp. 114–158, Burgess Publishing Co., Minneapolis
- Vácha, J. & Seifert, J. (1975) *Biochem. Pharmacol.* **24**, 401–405
- Vichi, F. L. & Earle, D. P. (1970) *Proc. Soc. Exp. Biol. Med.* **135**, 38–41
- Willems, M., Musilova, H. A. & Malt, R. A. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **62**, 1189–1194
- Williams, G. E. G. (1961) *Br. J. Exp. Pathol.* **42**, 386–396
- Wu, R. S. & Soeiro, R. (1971) *J. Mol. Biol.* **58**, 481–487