DEVELOPMENT OF THE METANEPHRIC KIDNEY

Protein and Nucleic Acid Synthesis

G. C. PRIESTLEY and R. A. MALT

From the Surgical Services, Massachusetts General Hospital and the Department of Surgery, Harvard Medical School, Boston, Massachusetts 02114

ABSTRACT

The metanephric kidney was studied in fetal and older mice beginning at 16 days after mating of the parents. Polyribosomes from fetal kidneys labeled in vitro with ^{14}C-labeled amino acids had 10–20 times more acid-precipitable radioactivity associated with them than polysomes from adult kidneys similarly labeled. Between 3 and 6 days after birth the rate incorporation of labeled amino acids by polyribosomes from neonatal kidneys declined sharply to only twice the value found for adult kidneys. There was no change in the shape of the polyribosome profile with increasing age, but before birth few, if any, ribosomes were bound to membranes compared with 20% 2 days after birth and between 20 and 30% in the adult. Total protein represented less than 10% of the wet weight in the fetal kidney but increased to 17% of the wet weight in the adult kidney. There was a steady decline in the concentration of RNA and DNA with respect to dry weight throughout kidney development. DNA concentration declined more rapidly than RNA concentration, so that the milligram to milligram ratio of RNA to DNA increased. In males the RNA/DNA ratio was stable at 1.3 at 40 days after birth; but in females the decline in DNA concentration was more protracted, and at 200 days after birth the RNA/DNA ratio was only 0.99. Thus, total nucleic acids show only gradual changes in concentration throughout development of the kidney, but a sharp change in the synthetic activity of the ribosomes and in their binding to membranes occurs in kidneys soon after birth.

The biochemistry of development has been studied most extensively in echinoderm and amphibian eggs, in which changes in nucleic acid and protein metabolism can be related to distinct phases of morphogenesis. The heterogeneity and the complex stimuli governing the growth of most mammalian organs make them less convenient for biochemical investigation. Nonetheless, the synthesis of ribosomes, ribosomal RNA (rRNA), and messenger RNA (mRNA) during compensatory growth can be analyzed in an organ as heterogeneous as the kidney (1–3).

In this paper we describe the patterns of protein and nucleic acid synthesis in the metanephros of the mouse during rapid normal growth from fetal to adult life. An intense synthesis of protein on free, i.e. not membrane-bound, polysomes during late fetal and early neonatal life ends abruptly between 3 and 6 days after birth; at the same time, membrane-bound ribosomes increase at the expense of free ribosomes. Thereafter, protein accumulates with little increase in DNA and with production of RNA proportionate only to maintaining a stable concentration of RNA in the organ. A preliminary statement of some of these findings has appeared (4).

MATERIALS AND METHODS

Kidneys

Mice used in this study were Ham/ICR albinos (Charles River Breeding Laboratories, North

Wilmington, Mass.). For experiments with fetal and juvenile kidneys, a single group of females were mated 42 days after their birth. The appearance of a vaginal plug in the mother was regarded as the first day of fetal age; birth occurred on the 19th day in most animals. Adult kidneys used as standards for polysome preparations were taken from males between 45 and 55 days old.

Adult mice were sacrificed by overstretching their necks. Individual fetuses were removed from the uteri immersed in ice-cold Hanks' solution (5), where they were quickly decapitated and dissected. Kidneys from mice younger than 11 days after birth were not purposely decapsulated, but capsules were often damaged in dissection. Kidneys of older mice were decapsulated by slitting the capsule at one end and squeezing gently between finger and thumb. The drained kidneys were weighed on a torsion balance to the nearest milligram.

On each day when fetal kidneys were examined, fetuses from a single pregnant female were used for a polysome preparation, those from a second female for nucleic acid, protein, and free amino acid determination, and those from a third female for measurement of dry weight. Newborn and older mice were selected at random from different litters, and the kidneys of comparable groups were used for the various determinations performed on each date.

Polysome Preparations

Profiles of free, i.e. not membrane-bound, polysomes were prepared from kidneys of fetuses 16, 17, 18, and 19 days after mating, of newborn mice 1, 2, 3, and 6 days after birth, and of older mice at less frequent intervals up to 200 days after birth. The kidneys were placed in a 25 ml flask containing 5 ml of Hanks' solution (pH 7.4) and 20 μc of solid protein hydrolysate-^{14}C (640 μc/mg; Nuclear-Chicago Corporation, Des Plaines, Ill. and Radiochemical Centre, Amersham, Eng.). Two decapsulated adult kidneys (approximately 500 mg) in identical flasks served as standards for each experiment. The flasks were sealed and agitated gently at 37°C for 15 min after which incorporation of isotope was halted by adding about 10 ml of ice-cold Hanks' solution. Further processing was carried out at 4°C. The kidneys were rinsed in Hanks' solution and then in homogenization buffer and were transferred to a Dounce homogenizer containing 1.75 ml of homogenization buffer (0.25 M sucrose (pH 7.6), 0.1 M Tris, 0.025 M KCl, 0.0015 M $MgCl_2$), they were allowed to chill for 1 min and then were disrupted with three strokes of the loose pestle and one stroke of the tight pestle. The homogenate was centrifuged at 850 *g* for 5 min; of the supernatant, 0.7 ml was layered onto a 16 ml linear gradient of 15–30% sucrose containing RSB (reticulocyte standard buffer: 0.01 M Tris, 0.01 M KCl, 0.0015 $MgCl_2$, pH 7.6 at 4°C) for centrifugation at 24,000 rpm for 210 min (Spinco SW 25.3 rotor, 4°C).

Fractions were removed at constant flow from the bottom of the tubes. Optical density (OD) was monitored continuously at 260 mμ in a Gilford (Gilford Instrument Company, Oberlin, Ohio) recording spectrophotometer. Fractions of approximately 1.0 ml were collected for evaluation of the incorporation of amino acid into protein by coprecipitation with carrier protein and 10% trichloroacetic acid after digestion in 1 N NaOH. Precipitates were retained on Millipore filters, dried, and counted in a Nuclear-Chicago Corporation gas flow counter (background 2 cpm). Yields of polysomes were estimated by measuring the area under the optical density profile on the recorder chart with a compensating planimeter and by adjusting the results to a value equivalent to 500 mg wet kidney. In the calculation of the proportion of ribosomes existing as polysomes (ratio P/P + M, where *P* represents polysomes and *M* represents monomeric ribosomes) (6), dimers were included with single ribosomes. The radioactivity incorporated by polysomes was assessed by dividing the corrected total cpm of the polysome fractions by their total area of OD on the recorder chart (cpm/cm^2 OD).

Membrane-Bound Ribosomes

In a separate series of experiments, the relative proportions of free and membrane-bound ribosomes and their respective capacities to incorporate radioactive amino acids in vitro were examined from kidneys of fetuses 17 days after mating of the parents, newborns 2, 5, and 15 days after birth, and older animals at intervals. In this series of experiments free ribosomes were operationally defined as those suspended in the gradients after zonal centrifugation of postmitochondrial supernatants not treated with detergent, and membrane-bound ribosomes were defined as those found in the pellet at the bottom of the ultracentrifuge tube. Later experiments (manuscript in preparation) have shown this assumption to be substantially correct.

Kidneys were collected, labeled in vitro, and homogenized as described above. The homogenate was centrifuged at 20,000 *g* for 5 min (Servall RC2-B centrifuge, 4°C). 1.5 ml of the supernatant were layered onto a 26 ml linear 15–30% sucrose-RSB gradient and centrifuged at 24,000 rpm for 150 min (Spinco SW 25.1 rotor, 4°). Fractions containing free polysomes were collected, pooled, and precipitated with 2 volumes of absolute ethanol at −20°C overnight. The pellet from the bottom of the sucrose gradient was drained, the upper part of the tube was cut off, and the lower part was wiped clean. The pellet and ethanol precipitate were each dissolved in 0.5% sodium dodecyl sulfate (SDS) buffer (0.005 M Tris,

0.1 M NaCl, pH 7.4 at 23°C). Aliquots of these solutions were precipitated and counted for radioactivity as described above. The remaining solutions were precipitated with cold ethanol, and the large-molecular-weight RNA was reprecipitated from solution in 2 M LiCl (7). Aliquots were dissolved in 0.1 M NaCl for measurement of OD at 260 mμ.

Chemical Determinations

Nucleic acid and protein contents were measured in a single tissue homogenate. Several kidneys were homogenized thoroughly in ice-cold 0.2 N perchloric acid (PCA), and the total volume of homogenate was adjusted to a standard volume. RNA was estimated by alkaline hydrolysis and ultraviolet spectrophotometry (32 μg RNA equivalent to 1.00 absorbance units) (8), and DNA by the method of Ceriotti (9), with a calf thymus standard (Calbiochem, Los Angeles, Calif.). Samples of the original PCA precipitate of the homogenate were frozen for determination of protein and free amino acids later. Aliquots were hydrolyzed in 0.3 N KOH for 1 hr, extracted with ether, and centrifuged at 12,000 *g* for 10 min. 4 ml of biuret reagent (Fisher Scientific Company, Pittsburgh, Pa.) were added to each 1.0 ml aliquot of supernatant, and the OD at 540 mμ was read 30 min later for comparison with a standard curve prepared with Labtrol standard protein solution (Dade Reagents Inc., Miami, Fla.).

Free amino acid concentrations in the thawed tissue homogenates were determined by using a method based on that of Moore and Stein (10). The methylcellulose–ninhydrin solution was diluted 1 : 1 with 2 N acetate buffer (pH 5.3) containing 0.16% $SnCl_2 \cdot 2 H_2O$, and 1 ml of the mixture was boiled with 1 ml of sample for 20 min. To the cooled reaction mixture were added 5 ml of absolute ethanol, and the OD of the solution at 570 mμ was compared with that of a standard 2 mM leucine solution processed in an identical manner.

Dry Weight Determinations

Groups of weighed kidneys were placed in a vacuum desiccator containing fresh Drierite and were dehydrated continuously for 48 hr by means of a vacuum pump. Thereafter, the tissue was maintained under vacuum and weighed at 2-day intervals until weights were stable.

Radioautography

Labeled kidneys were set aside during polysome preparations, washed in Hanks' solution, and fixed in 10% buffered formalin. Tissue sections 6 μ thick were prepared; some were stained in hematoxylin and eosin, or methyl-green pyronin; others were coated with Kodak NTB-3 liquid emulsion (11) and developed 2 or 3 wk later in D-19. The radioautographs were mounted unstained or were stained through the emulsion with Giemsa. Grain densities were counted on Giemsa-stained radioautographs with a × 100 objective and an eyepiece grid. Total counts from areas approximately 0.025 mm^2 in the center of each of four tissue sections were combined to give a mean value for each age. The mean background density was computed from counts of areas adjacent to the same tissue sections.

RESULTS

A prerequisite for investigations of protein synthesis by polyribosomes in the fetal kidney was the development of a technique for labeling the kidney with radioactive amino acids in vitro. The preliminary experiment described below demonstrates the reliability of the method and our choice of conditions for its use.

In Vitro Labeling

The mean polysome yield (cm^2 OD/500 mg wet kidney) from postnuclear supernatants of six control pairs of adult kidneys homogenized without prior incubation or decapsulation was set as an arbitrary 100%, with which to compare yields from a second group of incubated kidneys. The second group consisted of decapsulated, decapsulated-and-bisected, and untreated kidneys, all of which had been incubated, homogenized, and then centrifuged concurrently (Spinco SW 25.3 rotor). The results from duplicate determinations are shown in Table I; a repeat set of duplicate results is given in parentheses to show the reproducibility of the data. Standard errors are indicated.

The use of decapsulated, but not bisected, kidneys gave adequate labeling with little degradation of polysomes to single ribosomes, i.e. no decrease in P/P + M ratio (6), but yields of polysomes from all incubated preparations were low. Subsequent experiments showed that elimination of the bentonite (12) during incubation and homogenization increased the yields from decapsulated kidneys to about 85% of control values; therefore, bentonite was not used in the main series of experiments. The specific activities in Table I show that the effects of decapsulation or bisection are reproducible. The ratios of intact: decapsulated: decapsulated-and-bisected preparations are about the same in both experiments, but the generally higher activities obtained in the repeat experiment suggested that it would be prudent to include a standard preparation of adult

kidneys in each experiment with younger kidneys so that day-to-day variation in efficiency of labeling could be identified.

Kidney Weights

Wet and dry weights of mouse kidneys at different ages are shown in Fig. 1. The most rapid growth of the kidney began 3 days after birth and continued for a further 30–40 days, after which kidney weight increased more gradually. Dry weight increased most rapidly immediately after birth, and the rate of increase began to slow 2 days after birth. Dry weight represented about 25% of the adult wet weight.

Polysome Preparations

Specimen profiles of protein synthesis by free polyribosomes are shown in Fig. 2. There was no evidence of nonspecific aggregation of ribosomes at low temperature (13), and incubation with 1 μg ribonuclease (Worthington Biochemical Corporation, Freehold, N.J.) before layering transferred all the OD to the 80S peak. When differences in total OD due to the use of different weights of kidney were neglected, there was little gross change in the shape of the OD profile at any time. The high proportion of single ribosomes compared to polysomes in fetal and neonatal kidneys could be wholly or partly an artefact due

TABLE I

Effect of Incubation, Decapsulation, and Bisection of Kidneys on Yield of Polyribosomes and Incorporation of Amino Acids-C^{14} into Nascent Protein

Treatment	Polysome yield	Ratio	Mean cpm/cm² OD in polysome fractions
	%	*P/P+M*	
Controls	100 ± 12.2	0.72 ± 0.04	
Incubated intact	26 (45)	0.66	1.28 (1.60)
Decapsulated before incubation	38 (45)	0.75	2.44 (3.39)
Decapsulated and bisected before incubation	17 (29)	0.73	4.74 (5.11)

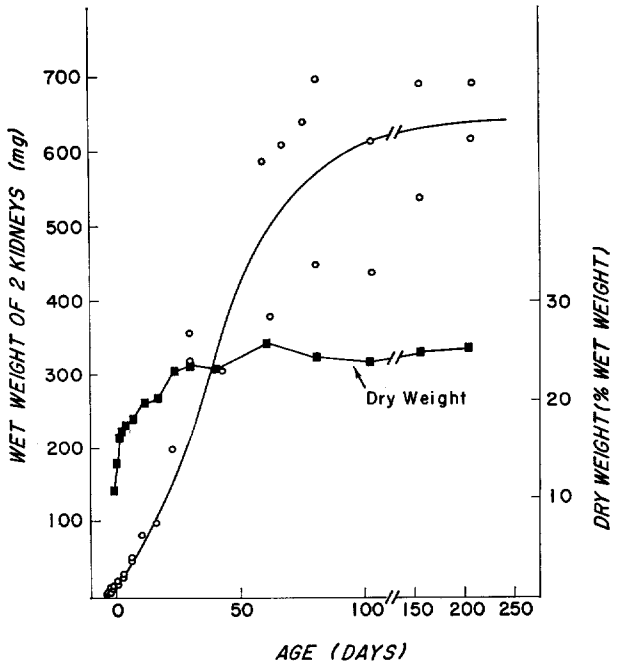

FIGURE 1 Increase in kidney mass with age. Kidneys from males and females were pooled up to 22 days of age; thereafter, they were weighed separately. The points above the wet weight curve are for males, those below for females. Data for dry weight showed no difference between sexes, and the values indicated are pooled means.

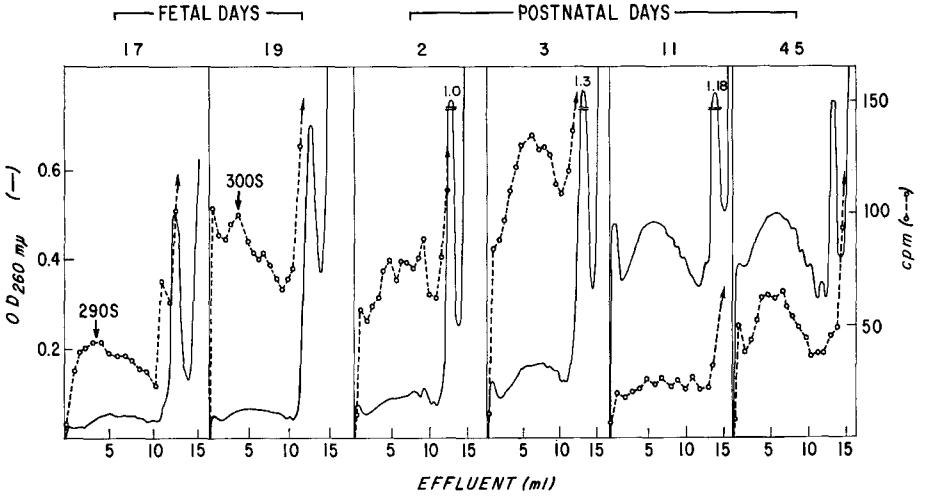

FIGURE 2 Polyribosome profiles from mouse kidneys of various ages that had been incubated in vitro with protein hydrolysate-^{14}C for 15 min. Direction of sedimentation is to the left. Differences in total OD are due to use of different concentrations of kidney homogenate. Sedimentation coefficients were calculated on the basis of a value of 80S for single ribosomes.

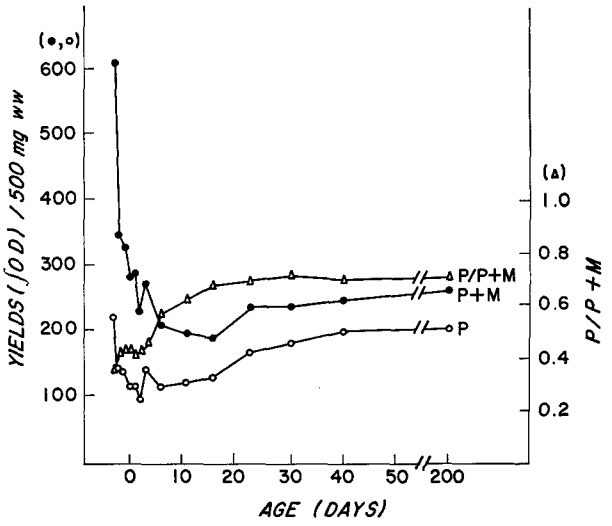

FIGURE 3 Yields of free polyribosomes (*P*) and free monomeric ribosomes (*M*) in analyses of mouse kidneys by density gradient centrifugation. *ww*, wet weight.

to degradation, but it probably is not because there is no concomitant increase in dimers and trimers. The plot of P/P + M (proportion of ribosomes sedimenting as polysomes; Fig. 3) showed a steady increase with age, which may be a consequence of the shorter time needed to harvest the older kidneys. The radioactivity curves for polysome profiles up to 2 days after birth showed a peak of highest activity associated with polysomes of medium size (10–15 ribosomes; ~ 270S–330S) that did not appear in the profiles from older mice, in which the maximum OD and radioactivity usually coincided. Incorporation of amino acids by polysomes at different ages, corrected for variation in the pool of free amino acids in each case, is shown in Fig. 4 *a*. Very high rates of incorporation occurred in fetal kidneys, with a maximum at full term and then a precipitous decline between the third and sixth days after birth. From 6 days after birth the rate decreased more gradually, and by 30 days it reached a low, stable level.

The over-all concentration of free amino acids

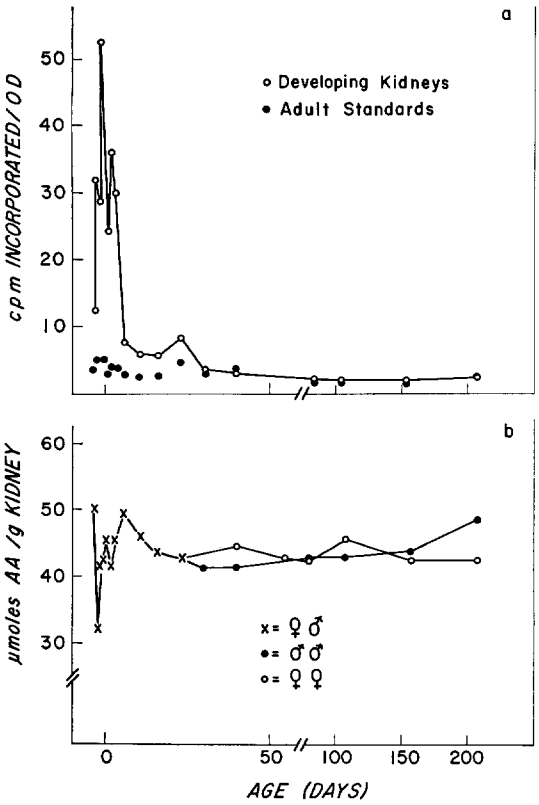

FIGURE 4 Incorporation of amino acids-^{14}C in vitro by free polyribosomes in mouse kidneys (*a*) corrected for variations in the pool of free amino acids (*b*). *AA*, amino acid.

in the kidney during its development (Fig. 4 *b*) was approximately stable. The values were used to adjust those for incorporation of radioactive amino acids given in Fig. 4 *a*.

The proportion of free ribosomes compared with bound ribosomes and the capacity of these classes of ribosomes to incorporate amino acids are shown in Fig. 5. Few bound ribosomes were found until 2 days after birth, when the proportion rose sharply to 20% of the total. After 2 days the proportion of bound ribosomes rose more gradually, reaching a stable level at about 30% of the total ribosomes in animals older than 50 days. In the older mice the females consistently had higher proportions of bound ribosomes than the males. The specific activities of the bound ribosomes were always somewhat greater than those of the free polysomes. Both curves in Fig. 5 show a sharp decrease soon after birth, apparently at a slightly earlier age than the decrease in rate of protein synthesis on free polysomes shown in Fig. 4.

Chemical Determinations

Concentrations of nucleic acids and of total protein in whole kidneys are plotted in Figs. 6 and 7, respectively, and as a percentage of dry weight in Fig. 8. The curves based on wet weight (Fig. 6, upper and center graphs) show nucleic acid concentrations increasing until 3 days after birth; but these peaks are not present in the curves based on dry weight (Fig. 8), which show nucleic acid concentration gradually decreasing with age. The values for RNA/DNA ratio (an index of RNA per diploid cell) are plotted in Fig. 6, lower graph. The ratio was lowest (0.6) during the 3 wk after birth, and then it increased. The increase was most rapid in male mice, which had a ratio of 1.0 at about 33 days and reached a plateau with ratios between 1.2 and 1.3 at 40 days. In the females, the decline in DNA concentration was more protracted than in the males, and the RNA/DNA ratio increased more slowly to only 0.9 at 260 days after birth.

Radioautography

In radioautographs of labeled kidneys the overall intensity of labeling decreased with age. Each radioautograph had a sharply defined marginal zone of high radioactivity (Fig. 9) corresponding to the outer cortex of the kidney, which surrounded an interior core. In this core, which corresponded to the inner cortex and medulla of the kidney, the label was less intense and uniformly distributed. There was no evidence of a decrease in the permeability of the tissue to isotope to explain the sudden decline in protein synthesis between 3 and 6 days after birth. In fact, counts of grain density (Table II) suggested that permeability increased after birth, the density over the center of each section being significantly above background level. This result suggested that the isotope had penetrated to the interior of even the largest (oldest) kidneys.

Summary of Results

2 or 3 days before birth the total protein content of the kidney is low (<8% of wet weight) but steadily increasing, while the fluid content is high (almost 90% of wet weight) but decreasing. Nearly all the ribosomes exist as free polysomes which are about 10–20 times more active in protein synthe-

sis than polysomes from adult kidneys; polysomes with sedimentation coefficients of ~ 300S appear to be the most active. Just before birth the fluid content of the kidney decreases sharply, and there is more of an increase in protein concentration than can be accounted for by the change in dry weight. The increased protein content probably results from the high rate of protein synthesis, which does not decline until several days after birth.

Concentration of nucleic acids expressed as proportion of dry weight decreases gradually and continually from the earliest age examined. By 5–6 days after birth the rate of protein synthesis slows to about twice the adult rate, although the kidney is then just entering its phase of most rapid increase in mass. About 10% of the wet weight and 50% of the dry weight are protein at this age; about one-fifth of the total ribosomes appears to be bound to membranes. From this age on, changes are more gradual. Total protein content continues to increase slowly and reaches a plateau at 16–17% of the wet weight (about 70% of dry weight) 45 days after birth. The rate of protein synthesis is then comparatively slow. Nucleic acid concentrations continue to fall and RNA/DNA ratios to increase, increasing more rapidly in males than in females. At 40 days after birth the RNA/DNA ratio is between 1.2 and 1.3 in males, but it is only 0.8 in females. All the variables, except the ratio of nucleic acid concentrations in the female, have reached equilibrium 40–50 days after birth, although some variables such as total protein continue to gain slightly. In the adult mouse little further change takes place, except that in the female the concentration of DNA falls slowly but never to the level seen in the male. There is no perceptible decrease in the rate of protein synthesis in the kidneys of the adult mice up to 200 days old.

Possible Sources of Error

Inevitable differences in individual mating and gestation times mean that the ages of mice used here are only correct to ±1 day; minor errors in wet weights may be caused by undrained Hanks' solution on the tissue. Estimations of the proportion of ribosomes bound to membranes are semi-quantitative. Because of preferential loss of membrane-bound ribosomes in preparing a postmitochondrial supernatant (14) and because of possible contamination of the pellets of bound ribosomes by the fastest sedimenting free polysomes, which might account for the 2–5% of the ribosomes designated "bound" in the fetal and neonatal kidneys (Fig. 5), our values do not describe the distribution in the original tissue homogenate. We cannot assess the discrepancy, but our estimate that 20–30% of the ribosomes are bound to membranes in the adult kidney has qualitative support both from electron micrographs of kidney cells (15, 16), which show the great majority of the ribosomes as free polysomes, and from further experiments now being prepared for publication.

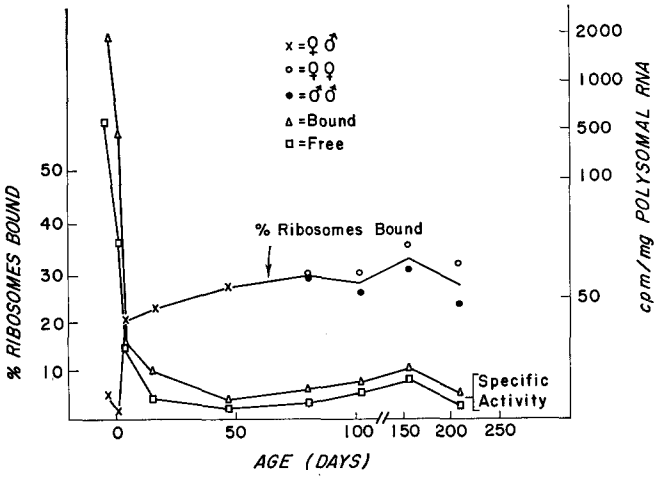

FIGURE 5 Incorporation of amino acids-^{14}C in vitro by free and membrane-bound ribosomes in mouse kidneys and proportion of the total ribosomes attached to membranes. RNA and radioactivity in the pellet at the bottom of the sucrose gradient were ascribed to bound ribosomes and compared with RNA and radioactivity in the pooled free polysome fractions collected from each gradient.

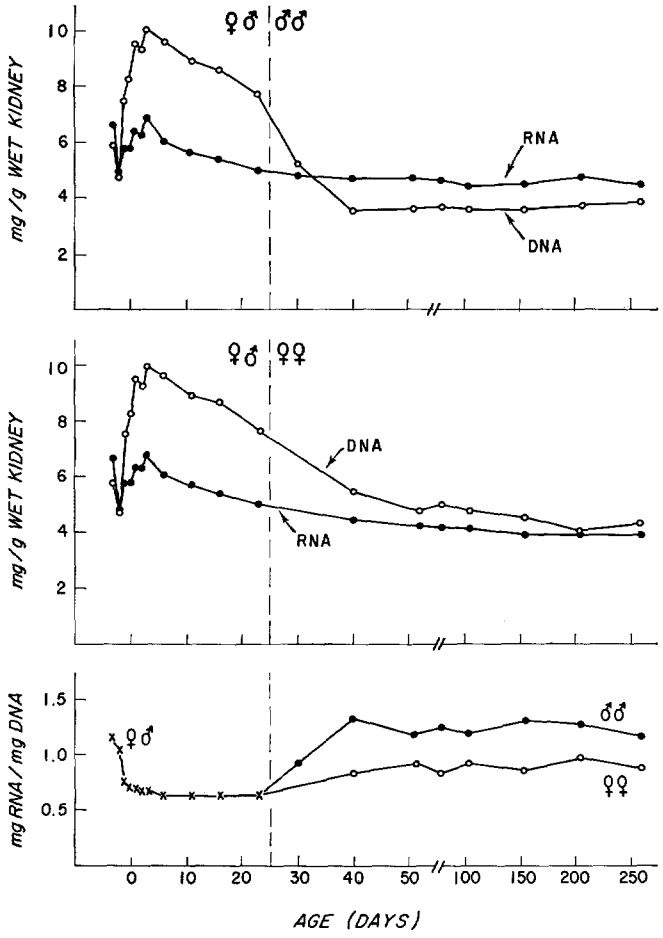

FIGURE 6 Concentration of nucleic acids in mouse kidneys at different ages. After 22 days of age, kidneys from males and females were analyzed separately; males are shown in the upper graph, females in the center graph, and the RNA/DNA ratios in the lower graph.

DISCUSSION

Weight Changes

A high water content is a characteristic of the mammalian embryo (17). In the developing mouse kidney the fluid content decreases progressively; in showing this decrease, our data for dry weight support those of Smith and Kissane (18) on rat kidney. The fluid content fell most abruptly in the newborn, presumably because of excretion of urine by the kidneys and of water by the lungs; and this change in the proportion of dry weight is reflected in curves for other variables based on wet weight. The apparent increases in nucleic acid concentrations up to 3 days after birth (Fig. 6) are a consequence of the dehydration; they are eliminated when the concentrations are expressed on a dry weight basis (Fig. 8).

Polyribosomes and Membrane-Bound Ribosomes During Development

The over-all profile of free polysomes remains unchanged throughout the major part of renal development, despite the presumably diverse and changing pattern of protein synthesis in this heterogeneous organ. Perhaps changes in the size of the polysomes in one cell type are nullified by reciprocal changes in the polysome complement of other cell types, or perhaps many different proteins are synthesized by polysomes of identical size. At present, we cannot identify the proteins synthesized during renal development. Little is known of the structural proteins present, and histochemical evidence of enzyme synthesis is still fragmentary; so far, only alkaline phosphatase (19), lactic dehydrogenase (18), and leucine aminopeptidase

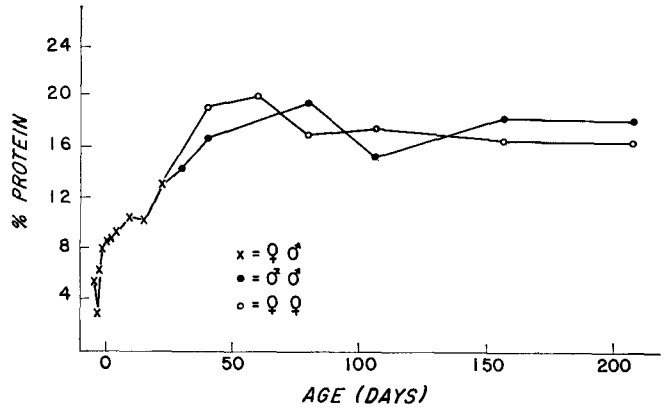

FIGURE 7 Concentration of total protein in mouse kidneys at different ages expressed as per cent of wet weight.

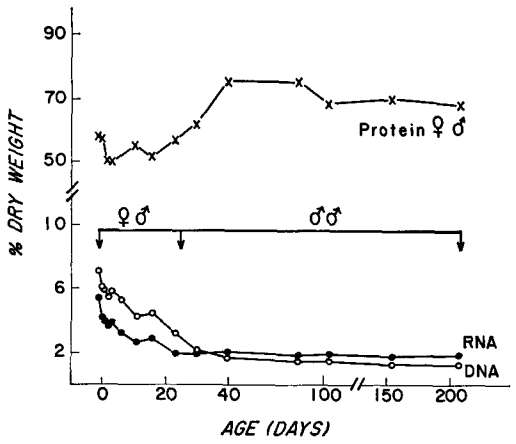

FIGURE 8 Total protein and nucleic acid concentration in mouse kidneys at different ages expressed as per cent of dry weight. Nucleic acid values are from pooled male and female kidneys up to 22 days of age; thereafter only male kidneys are illustrated.

(20) are definitely known to be active in the fetal metanephros.

These experiments indicate an important change in polysome activity soon after birth when 20–30% of the ribosomes, previously free, become bound to membranes. Clark's ultrastructural studies of mouse kidney (21) revealed that membranes appear in quantity at this time; probably the ribosomes attach to them immediately.

In liver (22) and pancreas (23) some, perhaps all, membrane-bound ribosomes synthesize protein for export, but it is not clear what proteins are exported from the kidney cell, except perhaps erythropoietin and renin. As it is unlikely that these two enzymes account for 20–30% of the total protein synthesis, either a substantial synthesis of other proteins for export occurs, or the bound ribosomes must make some protein for intracellular use. Certainly, until the role of membrane-bound ribosomes in general is better defined, it would be unwise to conclude from the data in Fig. 5 that after birth the kidney suddenly begins to make protein for export.

The finding that bound ribosomes incorporated more amino acids in vitro than the free ribosomes agrees with the results of two studies of rat liver (24, 25) but disagrees with other evidence from rat liver (26) and pigeon pancreas (27) which shows equal incorporation by the two classes of ribosomes. Incorporation of amino acid into ribosomal protein is not distinguished from incorporation into nascent protein in most of these experiments.

Protein Synthesis

Protein synthesis was 20 times faster in the fetal kidney than in the adult kidney (Fig. 4 *a*); Duck-Chong et al. (28) found a difference of a similar magnitude between fetal and adult liver in the chick. The apparent 100-fold difference between fetal and adult kidney shown in Fig. 5 is less certain because of possible contamination of these preparations with other radioactive cell constituents.

We have shown that the abrupt fall in incorporation of amino acids in vitro 3–6 days after birth is not a consequence of an increase in the pool of unlabeled amino acids. A second explanation for the phenomenon could be that 3 days after birth the kidney suddenly becomes much less permeable to amino acids, and, although this hypothetical change was not evident in radioautographs of

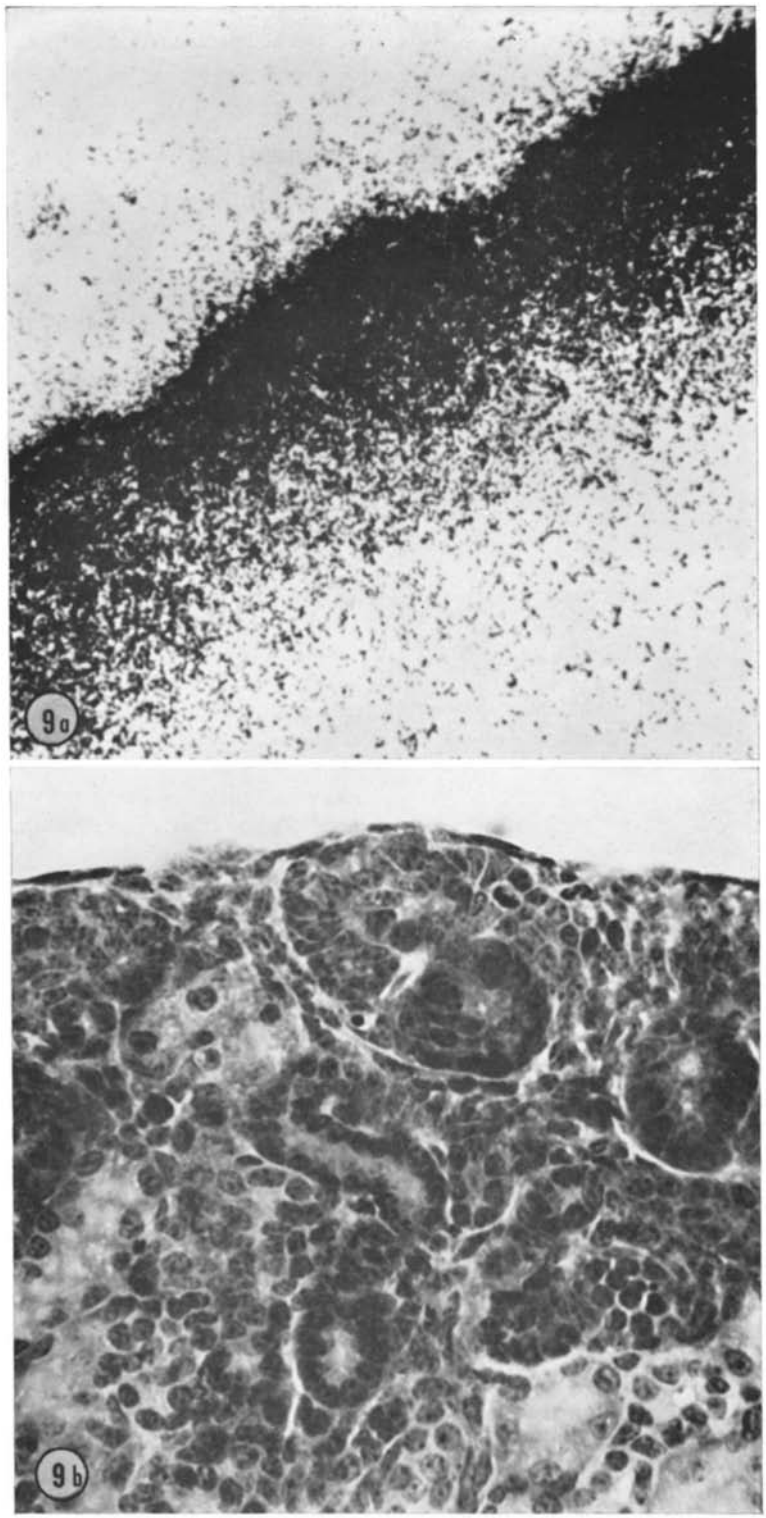

FIGURE 9 Uptake of amino acids-^{14}C by mouse kidney in vitro 3 days after birth. *a*, unstained radioautograph; *b*, histologic section of same kidney stained with hematoxylin and eosin. × 250.

the neonatal kidneys, it cannot be ruled out. On the one hand, changes in permeability would probably be gradual rather than abrupt, and on the other, active transport of amino acids by the tubules of the adult kidney (29) would probably offset any decrease in diffusion of amino acids into kidneys of older animals. Experiments to test these points are in progress.

Although the rate of protein synthesis decreases sharply so soon after birth, the highest rate of increase in kidney mass occurs later (Fig. 1: wet weight). A possible explanation for the apparent paradox is that proteins made in the fetal kidney are rapidly catabolized but that after birth catabolism slows and the organ can maintain its optimum rate of growth by synthesizing protein at a rate only twice the adult rate. A decrease in protein synthesis soon after birth is not peculiar to the kidney; a similar decrease has been found in liver (30) and brain (31). In brain, experiments with cesarean-delivered pigs (31) suggest that the decrease is related to birth itself rather than to chronological age. In mouse kidney the decrease may not occur until 3–6 days after birth (Fig. 4), or it may follow birth more closely (Fig. 5); the uncertainty could be resolved if kidneys could be obtained from precisely timed and synchronized births. The fact that a pattern of protein synthesis similar to that in Fig. 4 was obtained with brain in vivo (31) suggests that our results also apply to the kidney in vivo. The obvious parallel between the gradually decreasing concentration of RNA in the kidney and the decreasing rate of protein synthesis throughout kidney development implies that total concentration of RNA is a key factor in controlling the rate of renal protein synthesis; but the sudden decrease in protein synthesis after birth has no parallel in the curves for RNA concentration and may result from the uncertain temperature regulation (32) and less regular food supply (33) of the newborn mouse compared to the fetus. Some of the biochemical changes described here may be concerned with the maturation of renal function, but the nature of the relation will not emerge until data are available on the postnatal age at which the mouse kidney begins to concentrate urine.

TABLE II

Comparison of Grain Density in Radioautographs of Kidneys Labeled in Vitro

Age	Kidney	Background
2 days before birth	370 ± 55.0	141 ± 7.8
3 days after birth	1196 ± 81.1	265 ± 6.9
40 days after birth	1311 ± 173.0	257 ± 28.7

Fig. 6 presents a comprehensive record of nucleic acid concentrations in the kidney. The tendency for nucleic acid concentrations to decline during development appears to apply generally; Baserga et al. (34) and Oliver et al. (35) have noted the same tendency in other tissues. In general, measurements of nucleic acids in tissue have been handicapped, until recently, by inadequacies in the techniques available (8), but differences in strains, sex, and age in the animals used must also have contributed to the disparities between published values for each tissue (36).

Fig. 6 also shows that age and sex have a considerable influence on the RNA content of the mouse kidney; the results give no support to the suggestion that the RNA/DNA ratios are constant for each organ throughout development (37). The sharp decrease in the curves for DNA per wet weight in males 23 days after birth agrees with Zumoff and Pachter's finding (38) that cell multiplication pauses at the onset of puberty. Puberty in Charles River mice has not been dated accurately to our knowledge, but 20–30 days seems to be an acceptable minimum age for the onset of puberty in albino strains (39). Differences between nucleic acid concentrations of males and those of females have been noted by several other authors particularly Kochakian et al. (40, 41), who showed that androgens stimulate both protein and nucleic acid syntheses in mouse kidney; they reported nucleic acid concentrations for normal adult males which agree well with our values.

We thank Miss Mary L. Pruyn for her excellent help.

This work was supported by an Established Investigatorship of the American Heart Association (R.A.M.) and by grants from the Shriners Burns Institute, the American Heart Association, and the National Institutes of Health (HD-01988).

Received for publication 27 November 1967, and in revised form 12 February 1968.

REFERENCES

1. MALT, R. A., and W. L. MILLER. 1967. Sequential changes in classes of RNA during compensatory growth of the kidney. *J. Exptl. Med.* **126**:1.
2. MALT, R. A., and D. A. LEMAITRE. 1967. Differential labeling of 28-S RNA in free and membrane-bound ribosomes of kidney. *Biochim. Biophys. Acta.* **145**:190.
3. HALLIBURTON, I. W., and R. Y. THOMSON. 1965. Chemical aspects of compensatory renal hypertrophy. *Cancer Res.* **25**:1882.
4. PRIESTLEY, G. C., M. L. PRUYN, and R. A. MALT. 1967. Nucleic acid and protein synthesis in fetal and growing mouse kidneys. *J. Cell Biol.* **35**:107A. (Abstr.)
5. HANKS, J. H., and R. E. WALLACE. 1949. Relation of oxygen and temperature in the preservation of tissues by refrigeration. *Proc. Soc. Exptl. Biol. Med.* **71**:196.
6. LECOCQ, R. E., and J. E. DUMONT. 1966. Polysomes from thyroids incubated in vitro. *Biochim. Biophys. Acta.* **129**:421.
7. BARLOW, J. J., A. P. MATHIAS, R. WILLIAMSON, and D. B. GAMMACK. 1963. A simple method for the quantitative isolation of undegraded high molecular weight ribonucleic acid. *Biochem. Biophys. Res. Commun.* **13**:61.
8. MUNRO, H. N., and A. FLECK. 1966. Recent developments in the measurement of nucleic acids in biological materials. *Analyst.* **91**:78.
9. CERIOTTI, G. 1952. A microchemical determination of desoxyribonucleic acid. *J. Biol. Chem.* **198**:297.
10. MOORE, S., and W. H. STEIN. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem.* **176**:367.
11. PRESCOTT, D. M. 1964. Autoradiography with liquid emulsion. *In* Methods in Cell Physiology. D. M. Prescott, editor. Academic Press Inc., New York. 365.
12. FRAENKEL-CONRAT, H., B. SINGER, and A. TSUGITA. 1961. Purification of viral RNA by means of bentonite. *Virology.* **14**:54.
13. BYERS, B. 1967. Structure and formation of ribosome crystals in hypothermic chick embryo cells. *J. Mol. Biol.* **26**:155.
14. BLOBEL, G., and V. R. POTTER. 1967. Studies on free and membrane-bound ribosomes in rat liver. I. Distribution as related to total cellular RNA. *J. Mol. Biol.* **26**:279.
15. LEAK, L. V., and V. R. ROSEN. 1966. Early ultrastructural alterations in proximal tubular cells after unilateral nephrectomy and x-irradiation. *J. Ultrastruct. Res.* **15**:326.
16. DALTON, A. J., and F. HAGUENAU, editors. 1967. The Ultrastructure of the Kidney. Academic Press Inc., New York.
17. WIDDOWSON, E. M. 1964. Changes in the composition of the body at birth and their bearing on function and food requirements. *In* The Adaptation of the Newborn Infant to Extra-Uterine Life. J. H. P. Jonxis, H. K. A. Visser, and J. A. Troelstra, editors. Charles C Thomas, Publisher, Springfield, Ill. 1.
18. SMITH, C. H., and J. M. KISSANE. 1963. Distribution of forms of lactic dehydrogenase within the developing rat kidney. *Develop. Biol.* **8**:151.
19. VETTER, M. R., and C. W. GIBLEY. 1966. Morphogenesis and histochemistry of the developing mouse kidney. *J. Morphol.* **120**:135.
20. HOPSU, V. K., S. RUPONEN, and S. TALANTI. 1961. Leucine aminopeptidase in the foetal kidney of the rat. *Experientia.* **17**:271.
21. CLARK, S. L. 1957. Cellular differentiation in the kidneys of newborn mice studied with the electron microscope. *J. Biophys. Biochem. Cytol.* **3**:349.
22. CAMPBELL, P. N., and J. R. SARGENT. 1964. Morphological structure and protein synthetic activity in rat liver. *Biochem. J.* **91**:18P.
23. CARO, L. G., and G. E. PALADE. 1964. Protein synthesis, storage, and discharge in the pancreatic exocrine cell. An autoradiographic study. *J. Cell Biol.* **20**:473.
24. HALLINAN, T., and H. N. MUNRO. 1965. Protein synthesis and RNA turnover in rat liver microsome subfractions. *Biochim. Biophys. Acta.* **108**:285.
25. HENSHAW, E. C., T. B. BOJARSKI, and H. H. HIATT. 1963. Protein synthesis by free and bound rat liver ribosomes in vivo and in vitro. *J. Mol. Biol.* **7**:122.
26. BLOEMENDAL, H., W. S. BONT, M. DEVRIES, and E. L. BENEDETTI. 1967. Isolation and properties of polyribosomes and fragments of the endoplasmic reticulum from rat liver. *Biochem. J.* **103**:177.
27. SZÉKELY, M., L. BENEY, Ö. GAÁL, and S. VINCZE. 1966. Electrophoretic studies on proteins and RNAs of free and membrane-bound ribosomes. *Biochim. Biophys. Acta.* **123**:574.
28. DUCK-CHONG, C., J. K. POLLACK, and R. J. NORTH. 1964. The relation between the intracellular RNA distribution and amino acid incorporation in the liver of the developing chick embryo. *J. Cell Biol.* **20**:25.
29. ROSENBERG, L. E., A. BLAIR, and S. SEGAL. 1961. Transport of amino acids by slices of rat kidney cortex. *Biochim. Biophys. Acta.* **54**:479.
30. FRIEDBERG, F., M. P. SCHULMAN, and D. M.

GREENBERG. 1948. The effect of growth on the incorporation of glycine labeled with radioactive carbon into the protein of liver homogenates. *J. Biol. Chem.* **173:** 437.

31. SCHAIN, R. J., M. J. CARVER, J. H. COPENHAVER, and N. R. UNDERDAHL. 1967. Protein metabolism in the developing brain: influence of birth and gestation age. *Science.* **156:**984.
32. FITZGERALD, L. R. 1953. The oxygen consumption of neonatal mice. *J. Exptl. Zool.* **124:**415.
33. WIDDOWSON, E. 1961. Metabolic effects of fasting and food. *In* Ciba Foundation Symposium on Somatic Stability in the Newly Born. G. E. W. Wolstenholme and M. O'Connor, editors. Little, Brown and Company, Boston. 39.
34. BASERGA, R., R. O. PETERSEN, and R. D. ESTENSEN. 1966. RNA synthesis in liver and heart of growing and adult mice. *Biochim. Biophys. Acta.* **129:**259.
35. OLIVER, I. T., F. J. BALLARD, J. SHIELD, and P. J. BENTLEY. 1962. Liver growth in the early postpartum rat. *Develop. Biol.* **4:**108.
36. LESLIE, I. 1955. The nucleic acid content of tissues and cells. *In* The Nucleic Acids. E. Chargaff and J. N. Davidson, editors. Academic Press Inc., New York. **2:**1.
37. WINICK, M., and A. NOBLE. 1965. Quantitative changes in DNA, RNA and protein during prenatal and postnatal growth in the rat. *Develop. Biol.* **12:**451.
38. ZUMOFF, B., and M. R. PACHTER. 1964. Studies of rat kidney and liver growth using total nuclear count. *Am. J. Anat.* **114:** 479.
39. BRONSON, F. H., C. P. DAGG, and G. D SNELL. 1966. Reproduction. *In* Biology of the Laboratory Mouse. E. L. Green, editor. McGraw-Hill Book Company, New York. 187.
40. KOCHAKIAN, C. D., J. HILL, and S. AONUMA. 1963. Regulation of protein biosynthesis in mouse kidney by androgens. *Endocrinology.* **72:**354.
41. KOCHAKIAN, C. D., and D. G. HARRISON. 1962. Regulation of nucleic acid synthesis by androgens. *Endocrinology.* **70:**99.