Histological Changes in Normal and Drug-Induced Development of Renal PAH Transport[1] (35525)

G. H. HIRSCH,[2] D. F. COWAN, AND J. B. HOOK

Departments of Pharmacology and Pathology, Michigan State University, East Lansing, Michigan 48823

One of the most thoroughly studied functions of the immature kidney is the ability of renal cortical slices to actively accumulate the organic anion para-aminohippuric acid (PAH). New *et al.* (1) observed that renal cortical slices from newborn rabbits accumulated significantly less PAH than did slices from adults. Rennick *et al.* (2) observed similar age differences between newborn dogs and pigs and their adult counterparts. These authors extended their work to demonstrate that slices from the outer cortex of the newborn accumulated less PAH than did slices from the deeper cortex and this was correlated with a greater degree of histological maturation of the deeper cortex (2).

Recent reports from this laboratory demonstrated that treatment of young rats and rabbits with certain substrates of the organic anion transport system (penicillin, triiodothyronine, PAH) resulted in increased accumulation of PAH by renal cortical slices from these animals (3–5). Although this stimulation of PAH accumulation appeared specific for the organic anion transport system (base transport was not affected), drug treatment was often accompanied by an increase in kidney weight (5, 6). Thus, at least part of the effect could have been secondary to nonspecific stimulation of renal growth or maturation caused by the treatment.

It was, therefore, of interest to examine the relationship between renal histological development and the ability of renal cortical slices to accumulate PAH. This was approached in 2 ways: (i) by measuring PAH uptake by renal cortical slices from newborn of 3 species with histologically immature kidneys (rabbits, dogs, and cats) and comparing this with uptake by newborn guinea pig kidneys, which are histologically mature at birth (7); and (ii) by determining the effect of penicillin treatment of young rabbits on PAH accumulation and histological development of the renal cortex.

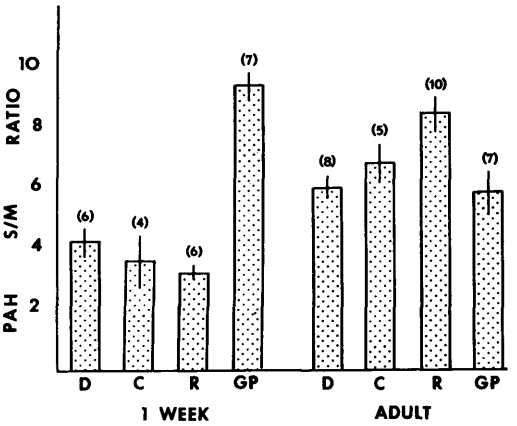

FIG. 1. Accumulation of PAH by renal cortical slices (*S/M*) from 1-week-old and adult dogs (D), cats (C), rabbits (R), and guinea pigs (GP): Each bar represents the mean (± SE) of triplicate determinations from the number of animals given in parentheses. The adult PAH *S/M* ratios for D, C, and R are significantly greater than the respective values at 1 week, while the reverse is true for GP ($p<.05$), group comparison.

Methods. New Zealand white rabbits, mongrel cats and dogs, and guinea pigs were used in these studies. Adult cats and dogs were anesthetized with pentobarbital. All other animals were killed by cervical dislocation. The kidneys were removed immediately and placed in cold saline. Renal cortical slices were prepared freehand and incubated in 2.7 ml of the phosphate buffer medium described

[1] Supported in part by U.S. Public Health Service Grant AM 10913.

[2] Supported in part by a Predoctoral Training Grant, Food and Drug Directorate, Canada.

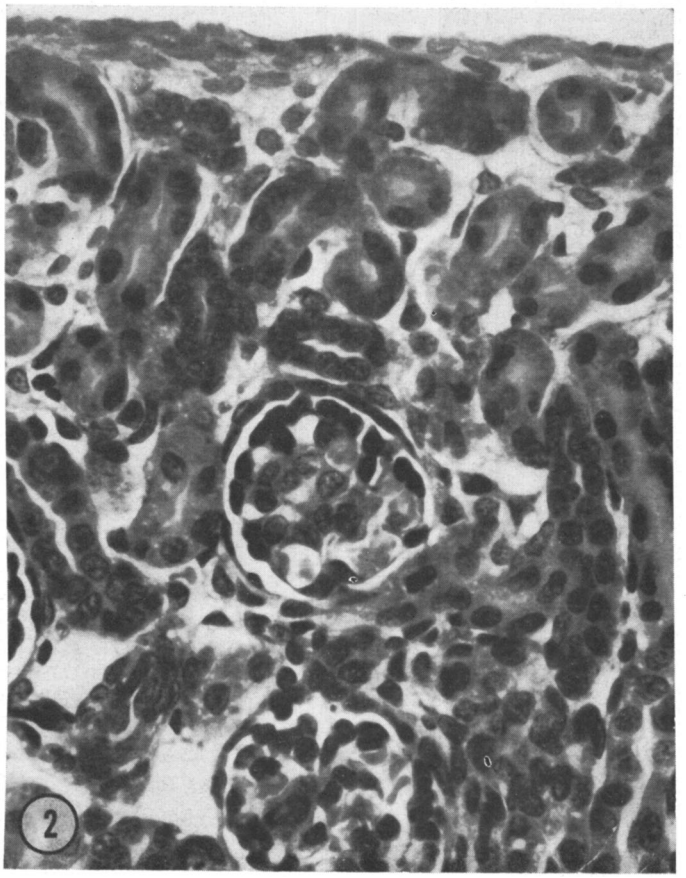

FIG. 2. Kidney from typical 2-week-old saline-control rabbit, ×640: hematoxylin–eosin stain. Glomerulus is small and densely cellular with prominent peripheral nuclei. Cortical tubules are small with crowded nuclei, limited amounts of cytoplasm, and inconspicuous tubular lumens. Measured PAH *S/M* ratio, 6.62.

by Cross and Taggart (8), which contained 7.4×10^{-5} *M* PAH. All incubations were carried out in a Dubnoff apparatus at 25° under a gas phase of 100% oxygen for 90 min. At the end of the incubation period the slices were quickly blotted and weighed. Both the tissue and a 2-ml aliquot of medium were treated as outlined by Cross and Taggart (8) and Hook and Munro (9). PAH was estimated by the method of Smith *et al.* (10). Guinea pig renal tissue acetylates PAH (8), so all analyses of guinea pig samples were done after hydrolysis in 0.2 *N* HCl to afford 100% recovery of PAH. Results are expressed as the slice/medium (*S/M*) ratio, where *S* equals milligrams of PAH per gram of tissue and *M* equals milligrams of PAH per milliliter of medium.

Procaine penicillin G (Eli Lilly and Company, Indianapolis, Ind.) was administered subcutaneously to 11-day-old rabbits in a dose of 60,000 international units (IU) twice daily for 3 days. Control littermates received saline, and all rabbits were killed 24 hr after the last injection. One-half of each kidney was used for histological study, while PAH accumulation was determined in cortical slices prepared from the remaining kidney tissue. Kidneys were fixed in Zenker's solution and placed in coded vials containing 70% ethanol. After imbedding the kidneys in paraffin, sections were cut at 4 μ, stained with hematoxylin–eosin, and photographed at 640× magnification. Histological sections of kidneys from untreated 4-week-old and adult rabbits were similarly prepared. Identifica-

TABLE I. Effect of Penicillin on PAH Accumulation by Rabbit Renal Cortical Slices.[a]

Saline control			Penicillin treated		
Rabbit	PAH *S/M*	Kidney wt (g)	Rabbit	PAH *S/M*	Kidney wt (g)
A	7.13[b]	3.280	D	12.98	2.922
B	6.62	2.736	E	12.62	3.884
C	7.09	3.582	F	15.03	3.050
Mean	6.90	3.199		13.54[c]	3.285
±SE	0.15	0.247		0.75	0.301

[a] Eleven-day-old rabbits were treated subcutaneously twice daily for 3 days with procaine penicillin G. Control littermates received saline; and all animals were killed 24 hr after the last injection.

[b] Values represent the means of duplicate determinations from individual animals from a typical litter.

[c] Significantly different from control ($p < .05$).

tion of slides was made on a single-blind basis. Data were analyzed statistically by Student's *t* test (8). The .05 level of probability was used as the criterion of significance.

Results. Accumulation of PAH by renal

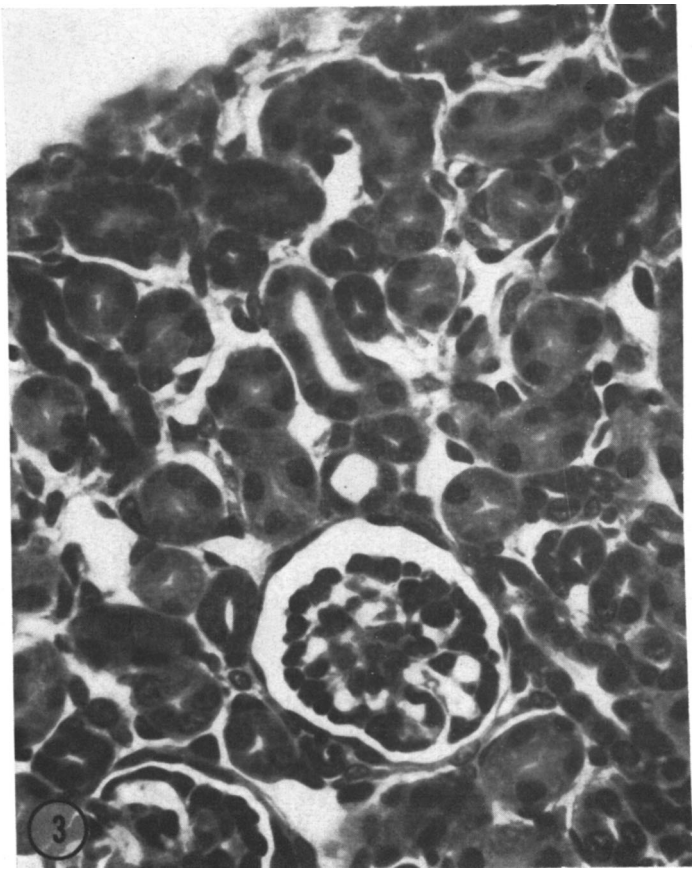

FIG. 3. Kidney from typical 2-week-old penicillin-treated rabbit, ×640: hematoxylin–eosin stain. Tubules and glomeruli are indistinguishable from those in Fig. 2. Measured PAH *S/M* ratio, 12.98.

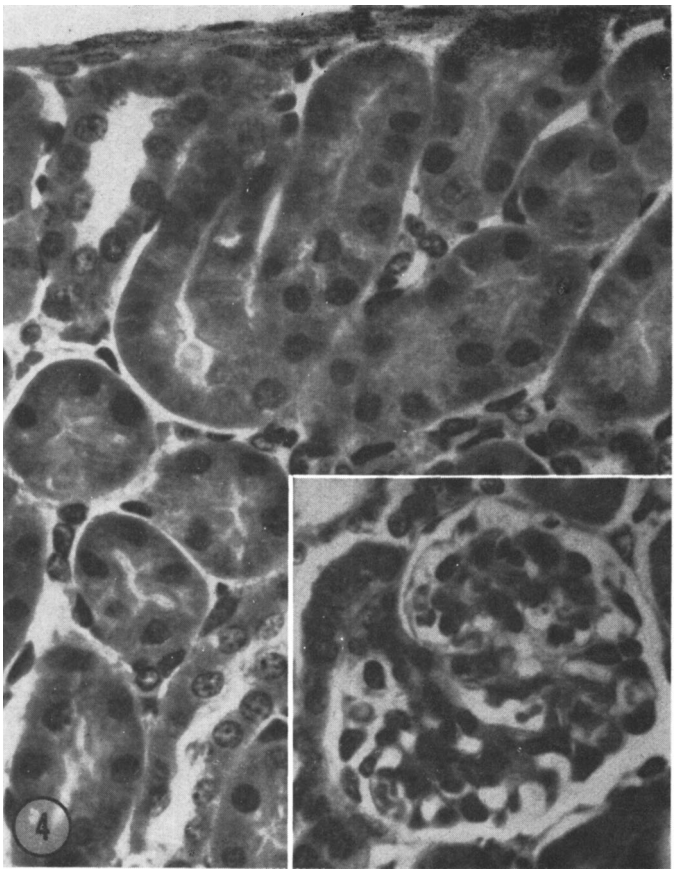

FIG. 4. Kidney from typical 4-week-old rabbit, ×640: hematoxylin–eosin stain. Cortical tubules are almost adult size and contain ample cytoplasm. (insert) Glomerulus greatly expanded and relatively less cellular than in kidney from 2-week-old rabbit. Measured PAH *S/M* ratio, 14.51.

cortical slices from 1-week-old dogs, cats, rabbits, and guinea pigs was compared to PAH accumulation by adult animals of these species (Fig. 1). Relative to the adult values, low PAH *S/M* ratios were obtained in dogs, cats, and rabbits at 1 week of age. In contrast, PAH *S/M* ratios were higher in slices from 1-week-old guinea pigs than from adults.

Treatment of 2-week-old rabbits with penicillin significantly elevated the PAH *S/M* ratios without increasing kidney weight (Table I). The ability of renal cortical slices to accumulate PAH was increased to the same capacity as that normally seen in 4-week-old rabbits (4). When examined on a single-blind basis however, kidneys from penicillin-treated rabbits could not be distinguished histologically from kidneys of control littermates. Kidneys from 2-week-old rabbits were characterized by the presence of small densely cellular glomeruli with prominent peripheral nuclei. Cortical tubules of kidneys from both treated and control rabbits were small, with crowded nuclei, limited amounts of cytoplasm, and inconspicuous tubular lumens. The presence of a nephrogenic zone in the outer cortex was exemplified by the histologically undifferentiated or immature tubular cells (Figs. 2 and 3). Normal histological development could be detected since distinct differences between kidney sections from 2- and 4-week rabbits and adults could readily be observed. By 4 weeks of age (Fig. 4) the renal tubules were approaching adult size and maturity (Fig. 5). Rapid growth

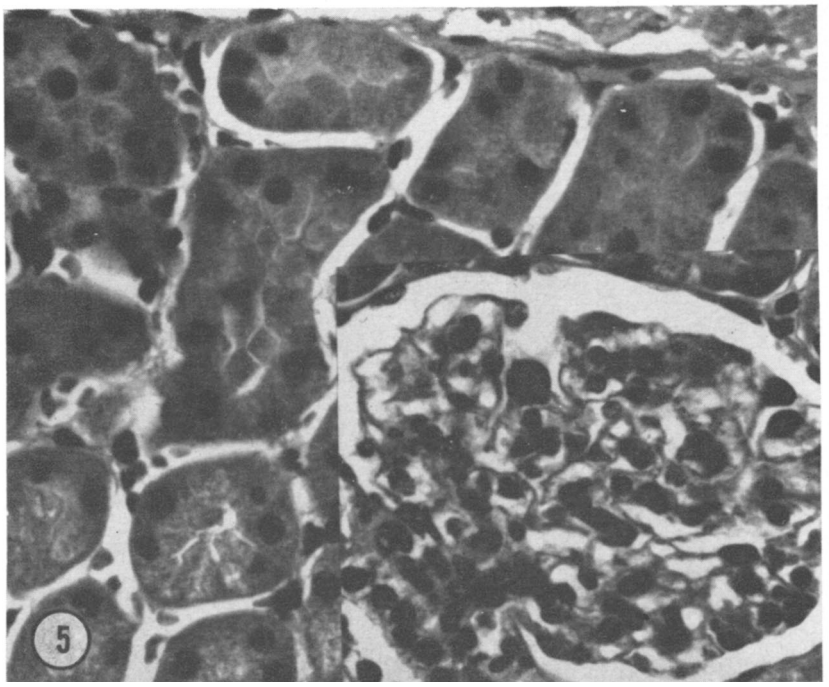

FIG. 5. Kidney from typical adult rabbit, ×640: hematoxylin–eosin stain. Cortical tubules are full sized. (insert) Fully expanded, normally cellular glomerulus with only the occasional peripheral nucleus. Measured PAH *S/M* ratio, 8.98.

between 2 and 4 weeks of age was indicated by the increased cell size and increased distance of the glomeruli from the cortical surface. The age-related increase in kidney size is evident since all histological sections were photographed at the same magnification. Thus, even though normal development can be readily demonstrated both physiologically and histologically, no histological changes can be seen when physiological development is accelerated by penicillin.

Discussion. The correlation between histological immaturity of the kidney cortex and low PAH accumulation observed by Rennick *et al.* (2) in puppies also is evident in the results present in Fig. 1. Kidneys from rabbits, puppies, and kittens are histologically immature at birth, and, at 1 week of age, low PAH *S/M* values are obtained (2, 7). After histological maturity is reached, higher PAH *S/M* values are observed. In contrast to the results obtained with other species, accumulation of PAH by renal cortical slices from 1-week-old guinea pigs was greater than in slices from adult animals (Fig. 1). The guinea pig has a relatively long gestation period and the kidneys of this species are histologically mature at birth (7). Zorzoli (12) reported results comparable to those presented here in that she found the activity of renal gluconeogenic enzymes to be higher in newborn than in adult guinea pigs. Thus the data suggest a correlation between histological maturity and cellular function. It was therefore reasoned that the stimulatory effect of penicillin on PAH accumulation by renal cortical slices previously described (3, 5, 6) may be associated with enhanced histological maturation of the kidney. However, this does not appear to be the case since no apparent morphologic differences between kidneys from treated and control rabbits could be observed (Figs. 2 and 3). Since the kidney has other specific functions in addition to secretion of organic acids, the lack of a direct correlation between physiological and anatomical changes in the present studies is not unexpected.

Previous studies suggested that the stimulatory effect of penicillin on PAH accumulation is due to substrate stimulation of the active concentrating mechanism mediating PAH secretion (5, 13). The specific effect of penicillin on the anionic transport system (5, 13), in conjunction with the results presented here, suggests that the stimulatory effect of penicillin involves specific subcellular changes. These changes may involve specific substrate stimulation of transport enzymes and may not be demonstrable using light microscopy. Stimulation of the particular enzymes associated with organic acid transport apparently is a more specific effect than the morphological changes associated with normal development. Precise changes such as these may require electron microscopy to delineate any structural alterations produced by penicillin while stimulating PAH transport.

Summary. Renal cortical slices from 1-week-old rabbits, dogs, and cats accumulated PAH to a lesser extent than slices from adult animals, while the values in 1-week-old guinea pigs were higher than those obtained in adults. Although penicillin treatment resulted in a significant increase in PAH accumulation, no histological differences between kidneys from treated and control rabbits could be observed using light microscopy. Normal histological maturation of newborn rabbit kidneys could be detected. It is concluded that the substrate-induced stimulation of PAH accumulation produced by penicillin involves specific subcellular changes (structural and/or biochemical) in the organic acid transport mechanism.

1. New, M., McNamara, H., and Kretchmer, N., Proc. Soc. Exp. Biol. Med. **102**, 558 (1959).

2. Rennick, B., Hamilton, B., and Evans, R., Amer. J. Physiol. **201**, 743 (1961).

3. Hirsch, G. H., and Hook, J. B., Science **165**, 909 (1969).

4. Hirsch, G. H., and Hook, J. B., Proc. Soc. Exp. Biol. Med. **131**, 513 (1969).

5. Hirsch, G. H., and Hook, J. B., J. Pharmacol. Exp. Ther. **171**, 103 (1970).

6. Hirsch, G. H., and Hook, J. B., J. Pharmacol. Histochem. Cytochem. **13**, 44 (1965).

7. Wachstein, M., and Bradshaw, M., J. Histochem. Cytochem. **13**, 44 (1965).

8. Cross, R. J., and Taggart, J. V., Amer. J. Physiol. **161**, 181 (1950).

9. Hook, J. B., and Munro, J. R., Proc. Soc. Exp. Biol. Med. **127**, 289 (1968).

10. Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., and Graber, M., J. Clin. Invest. **24**, 388 (1945).

11. Lewis, A. E., "Biostatistics," Reinhold, New York (1966).

12. Zorzoli, A., Develop. Biol. **17**, 400 (1968).

13. Hirsch, G. H., and Hook, J. B., Can. J. Physiol. Pharmacol. **48**, 550 (1970).

Received Nov. 20, 1970. P.S.E.B.M., 1971, Vol. 137.