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Accumulation of *para*-Aminohippurate by Slices of Kidney from Rabbits of Various Ages.* (25318)

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Previous investigations have shown a gradual increase in renal tubular function *in vivo* from fetal life to maturity. Williamson and Hiatt(1) observed rapid increase of phenolsulphonphthalein (PSP) excretion by rabbit kidney from 26th day of gestation to 10th day postnatally when PSP excretion attained adult levels. In the rat(2) ability of tubules to absorb an intravital dye such as trypan blue began at 12 days of age and reached a maximum at 28 days; intravital staining was directly correlated with acquisition of a brush border. Levine and Levine(3) working with fetal, newborn and adult rabbit also report a gradual increase in C_{PSP} until 1 month of age. Experiments on development of renal tubular function *in vitro* cover only the post-natal period. Deyrup(4) demonstrated a low S^{35} uptake in the newborn rat when compared to adult. Rennick(5) observed that transport systems for tetraethylammonium (TEA) and *para*-aminohippurate (PAH) developed at different ages in the puppy: that for PAH at 3-4 weeks of age and that for TEA at 7 weeks of age. This report extends investigation of development of renal tubular function *in vitro* into the fetal period. Accumulation of PAH and chlorphenol red (CPR) by kidney slices is used as a measure of tubular function from fetal life to maturity in the rabbit. Contrary to previous reports(1-5) these data show that instead of a gradual increase in function with age there is less PAH accumulation in the slice from newborn than from either fetus or adult.

Methods. Rabbits of various ages were used: fetuses of 26 to 28 days gestational age; nurslings from 6 hours to 22 days; adult males and females, pregnant and non-pregnant. Essentially methods described by Cross and Taggart(6) were utilized throughout. Slices weighing 200-300 mg(7) were incubated at 30°C for 60 minutes in Krebs-Ringer phosphate(8) with either PAH or chlorphenol red 7.4×10^{-5} M. Control flasks containing 5×10^{-5} M dinitrophenol were included in most experiments. At end of incubation, slices were removed, blotted and placed in 10% trichloroacetic acid for PAH determination. Aliquots of the medium and the filtrate were analyzed for PAH(9). When chlorphenol red was used slices were removed, blotted and smashed on glass slide for direct microscopic visualization. Nitrogen content of slices was determined by micro-Kjeldahl(10); dry weight by drying a minced slice first on steam bath and finally to constant weight over phosphorus pentoxide. Mitochondria were prepared by differential centrifugation of kidney homogenates in 0.25 M sucrose. Their capacity for oxidative phosphorylation (phosphorus/oxygen ratio) was measured(11) using α-ketoglutarate as substrate. Phosphorus was estimated colorimetrically(12) and oxygen consumption determined manometrically.

Results. Accumulation of PAH by slices: On the basis of wet weight, slices of fetal kidney showed greater accumulation of PAH than did those of the neonatal animal. Increased concentration of PAH in the medium (4.4×10^{-4} M) did not alter these results.

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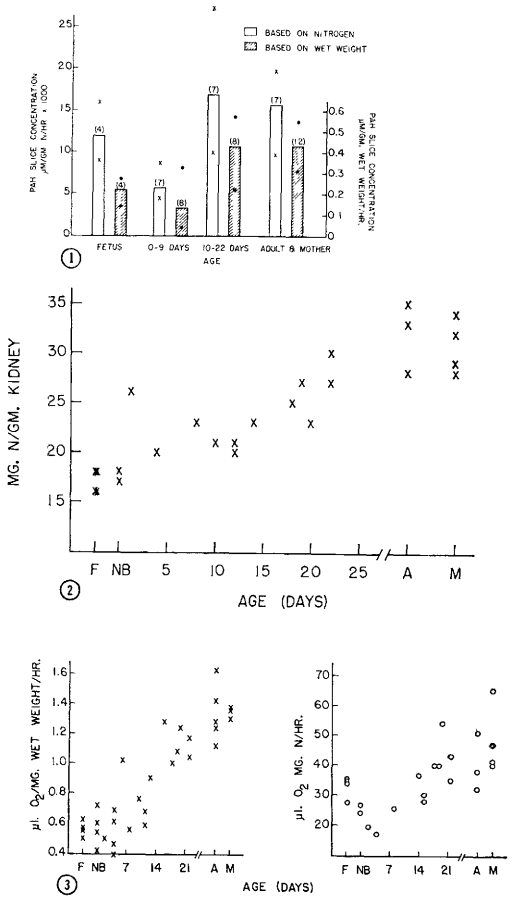

FIG. 1. Relation of age to accumulation of *para*-aminohippurate by slices of kidney from rabbits. Slices of kidney 0.3-0.5 mm thick weighing from 80-200 mg were incubated in Warburg flasks in an atmosphere of oxygen for 60 min. at 30°C. The medium contained a modified Krebs Ringer phosphate solution at pH 7.4 and PAH in a final concentration of 7.4×10^{-5} M.

FIG. 2. Concentration of nitrogen in kidney of rabbit at various ages.

FIG. 3. Consumption of oxygen by slices of rabbit kidney related to age.

Slices of kidney from animals 10 days and older showed the same amount of PAH uptake as those from adult rabbits (Fig. 1).

Accumulation of PAH by slices of kidney from the fetus was less than that of the adult when based on wet weight of slice, but values for the fetus and adult were similar when based on nitrogen content. This result was related to progressive increase in nitrogen content of kidney with age (Fig. 2).

Uptake of PAH can be expressed by the ratio of its concentration in the slice to its concentration in the medium(6), *i.e.*, $\frac{\mu\text{moles PAH/g wet weight slice}}{\mu\text{moles PAH/ml medium}}$. All groups showed active accumulation as evidenced by an S/M ratio greater than 1. Kidneys from the newborn and from animals up to 9 days of age showed minimal accumulation of PAH. When slices of kidney were used from animals 10 days or older, ratios of 8 to 11 were obtained which were similar to those reported for adult rabbits(6). Dinitrophenol added to control flasks resulted in S/M ratios of less than 1 with all age groups, indicating that there is an energy requirement for uptake of PAH into slices. When the S/M ratio was based on nitrogen content or dry weight, *i.e.*, $\frac{\mu\text{moles PAH/g N or g dry wt of slice}}{\mu\text{moles PAH/ml medium}}$ rather than wet weight of slice, the difference between values for the fetus and newborn was exaggerated while the difference between values from fetus and adult was decreased (Table I).

Droplets of chlorphenol red were visualized only in those preparations of tubular epithelial cells made from kidneys of animals 2 weeks of age or older.

Oxygen consumption by slices of rabbit kidney increased with age from newborn to adult whether based on nitrogen or wet weight of slice (Fig. 3). When based on nitrogen the lowest values for oxygen consumption were observed with slices of kidney from neonatal animals which also showed the least uptake of PAH. Other substrates, such as pyruvate, α-ketoglutarate, and succinate did not increase consumption of oxygen by the slices. Per mg of nitrogen, the slice from fetal kidney con-

TABLE I. Accumulation of *para*-Aminohippurate by Slices of Kidney from Rabbits of Various Ages, Expressed as S/M Ratio.*

Age of rabbit	S/M per g		
	Wet wt	N	Dry wt
Fetus	3.2	190	29
0- 8 days	2.0	76	10
10-22 "	10.2	330	46
Adult	8.4	250	39

* $\frac{\mu\text{moles PAH/unit wt of slice}}{\mu\text{moles PAH/ml medium}}$ after incubation of 60 min. at 30°C in oxygen.

sumed almost as much oxygen as the slice from the 10 to 21 day old rabbit.

Phosphorus/oxygen ratios: To determine whether differences in oxidative phosphorylation could account for the difference in PAH accumulation in slices from animals of various ages, mitochondrial phosphorus/oxygen ratios (P/O) were obtained. The P/O ratio in mitochondria isolated from kidneys of fetal, newborn, or adult rabbits was 1.9 to 2.9 indicating that decreased capacity for renal transport of PAH by neonatal animals does not result from uncoupling of oxygen and phosphorus.

Discussion. This study disagrees with previous reports which indicated a gradual continuous development of renal tubular function from fetal life to maturity. The data show that values of PAH accumulation *in vitro* for fetus and adult are similar when based either on dry weight or nitrogen content of kidney. The apparent difference in PAH accumulation between slices from the adult and fetal kidney (when based on wet weight) reflect an increased water content of the latter. Lack of visualization of CPR in slices of kidney from animals less than 2 weeks old may be the result of dilution of the dye. Tissue hydration does not, however, account for lack of accumulation of PAH in slices from the newborn kidney.

That oxygen consumption based on nitrogen content of the slice is lowest in the age group in which PAH accumulation is least may indicate some relationship between these 2 systems. Lack of enzymic development cannot explain a depressed accumulation of PAH by kidney of the newborn group, since the enzyme carrier system apparently is present in the fetus. Morphological development is gradual with no abrupt changes occurring at birth that might impair function(13-15). At present there is no explanation for the relative deficiency observed with slices of kidney in the newborn.

Summary. When the factor of high water content is eliminated, slices of kidney from the fetal rabbit accumulate as much PAH from surrounding medium as those from the adult. Slices of kidney from newborn rabbit are significantly deficient ($p = <.01$) in this function when compared to those from fetus or adult.

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