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SHORT COMMUNICATIONS

Development of *p*-aminohippurate transport and oxygen consumption in rabbit kidneys

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VARIOUS METABOLIC functions of the kidney, including the transport of organic acids, are not fully developed in many species at birth.[1–3] Utilizing the kidney slice technique of Cross and Taggart,[4] a similar developmental pattern *in vitro* of renal *p*-aminohippurate (PAH) transport has been reported in dogs,[5] pigs[2] and rabbits.[6] A gradual increase in the uptake of PAH (expressed as the slice/medium or S/M ratio) by renal cortical slices is observed, reaching a peak at 4 weeks of age and then gradually declining to adult values. The studies reported here were undertaken to determine if the characteristic increase in the PAH S/M ratio observed in studies *in vitro* at 4 weeks was accompanied by increased metabolic activity as measured by oxygen consumption, or if it could be attributed to the tissue slice preparation and the transport parameters affecting the degree of cellular accumulation.

New Zealand white rabbits were maintained with their litters in the laboratory animal quarters. The kidneys from rabbits killed at 1, 2 and 4 weeks, and adults, were quickly excised and placed in cold saline, whereupon either cortical slices or homogenates were prepared. Renal cortical slices were incubated in Warburg flasks in Cross and Taggart[4] media containing $7{\cdot}4 \times 10^{-5}$ M PAH as described by Kim *et al.*[7] Homogenates (10%, w/v) of renal cortex were prepared in 1·15% KCl with 10^{-4} M EDTA. One ml of homogenate was added to media consisting of $16{\cdot}7 \times 10^{-3}$ M KH_2PO_4, $4{\cdot}0 \times 10^{-3}$ M $MgCl_2$, $2{\cdot}0 \times 10^{-3}$ M ATP, and 20×10^{-3} M pyruvate, adjusted to pH 7·1. Oxygen consumption, for either slices or homogenates, was measured as described by Umbreit *et al.*[8] Incubations were for 1 hr at 25°. After incubation, 0·5 ml homogenate media was assayed for protein content by the biuret method as described by Gornall *et al.*,[9] and another 0·5-ml aliquot was dried to constant weight. The concentration of PAH in the slices and media was determined by the method of Smith *et al.*[10]

The net accumulation of PAH by renal cortical slices as measured by the PAH S/M ratio reached a maximum at 4 weeks of age (Table 1). Oxygen consumption by the slices, utilizing only endogenous substrates, was not significantly different at any of the ages, although the highest value was obtained at 4 weeks (Table 1). Tissue loss during incubation was not a factor, since quantitatively similar results were obtained at the various ages when oxygen consumption was calculated on the basis of initial slice weight. The addition of acetate significantly enhanced ($P < 0{\cdot}05$) both the PAH S/M ratio and oxygen consumption with respect to control values at all ages studied (Table 1). In contrast to the slice experiments, oxygen consumption of renal cortical homogenates from 4-week-old rabbits was significantly greater ($P < 0{\cdot}05$) than the values obtained in adults and 2-week-old rabbits. This may be a reflection of the experimental conditions used, or involve factors such as substrate availability. The protein content of rabbit kidney cortical homogenates was essentially the same at 2 weeks, 4 weeks and adults, with only the value at 1 week being significantly less than that observed in the adult ($P < 0{\cdot}05$).

Although there are many studies demonstrating that PAH uptake is an active energy-requiring process, an increase in PAH transport can occur without a measurable increase in the available energy supply to the kidney.[11] Studies employing techniques *in vivo* have demonstrated that renal function increases gradually with age.[1,5] The enhanced PAH S/M ratio observed at 4 weeks using the slice technique *in vitro* appears to be related to the various aspects of the transport system measured by this method. In this regard, Hook[12] has shown that at 4 weeks the rate of PAH uptake into rabbit kidney slices was greater than in adults. The increased PAH uptake rate along with the lower PAH runout constant reported by Hook[12] may explain the peak in the S/M ratio observed at 4 weeks. The lower PAH S/M ratio in adults relative to 4-week-old rabbits may also be related to the presence of endogenous inhibitors, which may be counteracted experimentally by acetate.[12] This is supported by the finding that the increase in PAH accumulation is relatively greater in adults (99 per cent) than at 4 weeks (50 per cent) after the addition of acetate (Table 1).

It therefore appears that the peak PAH S/M ratio observed at 4 weeks using rabbit kidney slices is related primarily to changes in the transport parameters involving uptake and runout as measured by the slice technique. This enhanced accumulation of PAH is not accompanied by increased metabolic activity in the presence of endogenous substrates, but the addition of acetate produces a significant increase

TABLE 1. EFFECT OF AGE ON PAH UPTAKE, OXYGEN CONSUMPTION AND PROTEIN CONTENT IN RABBIT RENAL CORTICAL SLICES OR HOMOGENATES*

	Slices				Homogenate	
	PAH S/M		Qo_2(μl/hr/mg tissue)		Qo_2	Protein
Age	Control	Acetate	Control	Acetate	(μl/hr/mg protein)	(mg/100 mg tissue)
1 week	4·0 ± 0·5 (4)		0·68 ± 0·07 (3)		5·65 ± 0·23 (6)	12·6 ± 1·0 (6)
2 weeks	4·6 ± 0·6 (7)	7·9 ± 1·2† (3)	0·67 ± 0·03 (6)	0·87 ± 0·04† (3)	5·38 ± 0·53 (5)	17·1 ± 1·9 (5)
4 weeks	12·3 ± 1·1‡ (8)	18·4 ± 1·9†,‡ (3)	0·73 ± 0·05 (8)	1·21 ± 0·04†,‡ (4)	7·23 ± 0·30‡ (9)	18·4 ± 1·4 (7)
Adult	6·5 ± 0·7 (8)	12·9 ± 1·8† (5)	0·64 ± 0·03 (8)	0·92 ± 0·05† (5)	5·18 ± 0·37 (6)	17·2 ± 1·2 (6)

* Rabbits were killed at the indicated ages and renal cortical slices or homogenates prepared. Incubations were at 25° for 60 min in Warburg flasks. The values represent means ± S. E. from the number of animals indicated in parentheses. PAH = *p*-aminohippurate.

† Acetate values significantly different from their respective controls ($P < 0·05$).

‡ Four-week values significantly different from their corresponding 2-week or adult values ($P < 0·05$).

in Q_{O_2} at 4 weeks. This observation, combined with the high Q_{O_2} in renal homogenates from 4-week-old rabbits, suggests that renal metabolic processes at this age are particularly susceptible to stimulation by substrates.

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Stoichiometry of drug metabolism in maturing male rats

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ANDROGENS are known to increase the rate of metabolism of type I drugs.[1–7] These reports indicate that androgens increase the binding of type I drugs to cytochrome P_{450} and thus increase the rate of reduction of cytochrome P_{450} and the rate of drug metabolism. However, recent studies by Hamrick *et al.*[8] on the relationship between NADPH oxidation and drug metabolism indicate that changes in the rate of metabolism of type I drugs are not always directly related to changes in the magnitude of type I spectral changes, the cytochrome P_{450} content, or the NADPH–cytochrome c reductase activity. These studies indicate that steroids such as testosterone, cortisone and spironolactone may regulate the rate of drug metabolism by altering the stoichiometric ratio between substrate-stimulated, carbon monoxide-inhibitable NADPH oxidation and drug metabolism.

The purpose of this study was to utilize the maturing male rat to study the effect of androgens on the stoichiometric ratio for ethylmorphine (EM) metabolism in the developing hepatic microsomal drug-metabolizing system, and to attempt to correlate the change in the stoichiometric ratio with changes in the binding and metabolism of EM. Since the testosterone concentration in the spermatic vein of male rats has been shown to increase from 0·42 μg/100 ml to 1·04 μg/100 ml between the age of 25 and 30 days and to increase from 0·96 μg/100 ml to 4·8 μg/100 ml between the age of 35 and 40 days,[9] the study was conducted in rats between the age of 3·0 and 6·5 weeks. Interestingly, the time of increase in testosterone concentration correlates well with previous observations that testicle size increases primarily between the age of 3 and 6 weeks,[10] that seminal vesicle size increases primarily between the age of 3 and 8 weeks,[10] that hexobarbital sleeping time decreases between the age of 4 and 5 weeks,[1] and that EM metabolism doubles between 3 and 5 weeks.[10] Thus, one might assume that the age of 3–5 weeks approximates puberty in the male rat.

For this investigation, a single group of male Sprague–Dawley rats weighing 40–50 g were obtained from Cherokee Lab Supply in Atlanta, Georgia, and they were utilized as they matured. As the animal