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1 P. JURTSHUK, C. R. BARRERA AND S. MANNING, *Can. J. Microbiol.*, 15 (1969) 321.
2 J. T. DUFF AND O. WYSS, *J. Gen. Microbiol.*, 24 (1961) 273.
3 P. JURTSHUK, S. MANNING AND C. R. BARRERA, *Can. J. Microbiol.*, 14 (1968) 775.
4 E. GOLDBERG, *Science*, 139 (1963) 602.

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Ontogenesis of microsomal ATPase in the rabbit kidney

The ability to concentrate urine is related to the build-up of an osmotic gradient along the renal medulla[1]. This osmotic gradient depends on a constant supply of Na^+ which is actively transported by epithelial cells along the nephron[2,3]. Thus, Na^+ deprivation results in a loss of concentrating ability[4].

The concentrating function of the kidney is poorly developed at birth and its full capacity is achieved only during extra-uterine development[5,6]. On the other hand, activity of a specific microsomal enzyme in the kidney, (Na^+, K^+)-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3), has been shown to be related to Na^+ reabsorption in the renal tubules[7]. The activity of this enzyme in the kidney is regulated by mineralocorticoids[8,9] and by Na^+ intake[10]. It seemed of interest, therefore, to study the ontogenetic development of this enzyme in the kidney during fetal development and after delivery, in relation to the maturation of the concentrating mechanism.

We have, therefore, investigated microsomal ATPase in kidneys of rabbit fetuses (28th day of gestation), on the day of birth, at the age of 10 days and in adult rabbits. Kidney tissue (approx. 1 g) was homogenized in 10 vol. of 0.25 M sucrose

TABLE I

ONTOGENESIS OF MICROSOMAL ATPASE IN THE RABBIT KIDNEY

Activities are expressed as mg P_i liberated per mg microsomal protein per h. n.s., difference between two consecutive stages of growth not significant.

Age	*Number of experiments*	*Mg^{2+}-ATPase activity*		*(Na^+, K^+)-dependent ATPase activity*	
Fetus	9	3.17 ± 0.17	$P < 0.01$	0.81 ± 0.13	n.s.
1 day	7	4.23 ± 0.07	$P < 0.001$	1.15 ± 0.24	n.s.
10 days	4	7.94 ± 0.17	$P < 0.05$	0.62 ± 0.24	$P < 0.01$
Adult	7	8.98 ± 0.27		2.05 ± 0.25	

containing 2 mM EDTA (disodium salt), and then centrifuged at 1000 × *g* for 10 min. The supernatant was centrifuged twice at 10 400 × *g* for 10 min, and finally the microsomes were separated from the soluble fraction by centrifugation at 105 000 × *g* for 30 min. The microsomes were resuspended in 2 ml of homogenizing medium. Total ATPase activity was assayed by incubating 0.2 ml of the microsomal suspension at 37° for 20 min in a medium containing 100 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, 4 mM ATP and 33 mM Tris buffer (pH 7.2); the final volume was 3 ml. To determine (Na^+,K^+)-dependent ATPase, ouabain (final concn., 0.1 mM) was added to the medium. The residual activity in the presence of ouabain is Mg^{2+}-ATPase or (Na^+, K^+)-independent ATPase. The reaction was stopped by the addition of 1 vol. of trichloroacetic acid (5%). P_i was determined according to BAGINSKI AND ZAK[11] and protein by the method of LOWRY *et al.*[12].

In kidneys of fetuses and those from newborn rabbits, the size of the medulla

TABLE II

DISTRIBUTION OF MICROSOMAL ATPASE IN CORTEX AND MEDULLA OF THE RABBIT KIDNEY

Activities are expressed as mg P_i liberated per mg microsomal protein per h. *P* values are for the difference in enzyme activity between 10-day-old and adult rabbits. n.s., difference between 10-day and adult rabbit not significant.

Age	*Number of experiments*	*Mg^{2+}-ATPase activity*		*(Na^+, K^+)-dependent ATPase activity*	
		Cortex	*Medulla*	*Cortex*	*Medulla*
10 days	4	6.91 ± 0.25	12.07 ± 0.17	0.78 ± 0.17	0.44 ± 0.47
Adult	7	7.36 ± 0.30	14.70 ± 0.22	1.67 ± 0.23	3.28 ± 0.36
		n.s.	$P < 0.01$	$P < 0.05$	$P < 0.01$

was very small, and, moreover, no clear demarcation could be seen between cortex and medulla; therefore, homogenates of whole kidneys were prepared. In kidneys from 10-day-old and adult rabbits, ATPase activity was assayed both in homogenates of whole kidneys and in homogenates of cortex and of medulla separately. The medulla included the external and internal medulla as well as the papilla.

In addition to ATPase activity in the kidney, osmolarity and composition of urine of 1-day-old, 10-day-old and adult rabbits were compared. Urine from adult male rabbits, on water and food *ad libitum*, was collected in metabolic cages. Urine from 1-day-old and 10-day-old rabbits was collected into a syringe directly from the urinary bladder.

Table I shows that microsomal Mg^{2+}-ATPase activity increased progressively with the development of the rabbit, both during intra-uterine and extra-uterine growth. The largest increase in this activity occurred between the 1st and the 10th day of life. On the other hand, (Na^+, K^+)-dependent ATPase activity showed no significant change during the first three stages of development (fetus to 10-day-old rabbit). However, in kidneys of adult rabbits, this activity was significantly higher (Table I).

Assay of ATPase activity in the cortex and medulla separately showed a striking difference between Mg^{2+}-ATPase and (Na^+, K^+)-dependent ATPase as seen in Table II. Thus, Mg^{2+}-ATPase increased only to a small extent from the age of 10

days to the adult, the increase being somewhat larger in the medulla. (Na^+, K^+)-dependent ATPase activity, on the other hand, was more than doubled in the cortex and in the medulla it increased 7-fold.

Interestingly, urine osmolality also doubled between 10-day-old and adult rabbits, as seen in Table III. Thus a clear divergence between the ontogenesis of kidney Mg^{2+}-ATPase and (Na^+, K^+)-dependent ATPase was observed. While Mg^{2+}-ATPase increased substantially between the 1st and 10th day of extra-uterine life, there was

TABLE III

OSMOLALITY OF RABBIT URINE AT DIFFERENT AGES

	1 day	*10 days*	*Adult*
Urine osmolality (mosM/kg)	556	545	985

no significant change in osmolality of urine between these 2 ages. (Na^+, K^+)-dependent ATPase activity increased significantly only beyond the age of 10 days, the increase being specifically evident in the medulla. It may be of significance that the increased activity of (Na^+, K^+)-dependent ATPase in the kidney coincided with an increase in urine osmolality and that the increased enzyme activity was most striking in the medulla, that part of the kidney which is mainly responsible for the concentration of urine. The possible significance of this observation is now being investigated in other species. Our finding that the activity of microsomal ATPase in renal medulla of the adult rabbit is about double that in the cortex corroborates the findings of BONTING *et al.* in the cat kidney[13] and in the dog kidney[14].

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1 H. WIRZ, B. HARGITAY AND W. KUHN, *Helv. Physiol. Pharmacol. Acta*, 9 (1951) 196.
2 C. W. GOTTSCHALK, *Am. J. Med.*, 36 (1964) 670.
3 W. KUHN AND A. RAMEL, *Helv. Chim. Acta*, 42 (1959) 628.
4 C. GOLDSMITH, H. K. BEASLEY, P. J. WHALLY, F. C. RECTOR AND D. W. SELDIN, *J. Clin. Invest.*, 40 (1961) 2043.
5 H. HELLER, *Arch. Disease Childhood*, 26 (1951) 195.
6 R. A. MCCANCE AND E. WILKINSON, *J. Physiol. London*, 106 (1947) 256.
7 A. I. KATZ AND F. H. EPSTEIN, *J. Clin. Invest.*, 46 (1967) 1999.
8 C. F. CHIGNELL AND E. TITUS, *J. Biol. Chem.*, 241 (1966) 5083.
9 E. J. LANDON, N. JAZAB AND L. FORTE, *Am. J. Physiol.*, 211 (1966) 1050.
10 P. L. JORGENSEN, *Biochim. Biophys. Acta*, 151 (1968) 212.
11 E. BAGINSKI AND B. ZAK, *Clin. Chim. Acta*, 5 (1960) 834.
12 O. H. LOWRY, N. J. ROSENBOUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
13 S. L. BONTING, K. A. SIMON AND N. M. HAWKINS, *Arch. Biochem. Biophys.*, 95 (1961) 416.
14 S. L. BONTING, L. L. CARAVAGGIO AND N. M. HAWKINS, *Arch. Biochem. Biophys.*, 98 (1962) 413.

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