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RENAL CORTICOPAPILLARY CONCENTRATION GRADIENT IN CALVES*

By

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ERIKSSON, LEA: *Renal corticopapillary concentration gradient in calves.* Acta vet. scand. 1972, *13*, 197—205. — From 31 calves samples were taken from blood, urine and kidneys in a slaughterhouse. The calves were divided into three age groups: A. Newborn calves, B. Grazing calves, C. Bulls and heifers. The renal cortical and papillary potassium concentrations were higher in younger than in older calves. The corticopapillary urea and sodium concentration gradients during antidiuresis give good information about renal concentrating ability. The urea gradient was fully developed in the newborn calves. The sodium gradient, however, showed a small, but statistically significant difference between the age groups. Thus the papillary sodium content was lower in the newborn calves than in the older animals while the cortical sodium concentration was higher in the newborn and in the grazing calves than in bulls and heifers. Compared to the neonatal rat and rabbit the newborn calf has a high, but not fully developed corticopapillary concentration gradient.

kidney; concentration gradients; newborn calf.

Newborn animals of many species (e. g. man, rat, rabbit, cat and pig) are neither able to concentrate nor to dilute urine to the same extent as adults (*Vesterdal* 1961, *McCance* 1964). In contrast, the newborn guinea pig excrete urine with the same specific gravity as the adult (*Dicker & Heller* 1951). The neonatal calf also has a well-developed renal function. It concentrates urine well, excretes a water load readily, and its urea clearance is high (*Dalton* 1966, 1967, 1968 a, b).

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The formation of concentrated urine is dependent on a continuous increase of solutes, mainly sodium and urea, from the cortex towards the papilla. Thus renal tissue analyses during antidiuresis give good information about the concentrating capacity of the kidney. Such analyses have been performed in newborn rabbits (*Fleischaker et al.* 1960, *Yaffe & Anders* 1960, *Forrest & Stanier* 1966) and rats (*Yunibhand & Held* 1965). In these species the papillary urea content increases with age. In the newborn rabbit the sodium gradient resembles that of the adult, but in the rat the gradient shows a gradual postnatal rise.

The purpose of this study was to investigate to what extent the renal concentrating ability of the newborn calf is reflected in a corticopapillary osmotic gradient.

MATERIAL AND METHODS

Samples from 31 Ayrshire calves were collected in a slaughterhouse. Blood samples were taken into heparinized tubes at the beginning of bleeding, centrifuged and the plasma separated. After slaughter and evisceration a urine sample was taken from the bladder and the right kidney was cut free. The urine osmolality indicated if the animal was concentrating urine (in antidiuresis) or excreting dilute urine (in water diuresis). The animals were divided into the following groups:

A.	Newborn calves (all in antidiuresis)	8
B.	Grazing calves (carcass weight under 90 kg) (9 in antidiuresis)	12
C.	Bulls and heifers (carcass weight over 90 kg) (6 in antidiuresis)	11

45 min., on an average, passed after the death of the animal till the dissection of the kidney. In order to study if the renal concentration gradient changed with time after death, samples were taken from the kidneys of six calves 45 min., 75 min. and 105 min. after slaughter.

For the tissue analysis samples were taken from the cortical regions and the entire papillae. Tissue pieces were placed into previously weighed small test tubes, weighed at once and sealed with parafilm. The samples were stored frozen, until they were analysed.

In order to establish whether the concentration gradient was the same in different lobes of the cattle kidney, duplicate samples were taken from six lobes in each kidney. The samples were randomly paired and used for the determination of sodium, potassium, urea and water. Six animals were used.

For water content determinations weighed samples were dried for three days at 105°C and reweighed.

Osmolality of urine and plasma was measured by the freezing-point depression method with a Fiske osmometer.

Sodium and potassium were determined with a Jouan flame photometer. The weighed tissue samples were homogenized with an Ultraturrax-homogenizer in 2 % trichloroacetic acid and made up to volume. Homogenates were stored in a refrigerator for at least 24 hrs. and centrifuged. The standards were made up in 2 % TCA solutions (*Mounib & Evans* 1957).

Urea was determined using *Chaney & Marbach*'s method (1962). Since the determination was developed for plasma analysis, it had to be adapted to the analysis of tissue samples. These were homogenized in redistilled water and urea was determined by liberating ammonia from urea by urease. The ammonia formes a blue colour with phenol and hypochlorite in the presence of nitroprusside. The extinction at 630 nm is measured. The preformed ammonia was measured substituting water for the urease solution, and a correction was made by substracting this ammonia blank.

The results for sodium, potassium and urea concentration were expressed as meq. or mmol per 100 g fresh weight. The water content was expressed as g per 100 g fresh weight. All numerical data were given as m. ± s. e. m.

RESULTS

Methodology

1. Precision of tissue urea determination.

During cortical urea measurement the blanks without urease had a rather intense greenish colour. The blank extinction was almost ⅓ of sample extinction. The standard deviation and the coefficient of variation were calculated from duplicate tissue pieces using the difference between duplicates. For cortical urea concentration the coefficient of variation was 15.3 %. Without correction for blank estimation the coefficient of variation in cortex was 11.6 %. In the estimation for papillary urea content the coefficient of variation was 10.6 %. Here the "blank" variation did not interfere.

2. The effect of time after death on papillary concentrations.

The water content did not change between the samples taken 45 min., 75 min. and 105 min. after slaughter. The concentrations of urea and sodium fell, but the difference was significant only for sodium between the first and second samples ($P < 0.01$).

3. No difference in sodium, potassium, urea and water content between lobes could be found by analysis of variance.

Differences between the age groups of calves

The significance of any difference between the age groups of calves was calculated by using the analysis of variance.

The plasma and urine concentrations of the calves in antidiuresis are shown in Table 1.

The plasma sodium concentrations did not differ between the groups. The potassium concentrations in the newborn calves were higher than in the older ones ($P < 0.01$). The results agree with the ones of *Fisher* (1960). The plasma urea was lower in the newborn than in the other calves ($P < 0.05$).

Table 1. Composition of plasma and urine from calves in antidiuresis (m. ± s. e. m.).

	A Newborn calves	B Grazing calves	C Bulls and heifers
Number of animals	8	9	6
Plasma osmolality mOsm/kg	291 ± 2	289 ± 1	287 ± 1
Plasma sodium meq./l	140 ± 2	143 ± 2	136 ± 2
Plasma potassium meq./l	5.6* ± 0.3	5.1 ± 0.2	4.5 ± 0.1
Plasma urea mmol/l	3.1** ± 0.4	4.3 ± 0.3	4.8 ± 0.7
Urine osmolality mOsm/kg	750 ± 80	640 ± 60	690 ± 110
Urine sodium meq./l	58 ± 15	42 ± 9	20 ± 8
Urine potassium meq./l	110 ± 20	130 ± 10	90 ± 20
Urine urea mmol/l	350 ± 70	280 ± 30	390 ± 90

The statistical significance of the differences in plasma composition between the age groups is shown:

* A—C: $P < 0.01$

** A—B and A—C: $P < 0.05$

Table 2. Composition of kidney cortex and papilla in newborn and older calves (m. ± s. e. m.).

		A Newborn calves	B Grazing calves	C Bulls and heifers	The significance of differences A—B	 A—C	 B—C
Number of animals		8	9	6			
Water g/100 g fresh weight	Cortex	79.61 ± 0.26	79.92 ± 0.52	79.78 ± 1.00			
	Papilla	83.47 ± 0.33	85.97 ± 1.50	83.99 ± 0.63			
Sodium meq./100 g fresh weight	Cortex	6.07 ± 0.14	6.10 ± 0.12	5.57 ± 0.10		$P < 0.05$	$P < 0.01$
	Papilla	12.47 ± 0.36	15.57 ± 0.47	16.78 ± 1.02	$P < 0.001$	$P < 0.001$	
Potassium meq./100 g fresh weight	Cortex	7.72 ± 0.11	7.68 ± 0.12	7.07 ± 0.17		$P < 0.01$	$P < 0.01$
	Papilla	6.68 ± 0.26	6.18 ± 0.16	5.73 ± 0.32		$P < 0.05$	
Urea mmol/100 g fresh weight	Cortex	1.1 ± 0.3	1.0 ± 0.1	1.2 ± 0.1			
	Papilla	10.7 ± 1.5	11.7 ± 1.8	14.2 ± 1.8			

The newborn calves in the present investigation had been starved one—two days before slaughter. Their urine osmolality was 750 ± 80 mOsm/kg, which is in accordance with *Dalton*'s figures (1966). He found that in neonatal calves urine osmolality after 24 hrs. of starvation was on an average 580 ± 50 mOsm/l and after two days of starvation 930 ± 85 mOsm/l. The urine osmolalities in the other groups were slightly lower (grazing calves 640 ± 60 mOsm/kg, bulls and heifers 690 ± 110 mOsm/kg).

The results of the analyses of kidney cortex and papilla are presented in Table 2.

The water content showed no significant differences at different ages and was on an average 80 % in the cortex and 84 % in the papilla. However, the concentration of sodium differed between age groups. Cortical sodium concentration was higher in newborn ($P < 0.05$) and in grazing calves ($P < 0.01$) than in bulls and heifers, while papillary sodium content was lower in the newborn calves ($P < 0.001$) than in the older animals. Potassium also showed a difference between age groups. Cortical potassium was significantly lower in bulls and heifers than in the younger calves ($P < 0.01$) and papillary potassium was lower in bulls and heifers than in the newborn calves ($P < 0.05$). The urea content showed no significant difference between age groups.

Correlation between urine osmolality and papillary composition

All animals (both those in water diuresis and those in antidiuresis) were used for the determination of a possible correlation between the urine osmolality and papillary composition. Between the urine osmolality and papillary urea content a significant correlation was present ($r = 0.785$, d. f. 26). Papillary sodium, potassium and water content on the contrary, did not show any correlation with urine osmolality.

DISCUSSION

A. Methodology

The use of slaughter-house material has some disadvantages. The treatment of animals before slaughter can not be controlled. In this study the older calves could drink while in the slaughterhouse and were therefore not so dehydrated as the newborn animals. When, however, only animals in antidiuresis were taken into account, the mean urine osmolalities in all groups were so similar that the papillary concentrations should be comparable. The samples were obtained late after death. The corticopapillary concentration gradient starts to diminish immediately after death (*Roch-Ramel & Peters* 1967). Therefore kidney slices have to be dissected as soon as possible. In slaughter-house material, however, it took on an average 45 min. from death to dissection. It is possible that this delay reduced the papillary concentrations. In this study the fall in papillary concentrations after 45 min. was slow, and significant only for sodium. This fact does not, however, exclude the possibility that diffusion has been more rapid earlier.

B. Differences between the age groups of calves

Dalton (1967) investigated the effect of dehydration on newborn calves by keeping them without milk and water for four days. The urine osmolality after two days was on an average 930 mOsm/l and after four days over 1300 mOsm/l. *Weeth & Lesperance* (1965) studied heifers which were dehydrated and fasted during four days. After two days urine osmolality was about 1200 mOsm/kg and after four days 1300 mOsm/kg. Consequently, a newborn calf concentrates urine to the same extent as adult cattle. On the basis of these previous studies the corticopapillary concentration gradient in the newborn calf was expected to be approximately the same as in adult cattle. In the present study a positive correlation between urine osmolality and papillary urea content has been conformed. Furthermore, papillary water and urea content did not differ significantly between the age groups. The low papillary sodium concentration in the newborn calves on the other hand needs to be explained. If it is not an age difference, it is possible that diffusion after slaughter has reduced papillary sodium concentration more effectively in the newborn calves owing to the shorter diffusion distance. *Yunibhand & Held* (1965) found in rat that papillary urea concentration increased about sevenfold with age. The decrease in the water content and the increase in the sodium concentration were smaller. In the rabbit (*Forrest & Stanier* 1966) the increase in the corticopapillary urea gradient was most noticeable, but water and sodium content did not change significantly. Since newborn animals of these species do not concentrate urine to the same extent as adults, it seems that the urea concentration gradient best reflects the maturity of renal concentrating ability. From that point of view the concentration gradient in the newborn calf is nearly as well developed as in the adult cattle.

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SAMMANFATTNING

Renal kortikopapillär koncentrationsgradient hos kalvar.

Prov togs från 31 kalvar av blod, urin och njurar i ett slakthus. Kalvarna delades i tre åldersgrupper: A. Nyfödda kalvar, B. Mellan-kalvar, C. Tjur och kvigor. Kaliumkoncentrationen i njurbarken och papillan var högre hos de unga kalvarna än hos tjurarna och kvigorna. Den kortikopapillära urinämne- och natriumkoncentrationsgradienten i antidiures ger god information om njurarnas förmåga att utsöndra koncentrerad urin. Urinämnesgradienten var fullt utvecklad hos de nyfödda kalvarna. Natriumgradienten däremot visade en liten men statistiskt signifikant skillnad mellan åldersgrupperna. Natriumkoncentrationen i papillan var hos de nyfödda kalvarna lägre än hos de äldre djuren, medan den i njurbarken hos de yngre kalvarna var högre än hos tjurar och kvigor. Jämfört med den nyfödda råttan och kaninen har kalven genast efter födelsen en hög, om än ej fullt utvecklad kortikopapillär koncentrationsgradient.

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