Medullary Sodium and Urea Gradient of the Dog Kidney in Hypothermia* (32925)

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Although the renal concentrating operation is known to be impaired in hypothermia (1–3), the reason for the impairment has not been elucidated. It was postulated earlier that the magnitude of the osmolal gradient in the medulla might be diminished in hypothermia and, consequently, the renal concentrating ability lowered (2, 3). To test the above hypothesis, we determined the medullary gradient of electrolytes and urea as a reflection of the medullary osmolal gradient in euthermic and hypothermic dogs.

Methods. Experiments were carried out in 19 anesthetized dogs (10–15 kg) which were dehydrated, unless stated otherwise, for a period of 15 hours prior to the onset of experiments. After anesthetizing the dog with Nembutal (27 mg/kg iv), an endotracheal tube was inserted. A femoral artery was catheterized for blood sampling and a femoral vein for infusion. Both ureters were then exposed retroperitoneally and were cannulated. A telethermometer probe was inserted approximately 20 cm into the rectum for continuous reading of deep body temperature. A blood blank was drawn following which priming doses of inulin (50 mg/kg) and PAH (8 mg/kg) were administered intravenously. Sustaining doses of inulin (1.0 mg/min per kg) and PAH (0.4 mg/min per kg) were dissolved in 0.15 *M* NaCl which was infused at a rate of 1 ml/min throughout the euthermic experimental period.

After a 90-min period of equilibration, blood and urine samples were collected every 30 min for 120 min. At the end of the euthermic period, the infusion was stopped and the kidneys were removed in 11 dogs (Group 1) while the other 8 dogs (Group 2) were immersed in an ice-water bath until the rectal temperature reached approximately 27–28°C. At this point, the animal was removed from the water bath. Body temperature continued to decrease, but leveled off after 40–60 min at 24–26°C. During this steady state of hypothermia, collection of blood and urine was resumed for four periods as above. Immediately after removal of the dog from the ice-water bath, a second dose of inulin and PAH amounting to one-third of the first dose was administered and the infusion of 0.15 *M* NaCl containing sustaining doses of inulin (0.5 mg/min per kg) and PAH (0.2 mg/min per kg) was resumed. At the termination of the hypothermic experiment, the kidneys were removed.

The removal of the kidney was performed as described by Boylan and Asshauer (5), except for the freezing procedure which was omitted in this investigation. Four slices of the tissue, 1.0–1.5 mm in thickness, from papilla to cortex were cut by hand and each was divided into two approximately equal parts for duplicate measurements. The Na, K, and urea concentrations of the slice were determined in slice extracts prepared by the method described. The tissue slice was then dried overnight in an oven at 160°C for the determination of water content which was used to calculate the electrolyte concentrations of the slice. The concentration of electrolyte in tissue water was calculated by the formula used by the above authors (5).

Plasma filtrates and urine samples were analyzed for freezing point depression, PAH, inulin, Na, K, and urea. The freezing point depression was measured by a Fiske osmometer. The PAH was measured by Bratton and Marshall's method as modified by Smith *et al.* (6). Inulin was determined by modification of Schreiner (7). The Na and K were determined by a Patwin flame photometer and urea by microdiffusion method (8).

Results. A. Renal concentrating ability. In 8 dogs (Group 2) the renal concentrating ability in euthermia and in hypothermia was

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TABLE I. Various Renal Functions of Euthermic and Hypothermic Dogs (Mean ± SE).

	Group 1[a] (Euthermia)	Group 2[b]		
		Euthermia	Hypothermia	p
P_{Na} (meq/liter)	143 ± 2	142 ± 3	143 ± 4	NS[c]
P_K (meq/liter)	3.8 ± 0.3	3.1 ± 0.2	2.7 ± 0.3	NS
P_{osm} (milliosmols/kg)	307 ± 3	305 ± 4	313 ± 7	NS
V (ml/min)	0.62 ± 0.16	0.29 ± 0.04	0.27 ± 0.05	NS
C_{IN} (ml/min)	37 ± 3	37 ± 4	13 ± 2	<0.005
C_{PAH} (ml/min)	120 ±11	122 ±12	32 ± 6	<0.005
F. F. (%)	33.1 ± 2.2	30.8 ± 2.9	39 ± 4.5	NS
U_{Na} (meq/liter)	214 ±20	244 ±35	181 ±23	NS
U_K (meq/liter)	97 ±13	108 ±12	59 ±13	<0.01
U_{osm} (milliosmols/kg)	737 ±67	988 ±47	577 ±28	<0.005
Urea[d] (mM/liter)	115	244	97	
V/C_{IN} (%)	1.45 ± 0.26	0.76 ± 0.09	1.92 ± 0.26	<0.005
C_{osm}/C_{IN} (%)	3.1 ± 0.5	2.7 ± 0.5	3.9 ± 0.6	<0.01
C_{Na}/C_{IN} (%)	2.2 ± 0.4	1.7 ± 0.3	2.6 ± 0.3	NS
C_K/C_{IN} (%)	32.1 ± 5.3	28.5 ± 4.0	60.0 ±15.1	<0.05

[a] Group 1 (Euthermic control): Average on 11 dogs.
[b] Group 2: Average on 8 dogs.
[c] NS denotes nonsignificant difference ($p>0.05$).
[d] Estimates by $U_{osm}-2(Na+K)$.

compared following 15 hours water deprivation to confirm our earlier reports of impairment of the renal concentrating operation in hypothermia (1–3); the results are summarized in Table I. The plasma composition of these animals was not significantly different in euthermia and hypothermia. The clearances of inulin and PAH were significantly lowered in hypothermia, while urine flow was not significantly altered. On the average, urine osmolality was reduced from the control value of 988 to 577 milliosmols/kg in hypothermia ($p<0.005$), again indicating an impairment of renal concentrating ability in hypothermia. This reduction in urine osmolality in hypothermia was accomplished by overall reductions in the urinary concentrations of Na, K, and urea. Moreover, the fractional excretion of filtered water and solutes all increased approximately 2-fold in hypothermia.

B. Solute composition of renal tissue. Average values of water content and solute concentrations of renal tissue slices are summarized in Table II. Data for the euthermic condition are from the 11 dogs in Group I; the plasma and urine compositions of this group are summarized in Table I for reference. The extent of dehydration was made variable in this group in order that the normal relation between papillary solute concentration and urine osmolality could be studied. On the other hand, the data on hypothermic kidneys are from the 8 dogs in Group 2 in which both the euthermic and hypothermic experiments were performed and then the kidneys removed. Hence, the two series of data cannot be strictly compared but provide a basis for discussion.

The water content of comparable tissue slices was not significantly different in euthermic and hypothermic kidneys. As is well known (4, 5, 9–12), Na and urea concentrations were lowest in the cortex and increased gradually toward the papilla. However, we found that the concentration of Na in the medullary slice was somewhat greater in hypothermia than in euthermia. The K concentration was greatest in the cortex and was significantly lower in the medulla. On the average, the K concentrations of comparable tissue slices were similar in both hypothermia and euthermia.

Since the urine osmolality of the euthermic group was not comparable to that of the hypothermic, the former was subdivided into two

TABLE II. Water Content and Electrolyte Composition of Renal Tissue Slices (Mean ± SE).

	Euthermia (group 1)[a]	Hypothermia (group 2)[b]
(A) H_2O content (%)		
Cortex	80.3 ± 0.5	80.3 ± 0.6
Outer medulla	86.6 ± 0.5	84.8 ± 0.8
Inner medulla	87.3 ± 0.3	87.5 ± 0.4
Papilla	88.8 ± 0.6	87.8 ± 0.4
(B) Na concentration (meq/kg of H_2O)		
Cortex	154 ± 10	128 ± 3
Outer medulla	184 ± 8	241 ± 21
Inner medulla	186 ± 13	239 ± 15
Papilla	227 ± 13	300 ± 25
(C) K concentration (meq/kg of H_2O)		
Cortex	102 ± 21	104 ± 15
Outer medulla	66 ± 10	58 ± 3
Inner medulla	62 ± 5	65 ± 17
Papilla	69 ± 6	66 ± 13
(D) Urea concentration (m*M*/kg of H_2O)		
Cortex	24 ± 2	22 ± 5
Outer medulla	94 ± 16	64 ± 18
Inner medulla	154 ± 31	150 ± 38
Papilla	173 ± 37	177 ± 25

[a] Group 1: Average on 16 kidneys from 11 dogs.
[b] Group 2: Average on 11 kidneys from 8 dogs.

groups according to the level of urine osmolality, one with average U_{osm} of 947 ± 86 milliosmols/kg and the other with 527 ± 23. The papillary solute composition of these euthermic groups is summarized in Table III along with that of hypothermic. In euthermia, the difference in urine osmolality between the two groups was reflected by the difference in the papillary Na and urea concentration. Moreover, the total papillary solute concentration, as estimated by 2(Na + K) + urea, was reasonably close to U_{osm}. On the other hand, the papillary Na and urea concentrations in hypothermia were greater than the corresponding values obtained in euthermia with comparable U_{osm}. Thus, the total papillary solute concentration was markedly greater than U_{osm}, suggesting that there exists a disequilibrium across the collecting duct in hypothermia.

Discussion. As shown by many previous investigators (1–3, 13–15), hypothermia induced marked reductions in both GFR and RPF while urine flow rate was not significantly altered (Table I). This paradoxical effect is due to the fact that tubular reabsorption of solutes, especially Na and Cl and therefore of water, is suppressed in hypothermia (Table I). The present investigation confirms the earlier findings of Hong and Boylan (1–3) that the renal concentrating ability is impaired in hypothermia, as indicated by a reduction in the maximal urine osmolality (Table I) and sheds some light on the mechanism of this impairment.

On the basis of the counter-current theory (4, 16, 17) one might expect the medullary osmotic gradient to be reduced in hypothermia as a result of reduction in Na reabsorption in the loop of Henle; osmotic reabsorption of water in the collecting duct would be in turn reduced. However, papillary Na and urea concentrations at comparable urine osmolality were significantly higher in hypothermic kidneys than in euthermic (Table II). Moreover,

TABLE III. Comparisons between the Urine Osmolality and the Papillary Solute Concentration (Mean ± SE).

Rectal temp. (°C)	U_{osm} (milliosmols/kg)	Papillary solute conc (m*M*/kg of H_2O)				U_{osm} − [2(Na+K) + urea]
		Na	K	Urea	2(Na+K) + urea	
37 (8)[a]	947 ± 86	251 ± 17	63 ± 6	251 ± 43	879	68
37 (8)	527 ± 23	203 ± 17	75 ± 22	96 ± 13	652	−125
Av	737 ± 67	227 ± 13	69 ± 11	173 ± 37	765	− 27
25 (11)	577 ± 28	300 ± 25	66 ± 13	177 ± 25	909	−332

[a] No. of kidneys analyzed is in parentheses.

unlike euthermia, the total papillary solute concentration was markedly greater than U_{osm} in hypothermia (Table III). These observations suggest that the impairment of renal concentrating ability in hypothermia is at least in part attributable to a failure to reabsorb water osmotically in the collecting duct.

When solute excretion is low a marked reduction in GFR *per se* is known to lower urine osmolality by decreasing the delivery of Na salts and urea necessary to maintain hypertonicity of the medullary interstitial fluid (18). Since GFR is reduced in hypothermia by 50 to 60% (Table I), the role of this event in the observed reduction in renal concentrating ability in hypothermia had to be considered. However, the impairment of renal concentrating ability in hypothermia was not accompanied by a reduction in medullary osmotic gradient (Tables II and III).

The fact that the medullary osmotic gradient is maintained in the face of greatly reduced GFR in hypothermia may be attributed to the hypothermic inhibition of solute (Na salts and urea) reabsorption in the proximal tubule, as a result of which the delivery of solute to the ascending limb of Henle's loop for a given GFR is greater in hypothermia than in euthermia. Reabsorption of solutes in ascending Henle's loop may also be decreased in hypothermia, but sufficient amounts of solutes are still reabsorbed (3) to prevent a decrease in the medullary osmotic gradient. Furthermore, the marked reduction in RPF in hypothermia (Table I) could contribute to the maintenance of a relatively high medullary osmotic gradient by preventing the "rinsing effect" of the medullary blood flow (4). Although it is possible that the blood flow to the renal medulla may be more reduced than that to the cortex in hypothermia, no significant difference in PAH titration curve between euthermia and hypothermia was observed (3).

Since water permeability of the collecting duct is largely dependent upon ADH (16, 17, 19, 20), one may postulate that the action of ADH is less effective at low body temperature. In a previous study intravenous administration of ADH failed to increase significantly U_{osm} in hypothermia (3), so that failure of final urine to equilibrate with medullary tissue is not due to lack of endogenous ADH. Since the action of ADH on water permeability is dependent upon the supply of metabolic energy (21–23), it is tempting to speculate that a reduction in renal cellular metabolism during hypothermia may result in a partial loss of ADH action.

Summary. The kidneys were removed from 11 euthermic and 8 hypothermic (average rectal temp. 25°C) dogs and four slices of the tissue from papilla to cortex were taken from each kidney. The Na, K, and urea concentrations of the slice were then determined in slice extracts. Although urine osmolality was significantly reduced in hypothermia, the papillary Na and urea concentrations at a given urine osmolality were markedly higher in hypothermic kidneys than in euthermic, suggesting that the impairment of renal concentrating ability in hypothermia is partly attributable to a failure of the collecting duct urine to osmotically equilibrate with the surrounding media. On the basis of the latter finding, it is postulated that the action of ADH is less effective in hypothermia.

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Pre- and Postnatal Normal Mouse Blood Cell Counts* (32926)

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Blood cell counts at any moment may be affected by the rate of production (hemopoiesis), the rate of release, and the length of survival (1). Following the initial formation of blood islands in the 8–9 gestation day mouse embryo (2), there is accelerated formation of the circulatory system and the blood elements in preparation for the adjustments of birth. Nevertheless, the blood of the newborn mouse is quite different from that of the mature mouse of 2 or 3 months of age, with some elements lacking and others being in overabundance, and there are some species variations (3). Even sex is a factor (4). Most pertinent, possibly, is the fact that there are daily diurnal fluctuations in the blood picture so that any continuing study must be made at the same time of day (5–7). This study was designed to determine at weekly intervals the changes in the blood elements of the postnatal mouse, up to 9 weeks at which time a plateau is generally established. Two additional readings were planned, at 12 and at 24 months to see what progressive aging would do to these same blood elements. Studies were always made at the same time of day.

The CF1 strain of laboratory mouse is a readily accessible mammal whose blood maturing changes can be plotted easily. The prenatal mouse is so small, and its blood volume so inadequate for proper study, that samples are difficult to obtain except toward the period of delivery or after day 17. The postnatal data obtained are statistically significant because they were derived from a minimum of 25 mice of each sex, and the same mice were used for the first 3–9 weeks of study. At 12 months one group of 20, and at 24 months another of 13, was examined, all normal controls.

Materials and Method. For complete blood count: (CBC). Blood from mice of 3 or more weeks of age was taken from the tip of the tail. No anesthetic or restraining of the mouse was necessary. Samples for hemoglobin determination were taken first, then samples for white, and red blood cells, and platelets were taken in that order. (Other techniques are available for older mice (8)).

Taking sufficient blood from mice 2 weeks or younger posed some problems. The newborn mouse was held in the left hand, the index finger pressing the head back and the thumb on the lower abdomen. The prominent jugular vein was cut with very fine scissors and three previously prepared pipettes with rubber tubing could be successively controlled by the free hand. Blood is generally plentiful, rich and homogeneous and any mixing with lymph or tissue fluids seemed to be minimal. The total blood removed is about 0.5 ml.

By 2 weeks of age the mice were so strong

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