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RENAL FUNCTION IN DOGS WITH PYOMETRA

5. SODIUM CONTENT OF THE RENAL MEDULLA IN RELATION TO CONCENTRATING ABILITY

By

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Bitches with pyometra (chronic purulent endometritis) release normal amounts of ADH but are unable to concentrate urine to a normal degree (*Åsheim* 1963). The major explanation of this renal dysfunction seems to be a reduction in the resorbing capacity of the collecting tubules (*Åsheim* 1964). With present knowledge of the intrarenal processes governing urine concentration, the basic causes of a reduction in concentrating capacity are to be sought in 1) reduced hypertonicity in the renal medulla and 2) reduced permeability of the epithelium of the collecting tubules (*Epstein et al.* 1959). Either of these would result in a reduction in the volume of fluid diffusing from the lumina of the collecting tubules to the medullary interstitium and in this manner a reduction in the final concentration of the urine.

The establishment and maintenance of hypertonicity in the medulla results from active resorption of sodium from the loops of Henle and the collecting tubules. The countercurrent multiplier system formed by the loops of Henle makes possible the gradual increase in sodium content from the cortex to the papilla, the "sodium gradient", which is a feature of the normal kidney (*Gottschalk & Mylle* 1959). The sodium gradient can be measured by determining the sodium content in tissue slices taken at various levels in the cortex and medulla. If, as was done in the studies described here, the sodium gradient is compared with the osmolarity of the final urine, it should be possible to evaluate whether

and to what degree reduction in medullary hypertonicity is involved in the reduced concentrating ability known to exist in bitches with pyometra.

MATERIALS AND METHODS

The studies have been carried out on five normal bitches and three bitches with pyometra.

Water was withheld for 21 hours, the animals were injected with ADH during the latter part of this period, and urine and blood samples were taken for determinations of osmolarity. Immediately after these samples were obtained the left kidney was removed under barbiturate anaesthesia. Tissue samples were removed from the cortex and medulla of this kidney for determinations of sodium and water contents.

EXPERIMENTAL DETAILS

The three pyometra bitches, each with an anamnesis of greatly increased thirst, weighed 13, 18 and 21 kg and were 6, 8 and 9 years old. The diagnosis was confirmed by examination of the uterus after hysterectomy. The normal bitches were medium-sized (mean weight 17 kg) and aged from 1 to 3 years.

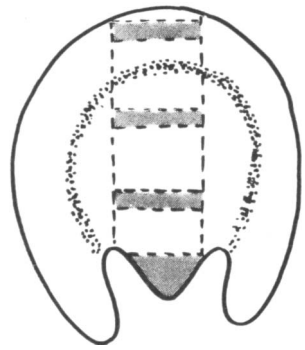
Water (and feed) were withheld. After 18 hours, 5 pressor units pitressin tannate in oil*) were injected i. m. After 20 hours the bladder was catheterised and emptied by manual pressure after the insufflation of air. One hour later another urine sample was taken. Urine osmolarity was determined by freezing-point depression (see Åsheim 1963). Sodium in the urine was determined with an EEL flame photometer.

The animals were anaesthetised with a barbiturate (Mebumal ®) immediately after the last urine sample was obtained. The left kidney was removed through a mid-line abdominal incision; the artery, vein and ureter were exposed and the kidney was removed within about 30 seconds after clamping.

The normal bitches and two of the pyometra bitches were killed by the i. v. injection of Mebumal ® immediately after nephrectomy. The remaining pyometra bitch was ovariohysterectomised and put through the concentration test again 16 days later, the right kidney removed under anaesthesia, and the animal killed.

For uniformity, the sodium and water contents in the kidney tissue were determined in samples taken from the same levels in every dog (Fig. 1). The renal pelvis was opened to expose the papilla and then the kidney was divided into thirds by two transverse cuts to separate the poles from the middle third. The middle third was then

*) Parke, Davis.



F i g. 1. Cross-section through a kidney illustrating the sites of sampling. The dashed lines represent the slice of tissue removed and the shaded areas the sites from which the tissue samples were taken.

sectioned longitudinally by two cuts running from each side of the base of papilla to the capsula. From this slice small tissue samples about 2 or 3 mm thick were taken from the cortex and at three levels of the medulla. Each of these samples were divided into four equal parts by slicing in a corticomedullary direction. Two of the four pieces from each level were used for determining the sodium content (wet ashing in HNO_3 and H_2O_2, EEL flame photometer) and two for water content (heating weighed tissue pieces at 105°C until constant weight was obtained).

The sodium and water contents at each level are given as the mean of the values for the two tissue pieces. The standard error was calculated by taking the difference (d) between the values for each pair of tissue pieces from the normal bitches and applying the formula

$$\sqrt{\frac{\Sigma\, d^2}{2\, n}} \qquad n = \text{no. of value pairs}$$

to give 8.9 mEq per kg tissue water for sodium and 0.9 g H_2O per 100 g wet tissue for water. The mean wet weights for the final tissue samples were 70 mg for the papilla, 125 mg for the inner medulla, 136 mg for the outer medulla, and 158 mg for the cortex.

RESULTS

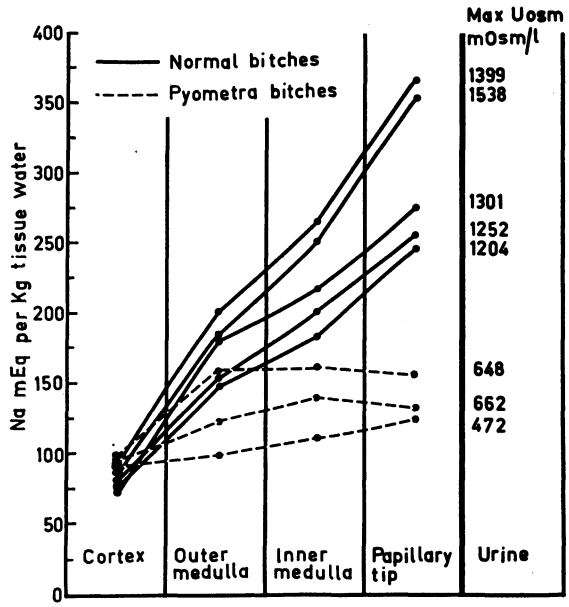
There were no differences in sodium content of the cortical samples from the normal and the pyometra bitches (Table 1). In the normal bitches the sodium content in the *medulla* rose steadily towards the tip of the papilla where it was about four times greater than in the cortex (Table 1). These results accord well with those reported by others for normal dogs (*Ullrich & Jarausch* 1956, *Levitin et al.* 1962). The medullary sodium content

Table 1. Sodium and water contents at different sites in the kidney after dehydration. Max. Uosm and urinary sodium levels in samples obtained immediately before nephrectomy are also listed.

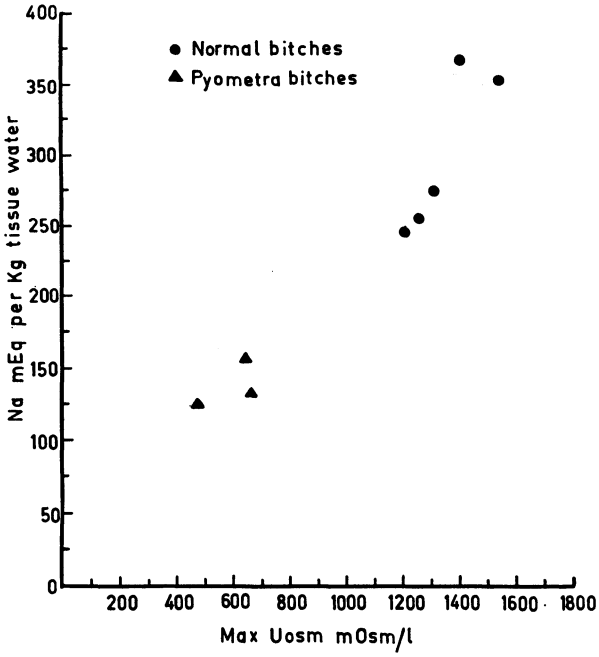
Normal bitches no.	Cortex			Outer medulla			Inner medulla			Papillary tip			Urine		Ratio
	Na mEq per kg tissue water	Na mEq per 100 g dry tissue	H_2O g H_2O per 100 g wet tissue	Na mEq per kg tissue water	Na mEq per 100 g dry tissue	H_2O g H_2O per 100 g wet tissue	Na mEq per kg tissue water	Na mEq per 100 g dry tissue	H_2O g H_2O per 100 g wet tissue	Na mEq per kg tissue water	Na mEq per 100 g dry tissue	H_2O g H_2O per 100 g wet tissue	Max. Uosm mOsm/l	Na mEq/l	Uosm / Nap*
P 18	93	31	77.0	204	124	85.8	265	124	82.4	368	131	78.2	1399	184	3.8
P 19	81	26	76.1	153	82	84.2	208	109	84.0	256	109	80.8	1252	204	4.9
P 20	79	24	75.1	148	70	82.5	182	106	85.3	245	136	84.6	1204	108	4.9
P 21	75	26	77.6	179	111	82.2	217	102	82.4	275	118	81.1	1301	179	4.7
P 25	87	31	78.0	182	99	84.5	251	149	85.4	353	176	83.2	1538	179	4.4
	83**	28	76.8	173	97	83.8	225	118	83.9	299	134	81.6	1339		4.5
	± 7	± 3	± 1.2	± 23	± 22	± 1.5	± 34	± 19	± 1.5	± 57	± 25	± 2.4	± 133		± 0.5
Pyometra bitches no.															
107	94	36	80.7	152	69	82.0	160	84	84.0	156	74	83.0	648	120	4.2
110	77	27	77.9	98	48	82.8	110	59	84.3	124	61	82.9	472	155	3.8
34	90	35	77.8	122	66	84.1	139	89	86.5	132	80	85.3	662	112	5.0
	87	33	78.8	124	61	83.0	136	77	84.9	137	72	83.7	594		4.3
	± 9	± 5	± 1.6	± 27	± 11	± 1.1	± 25	± 16	± 1.4	± 17	± 10	± 1.4	± 106		± 0.6

* Na_p = sodium level at the papillary tip (mEq per kg tissue water).

** Mean ± standard deviation.



F i g. 2. Sodium gradient of normal bitches and of bitches with polydipsia associated with pyometra during dehydration. Max. Uosm immediately before nephrectomy is also indicated.



F i g. 3. Max. Uosm as a function of sodium levels in the papillary tip of normal bitches and of bitches with polydipsia associated with pyometra.

Table 2. Pyometra bitch no. 34. Sodium and water contents at various sites in the kidneys at the time of ovariohysterectomy and 16 days later.

Cortex			Outer medulla			Inner medulla			Papillary tip			Urine		Ratio
Na mEq per kg tissue water	Na mEq per 100 g dry tissue	H_2O g H_2O per 100 g wet tissue	Na mEq per kg tissue water	Na mEq per 100 g dry tissue	H_2O g H_2O per 100 g wet tissue	Na mEq per kg tissue water	Na mEq per 100 g dry tissue	H_2O g H_2O per 100 g wet tissue	Na mEq pr kg tissue water	Na mEq per 100 g dry tissue	H_2O g H_2O per 100 g wet tissue	Max. Uosm mOsm/l	Na mEq/l	Uosm / Nap
90	35	77.8	122	66	84.1	139	89	86.5	132	80	85.3	662	112	5.0
86	29	76.9	228	118	83.8	286	107	79.0	343	127	78.6	1723	169	5.0

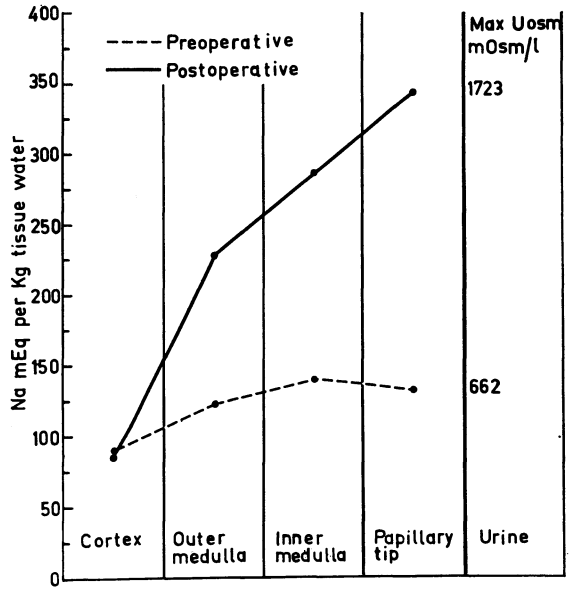


Fig. 4. Sodium gradient and max. Uosm immediately before and 16 days after ovariohysterectomy of pyometra bitch no. 34.

in the pyometra bitches was much lower than in the normal bitches and the differences between the two groups of animals increased towards the papillary tip (Table 1). Two of the pyometra bitches had lower sodium values in the papillary tip than in the inner medulla. The reduction in sodium gradient for the pyometra bitches was as evident when the sodium content was calculated on the basis of dry matter content as when the tissue water content was taken as the basis (Table 1).

The urinary sodium values for the pyometra bitches were in the same range as those obtained for the normal bitches (Table 1). Max. Uosm for the pyometra bitches, however, was greatly reduced. The ratio between max. Uosm and sodium content in the papillary tip kept within the same limits in both groups (3.8—4.9 for the normal bitches, 3.8—5.0 for the pyometra bitches) and the mean ratios for the groups were quite similar (4.5 and 4.3).

The practically linear correlation in both groups between max. Uosm and sodium content in the papillary tip is illustrated in Fig. 3. Similar results were also obtained for the pyometra bitch (no. 34) which was examined on two occasions and at two levels of concentrating ability (before and 16 days after ovariohysterectomy). These results are given in Table 2 and Fig. 4.

DISCUSSION

These studies were designed to find out whether reduced hypertonicity in the medulla or reduced permeability of the collecting tubules or both these in concert can explain the reduced capacity for water resorption of the collecting tubules in the kidneys of bitches with pyometra and, ultimately, the polydipsia.

What emerged from these studies was the inability of the kidneys from bitches with pyometra to maintain the normally high sodium level in the medulla (and the papillary tip). Furthermore, the pyometra bitches, like the normal bitches, displayed a practically linear correlation between the sodium content in the papilla and max. Uosm during antidiuresis. This correlation also held for a pyometra bitch examined at different levels of concentrating ability, i. e. before and after ovariohysterectomy. *Ullrich & Jarausch* (1956) have also demonstrated for normal hydropenic dogs that the final urine concentration is a linear function of the sodium content in the papillary tip.

From the results obtained it appears that reduction in the hypertonicity of the renal medulla is the major cause of the reduced capacity of the collecting tubules for resorption of water, a characteristic exhibited by most bitches with pyometra. If the permeability of the collecting tubules was also reduced, the max. Uosm should have been reduced to a greater extent than the reduction in the sodium content of the papillary tip. This was not the case. In considering the general validity of these results, however, the small number of animals examined has to be taken into account.

A renal dysfunction which has many points of similarity with that seen in pyometra bitches has been demonstrated in dogs with experimentally-induced potassium deficiency (*Smith et al.* 1950, *Manitius et al.* 1960). These dogs also had a reduced sodium gradient in the medulla. Since the reduction in concentrating ability was relatively greater than the reduction in the sodium content in the papillary tip, it was concluded that as well as the reduced sodium gradient a reduction in the permeability of the collecting tubules was also involved in producing the reduced resorption capacity. Experimental potassium deficiency in rats is also associated with a reduced medullary sodium gradient and concentrating ability (*Manitius et al.* 1960) but *not* with a reduction in the permeability of the collecting tubules for water (*Eigler et al.* 1962, *Bray* 1963).

In summing up it seems that the reduced resorption capacity of the collecting tubules in the kidneys of pyometra bitches is mainly the result of a reduction in the sodium gradient in the renal medulla since signs of a major defect in the permeability of the collecting tubules were not demonstrated. In view of the nature of the renal dysfunction in experimentally-induced potassium deficiency, study of the possible pathogenic significance of potassium deficiency for the renal dysfunction associated with pyometra is a logical extension of the results presented here. In fact, results now available for 38 pyometra bitches show a significant drop in plasma potassium levels in comparison with normal values.

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SUMMARY

The tissue sodium content in various parts of the kidney was determined for normal bitches and for bitches with polydipsia in association with pyometra after dehydration for 21 hours and the administration of ADH. The values obtained were plotted against max. Uosm for the same animals.

Under these experimental conditions the normally rising sodium gradient towards the tip of the papilla was less distinct or practically indiscernible in the pyometra bitches. Both the normal and the pyometra bitches, however, displayed a linear correlation between max. Uosm and sodium content in the papillary tip. From these results it appears that reduction in the normal medullary hypertonicity is a major component in the pathogenesis of the reduced capacity of the collecting tubules to resorb water which is seen in pyometra bitches. There did not appear to be any significant reduction in the permeability of the collecting tubules. The possible pathogenic significance of a potassium deficit for the renal dysfunction is discussed.

ZUSAMMENFASSUNG

Die Nierenfunktion bei Hunden mit Pyometra.

5. Natriumgehalt der Nieren bei verminderter Konzentrationskapazität.

Der Verfasser hat bei normalen Hunden und bei Hunden mit Pyometra-Polydipsie-Syndrom, den Na-Gehalt in verschiedenen Nierenteilen nach einer Durstperiode von 21 St. und Zufuhr vom ADH, untersucht. Dabei erhaltene Werte wurden mit max. Uosm bei denselben Tieren verglichen.

Die unter angegebenen Bedingungen normal vorkommende stufenweise Steigerung des Na-Gehaltes in der Richtung gegen den Papillenspitzen (Na-Gradient), war bei den Tieren mit Pyometra weniger ausgeprägt oder fehlte beinahe ganz. Dagegen bestand bei Tieren mit Pyometra beinahe gleiche lineäre Korrelation zwischen max. Uosm und Na-Gehalt in den Papillenspitzen die bei normalen Tieren charakteristisch sind. Das Resultat scheint den Beschluss zu rechtfertigen, dass bei Hunden mit Pyometra eine Verminderung der normalen osmotischen Hypertonizität im Nierenmark, wahrscheinlich eine wesentliche pathogenetische Bedeutung für die verminderte Fähigkeit der Nieren das freie Wasser zurückzuresorbieren, hat. Gleichzeitig jedoch eine hochgradige Permeabilitätsverminderung der Sammelröhren wäre nicht vorhanden. Die eventuelle pathogenetische Bedeutung des K-Defizits beim Entstehen der Funktionstörungen wurde diskutiert.

SAMMANFATTNING

Njurfunktionen hos hundar med pyometra.

5. Njurarnas natriuminnehåll vid nedsatt koncentrationsförmåga.

Hos normala hundar och hos hundar med pyometra-polydipsisyndromet har författaren undersökt Na-innehållet i njurens olika

delar efter en törstperiod på 21 timmar och tillförsel av ADH. De därvid erhållna värdena har jämförts med max. Uosm hos samma djur.

Den under angivna betingelser normalt förekommande successiva stegringen av Na-halten i riktning mot papillspetsen (Na-gradienten) var hos pyometradjuren tydligt mindre utpräglad eller saknades nästan helt. Däremot förelåg det hos pyometradjuren ungefär samma lineära korrelation mellan max. Uosm och Na-halten i papillspetsen som karakteriserar den normala njuren. Resultaten synes motivera den slutsatsen att hos pyometrahundarna en reduktion av den normala osmotiska hypertoniciteten i njurens märg synes spela en väsentlig patogenetisk roll för njurarnas reducerade förmåga till återresorption av fritt vatten medan däremot någon höggradig minskning av samlingsrörens permeabilitet ej synes föreligga. Den eventuella patogenetiska betydelsen av ett K-deficit för uppkomsten av funktionsstörningen diskuteras.

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