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RENAL FUNCTION IN DOGS WITH PYOMETRA

4. MAXIMUM CONCENTRATING CAPACITY DURING OSMOTIC DIURESIS

By

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In bitches with pyometra (chronic purulent endometritis) the concentrating ability of the kidneys is reduced in spite of the presence of antidiuretic hormone (*Åsheim* 1963). Since the reduction in concentrating ability is greater than can be explained by an increase in solute excretion rate per nephron (*Åsheim* 1964), it probably reflects dysfunction in those parts of the nephron where the final concentration takes place.

In the renal medulla an intricate physiological process establishes an osmotic gradient with the greatest osmotic pressure in the tissues of the papillary tip (*Hargitay & Kuhn* 1951, *Wirz et al.* 1951). The osmotic differences result from active resorption of sodium from fluid in the ascending portion of Henle's loop resulting in the fluid being hypotonic during its passage through the loop. In the distal tubules water passes into the interstitium of the cortex which is isotonic with plasma. With maximum antidiuretic effect water passes into the interstitium in such a quantity that an osmotic equilibrium is attained and the fluid which enters the collecting tubules is isotonic with the plasma (*Wirz* 1956, *Gottschalk & Mylle* 1959). Final concentration of the urine takes place in the collecting tubules by resorption of "free" water from the urine to the hypertonic interstitium of the medullary until osmotic equilibrium is attained (*Ullrich et al.* 1955, *Gottschalk & Mylle* 1959). Under physiological conditions, there is resorption capacity in reserve. By inducing an excessive osmotic load, i.v. infusion of mannitol for example, resorption of the glomerular filtrate in the proximal tubules is reduced. The volume of fluid reaching the collecting tubules then increases, and resorption of "free" water attains a maximum.

The present studies are concerned with this maximum resorption capacity in the distal and collecting tubules for "free" water during osmotic loading of bitches with polydipsia in association with pyometra.

Physiology of final urine concentration

Fluid passing from the distal to the collecting tubules is assumed to be isotonic with plasma. The formation of a concentrated urine requires the removal of "free" water during passage through the collecting tubules. The amount of "free" water ($T^c_{H_2O}$) removed in this concentration process is calculated as the difference between urine volume (V) and the volume in which the substances excreted in the urine must be dissolved to give a solution isotonic with plasma (osmolar clearance, Cosm). The formula of *Wesson Jr. & Anslow Jr.* (1952) expresses this as

$$T^c_{H_2O} = \text{Cosm} - \text{V (ml per min.)}$$

$$\text{in which Cosm} = \frac{\text{Uosm} \times \text{V}}{\text{Posm}} \text{ (ml per min.)}$$

Uosm = urine osmolarity (mOsm per litre)
Posm = plasma osmolarity (mOsm per litre)

There is a physiological reserve in the capacity to resorb "free" water. By the continuous infusion of mannitol the volume of fluid reaching the collecting tubules can be increased (osmotic diuresis) to such a degree that the resorption capacity of the collecting tubules is fully engaged provided that sufficient amounts of ADH are released or administered. Under these conditions $T^c_{H_2O}$ gradually attains a constant maximum value ($T^cm_{H_2O}$). Fig. 1 illustrates V as a function of Cosm during osmotic loading in this manner. When $T^cm_{H_2O}$ is attained, the corresponding values for Cosm and V lie on a straight line parallel with the line representing the volume of isosmotic urine at the corresponding Cosm (the isosmotic parameter). The horizontal distance between the two lines represents $T^cm_{H_2O}$.

The line representing the relation between Cosm and V in the final urine can, if the osmotic load is increased, bend and cross the isosmotic line (point of crossover). This implies that the urine, in-

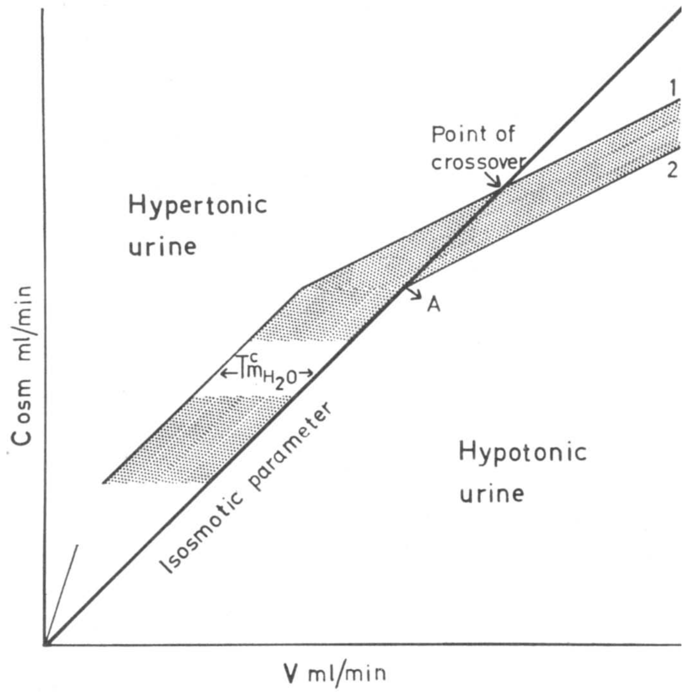

Fig. 1. Line 1 gives the correlation between Cosm and V in the final urine during infusion of mannitol in increasing amounts. The horizontal extension of the shadowed area represents the capacity for free water resorption of the collecting tubules.

For Cosm values below the level of point A the volume of the fluid delivered from the distal tubules is — according to current concepts — isotonic with the plasma i. e. its volume is given by the isosmotic parameter. For Cosm values above the level of "A" the distal tubular fluid is hypotonic and its hypothetical volume is given by line 2. The fluid leaving the distal tubules at these high Cosm values consists — hypothetically — of a) isotonic fluid with a volume represented by the isosmotic parameter, b) a free water component represented by the horizontal distance between the isosmotic parameter and line 2. The free water component of the fluid increases to obscure to an ever-increasing extent the resorption capacity of the collecting tubules. When the free water corresponds to the resorption capacity of the collecting tubules the point of crossover occurs. Beyond this point the final urine becomes hypotonic.

stead of being hypertonic, becomes hypotonic in relation to plasma (Fig. 1). This change is believed to result from the rate of flow through the distal tubules ultimately increasing to such a degree that osmotic equilibrium does not have time to become established with the result that hypotonic fluid passes into the collecting tubules (*de Wardener & del Greco* 1955, *Raisz* et al. 1959, *Kleeman* et al. 1960). Theoretically, this hypotonic fluid can be divided into two components — one isotonic with plasma and the other representing "free" water. At osmotic loads of this magnitude the capacity of the collecting tubules for resorption will partly be occupied with the "free" water component of the fluid delivered. This reduces the over-all results of the concentrating processes and means that the line in Fig. 1 representing the relation between Cosm and V in the final urine (line 1) is deflected towards the isosmotic parameter. As the osmotic diuresis steadily increases, the resorption capacity of the collecting tubules will — at a certain value of Cosm — be fully occupied with "free" water, i. e. the point of crossover has been reached to give a final urine isotonic with plasma. If the osmotic load is increased still further, the final urine will be hypotonic with respect to the plasma.

In consideration of the discussion below it should be pointed out that even a *constant* hypotonicity of the fluid delivered to the collecting tubules will result in a deviation of the line 1 in Fig. 1. This takes place because the absolute values of the "free" water component of the delivered hypotonic fluid will increase with increasing osmotic diuresis. The only condition that will give parallelism between line 1 and the isosmotic parameter is isotonicity of the fluid delivered to the collecting tubules.

CALCULATIONS

$T^c m_{H_2O}$ ought to be expressed in relation to the functioning renal mass. The glomerular filtration rate (GFR) can be used as a measure of the functional renal mass. In pyometra bitches, however, there is a tendency towards a reduction in the filtration fraction (FF) to give values for $T^c m_{H_2O}$ per 100 GFR somewhat *greater* than the actual functional activity in the distal portion of the nephron (*Åsheim* 1963). For this reason, $T^c m_{H_2O}$ has also been calculated in relation to body surface area. Since the body surface is not tied to a reduction in renal mass, the values for $T^c m_{H_2O}$ calculated in this manner will be somewhat *smaller* than the actual functional activity in the distal nephron if the functioning renal mass is reduced.

In experiments on normal dogs $T^c m_{H_2O}$ per 100 GFR has been given as 4.6 ± 1.5 ml/min. (*Goldsmith et al.* 1961). In dogs the GFR per m^2 body surface is about 80 ml/min; recalculation of the value given by *Goldsmith et al.* on the basis of body surface, would give a $T^cm_{H_2O}$ of about 3.7 ml/min./m².

According to the studies by *Goldsmith et al.* (1961), the point of crossover can be reached at Cosm values as low as 20 ml/min. This result could not be verified in the experiments to be described below; in normal dogs Cosm values of up to 53 ml/min./m² were produced by infusing mannitol without the urine becoming hypotonic. In view of the results of *Goldsmith et al.,* however, values for the point of crossover greater than 25 ml/min./m² or 30 ml/min./100 GFR will be considered normal.

In this paper, however, $T^cm_{H_2O}$ will be used to refer to the maximum value for resorption of "free" water ($T^c_{H_2O}$) whether or not a linear correlation existed between Cosm and V during the experiment. As will be seen later, there was no linear correlation in some of the pyometra bitches.

MATERIALS AND METHODS

The test animals, 10 normal bitches and 17 bitches with pyometra, are part of a series which has been described previously (*Åsheim* 1964). Details of the techniques employed have also been described. For these studies the animals were dehydrated and ADH administered. Continuous infusion in steadily increasing amounts 5 or 10 per cent mannitol solution with 100 mEq NaCl added per litre gave diuresis of sufficient magnitude for maximum utilization of the capacity for resorption of "free" water and permitted calculation of $T^cm_{H_2O}$.

Ovariohysterectomy was performed after the renal function studies were completed. The function studies were repeated on nine of the animals at different times after operation.

RESULTS

Table 1 contains the $T^cm_{H_2O}$ values for the ten normal bitches. The mean $T^cm_{H_2O}$ $\pm$ standard deviation was 4.9 ± 1.1 ml/min./m² body surface or 6.1 ± 1.1 ml/min./100 GFR. In all these experiments the relation between Cosm and V formed a straight line parallel or nearly parallel with the isosmotic parameter (Fig. 2). None of the normal bitches excreted a hypotonic urine in spite of the heavy osmotic load which in two experiments (one of these is illustrated in Fig. 2) gave Cosm values of 45 and 53 ml/min./m².

Table 1. Maximum resorption capacity ($T^c m_{H_2O}$) and the point of crossover in normal bitches during the infusion of mannitol. The maximum osmotic U/P ratio represents the value obtained after dehydration for 24 hours before infusion was begun.

Dog no.	Surface area m^2	No. of experimental periods	C In * ml/min. per m^2	$T^c m_{H_2O}$ ml/min.		Point of crossover		Max. osmotic U/P ratio
				per m^2	per 100 GFR	per m^2	per 100 GFR	
F 7	0.79	14	72	4.3	6.0	> 25	> 30	4.7
F 8	0.84	14	88	6.3	7.2	> 25	> 30	5.4
F 9	0.98	12	52	3.0	5.8	> 25	> 30	6.9
F 12	0.75	10	83	4.8	5.8	> 25	> 30	5.6
P 9	0.90	11	97	6.1	6.3	> 25	> 30	4.9
P 12	1.03	12	97	4.3	4.4	> 25	> 30	5.2
P 15	0.61	12	67	5.9	7.7	> 25	> 30	4.9
P 16	0.78	14	55	3.8	7.0	> 25	> 30	5.8
P 17	0.77	13	101	4.7	4.6	> 25	> 30	5.3
P 18	0.74	19	87	5.5	6.3	> 25	> 30	6.2
		Mean**	80 ± 18	4.9 ± 1.1	6.1 ± 1.1			5.5 ± 0.7

* Mean C_{In} for all periods in each experiment.
** Mean ± standard deviation.

Table 2. Maximum resorption capacity ($T^c m_{H_2O}$) and the point of crossover in pyometra bitches during the infusion of mannitol. The maximum osmotic U/P ratio represents the value obtained after dehydration for 21 hours before infusion was begun.

Dog no.	Surface area m^2	No. of experimental periods	CIn * ml/min. per m^2	$T^c m_{H_2O}$ ml/min.		Point of crossover		Max. osmotic U/P ratio
				per m^2	per 100 GFR	per m^2	per 100 GFR	
10	0.74	8	86	1.3	1.5	11	12	3.6
12	0.70	11	79	0.8	1.0	2	4	3.6
13	1.04	12	61	1.3	2.1	5	8	3.5
16	0.51	10	61	1.5	2.5	—	—	3.1
20	1.07	14	79	1.3	1.6	5	6	3.0
29	0.54	8	35	0.4	1.1	6	17	2.6
30	0.63	13	92	2.0	2.1	16	18	2.3
31	1.34	11	74	1.6	2.2	—	—	2.4
32	0.67	11	80	1.6	2.0	12	16	2.3
33	0.63	11	32	1.3	3.3	8	25	2.3
39	0.47	11	41	1.0	2.4	13	29	1.9
40	0.90	11	62	0.8	1.3	4	5	1.9
44	0.64	10	22	0.3	1.4	7	30	1.4
46	0.73	10	27	0.6	2.2	5	22	1.4
47	0.43	7	14	0.1	0.8	1	8	1.4
50	1.13	12	50	1.0	1.6	3	6	1.1
52	0.91	11	58	0.1	0.1	1	2	1.0
		Mean**	56 ± 24	1.0 ± 0.6	1.7 ± 0.8			2.2 ± 0.8

* Mean C_{In} for all periods.
** Mean ± standard deviation.

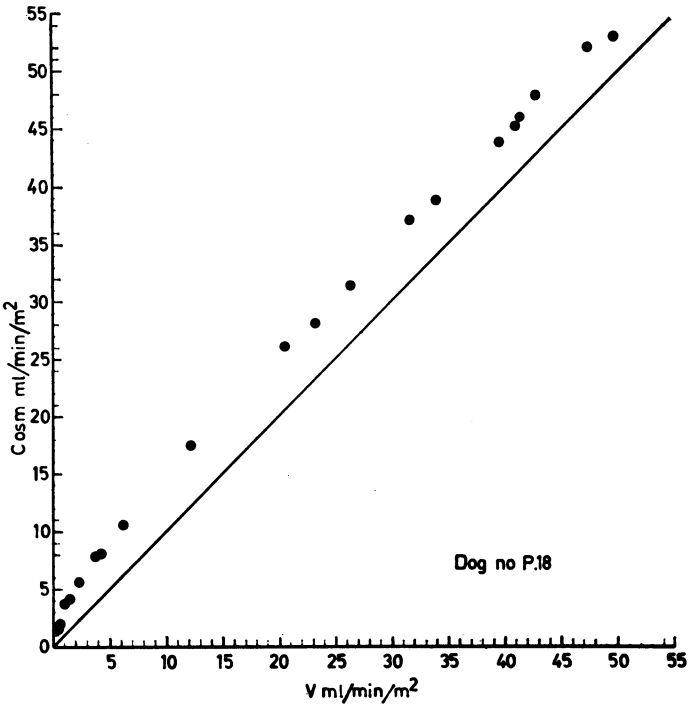

Fig. 2. Correlation between Cosm and V for a normal hydropenic bitch during the infusion of mannitol and the administration of ADH.

For comparison with $T^c m_{H_2O}$. Table 1 also contains the maximum values for osmotic U/P ratio obtained during the dehydration period before the infusion of mannitol. Mean maximum U/P ratio for the normal dogs was 5.5 ± 0.7.

Pre-operative $T^c m_{H_2O}$ values for the pyometra bitches, mean 1.0 ± 0.6 ml/min./m² or 1.7 ± 0.8 ml/min./100 GFR, were invariably lower than the lowest normal value (Table 2). The maximum osmotic U/P ratio was also reduced to give a mean of 2.2 $\pm$ 0.8.

In all instances in which the studies could be completed, the value for Cosm at which urine became hypotonic, the point of crossover, was invariably lower (Table 2) than the Cosm values obtained for normal bitches without causing deviation of the Cosm-V line. Two of the studies on pyometra bitches had to be interrupted before the Cosm reached values which would make the urine hypotonic. Fig. 3 A-F illustrates the relation between Cosm and V on six of the animals included in Table 2. These six

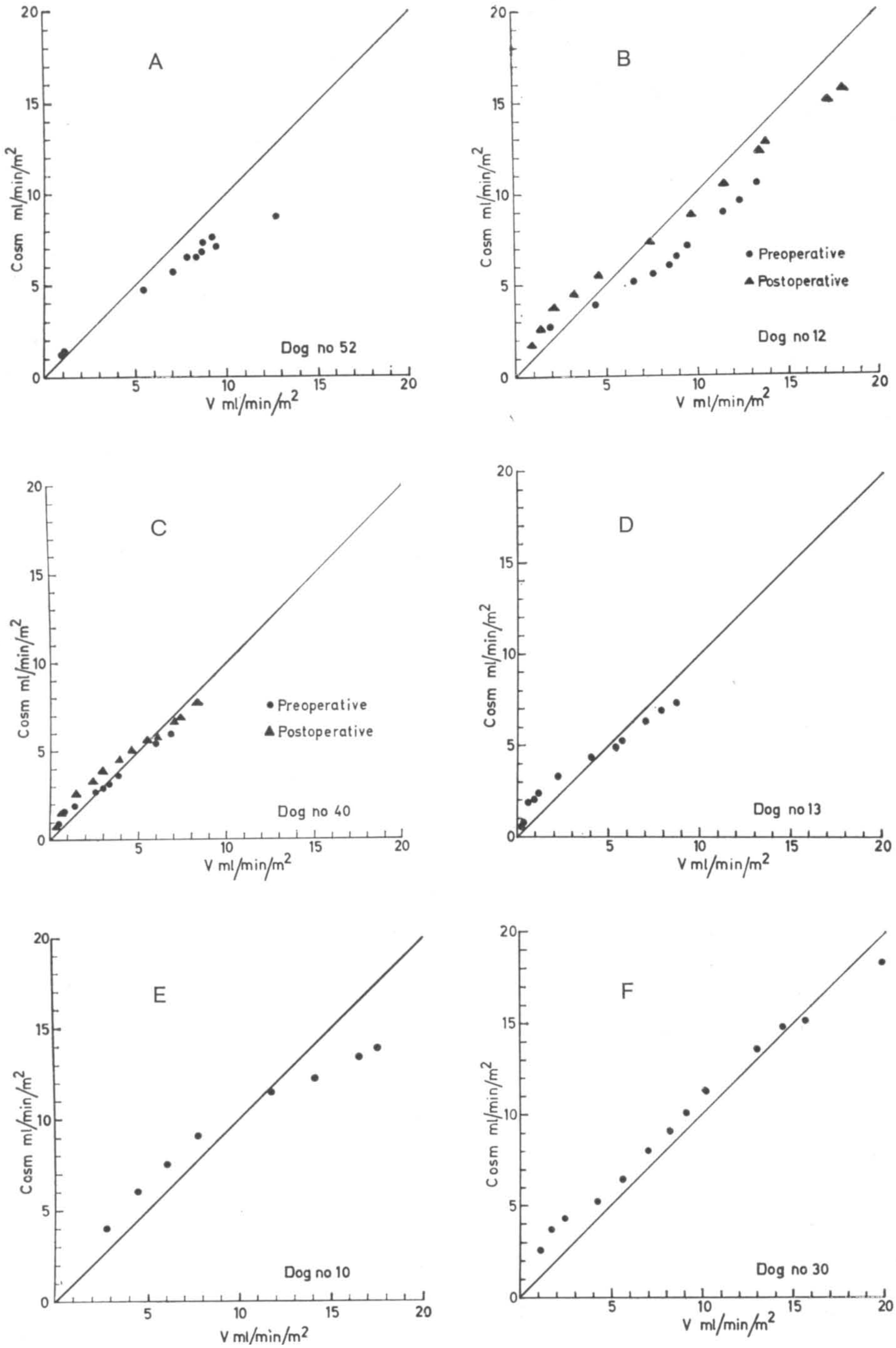

Fig. 3 A—F. Cosm and V for hydropenic pyometra bitches during the infusion of mannitol in increasing amounts and the administration of ADH (Table 2). In Figs. 3 B and 3 C are given both preoperative and postoperative results.

Table 3. Maximum resorption capacity ($T^c m_{H_2O}$) and the point of crossover in pyometra bitches during the infusion of mannitol before and after ovariohysterectomy. The maximum osmotic U/P ratio represents the value obtained after dehydration for 21 hours before infusion was begun.

Dog no.	Surface area m^2	No. of days after ovario-hysterectomy	No. of experimental periods	CIn ml/min. per m^2	$T^c m_{H_2O}$ ml./min.		Point of crossover ml/min.		Max. osmotic U/P ratio
					per m^2	per 100 GFR	per m^2	per 100 GFR	
10	0.74	pre-op.	8	86	1.3	1.5	11	12	3.6
	0.74	12	11	100	4.4	4.4	15	15	4.3
12	0.70	pre-op.	11	79	0.8	1.0	2	4	3.6
	0.70	14	12	77	1.8	1.8	7	9	3.6
20	1.07	pre-op.	14	79	1.3	1.6	5	6	3.0
	1.08	49	16	44	2.4	5.4	10	22	4.6
29	0.54	pre-op.	8	35	0.4	1.1	6	17	2.6
	0.51	35	12	53	2.3	4.3	25	30	4.4
30	0.63	pre-op.	13	92	2.0	2.1	16	18	2.3
	0.64	44	11	65	1.8	2.3	17	21	2.5
31	1.34	pre-op.	11	74	1.6	2.2	—	—	2.4
	1.34	13	12	69	5.3	7.7	—	—	4.0
33	0.63	pre-op.	11	32	1.3	3.3	8	25	2.3
	0.62	41	13	61	3.8	6.1	—	—	4.2
40	0.90	pre-op.	11	62	0.8	1.3	4	5	1.9
	0.86	12	12	64	1.6	2.6	6	9	2.0
46	0.73	pre-op.	10	27	0.6	2.2	5	22	1.4
	0.72	12	10	42	1.2	3.1	—	—	2.7
	0.72	27	10	47	2.6	6.3	17	30	3.1
	0.74	65	11	39	2.8	7.0	18	30	3.4

bitches were chosen as examples of different degrees of renal dysfunction. In the experiment represented in Fig. 3 A, the urine became hypotonic under a slight osmotic load. The other curves in Fig. 3 demonstrate that the point crossover can occur at different values for Cosm.

In those instances in which the calculated point of crossover occurred at low values, so few observations could be made prior to that point that it was impossible to demonstrate a linear correlation between Cosm and V (Fig. 3, A-C). When the point of crossover occurred at higher values, enough observations were made to establish a graphical linear correlation between Cosm and V which was nearly parallel with the isosmotic parameter (Fig. 3, D-F).

There was no obvious correlation between the value for $T^{c}m_{H_2O}$ and the value for the point of crossover. Fig. 3 F illustrates how the point of crossover occurred at a relatively high value for Cosm although $T^{c}m_{H_2O}$ was greatly reduced.

Most of the bitches which were re-examined after ovariohysterectomy had an increase in $T^{c}m_{H_2O}$ and displacement of the point of crossover towards a greater osmotic load (Table 3). In Figs. 3 B and 3 C, curve I represents the pre-operative results and curve II the post-operative results. Both pre-operative results showed such a rapid changeover from hypertonic to hypotonic urine that no linear correlation between Cosm and V could be demonstrated. Post-operatively, the point of crossover occurred at higher values so that a line representing the correlation between Cosm and V could be demonstrated. The line obtained does not seem to deviate greatly from the isosmotic parameter.

The post-operative increase in the max. osmotic U/P ratio resulting from the increase in $T^{c}m_{H_2O}$ is shown in Table 3. In the animals with little or no increase in $T^{c}m_{H_2O}$ the concentrating capacity of the kidneys has remained depressed.

DISCUSSION

Osmotic loading of the pyometra bitches gave wide individual differences in the relation between Cosm and V. In some bitches, for example those illustrated in Figs. 2 E and 2 F, this relation can be represented by a straight line which is probably parallel with the isosmotic parameter. From what has been said in the section of this paper dealing with the physiology of concentration, it can be assumed that this state of parallelism means that the urine flowing from the distal to the collecting tubules is isotonic with plasma. Under these conditions the horizontal distance between the two lines ($T^{c}m_{H_2O}$) is a measure of the resorption of "free" water in the collecting tubules. Consequently the reduction in $T^{c}m_{H_2O}$ which could be demonstrated in some pyometra animals fulfilling these prerequisites implies that the ability of the collecting tubules to resorb "free" water is reduced.

If the osmotic load for the pyometra animals is increased still more, the final urine will ultimately be hypotonic. Since it is not known that the collecting tubules can resorb solute in an excess of water, a prerequisite for the formation of a hypotonic final urine is that the fluid passing from the distal tubules to the collecting tubules is hypotonic in relation to plasma (*Goldsmith et al.* 1961). This state can probably occur in normal animals under a relatively heavy osmotic load. The most probable explanation for hypotonic urine entering the collecting tubules from the distal tubules during slight osmotic diuresis — as is the case in pyometra animals — is that the distal tubules are incapable, in spite of maximum ADH influence, of increasing their permeability for water to the same extent as in normal bitches (*Orloff et al.* 1958, *Buchborn et al.* 1959).

The lack of an obvious correlation between $T^{c}m_{H_2O}$ and the value for Cosm which corresponds to the point of crossover indicates that reduction in permeability in the distal tubules is not necessarily paralleled by changes in the resorption capacity of the collecting tubules.

In some of the pyometra bitches hypotonic urine was formed under only a very slight osmotic load. In these animals there is often no linear correlation between Cosm and V. According to the discussion above this makes it impossible to know whether the urine delivered by the distal tubules to the collecting tubules is hypotonic even in a very early stage of the experiment. The values obtained for $T^{c}m_{H_2O}$ in these animals, then, cannot be accepted as an accurate measure of water resorption in the collecting tubules because the resorption taking place here can be partially obscured by hypotonicity of the fluid delivered by the distal tubules. It is conceivable that in these instances a slower increase in the rate of mannitol infusion of these animals would result in curves in which the relation between Cosm and V would be linear and parallel with the isosmotic parameter. Should that be the case it would be possible to distinguish between dysfunction of the distal tubules and dysfunction of the collecting tubules in these animals.

The results obtained in these studies suggest that the reduction in concentrating ability of bitches with pyometra results from

1) reduced permeability for water in the distal tubules, and
2) reduced resorption of water from the collecting tubules.

Renal dysfunction which — like the dysfunction described for the pyometra bitches — is characterized by reduced permeability for "free" water in the distal tubules and reduced resorption of water from the collecting tubules can be induced experimentally in dogs by a potassium deficit (*Giebisch & Lozano* 1959, *Manitius et al.* 1960). There was a significant drop in plasma potassium levels, a sign of potassium deficit, in a series of bitches with polyuria and polydipsia in association with pyometra; these results will be published in another context. Abnormal potassium metabolism, then is possibly involved in the pathogenesis of the renal dysfunction which often accompanies pyometra.

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SUMMARY

The ability of the kidneys to conserve water during osmotic loading with mannitol has been studied in bitches with pyometra associated with polyuria and polydipsia. The results were compared with those obtained for normal bitches.

All the pyometra bitches had a reduced concentrating ability. From the values obtained it appears that this dysfunction results from reduced permeability for water in the distal tubules and reduced resorption of "free" water by the collecting tubules.

The concentrating ability usually improved after ovariohysterectomy. The improvement apparently resulted from increased permeability in the distal tubules and greater resorption of water by the collecting tubules.

Abnormal potassium metabolism in possibly involved in the reduction of renal concentrating ability in bitches with pyometra.

ZUSAMMENFASSUNG

Die Nierenfunktion bei Hunden mit Pyometra.

4. Die maximale Konzentrationskapazität bei osmotischer Diurese.

Der Verfasser hat bei Hunden die an Polyurie und Polydipsie infolge einer Pyometra gelitten haben, die Fähigkeit der Nieren die unter osmotischer Belastung durch Mannitol standen, das Wasser reabsorbieren zu können, untersucht. Die Resultate wurden mit den Werten die an normalen Hunden unter gleichen Versuchsbedingungen erhalten wurden, verglichen.

Bei allen untersuchten Hunden mit Pyometra, war die Konzentrationsfähigkeit vermindert. Durch eine nähere Untersuchung der Versuchsresultaten wurde hervorgebracht, dass die mangelnde Konzentrationsfähigkeit die für die Nierenfunktion bei Pyometra charakteristisch ist, entsteht teils durch die verminderte Permeabilität für

Wasser in den distalen Tubuli, teils durch die verminderte Reabsorptionskapazität vom freien Wasser aus dem Sammelrohr.

Untersuchungen die nach einer Hysterektomie durchgeführt wurden zeigten, dass sich in der Regel die Konzentrationsfähigkeit verbessert hat. Diese Verbesserung beruht wahrscheinlich teils auf einer erhöhten Permeabilität in den distalen Tubuli, teils auf einer erhöhten Reabsorption vom Wasser im Sammelrohr.

Zuletzt wurde die Möglichkeit einer Störung im Kaliumstoffwechsel die von Bedeutung wäre für die verminderte Konzentrationsfähigkeit der Nieren bei Pyometra, diskutiert.

SAMMANFATTNING

Njurfunktionen hos hundar med pyometra.

4. Maximala koncentrationskapaciteten vid osmotisk diures.

På hundar med polyuri och polydipsi i samband med pyometra har författaren studerat njurarnas förmåga att resorbera vatten under osmotisk belastning med mannitol och under tillförsel av ADH. Resultaten har jämförts med de värden som erhållits på normala hundar under samma försöksbetingelser.

Hos samtliga undersökta pyometrahundar var koncentrationsförmågan nedsatt. En närmare analys av försöksresultaten talar för att den bristande koncentrationsförmåga, som karakteriserar njurfunktionen vid pyometra, betingas av dels en minskad permeabilitet för vatten i distala tubuli, dels en minskad resorptionskapacitet för fritt vatten från samlingsrören.

De undersökningar som utförts efter företagen hysterectomi visar att koncentrationsförmågan i regel förbättras postoperativt. Förbättringen synes bero på dels en ökad permeabilitet i distala tubuli, dels en ökad återresorption av vatten i samlingsrören.

Slutligen diskuteras möjligheten av att en störning i kaliumomsättningen kan vara av betydelse för njurarnas nedsatta koncentrationsförmåga vid pyometra.

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