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RENAL FUNCTION IN DOGS WITH PYOMETRA

1. STUDIES OF THE HYPOTHALAMIC-NEUROHYPOPHYSEAL SYSTEM

By

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Polydipsia is often noticed in bitches with pyometra (*Dow* 1959, *Rieck* 1961).

Talanti (1959) attempted to ascertain the cause of this polydipsia by histochemical examinations for the presence of neurosecretory material in the hypothalamic-neurohypophyseal system of 6 bitches with pyometra. He found that in some parts of the system the amount of neurosecretory material was reduced in comparison with that present in normal dogs. One possible explanation advanced was that this change in the neurosecretory system represented a reduction in the formation and release of the antidiuretic hormone to such a degree that a state of diabetes insipidus resulted.

The purpose of the studies reported here was to study the formation and release of antidiuretic substances in bitches with polydipsia associated with pyometra. The study falls into two main parts.

A. Biological tests for the presence of antidiuretic substances in the urine.

B. Histochemical examination of the hypothalamic-neurohypophyseal system for the presence of neurosecretory substance.

The dogs examined were selected from a clinical material on the basis of clinical signs of pyometra (chronic purulent endometritis) — enlarged uterus during metoestrus and polydipsia among others. The diagnosis was confirmed histologically after ovariohysterectomy.

A. BIOLOGICAL TESTS FOR ANTIDIURETIC SUBSTANCES

Materials and methods

Urine was available from 4 bitches with polydipsia in association with pyometra. According to the anamneses, water consumption was 3 to 5 times greater than normal. The animals were dehydrated by withholding water for 18 to 21 hours. One hour before the end of the dehydration period the bladder was thoroughly emptied by catheterisation after the insufflation of air. The urine for the last hour was collected and measured. One-quarter of the urine, *i. e.* the amount produced during 15 minutes, was used in a biological test to ascertain the amount of andidiuretic hormone (ADH) contained.

The biological tests were carried out on 2 dogs which had developed persistent polydipsia and polyuria after section of the pituitary stalk (*Aschner* 1912). When water consumption had levelled off about 3 weeks after the operation, dog F 1 (16 kg.) drank 4 to 5 litres daily and dog P 10 (18 kg.) drank 2 to 3 litres daily. Fourteen days before being used in the tests, *i. e.* 7 and 2 months after operation, concentration tests were carried out. After 18 hours without water the osmolarity of the urine (Uosm) for F 1 was 259 mOsm/l. and for P 10 633 mOsm/l. Each dog then received an i. m. injection of 5 pressor units pitressin tannate in oil[1]) (PTO). The dehydration period was continued and the bladder emptied each hour for 3 hours. The maximum Uosm values recorded were 725 mOsm/l. for F 1 and 1158 mOsm/l. for P 10.

The dogs had been trained to stand quietly without any sedation in a sling specially constructed for the tests. The day before the tests were carried out polythene catheters were inserted into a vein and into the femoral artery using the method described previously (*Helander et al.* 1958). The dogs were starved for the 12 hours preceding the test. The experiment was divided into periods of 5 minutes each; the periods before the actual injection of the test substances will be referred to as control periods. A permanent Foley catheter was introduced into the bladder and urine collected in graduated vessels. To ensure complete emptying, air was injected and the bladder compressed manually through the abdominal wall. All material for assay was injected intravenously. A standardised vasopressin, Pitressin®[1]) dissolved

[1]) Parke, Davis.

in physiological saline solution immediately before use served as comparison material. The dogs were allowed free access to water until one hour before the experiments began. They then received water through a stomach tube in an amount corresponding to 25 ml./kg. The positive water balance attained in this way was maintained by the intravenous injection of 3 per cent glucose solution at a rate sufficient to result in the formation of about 5 ml. urine per minute during the control periods. During the course of the experiment the amount of fluid injected was adjusted to correspond approximately to the urine volume.

After 3 or more control periods pitressin in a dose of 0.2 mU/kg. bodyweight was injected. When the urine volume had returned to the values for the control periods the test urine was injected. The experiments were arranged so that when the bladder of a dehydrated polydipsic dog was emptied for the last time the assay dog was ready to be injected with the urine. In no instance did more than 30 minutes elapse between collection of the urine and its injection. Prior to injection the urine was mixed with physiological saline solution to a final volume of 20 ml. The experiment was concluded when the urine volume for the experimental periods returned to the initial values.

During the experiments 4 to 6 samples of heparinised arterial blood were taken (1 mg. heparin, 10 ml. blood).

Inulin was injected to determine the glomerular filtration rate (GFR). An initial dose of 40 mg. per kg. bodyweight was given. Infusion of inulin was then begun at least 45 minutes before the first control period at a rate estimated to maintain a plasma level of about 20 mg./100 ml.

The inulin and sodium levels as well as osmolarity were determined for the blood and urine samples. The method for inulin determination was *Josephson & Godin*'s (1943) modification of *Corcoran & Page*'s (1939) method. Sodium levels were determined by flame photometry (EEL) and osmolarity by freezing-point depression using a thermistor coupled to a Wheatstone bridge. All values are given as the mean of double determinations. Coefficients of variation of 0.22 to 0.38 per cent were obtained from 15 determinations on each of four standard solutions containing 100, 300, 500 and 750 mOsm/l. respectively.

Osmotic clearance (Cosm) and free water clearance (C_{H_2O}) were calculated from the formulae

$$Cosm = \frac{Uosm \cdot V}{Posm} \qquad C_{H_2O} = V - Cosm$$

in which Cosm and C_{H_2O} are expressed in ml./min. and Uosm (mOsm/l.) represents the urine osmolarity, Posm (mOsm/l.) the plasma osmolarity and V (ml./min.) the rate of urine formation. C_{H_2O} expresses the volume (ml.) of water which must be removed from the hypotonic urine formed during a minute so that the urine will become isotonic with plasma.

Results

All 4 polydipsic dogs had a distinct rise in plasma osmotic pressure (Posm) in relation to initial values as a sign of the dehydration which developed during the 18 to 21 hours without access to water (Table 1). The urine samples collected at the conclusion of the thirst period produced in each instance an antidiuretic effect when injected into the test dogs (Table 1). The urine sample from one animal representing the amount formed in 15 minutes, had a greater antidiuretic effect than the standard and the samples from the other 3 animals had an effect roughly comparable with that of the standard. There were no significant changes in Cosm.

Table 1. Injection of pitressin standard (ADH) and urine from dehydrated polydipsic dogs with pyometra into test dogs with experimental diabetes insipidus.

Pyometra bitches			Test dogs				
	Posm			Minimum Uosm during control period	Maximum Uosm		Amount ADH injected mU
No.	before dehydration	after dehydration	No.		injection of ADH	injection of urine	
261	309	316	F 1	59	188	192	3.2
297	293	306	F 1	67	165	157	3.2
1415	294	305	P 10	58	128	108	3.6
1453	276	294	P 10	61	153	316	3.6

Sodium excretion increased in each instance after the injection of pitressin. Two of the urine samples injected gave a similar or even greater increase in sodium excretion. The other two did not cause any obvious deviations from the control values for sodium excretion.

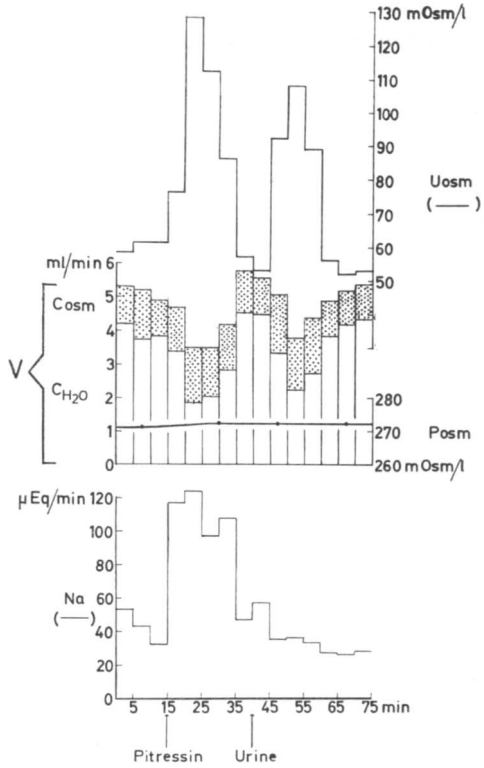

Fig. 1. Effects of the injection of ADH (pitressin 0.2 mU/kg.) and urine (15 min. sample) from a polydipsic dog into a test dog with experimental diabetes insipidus.

The upper diagram represents the changes in urine osmolarity (Uosm), plasma osmolarity (Posm), and urine flow (V). V is the sum of osmolar clearance (Cosm) and free water clearance (C_{H_2O}). The lower diagram represents the changes in sodium excretion (Na).

GFR was practically constant during one of the experiments and rose somewhat during the course of the other three.

The results of an experiment (dog no. 1415) are illustrated in Fig. 1. The oral dose of water and the infusion of 3 per cent glucose i. v. resulted in an urine flow of about 5 ml./min. during the control periods. The injection of pitressin produced a marked reduction in urine flow and at the same time an increase in Uosm to a value of 128 mOsm/l. As the effects of pitressin regressed the test urine was injected. This resulted in a sharp reduction in urine flow and an increase of Uosm to 108 mOsm/l. The anti-diuretic effect of the urine injection was somewhat less than that

of the pitressin standard. There were only slight variations in solute excretion (Cosm) during the experiment. Sodium excretion increased strongly after the injection of pitressin but was practically unaffected by the injection of urine. GFR rose during the course of the experiment from 56 to 71 ml./min./m^2 body surface.

B. HISTOCHEMICAL EXAMINATION OF THE HYPOTHALAMIC AND NEUROHYPOPHYSEAL SYSTEM

Materials and methods

Four other bitches with polydipsia associated with pyometra were examined. According to the anamneses 2 of the bitches drank about 5 times and 2 about twice the normal amount of water. All bitches were subsequently autopsied and the diagnosis confirmed.

The bitches were killed with Mebumal® (barbiturate) given intravenously and immediately decapitated. The head was perfused through the carotid arteries with Ringer's solution followed by Bouin's fixative. The hypothalamus together with the hypophysis was embedded in paraffin and 5 μ-thick serial sections cut. The sections were stained with *Gomori*'s (1941) chrome haematoxylin phloxine as modified by *Bargmann* (1950). Corresponding sections from 2 normal dogs were prepared in the same way for comparison.

Results

Material stainable by Gomori's method was present in the cells of the neurosecretory hypothalamic nuclei of the dogs with polydipsia (Fig. 2) to about the same extent as in the corresponding sections from normal dogs (Fig. 4). The neurohypophysis also contained a fairly abundant amount of Gomori-positive material, some of it in typical Herring bodies (Fig. 3).[1])

[1]) I should like to thank Dr. *Bengt Andersson* for his help in preparing the diabetes insipidus dogs and in evaluating the histological sections.

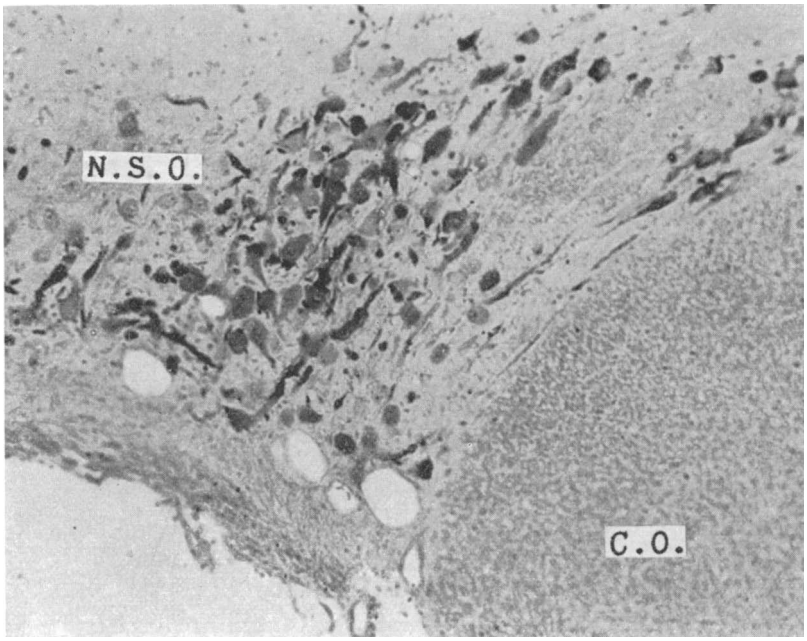

Fig. 2. The anterior supraoptical nucleus (N.S.O.) and adjacent structures (optic chiasma, C.O.) from a bitch with polydipsia associated with pyometra. Sagittal section. Abundant intracellular Gomori-positive material. Gomori's chrome haematoxylin phloxine stain. 5μ. x 160.

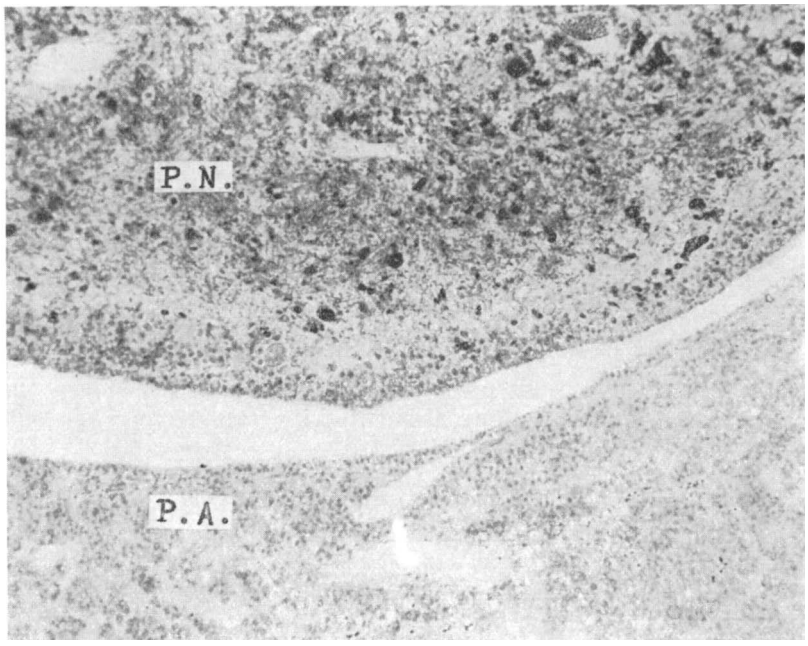

Fig. 3. Sagittal section through the neurohypophysis of the same bitch as in fig. 2 (pars neuralis = P.N., pars anterior = P.A.). Fairly abundant Gomori-positive material, some of it as Herring bodies. Gomori's chrome haematoxylin phloxine stain. 5μ. x 100.

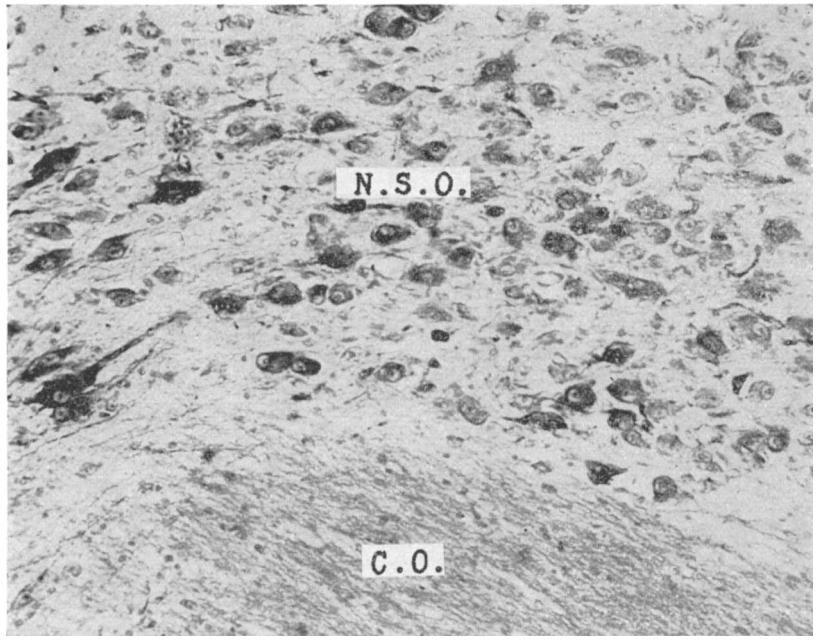

Fig. 4. The anterior supraoptical nucleus (N.S.O.) and adjacent structures (optic chiasma, C.O.) from a normal dog. Horizontal section. Abundant intracellular Gomori-positive material. Gomori's chrome haematoxylin phloxine stain. 5μ. x 160.

DISCUSSION

All urine samples from the dehydrated polydipsic dogs had an antidiuretic effect when injected intravenously into dogs with experimental diabetes insipidus. When evaluating this observation there are also factors to be taken into account which can alter the concentrating capacity of the kidneys but which are not caused by variations in the amount of circulating ADH. These are

1. changes in the solute excretion rate (*Berliner et al.* 1958, *Kleeman et al.* 1960).
2. changes in the glomerular filtration rate (*Levinsky et al.* 1959).

Since both GFR and Cosm remained fairly constant during the experiments neither is likely to have influenced the antidiuretic effect. The antidiuretic effect observed, then, must be ascribed to ADH in the injected urine (*Ames et al.* 1950, *Thorn* 1958).

The concentrating ability of normal kidneys is affected by even very small doses of ADH. *Hare et al.* (1941) demonstrated on dogs with experimental diabetes insipidus that the intravenous injection of 0.1 mU ADH had a measurable antidiuretic effect. Under the same experimental conditions and using medium-sized dogs (12—20 kg.) *Hare et al.* (1945) obtained a distinct effect with 0.5 mU and a maximum antidiuretic effect with 3.0 mU ADH given intravenously. *Shannon* (1942) obtained maximum antidiuresis in dogs weighing 10—15 kg. with 5 mU ADH per hour intravenously. His results coincide with those of *Verney* (1947) who found that 1.17 μU ADH intravenously per second, or about 4.2 mU per hour, gave strong antidiuresis.

The urine from the polydipsic dogs had an antidiuretic effect which in one instance was greater and in the other 3 the same or slightly less than the effect of the pitressin standard. This standard was given as 0.2 mU/kg. representing total doses of 3.2 and 3.6 mU ADH for the 2 test dogs. This implies that the urine formed during 15 minutes had an antidiuretic effect corresponding to about 3 mU. According to the references cited above strong antidiuresis results from the administration of 4 to 5 mU ADH per hour; this makes it unlikely that the polydipsia in bitches with pyometra is caused by a state comparable to diabetes insipidus.

There was a distinct increase in sodium excretion after the injection of the test dogs with the pitressin standard. Since vasopressin does not affect sodium excretion (*Ali* 1958, *Brooks & Pickford* 1958) but oxytocin does (*Brooks & Pickford* 1958), the increase in sodium excretion probably reflects the presence of small amounts of oxytocin in the pitressin. The samples of urine injected had different effects upon sodium excretion; in 2 instances there was an increase comparable to that occurring after the injection of pitressin. While there is no evidence concerning the nature of this effect of the urine injections it is conceivable that the urine as well had an oxytocin-like effect.

In summing up it can be pointed out that the antidiuretic substance in the urine and the Gomori-positive material in the hypothalamus and neurohypophysis were present in such amounts that it is unlikely that disturbances in the neurosecretory system are of primary importance for the occurrence of the polydipsia which often accompanies pyometra in bitches.

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SUMMARY

Bitches with polydipsia and pyometra (chronic purulent endometritis) were examined for ADH in the urine and the presence of Gomori-positive material in the hypothalamus and neurohypophysis.

There were no significant deviations from the normal in these respects. From this it appears that the cause of the polydipsia which often occurs in bitches with pyometra cannot be referred to disturbances in the hypothalamic-neurohypophyseal system.

ZUSAMMENFASSUNG

Die Nierenfunktion bei Hunden mit Pyometra.

1. Untersuchungen des hypothalamome-neurohypophysären System.

Hündinnen mit Pyometra (Endotritis purulenta chronica) und Polydipsie wurden auf Ausscheiden von ADH mit dem Urin als auch auf das Vorkommen vom Material im Hypothalamus und Neurohypophyse das sich nach Gomori färben lässt, untersucht.

Da sich die Tiere in erwähnter Hinsicht von den normalen Tieren unbedeutend unterscheiden, scheint es unwahrscheinlich, dass die Ursache solcher Polydipsie, die oft im Zusammenhang mit der Pyometra bei Hündinnen vorkommt, den Störungen im Hypothalamo-Neurohypophysären System, zuzuschreiben wäre.

SAMMANFATTNING

Njurfunktionen hos hundar med pyometra.

1. Undersökning av det hypothalama-neurohypofysära systemet.

Tikar med pyometra (kronisk purulent endometrit) och polydipsi har undersökts på utsöndringen av ADH med urinen samt på förekomsten av Gomori-färgbart material i hypothalamus och neurohypofysen.

Då djuren i ovannämnda avseenden ej påtagligt avveko från normala djur förefaller det osannolikt att orsaken till den polydipsi, som ofta förekommer i samband med pyometra hos tik, skulle vara betingad av störningar i det hypothalama-neurohypofysära systemet.

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