SOME FACTORS MODIFYING THE ACTIONS OF POSTERIOR PITUITARY HORMONES ON RENAL FUNCTION

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Doctor of Philosophy

by

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The experiments described in Section VIII have already been published in the British Journal of Pharmacology and Chemotherapy, September 1958, vol 13, No. 3, p 315. Some of the work described in Section I formed part of a paper presented by Dr. M.N. Ali, Dr. L.M. Pickford and myself in the Journal of Physiology 1958, vol. 141, No. 1, p 177.

PROLOGUE

The central theme of this thesis is an investigation of the effect of posterior pituitary hormones on the excretion of electrolytes by the dog. For the sake of simplicity the findings are presented and discussed under eight separate sections, but this has, on occasion, necessitated the presentation of observations out of their logical context. Thus Section I is made up of all the control obseravtions made throughout the entire study and contains data obtained from each dog under all the conditions under which it was subsequently studied.

In Section II the actions of the posterior pituitary hormones on electrolyte excretion were studied in normal dogs on normal diets. The next section describes how the relative proportions of the various urinary electrolytes were varied in an attempt to determine whether the posterior pituitary hormones exert a specific effect on the excretion of any particular ion. A more detailed analysis of the same data in Section IV appears to provide some clue as to the site of action of vasopressin.

The sections concerning the adrenalectomized dog and the dog with denervated kidneys are presented as evidence that vasopressin exerts its action directly on the renal tubules. The description of the renal action of Intermediate Lobe Hormone is included because this substance is a possible contaminant of all posterior pituitary extracts.

CONTENTS

SECTION I

ELECTROLYTE EXCRETION DURING WATER DIURESIS AND DURING THE NON-DIURETIC STATE

INTRODUCTION

The basic experimental method underlying all the studies recorded below is an observation of the simultaneous excretion of water, Na, K, and Cl by a dog during a standard two hour period. The excretion pattern was modified by the injection of various substances into the dog during the observation period, or by varying the metabolic state of the animal before the observations began.

In many cases two or more experimental conditions were varied at the same time. It follows that if any firm conclusions are to be drawn from the manner in which the excretory pattern was modified by the experiment, the normal excretory pattern must be clearly defined.

In Section I therefore, a detailed investigation of the relationship between water excretion and electrolyte excretion has been undertaken. Consideration has also been given to the underlying mechanisms whereby the concentrations of urinary electrolytes may be varied.

Although the relationship between urine flow rates and electrolyte excretion has been extensively studied there are unfortunately many conflicting results which cannot be explained by species differences.

There is considerable evidence that despite the formation of a more dilute urine at higher rates of urine

flow, the absolute amount of electrolyte excreted in any minute actually increases as urine flow increases. Thus Friberg, Karvonen and Leppanen (1950) demonstrated a strong positive correlation between Na excretion and urine flow in dogs during an acute water diuresis.

A similar correlation was found in humans by Roscoe (1956), who maintained his subjects in a standard state for many days and analysed a urine sample collected at a standard time each day. By regulation of water intake the minute volume was varied from very low rates of flow to moderate rates, but a maximal water diuresis was not studied. Roscoe found that the positive correlation between water excretion and electrolyte excretion was best demonstrated at low rates of flow. At a critical moderate rate of flow the slope of the correlation curve flattened out, and there was some evidence that electrolyte excretion could remain at a steady level while urine flow rates further increased.

In an experiment on men where a water diuresis was maintained for 11 days by the daily ingestion of 10 l of water, de Wardner and Herxheimer (1957) found that one of their subjects developed a large Na deficit, despite an increased Na intake.

At the opposite extreme there are reports that the absolute excretion of electrolytes decreases during water diuresis. In the human, Eggleton (1943) found that Cl excretion fell progressively as the water diuresis developed. Similar findings had previously been recorded by Adolph and Ericson (1926), who also noted that the Cl

excretion continued to fall after the peak of diuresis had passed.

In both humans and dogs, Marshall (1920) observed a preliminary increase in Cl excretion at the onset of a water diuresis. By the time the peak of diuresis had been achieved the Cl excretion had always fallen below the starting level. Priestley (1921) found a similar biphasic response in humans on a normal diet. When the diet was low in Cl, or if the diuresis was initiated after a period of fasting, the initial increase in Cl excretion was not seen and Cl output fell progressively from the outset.

A third possibility was noted by Klisiecki, Pickford, Rothschild and Verney (1932) who found that in dogs absolute Cl excretion did not alter during water diuresis.

Probably most of the confusion in the above results arises in the lack of standardization of experimental conditions. The following study therefore aims to define clearly the response seen under the highly standardized conditions used in this particular series of investigations.

METHODS

A total of 263 observations were made on 7 dogs. Of these, 42 were designed primarily as control observations of the unmodified excretory pattern. In the remaining experiments the urine flow was observed for 40 to 50 min before some additional experimental procedure was undertaken. Thus in all cases some observations of the unmodified excretory pattern are available for analysis.

The animals used were adult bitches weighing from 9.1 kg to 33 kg. At a preliminary operation the perineum had been split to display the urethral orifice for easy catheterisation. All dogs had fully recovered from any operative procedure before any observations were made. One dog (Jill) had been subjected to bilateral ovariectomy 10 months before the observations were made.

Five of the dogs were fed a stew prepared from bread and meat as provided to all stock dogs in the animal house. To the stew pot a standard measure of salt was added each day, resulting in a ration of NaCl of about 2gm/dog/day. This diet varied considerably in electrolyte content from month to month, but was fairly constant over shorter periods. One dog (Jill) was fed a standard diet prepared from Red Heart canned dog food and bread supplemented by uniform dog biscuits. This standard diet was also fed to an adrenalectomized dog. The dogs were fed once daily at 5p m.

On the day of an experiment a dose of water (200 to 300 ml depending on the size of the animal) was administered by stomach tube at 11.45 am. The observation

period commenced at about 2.30 pm when a second water dose of the same volume was administered. When the non-diuretic state was to be observed, this afternoon dose was omitted. During the observation period the dog rested quietly on its side on a table. The bladder was catheterized, and urine drained into an open measuring cylinder below the level of the dog. A T piece in the catheter close to the vulva allowed air to be blown through the external collecting system at the end of each collecting period. Most of the dogs developed a reflex whereby the bladder was emptied when the tail was manipulated. Occasionally slight suprapubic pressure was required. Urine was collected every 10 min.

Na and K were measured by means of an E.E.L. flame photometer. Cl was estimated by the method of Prout Winter. (Cole, 1919).

RESULTS

(1) ELECTROLYTE EXCRETION BY THE NORMAL DOG

Unmodified water diuresis.

(a) Rising phase of diuresis. In 75 experiments on seven dogs a simple water diuresis was induced. In 47 of these the absolute excretion of all three electrolytes (Na, K and Cl) fell progressively while the urine flow was increasing; i.e. there was a negative correlation between urine flow and electrolyte excretion. Some dogs reproduced this pattern more regularly than others, as is shown in table I. The magnitude of the fall varied from 100% changes to changes of 10%.

Table I

Dog	Total experiments	Typical responses	% Typical
Big Claus. 25.5kg	25	18	72
Spot. 9.1kg	8	7	88
Popsey. 18kg	10	9	90
Susy. 13kg	12	3	25
Fernitickles. 13kg	13	10	77
Lady. 33kg	1	0	0
Jill. 13.6kg	6	0	0
Totals	75	47	63

Note: A typical response is one in which the excretion of Na, K and Cl consistently falls during the rising phase of diuresis.

Table II shows the 28 atypical results expressed in a manner which illustrates the changes in the individual

electrolytes in each dog. From the totals it may be seen that Cl excretion most consistently decreased with increasing urine flow (60% of cases). Of the three electrolytes measured Na excretion most frequently increased during the diuresis (25% of cases). The most frequent atypical response, however, took the form of the electrolyte excretion remaining unaltered throughout the diuresis.

Table II

Dog	Total	Na			K			Cl		
		--	0	+	--	0	+	--	0	+
Big Claus	7	2	1	4	5	1	1	6	0	1
Spot	1	1	0	0	0	1	0	1	0	0
Popsey	1	1	0	0	0	1	0	1	0	0
Susy	9	6	3	0	1	4	4	6	1	2
Fernitickles	3	0	2	1	3	0	0	1	1	1
Lady	1	0	1	0	0	1	0	0	1	0
Jill	6	0	4	2	2	3	1	2	2	2
Totals	28	10	11	7	11	11	6	17	5	6

-- Excretion consistently decreasing with increasing urine flow.

0 Excretion unchanged with increasing urine flow, or change not consistent in direction.

+ Excretion consistently increasing with increasing urine flow.

Of the 28 atypical responses there were 17 cases where the excretion rates of two of the electrolytes decreased with increasing urine flow. In 9 of these 17 experiments the excretion of the third electrolyte was unchanged by the diuresis, and in 8 cases the excretion

of the third electrolyte increased during the rising phase of the diuresis. In only four experiments did the excretion rate of more than one electrolyte increase with increasing urine flow, and on only two occasions did all three electrolytes show this response.

There is no obvious reason why these atypical responses occurred. They did not depend on the initial rates of electrolyte excretion. In this series of 75 experiments the Na and Cl excretion rates varied from less than 10uEq/min to over 100 uEq/min, while the K excretion ranged from below 5 uE/min to over 40 uEq/min. The atypical responses were distributed evenly throughout this range. The Na/K ratio did not appear to influence the response.

(b) Declining phase of diuresis. In 14 experiments the water diuresis was allowed to progress until the urine flow returned to normal levels. In all cases the fall in electrolyte excretion continued beyond the peak of diuresis; i.e. there was a period when both urine flow and electrolyte excretion were decreasing at the same time. In 11 of these 14 observations the electrolyte excretion began to rise again within 30 min after the peak of diuresis, despite a continued fall in urine flow (Fig 1). In one case this increase was delayed for 60 min, and twice it had not occurred when the experiment ended 40 min after the peak of diuresis.

In six experiments the water diuresis was induced after a preliminary period of observation. In all six cases the onset of diuresis was marked by an increase in

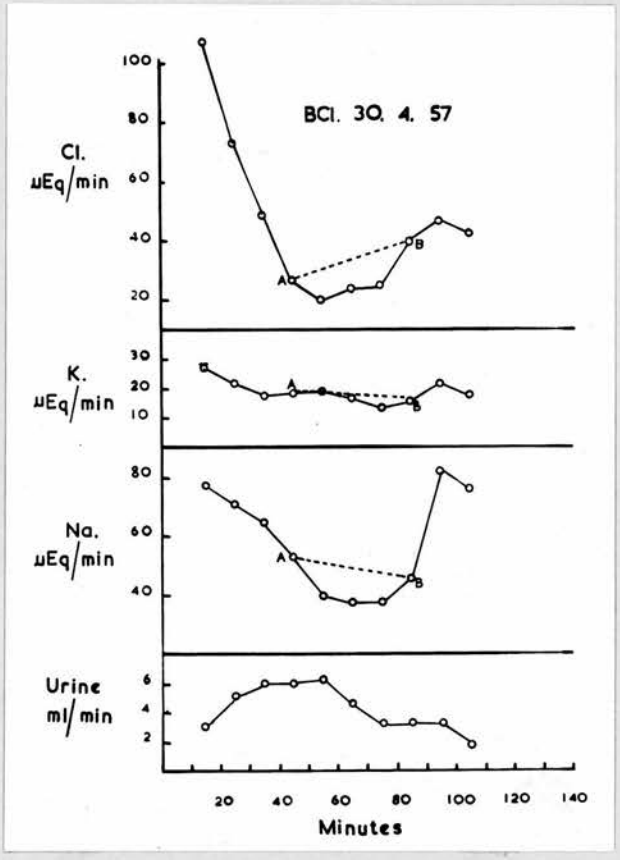

Fig. 1. B.Cl 30.4.57. Water diuresis in a normal dog. 300 ml water at 0 time. The rate of electrolyte excretion is lowest in the period immediately following the peak of diuresis.

electrolyte excretion, but as the urine flow subsequently increased the electrolyte excretion began to fall again, giving rise to the typical response described above. In only one case, however, did the electrolyte excretion during the diuresis fall below that observed during the preliminary control period (Fig 2).

Non diuretic state.

In 32 experiments on the same seven dogs the urine flow was observed throughout the usual afternoon period, but with the omission of the afternoon dose of water. No correlation between urine flow and electrolyte excretion was apparent. The rates of excretion of Na, K and Cl either remained steady or fell slightly throughout the observation period, and within wide limits were independant of urine flow. However, large fluctuations in the urine flow of the order of 100% change in minute volume between samples did frequently cause the excretion rates of all the electrolytes to move in the same direction (Fig 3).

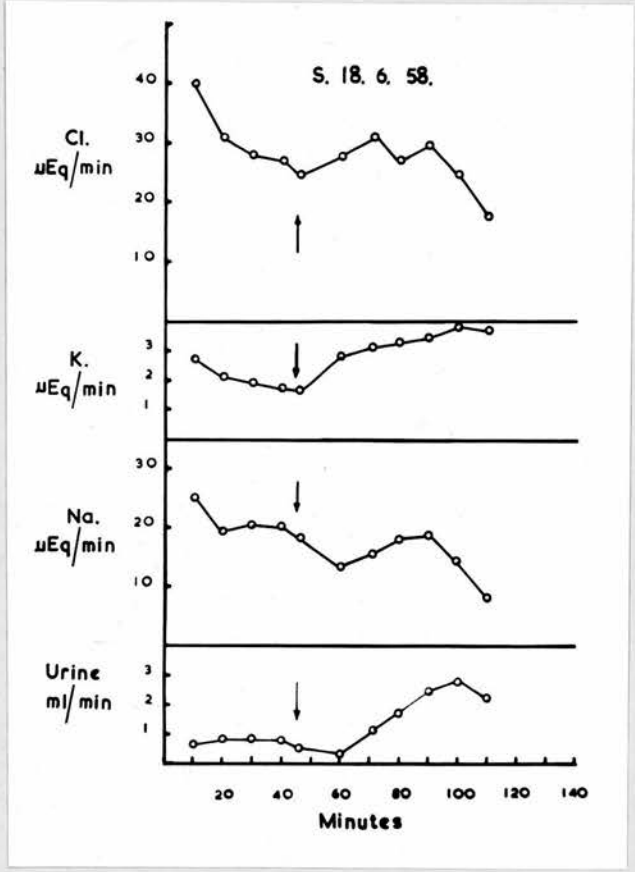

Fig. 2. S. 18.6.58 Water diuresis in a normal dog. 250 ml water administered at 44 min (arrow). The rate of excretion of Na and Cl during the diuresis falls below the pre hydration rate. Note that K is plotted on a magnified scale.

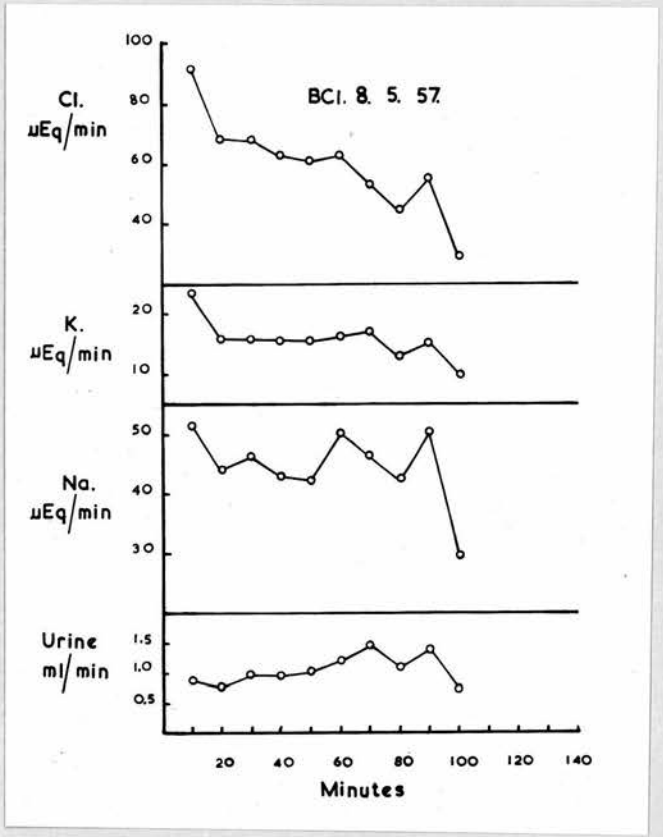

Fig. 3. B.Cl 8.5.57 Non diuretic urine flow in a normal dog. There was no correlation between electrolyte excretion and urine flow except terminally, where a sudden decrease in flow rate was accompanied by a decreased electrolyte output.

(II) ELECTROLYTE EXCRETION BY THE NORMAL HUMAN

In four experiments on two human subjects a water diuresis was induced by drinking one litre of water three hours after the subjects usual breakfast. In two experiments on an adult male the electrolyte excretion remained unchanged during the rising phase of the diuresis. In two experiments on an adult female the electrolyte excretion increased as the urine flow increased.

In two experiments on the male subject the urine flow was observed at the same time of day, but without water loading. Under these circumstances electrolyte excretion could not be correlated with the rate of urine flow.

(III) ELECTROLYTE EXCRETION AFTER RENAL DENERVATION

Method.

One dog was subjected to denervation of both kidneys at a single stage operation in which both kidneys were mobilized and the renal vessels stripped. Observations were made over the period ranging from one to twenty weeks after the operation. This animal had also been extensively studied before the operation.

Experimental.

(a) Water diuresis. In the dog subjected to renal denervation 21 observations of simple water diuresis were made. In seven experiments the rate of excretion of all three electrolytes decreased during the rising phase of the diuresis, and in another three experiments the rate of excretion of two of the electrolytes decreased while the third remained unchanged. In all the other

experiments the rate of excretion of the electrolytes either remained unaltered by the diuresis, or the excretion of individual electrolytes changed in different directions in the same experiment. Table III shows the frequency of each type of response for the individual electrolytes. The nature of the response could not be correlated with the time after operation at which the observation was made.

TABLE III.

Na			K			Cl		
--	0	+	--	0	+	--	0	+
7	8	6	17	3	1	13	7	1

(b) Non diuretic state. On five occasions after renal denervation the non diuretic urine flow was observed. Four of the observations were made within three weeks of the operation, and the fifth was made 16 weeks later. In no case was there any correlation between urine flow and electrolyte excretion. Figure 4 illustrates a typical experiment. This pattern of electrolyte excretion was the same as that seen in the intact animal.

(IV) WATER AND ELECTROLYTE EXCRETION AFTER ADRENALECTOMY.

Method.

One dog was subjected to bilateral adrenalectomy at a two stage transabdominal operation. It was maintained on 0.7mg D.C.A. and 8mg hydrocortisone daily. The diet consisted of Red Heart canned dog food (two tins per day) mixed with bread. This was supplemented with 200ml of cows milk, four small standardized dog biscuits, and

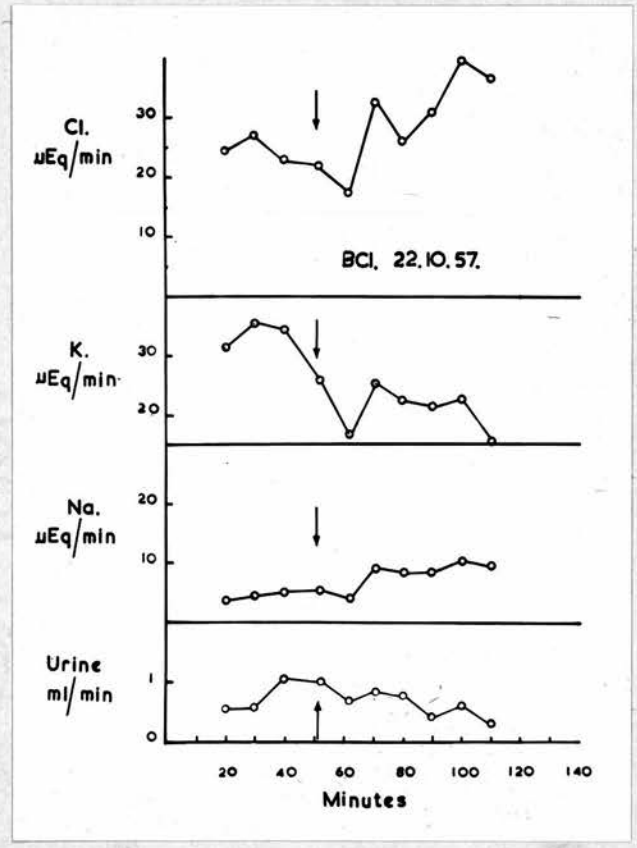

Fig. 4. B.Cl 22.10.57. Non diuretic urine flow in a dog with denervated kidneys. 1.0 ml of 0.9% NaCl administered i.v. at 51 min (arrow). There was no correlation between electrolyte excretion and urine flow.

1.2 gm NaCl daily. This regime was judged to be adequate in that the dog appeared well, and maintained a constant body weight and normal serum electrolyte levels.

Experimental.

(a) Water excretion. In 30 experiments after adrenalectomy a simple water diuresis was induced. The maintenance dose of steroids was sufficient to enable a normally brisk diuresis to occur. In five cases the daily dose of D.C.A. was increased to twice or three times the maintenance level, and on five other occasions the dose of D.C.A. was either reduced to half or omitted. It would be of interest to compare the magnitude and rapidity of onset of the diuresis in these two groups. Unfortunately the original plan of the experiments involved making an injection just before the predicted peak of diuresis, so that the complete unmodified diuretic curves are not available for comparison.

In the cases in which high doses of D.C.A. had been given, the last unmodified minute volumes had been recorded at times ranging from 44 to 49 min (mean 46 min) after the water loading. In the D.C.A. deprivation experiments the corresponding observations had been made at times ranging from 42.5 min to 49 min (mean 46.3 min) after water loading. The two groups are therefore comparable.

In the high steroid experiments the mean minute volume at this last reading was 5.83 ($\pm$ 0.89) ml/min. At the corresponding time in the low steroid experiments the minute volume had achieved a mean value of only

4.65 (±0.605) ml/min. This difference is significant at the 5% level. (t = 2.33). Excessive D.C.A. therefore appears to promote a larger and more brisk diuresis.

(b) Electrolyte excretion. Of the 20 experiments carried out during normal steroid maintenance 7 showed a fall in the excretion of Na, K and Cl during the phase of increasing urine flow. In 9 cases there was a preliminary increase followed by a decrease in the excretion of all electrolytes (Fig 5). In the remaining cases there was no correlation between electrolyte excretion and urine flow.

Of the five experiments in which D.C.A. dosage was excessive, four showed decreasing Na, K and Cl excretion during the rising phase of the diuresis while the fifth showed the biphasic response described above. When the D.C.A. dosage was low three of the five cases showed decreasing excretion of Na, K and Cl as urine flow increased, while in the other two experiments the diuresis did not alter the excretion of electrolytes.

In three of the experiments during normal steroid maintenance, and one during low steroid therapy, the water load was given after a preliminary observation period. In all four cases the electrolyte excretion rose at the beginning of the diuresis but fell again before the peak of diuresis was reached. As in the normal animal the electrolyte excretion during the diuresis did not fall below the level seen in the preliminary observation period. As also with the normal dog, the electrolyte excretion rates continued to fall for some time after the

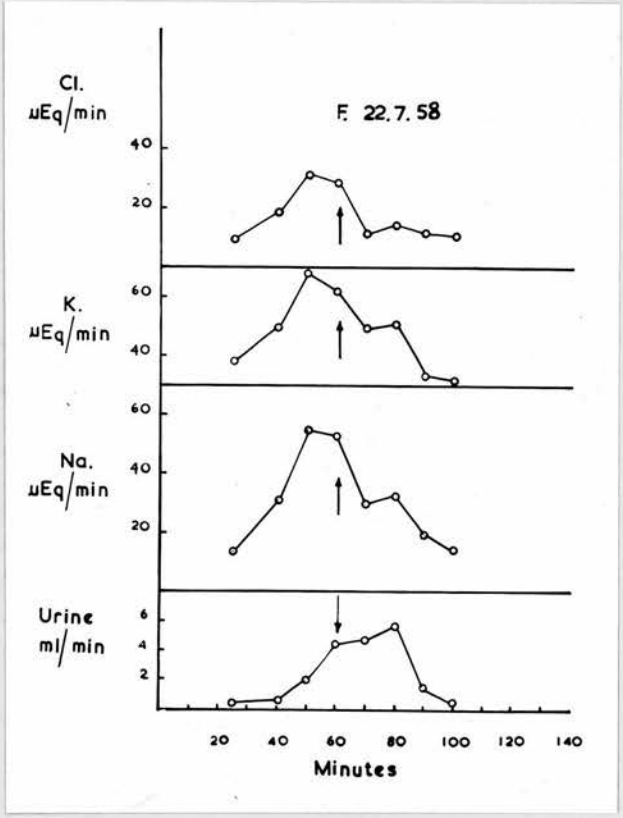

Fig. 5. F. 22.7.58 Water diuresis in an adrenalectomized dog. 250 ml water at 0 time. 1.0 ml of 0.9% NaCl administered i.v. at 61 min (arrow). 8 mg hydrocortisone and 0.7 mg D.C.A. had been administered 3 hours before. There was a preliminary increase in electrolyte excretion at the beginning of the diuresis, followed by a subsequent fall in the rate of electrolyte excretion at the peak of diuresis.

peak of diuresis.

High D.C.A. dosage resulted in an initially low Na/K ratio (mean 1.66), and low D.C.A. a high Na/K ratio (mean 5.00). These ratios remained fairly constant throughout the diuresis, the fall in output of any particular electrolyte being proportional to its initial value.

(c) Non diuretic state following adrenalectomy. In 9 experiments during normal steroid maintenance, and in one experiment where the steroid dose had been reduced to half, the resting urine flow was observed. No correlation between urine flow and electrolyte excretion was detected.

(V) EFFECT OF PREVIOUS LOADING WITH VARIOUS SALTS ON ELECTROLYTE EXCRETION.

Method

Originally the intention of the experiment was to study the effect of including in the morning water load a dose of approximately 1.5 gm NaCl. This amount had been arbitarily decided upon as being equivalent to the NaCl content of an average meal, and contained the inconvenient number of 255 mEq of Na and Cl. In later experiments doses of other salts were calculated to contain the same number of mEq of the electrolyte under investigation.

Experimental.

(a) NaCl loading. In 21 experiments on six normal dogs the morning dose of water contained 1.5 gm NaCl. In 14 of these experiments the excretion of all three electrolytes fell during the rising phase of the diuresis.

These cases included two control experiments where it was seen that the electrolyte excretion did not rise again until 30 min after the peak of diuresis. Tables IV and VII illustrate the frequency and type of response of the individual electrolytes.

Table IV

Na			K			Cl		
--	0	+	--	0	+	--	0	+
15	3	3	18	2	1	19	2	0

The Na excretion is seen to increase most frequently with increasing urine flow, whereas the Cl excretion never increases.

The experiment was repeated four times on the dog with denervated kidneys. In three of these cases the excretion of all electrolytes decreased as urine flow increased, but in the fourth experiment Na excretion increased while the excretion of K and Cl decreased.

Only twice was the non diuretic state observed after a morning dose of NaCl. One experiment was in the normal dog and one in the dog with denervated kidneys. In neither case could the electrolyte excretion be correlated with urine flow.

(b) KCl loading. In five experiments on four normal dogs the morning dose of water contained 1.9 gm KCl. In three of these experiments the excretion of all three electrolytes decreased during the rising phase of the diuresis. In one case the excretion of Na and Cl fell while that of K remained stationary. In the other experiment the excretion of all electrolytes was unaltered

by the diuresis.

In one experiment in which the non diuretic state was observed after a morning dose of KCl the excretion of all three electrolytes decreased when urine flow increased. However, the minute volumes in this experiment were unusually high (1.00-1.4 ml/min).

(c) $NaHCO_3$ loading. In 16 experiments on four normal dogs the morning water dose contained 2.15 gm $NaHCO_3$. In 10 cases the excretion of all three electrolytes fell during the diuresis. In two experiments all three electrolytes increased during the diuresis, and in the remaining cases there was no consistent change in electrolyte excretion. Tables V and VII summarize the response of each electrolyte.

Table V

Na			K			Cl		
−	0	+	−	0	+	−	0	+
11	3	2	12	2	2	11	3	2

(d) $KHCO_3$ loading. In four experiments on three normal dogs the morning dose of water contained 3.35 gm $KHCO_3$. In all cases the output of all three electrolytes fell during the diuresis.

(e) $NaHCO_3$ and $KHCO_3$ loading combined. In one animal (Jill, ovariectomised) six experiments were conducted in which the morning dose of water contained 1.5 gm $NaHCO_3$ and 0.5 gm $KHCO_3$. Except for two cases where the excretion of Cl fell during the rising phase of the diuresis the excretion of electrolytes did not appear

to be related in any way to urine flow.

In one experiment where the water loading was made after a 40 min preliminary period of observation the electrolyte excretion rates rose immediately after loading, fell a little as the peak of diuresis was approached, and were still falling 30 min after the peak of diuresis, by which time the Na excretion had fallen below the control levels (Fig 6). In another control experiment the prolonged fall in electrolyte excretion was still present 40 min after the peak of diuresis.

The only observation on resting rate made under these circumstances was the preliminary observation period illustrated in Fig 6. Here there was no correlation between urine flow and electrolyte excretion.

(f) NH_4Cl loading. In 18 experiments on five normal dogs the morning water dose contained 1.4 gm NH_4Cl. In 10 cases the excretion of all three electrolytes fell during the diuresis. In the remaining experiments the response was mixed. Tables VI and VII illustrate the frequency and type of response of the individual electrolytes.

Table VI

Na			K			Cl		
—	0	+	—	0	+	—	0	+
11	3	4	15	2	1	16	2	0

The table shows that Cl excretion is most frequently decreased with increasing urine flow, and that the most frequent pattern is for all the excretion rates to decrease during the diuresis.

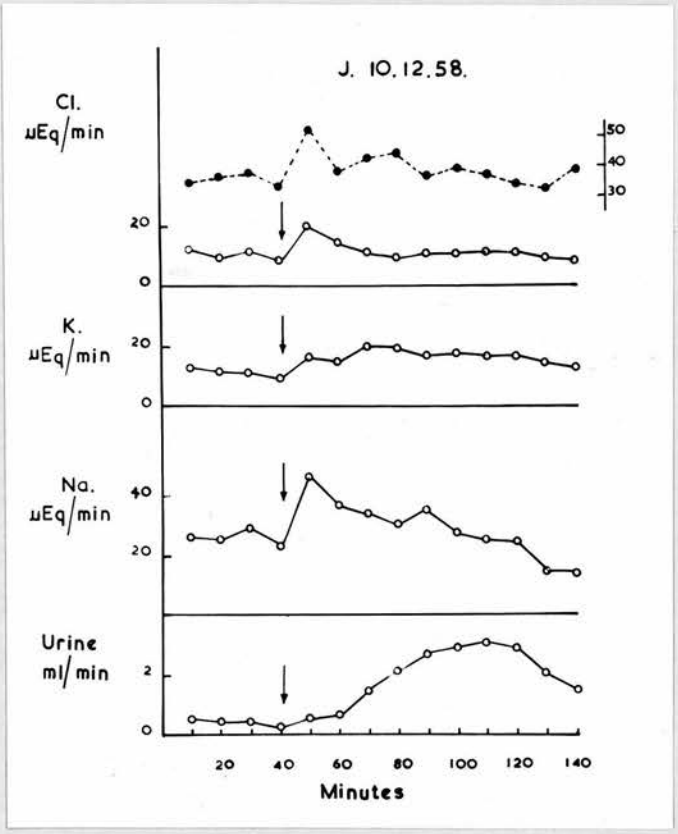

Fig. 6. J. 10.12.58 Water diuresis in a normal dog. 1.5 gm $NaHCO_3$ and 0.5 gm $KHCO_3$ administered with the morning water load. 250 ml water administered at 41 min (arrow). Upper graph (solid circles) shows excretion of creatinine in mg/min. There was an increase in electrolyte excretion at the beginning of the diuresis followed by a subsequent decrease.

In one control experiment the excretion rate of all the electrolytes increased immediately after water loading but fell again as the diuresis was established and continued to fall for 30 min after the peak of diuresis. In this experiment the final excretion of Cl was less than that observed during the preliminary control period. During this control period there was no correlation between urine flow and electrolyte excretion.

In one experiment the dog with denervated kidneys was given a morning load of NH_4Cl. During the subsequent water diuresis the excretion of Na, K and Cl decreased.

(g) Loading with sundry salts. In two normal dogs a series of single experiments were carried out in which the morning dose of water contained equimolar doses of Na citrate, K citrate, Na_2SO_4, $(NH_4)_2SO_4$, and NH_4HCO_3. In all cases the excretion of Na, K and Cl fell during the diuresis. (Table VII).

Table VII

Loading Salt	Na			K			Cl			Total
	--	0	+	--	0	+	--	0	+	
NaCl	15	3	3	18	2	1	19	2	0	21
KCl	4	1	0	3	2	0	4	1	0	5
$NaHCO_3$	11	3	2	12	2	2	11	3	2	16
$KHCO_3$	4	0	0	4	0	0	4	0	0	4
$NaHCO_3$ + $KHCO_3$	0	6	0	0	6	0	2	4	0	6
NH_4Cl	11	3	4	15	2	1	16	2	0	18
Na citrate	1	0	0	1	0	0	1	0	0	1
K citrate	2	0	0	2	0	0	2	0	0	2
Na_2SO_4	1	0	0	1	0	0	1	0	0	1
$(NH_4)_2SO_4$	1	0	0	1	0	0	1	0	0	1
NH_4HCO_3	1	0	0	1	0	0	1	0	0	1

-- Excretion consistently decreasing with increasing urine flow.

0 Excretion unchanged with increasing urine flow, or change not consistent in direction.

+ Excretion consistently increasing with increasing urine flow.

(VI) EFFECT OF DIAMOX ON ELECTROLYTE EXCRETION.

In 10 experiments on three normal dogs the water diuresis was observed after a morning dose of 100mg Diamox. The urine flow reached much higher levels than usual (up to 8.15 ml/min in one dog which had otherwise never exceeded 6.0 ml/min). There was no obvious correlation between urine flow and electrolyte excretion, the general trend being for the electrolyte excretion to remain steady. Table VIII lists the responses of the individual electrolytes.

Table VIII

Na			K			Cl		
—	0	+	—	0	+	—	0	+
2	6	2	1	5	4	4	4	2

In one experiment after Diamox the non diuretic state was observed. There was no correlation between urine flow and electrolyte excretion.

(VII) CHANGES IN URINARY pH DURING WATER DIURESIS.

Method.

In this series of experiments the urine was collected by the usual method of open drainage and the pH was estimated immediately after the completion of each 10 min collecting period, using B.D.H. universal indicator. In four experiments the urine was collected under paraffin and the pH estimated both by indicator and by glass electrode pH meter. These more accurate experiments confirmed the trend of results obtained by the more crude methods. Doses of $NaHCO_3$ and NH_4Cl were as described above.

Experimental.

The pH was measured during a water diuresis in 21 experiments on five normal dogs. In 10 experiments the dog was on a normal diet. In six experiments the urine had been rendered alkaline by a morning dose of $NaHCO_3$, and on four occasions the urine was acid as a result of NH_4Cl loading. One experiment was conducted 30 hours after a dose of Diamox. In all cases the urinary pH either increased or decreased from its initial value to approach pH 7.0 at the peak of diuresis.

(VIII) CHANGES IN URINARY TITRATABLE ACIDITY DURING WATER DIURESIS.

Method.

Urine was collected under paraffin and the titratable acidity was estimated by adding a known volume of N/10 NaOH and back titrating with N/100 HCl, using phenol phthalein as an indicator. The volume of urine available for the estimation was frequently only 1 ml, so that the accuracy of the experiment was limited.

Experimental.

The excretion of titratable acid was observed during a water diuresis in eight experiments on one dog. In five cases the urine was acid and in three cases the urine was alkaline. The acid excretion remained steady throughout the diuresis, and could not be correlated with urine flow. However in this particular series of experiments the excretion of Na, K and Cl also remained steady throughout the diuresis.

(IX) AMMONIA EXCRETION DURING WATER DIURESIS

Method.

In all cases where NH_3 was to be estimated the procedure was undertaken within two hours of the collection of the urine to avoid false values due to activity of urea splitting organisms. A protein free filtrate of urine was made by first precipitating with $ZnSO_4$ and NaOH, diluting 1/50 and filtering. The NH_3 content was estimated by the method described by King and Wootton (1956), wherein 5 ml of the filtrate together with 5 ml of Nessler's reagent were diluted to 50 ml and the resultant colour compared with the colour developed by a similarly treated standard solution of NH_4Cl. Readings were made on a Spekker twin cell photometer using a blue filter.

Experimental.

NH_3 excretion was observed during water diuresis on five occasions in one dog. In three experiments the urine was acid, and in two experiments the urine was alkaline. In all cases the excretion of NH_3 remained steady and independent of urine flow. However, in these experiments the excretion of Na, K and Cl also remained steady and independent of urine flow.

(X) TOTAL PHOSPHATE EXCRETION DURING WATER DIURESIS

Method.

Total urinary phosphate was estimated by the method described by King and Wootton (1956), wherein the phosphate in the presence of a reducing agent (ascorbic

acid) and perchloric acid developes a blue colour with ammonium molybdate. Colours were compared on the Spekker photometer using a red filter.

Experimental.

Urinary phosphate excretion was observed during a water diuresis on three occasions. Twice the urine was alkaline and once it was acid. On one occasion during an alkalosis the excretion of phosphate fell during the diuresis, but in the other two cases the phosphate excretion remained steady and independent of urine flow.

(XI) CHANGES IN GLOMERULAR FILTRATION RATE DURING WATER DIURESIS

Method.

Changes in the glomerular filtration rate were observed by measuring the excretion rate of endogenous creatinine. Over the short course of these experiments (two hours) the blood level of creatinine could reasonably be expected to remain steady (Marshall, 1920) with perhaps some slight decrease as a result of assimilation of the water load. If one makes the additional assumptions that none of the filtered creatinine is subsequently reabsorbed, and that none is excreted by the tubules (Smith, 1956), changes in the rate of excretion of creatinine are seen to provide an indirect measure of changes in the glomerular filtration rate.

Urinary creatinine was estimated by the method of Folin (King and Wootton, 1956) which depends on the development of an orange colour by creatinine and picric acid in the presence of NaOH. Colours were compared in

the Spekker photometer using a green filter.

Experimental.

On 10 occasions on two dogs the excretion of endogenous creatinine was measured during a water diuresis. In two of these experiments the excretion of creatinine fell at the peak of diuresis, but in all the other experiments creatinine excretion remained fairly steady and independent of urine flow. Creatinine excretion could not be correlated with electrolyte excretion.

On three occasions when the water diuresis was induced after a preliminary period of observation, there was an increase in creatinine excretion at the time of intubation. In two of these experiments the creatinine excretion fell again as diuresis was established (Fig 6), but in the third case the increase in creatinine excretion was maintained for the remainder of the experiment.

(XII) APPENDIX: EFFECT OF NOR-ADRENALINE ON ELECTROLYTE EXCRETION DURING WATER DIURESIS

On two occasions in one dog a small dose of nor-adrenaline was administered during a water diuresis. In one case a single intravenous dose of 1 ug nor-adrenaline resulted in a 30% increase in the excretion rates of both Na and Cl during the next 10 min. In another experiment the infusion of 7 ug administered intravenously over 14 min resulted in a 50% increase in Na excretion for the duration of the infusion. In neither case was the diuresis inhibited. The glomerular filtration rate did not appear to be significantly altered by the nor-adrenaline.

DISCUSSION

The pattern of water excretion in dogs was uniform throughout all the experiments carried out during water diuresis, and agreed with all previously published results. The urine flow rate usually began to increase within 20 min of the ingestion of water, and the peak of diuresis was usually achieved within 50 min. The flow rate in almost all experiments had returned to resting levels within 120 min of the water loading.

In these experiments on water diuresis the excretion of electrolytes also showed a fairly uniform pattern. In most cases the rate of excretion of electrolytes fell progressively up to the peak of diuresis and for 30 min after. This was true for all experimental conditions except Diamox diuresis.

The tendency not to conform to this pattern was more obvious in some animals.Thus one dog (Jill, which had been ovariectomized) contributed most of the aberrant responses and few of the normal responses. However, as the experiments were made on only one animal at a time, and as the observations on each animal frequently extended over many consecutive weeks, it may be that some unrecognized environmental or seasonal factor (rather than individual variations between dogs) contributed to the abnormal behaviour. Nevertheless, even in the aberrant responses the electrolyte excretion rarely increased during a diuresis, and was usually at its lowest level 30 min after the peak of diuresis.

Under the conditions of the water diuresis

experiments described above, therefore, it may be fairly safely predicted that the rate of excretion of Na, K, and Cl at any time between 50 min and 80 min after the ingestion of water will be less than the excretion rate at 40 min. i.e. if the excretion rate of an electrolyte were plotted graphically against time, a straight line joining the 40 min excretion rate to the 90 min excretion rate would almost invariably pass above the intervening points on the plot. (Fig| line AB). This statement forms the basis upon which all succeding sections of this study rest.

These findings obtained in dogs would appear to be in complete agreement with those of Adolph and Ericson (1926) and Eggleton (1943). Unfortunately in all these studies observations were not started until after the hydration. Therefore it is probably incorrect to assume that the excretion rate of electrolytes in the first samples represents the excretion rate in the nondiuretic state. Where a preliminary observation period preceded the water loading, the excretion of electrolytes was found to increase at the time of water loading. This preliminary increase was observed by Priestley (1921), who referred to the phenomenon as "washing out of Cl" by the increasing urine flow. In the present studies however, the increased electrolyte excretion usually preceded the onset of diuresis. As the urine flow increased the electrolyte excretion usually decreased.

In orded to demonstrate a true decrease in electrolyte excretion during a water diuresis (as compared with

the pre-hydration rate) it would be necessary to extend the period of observation to include a preliminary period before the administration of water, and the entire water diuresis until the urine flow had returned to resting levels. It would be necessary to show that during the diuresis the excretion of electrolytes fell below the pre-hydration level, and that as the urine flow decreased again the electrolyte excretion increased.

In three experiments the electrolyte excretion did fall below control levels, but unfortunately in none of these cases was the observation period long enough to observe a terminal increase in electrolyte output as the urine flow subsided. Priestley (1921) demonstrated that in the fasting human a progressive decline in the level of Cl excretion is to be expected. Thus it is possible that any decrease in electrolyte excretion observed during an experiment is spontaneous and unrelated to the water loading. To satisfy the hypothesis that electrolyte excretion is reduced during a water diuresis it would be necessary to demonstrate an increase in electrolyte excretion at the end of the diuresis. This late rise has been observed in many experiments where no preliminary observations were made before water loading (Fig I).. Although the entire phenomenon has not been demonstrated in a single experiment, it seems probable that during a water diuresis the excretion of electrolytes is reduced, and that a subsequent increase in electrolyte excretion is to be expected when urine flow returns to resting levels.

Results obtained from the human experiments are even less conclusive. No study was carried out past the peak of diuresis. One subject consistently showed an increased electrolyte excretion during the rising phase of the diuresis, while in the other subject the electrolyte excretion remained constant. These findings are in accord with those of Priestley (1921).

The stimulus for the production of a water diuresis is probably the absorbtion of water from the gut. Berkhin (1956) has demonstrated that nervous reflexes arising from the mouth cause water diuresis to be more prompt if water is administered by the mouth rather than by stomach tube. In these present experiments the water diuresis was always prompt after water loading by stomach tube. Control observations were made to demonstrate that the diuresis was not a conditioned response to the passage of the stomach tube. On one occasion gastric distension was produced by air and no diuresis resulted.

The absorbtion of water is rapid, a dose of 250 ml being absorbed by dogs in 35 min (Klisiecki, Pickford, Rothschild and Verney, 1932). This water causes a dilution of plasma electrolytes which is maximal some 30 min before the peak of diuresis (Rioch, 1930). Theoretically the plasma electrolytes could be diluted without the absorbtion of water, by the passage of electrolytes into the hypotonic fluid in the gut. However, by means of isotopic studies, Visscher, Fletcher, Carr, Gregoe, Bushey and Barker (1944) demonstrated that hypotonic fluid introduced into the gut does not attract

electrolytes from the blood as readily as had previously been supposed.

The probable mechanisms by means of which an increased water content of the body might produce a water diuresis are generalized changes in haemodynamics leading to changes in G.F.R. and R.P.F., nervous mechanisms involving the renal nerves, and hormonal changes.

Renal denervation has been reported to lead to increased water and electrolyte excretion (Kaplan, West and Foman, 1953). The urine elaborated by such kidneys has been found to be of constant electrolyte concentration (Sartorius and Burlington, 1956). Therefore it was surprising to find that after renal denervation water diuresis proceeded quite normally, with the electrolyte excretion declining as the urine flow increased.

Changes in renal haemodynamics may be implicated. Sellwood and Verney (1954) have demonstrated a slight rise in G.F.R. and R.P.F. during water diuresis. Although increases in G.F.R. at the onset of diuresis have been found in this present study, the subsequent changes in G.F.R. did not parallel the increased urine flow or the decreased electrolyte excretion.

The role of the adrenal gland must be considered. There is much evidence that diuresis cannot proceed in the absence of adrenal steroids (Gaunt, 1951). This phenomenon has been used as a test of adrenal insufficiency (Robinson, Power and Kepler, 1941). This present study illustrates that diuresis is normal in the

adrenalectomized animal provided the steroid maintenance is adequate. This proves that diuresis does not depend upon any modification of adrenal function.

The role of the posterior pituitary must also be considered. Evidence will be presented in Section II that vasopressin acts to decrease the flow of urine and to increase electrolyte excretion. This suggests that water diuresis may be due merely to a decreasing level of circulating vasopressin. Verney (1947) has shown that the secretion of vasopressin is regulated by the plasma osmotic pressure. Therefore the reduction of serum electrolyte concentration produced by the absorption of a water load could be expected to decrease vasopressin secretion. There is good evidence, however, that alteration in urine volume and urinary concentration can occur in the absence of a posterior pituitary (Brodsky and Rapoport, 1951). The electrolyte excretion during water diuresis in a dog with diabetes insipidus follows the same pattern as in normal dogs (Baird and Pickford, 1958). Water diuresis cannot therefore be looked upon as merely a cessation of the action of vasopressin.

The tubular pre-urine has been found to be approximately isotonic to plasma in the distal convoluted tubule (Wirz, 1957). During a water diuresis osmotically free water can be excreted only by diluting this tubular fluid. This dilution could be achieved either by removing solute from the tubule (Smith, 1956) or by adding water (Roscoe, 1956)(Brodsky and Rapoport, 1951) or both. It was hoped that these experiments would lend support to

one or other of these two opposing theories.

If urinary free water is generated by solute resorption it follows that for any given G.F.R. the excretion of electrolyte must decrease as the concentration of the urine decreases. Under these circumstances the rate of urine flow would be determined by the existing G.F.R. (Kleeman, Epstein and White, 1956). Although this present study did not demonstrate any increase in G.F.R. with increasing urine flow, such changes have previously been reported (Sellwood and Verney, 1954).

If, on the other hand, osmotically free water were generated by secretion of water into the tubule, increases in urine flow would depend upon the rate of this secretion, and it would not be necessary to postulate increases in G.F.R. during the diuresis. The excretion of electrolyte would remain steady throughout the diuresis unless there was some change in G.F.R. The findings in these experiments are consistent with either theory, and are not extensive enough to favour any particular one.

DISCUSSION OF APPENDIX

The study recorded in the appendix was designed to investigate the increase in electrolyte excretion seen immediately after water loading. This was usually accompanied by an increase in G.F.R. and it was thought probable that the increase in electrolyte excretion was due to the increase in G.F.R. An attempt was made to

produce a small increase in G.F.R. by the injection of minute doses of nor-adrenaline. This substance was selected because its circulating level was probably increased by the disturbance of water loading. The aim of the experiment (to produce a small increase in G.F.R.) was not achieved, but a transitory increase in electrolyte excretion did result.

These findings served to indicate the complexity of the problem. It is obvious that large rapid changes in electrolyte excretion can occur in the absence of changes in G.F.R.

SECTION II

EFFECT OF VASOPRESSIN AND OXYTOCIN ON ELECTROLYTE EXCRETION

INTRODUCTION

Although the role of the posterior pituitary in controlling urine volume appears to be well established, there is still considerable argument as to whether posterior pituitary extracts have any effect on electrolyte excretion. During experiments on the isolated dog kidney, Starling and Verney (1925) found that when posterior pituitary extracts were added to the perfusing fluid not only did the urine volume fall but the rate of excretion of Cl (as well as the urinary concentration of Cl) increased. This action of crude extracts of the posterior pituitary has also been demonstrated in the intact rat by Unna and Walterskirchen (1936) and Jacobson and Kellogg (1956). However, there appears to be no agreement as to the relative importance of the two posterior pituitary fractions (vasopressin and oxytocin) in producing this effect.

In a recent review Thorn (1958) claimed that there was little evidence that vasopressin could increase electrolyte excretion. This statement is probably accurate if confined to human experiments, for although Little, Wallace, Whatley, and Anderson (1947) were able to produce increases in Cl excretion in humans with doses of vasopressin too small to produce antidiuresis, no such increases were ever produced by a full antidiuretic dose

of vasopressin. These negative findings in the human were confirmed by Holland and Stead (1951) and by Chalmers, Lewis and Pawan (1951).

There is good evidence that in the dog Pitressin is chloruretic. Shannon (1942) found that Pitressin increases the excretion of Cl in normal dogs undergoing a water diuresis, and in dogs suffering from diabetes insipidus. Similar findings were reported by Sartorius and Roberts (1949) and by Anslow and Wesson (1955), who found that the effect was most obvious if the dog had been loaded with NaCl. More recently similar findings have been reported by Brooks and Pickford (1957) and Ali (1958). However not all workers are agreed, and it has been claimed that posterior pituitary extracts are without any effect upon electrolyte excretion in the dog. (O'Connor, 1948)

The rat has also been reported to respond to vasopressin by increasing electrolyte excretion. This action has been used by Ralli, Raisz, Leslie, Dumm and Laken (1950) as an index of vasopressin activity. This effect is more pronounced if the rat has been previously overloaded with NaCl (Jacobson and Kellogg, 1956), in which case the chloruretic effect may predominate over the antidiuretic effect and diuresis may occasionally result. Similar findings have been reported by Friedman,Nakashima and Friedman (1955) but Dicker (1957) is convinced that in the rat vasopressin acts only on water reabsorbtion and is without effect on electrolyte excretion. Croxatto, Rosas and Barnafi (1956) found that Pitressin decreased

the excretion of Na in rats. During Pitressin antidiuresis in the rat Schlegel and Stone (1957) found that solute excretion was directly proportional to urine flow. This finding would appear to be in conflict with those where electrolyte excretion was found to increase as urine flow decreased.

The role of oxytocin would appear to be more clearly defined. In the rat, Fraser (1937) found that oxytocin always increased Cl output and was occasionally diuretic. These findings were confirmed by Kuschinsky and Bundschuh (1939) and by Dicker (1957) who found the effect to be most obvious at low rates of urine flow. Jacobson and Kellogg (1956) found that oxytocin was always chloruretic in the rat, but especially if the animal was loaded with NaCl. Similar findings have been reported by Croxatto, Rosas and Barnafi (1956).

In the dog oxytocin has been found to increase electrolyte excretion when administered to the non-hydrated animal (Brooks and Pickford, 1957). These findings differ from those of Anslow and Wesson (1955) who however used smaller doses. The problem has not been very adequately investigated in the human, although there are two reports of oxytocin being without effect on electrolyte excretion in the human. (Thomson, 1959; Brunner, Kuschinsky, Munchow and Peters, 1957).

METHODS

Most of the experiments described in this section are merely continuations of experiments already reported in Section I. Following the afternoon dose of water the diuresis was allowed to progress for 40 to 50 min. At this time (which was calculated to be just prior to the peak of diuresis) an intravenous injection of the test substance was made.

Urine samples were collected over 10 min periods except for the samples immediately before and immediately after the injection, which were usually 5 min. By this means any short term effects due to the disturbance of the injection were confined to two samples and were thus more obvious. No samples were discarded.

Doses of vasopressin and Pitressin were chosen which reduced the urine flow to about 0.5ml/min. Slower urine flows were avoided because they introduced proportionately larger collection errors. These doses ranged from 1mU to 4mU depending on the size and responsiveness of the dog. If a dog failed to respond to this order of dosage the vasopressin was administered by slow intravenous infusion. In this case the dose was of the order of 1mU as a priming dose followed by 0.1mU/min for 10 to 15 min.

The doses of oxytocin or Pitocin were of the same order as those used by Brooks and Pickford (1957) in similar experiments, and ranged from 80mU to 150mU. This dose was usually small enough not to depress a water diuresis to any great degree, but sufficient to

demonstrate changes in electrolyte excretion at low rates of urine flow. If an animal failed to show any response to single injections of this magnitude, slow intravenous infusions were made. The dose in this type of experiment was 80mU to begin with followed by 2mU/min for 10 to 15 min.

The vasopressin used was a highly purified arginine vasopressin prepared by Dr. V du Vigneaud. Pitressin (Parke Davis) provided an alternative preparation of adequately purified vasopressin. The oxytocin used was the synthetic product "Syntocinon" of Messres Sandoz.

In the water diuresis experiments the changes in electrolyte excretion brought about by the injection were estimated by calculating the average rate of excretion of electrolytes for the period following the injection, and comparing this value with the one expected from a knowledge of control experiments. The average excretion rate after the injection was compared with the excretion rate seen at the last full collecting period before the injection was made. The post injection period included all samples collected after the injection and until the electrolyte excretion resumed its normal pattern. This frequently included a preliminary decrease in electrolyte excretion followed by a rise. This biphasic response was almost certainly due to a dead space error which is probably best ignored. The information required of these experiments is the amount of electrolyte excreted in a given period after the injection. The dead space is usually cleared within 2 min (Morales, Crowder, Fishman,

Maxwell and Gomez, 1950). If the period of observation is long enough the dead space error becomes insignificant.

RESULTS

A. EXPERIMENTS ON DOGS.

(I) Experiments conducted during water diuresis

(a) Effect of arginine vasopressin. In six experiments on five normal animals arginine vasopressin was administered during a water diuresis. In all cases there was an antidiuresis lasting about 30 min. In all six of the experiments the excretion of Cl was increased during the antidiuresis, in five cases the excretion of Na was increased, but only in three cases was the excretion of K also increased. These findings are expressed in Tables IX and XIII. An increase in K excretion was never seen in the absence of an increase in Na excretion.

Table IX

Na			K			Cl		
+	0	--	+	0	--	+	0	--
5	0	1	3	1	2	6	0	0

\+ Increased excretion.

0 No change.

-- Decreased excertion.

The magnitude of the increase in electrolyte excretion varied. The absolute amount of the increase was greatest in those experiments where the electrolyte excretion was high at the start of the experiment, but the percentage increase was sometimes higher in those experiments where the electrolyte excretion began at low levels. This was not a constant finding.

The mean increase in electrolyte excretion expressed as a percentage was approximately 50%. The size of the electrolyte increase did not appear to be related to the degree of antidiuresis produced.

The peak of Na and Cl excretion usually occurred together within 20 min of the onset of antidiuresis. The peak of K excretion might occur in the earlier or later sample. The elevation in electrolyte excretion persisted for about 40 min, which roughly corresponded to the duration of antidiuresis.

In four cases there was a preliminary decrease in electrolyte excretion at the onset of antidiuresis,(Fig 7), but in two cases where the percentage increase in electrolyte excretion was large (100%) the electrolyte excretion was elevated in the first sample collected during the antidiuresis (Fig 8).

(b) Effect of Pitressin. In 22 experiments on five normal dogs Pitressin was administered during a water diuresis. Antidiuresis was always produced. In 16 cases the level of Cl excretion during the antidiuresis was greater than the Cl excretion at the peak of diuresis. On 16 occasions the excretion of Na increased during the antidiuresis, and in 16 experiments the excretion of K was also increased (Fig 9). These findings are shown in Tables X and XIII.

Table X

Na			K			Cl		
+	0	--	+	0	--	+	0	--
16	0	6	16	2	4	16	2	4

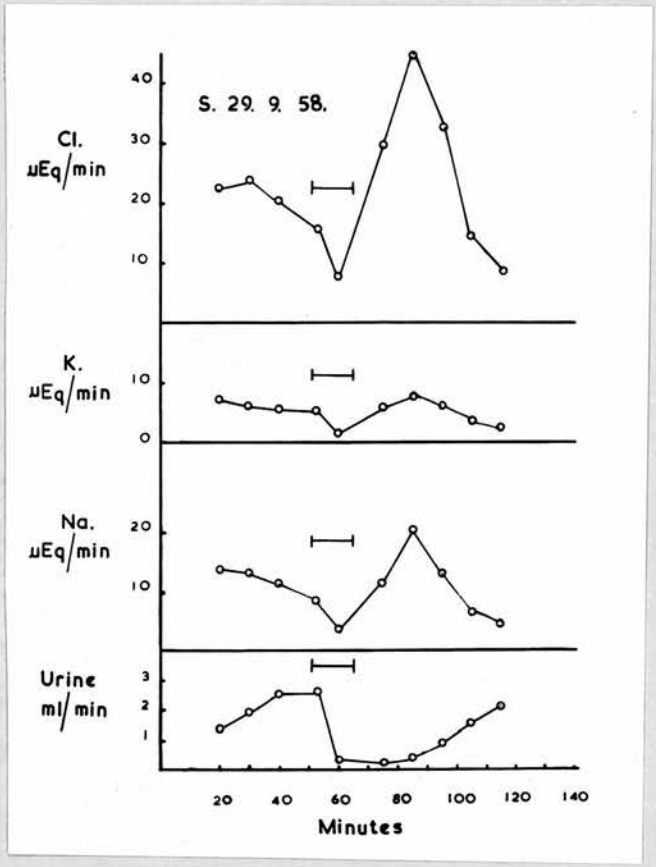

Fig. 7. S. 29.9.58 Water diuresis in a normal dog. 200 ml water at 0 time. 1.6 mU of arginine vasopressin administered i.v. at 51 min, followed by an infusion of 0.1 mU for 14 min.

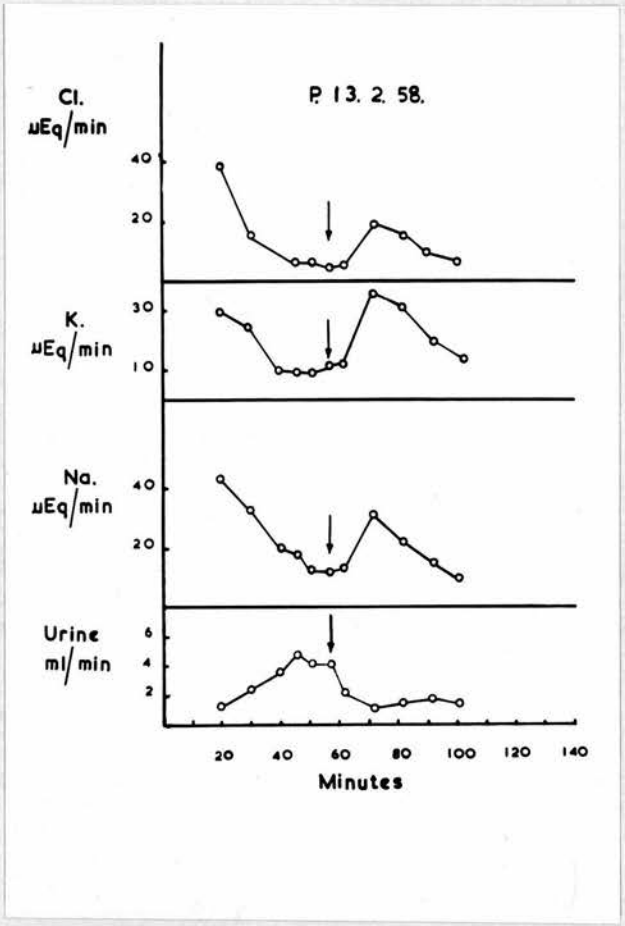

Fig. 8 P 13.2.58 Water diuresis in a normal dog. 250 ml water at 0 time. 2mU arginine vasopressin administered i.v. at 56 min (arrow). The electrolyte excretion was increased at the first sample collected during the antidiuresis.

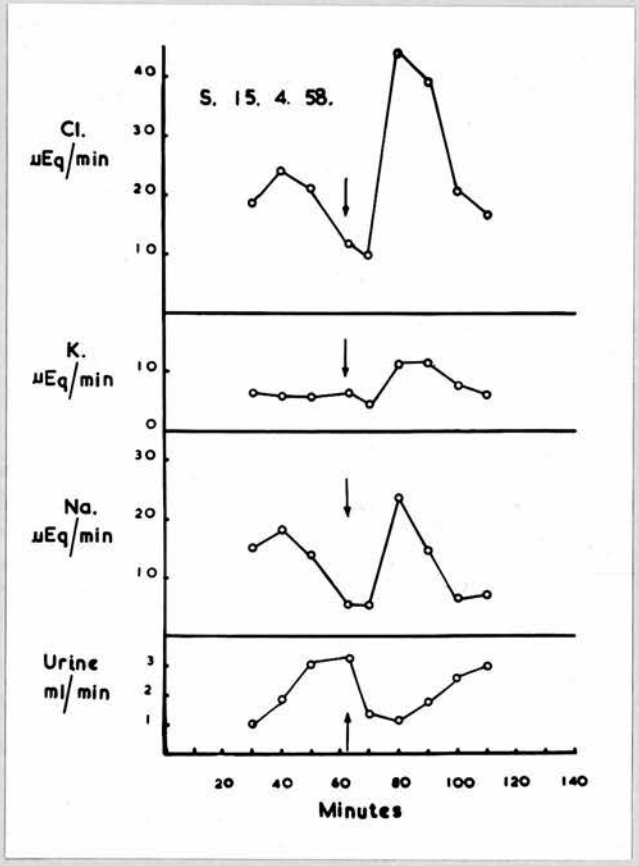

Fig. 9. S 15.4.58 Water diuresis in a normal dog. 250 ml water at 0 time. 2mU Pitressin administered i.v. at 63 min (arrow).

In only two experiments did none of the electrolyte excretion rates increase during the antidiuresis, but even in these cases Pitressin was seen to have an effect in that the progressive decrease in electrolyte excretion usually seen during a water diuresis was arrested during the antidiuresis.

The absolute increase in electrolyte excretion was greatest when electrolyte excretion was high at the beginning of the experiment, but the largest percentage increases were seen when the initial electrolyte excretion was low. The percentage increase in electrolyte excretion usually lay between 50% and 100%, but the actual figure was extremely variable. The magnitude of the increase in electrolyte excretion could not be correlated with the degree of antidiuresis produced.

In 14 experiments the electrolyte excretion was diminished in the first sample collected during the antidiuresis, but in the remaining experiments the increase in electrolyte excretion was immediate. The peak of Na and Cl excretion usually occurred together within 20 min of the onset of antidiuresis, but the peak of K excretion frequently occurred in an earlier or later sample. The elevation of electrolyte excretion usually persisted for about 35 min, which corresponded approximately to the duration of the antidiuresis.

In one experiment where 100 times the usual dose of Pitressin was administered the increase in electrolyte excretion was fivefold, and was maintained for more than 60 min (when the experiment ended). The antidiuresis in

this case was neither excessive nor prolonged, and the diuresis was progressing again within 40 min. (Fig 10).

(c) Effect of oxytocin. In 12 experiments on four normal dogs oxytocin was administered during a water diuresis. Tables XI and XIII list the frequency and type of response of the individual electrolytes.

Table XI

Na			K			Cl		
+	0	--	+	0	--	+	0	--
8	1	3	5	0	7	6	2	4

\+ Increased excretion. 0 No change.

-- Decreased excretion.

These increases in electrolyte excretion were usually less than 20% except in one case (Fig 11) where the dog had been accidently fed before the morning dose of water had been administered.

In all the experiments there was some inhibition of the water diuresis, which varied from a mild transient decrease in the rate of urine flow to an antidiuresis almost of the same magnitude as that produced by vasopressin. The electrolyte response could not be correlated with the degree of antidiuresis produced. Of five cases in which oxytocin produced an obvious increase in the excretion of Na, K and Cl, three cases showed marked antidiuresis while in two cases there was only a slight inhibition of urine flow. In the four experiments where oxytocin did not cause an increase in electrolyte excretion, two were associated with obvious antidiuresis,

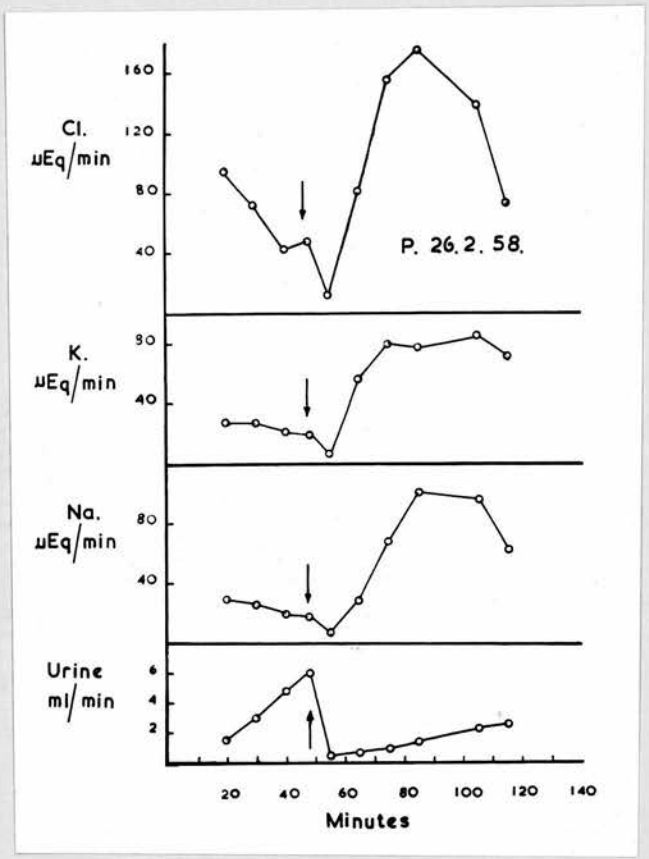

Fig. 10. P 26.2.58 Water diuresis in a normal dog. 300 ml water at 0 time. 300mU Pitressin administered i.v. at 48 min (arrow). There was a large increase in electrolyte excretion and a moderate antidiuresis.

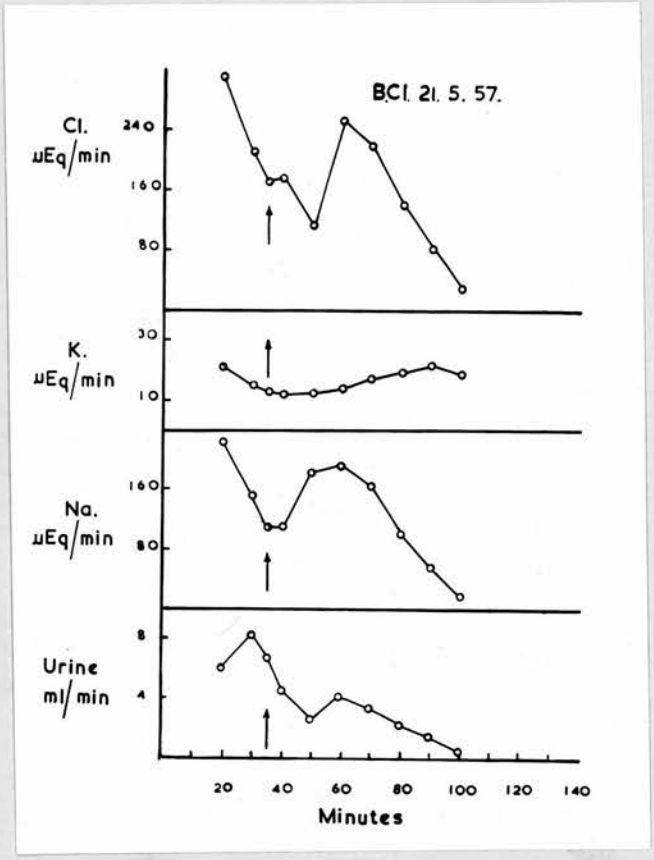

Fig. 11. B.Cl 21.5.57 Water diuresis in a normal dog. 250 ml water at 0 time. 150mU oxytocin administered i.v. at 35 min. The large increase in electrolyte excretion was thought to be related to the fact that the dog had been accidentally fed four hours before.

one showed a slight decrease in urine flow, while in the other case the change in urine flow was not significant.

When an increase in electrolyte excretion was produced it tended to be of longer duration than that produced by vasopressin (Fig 12). In four cases the increase persisted for more than 60 min, in two cases it lasted 40 min, while in the remaining cases the changes were small and did not persist for more than 20 min.

(d) Effect of Pitocin. In two experiments Pitocin was administered to a normal dog during water diuresis. In both cases there was an increase in the excretion rates of all three electrolytes with only a moderate inhibition of urine flow. (Fig 13). This increase was of the order of 20% and lasted for 30 min in one experiment and 40 min in the other.

(e) Effect of mixtures of oxytocin and vasopressin. In four experiments on one dog a mixture of oxytocin and arginine vasopressin was administered during a water diuresis. The mixture consisted of 1/2 the normal dose of oxytocin plus 1/3 the normal dose of vasopressin (80mU oxytocin plus 0.6mU vasopressin).

This mixture was as strongly antidiuretic as the usual dose of vasopressin. The electrolyte responses are shown in tables XII and XIII.

Table XII

Na			K			Cl		
+	0	--	+	0	--	+	0	--
1	1	2	1	0	3	1	0	3

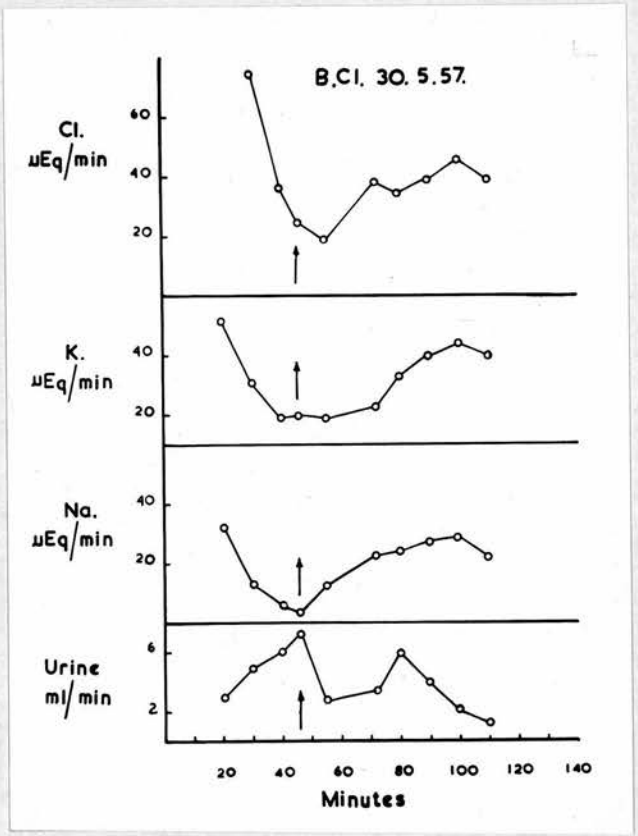

Fig. 12. B.Cl 30.5.57 Water diuresis in a normal dog. 300 ml water at 0 time. 150mU of oxytocin administered i.v. at 46 min (arrow). The increase in electrolyte excretion was small, but was of longer duration than that produced by vasopressin.

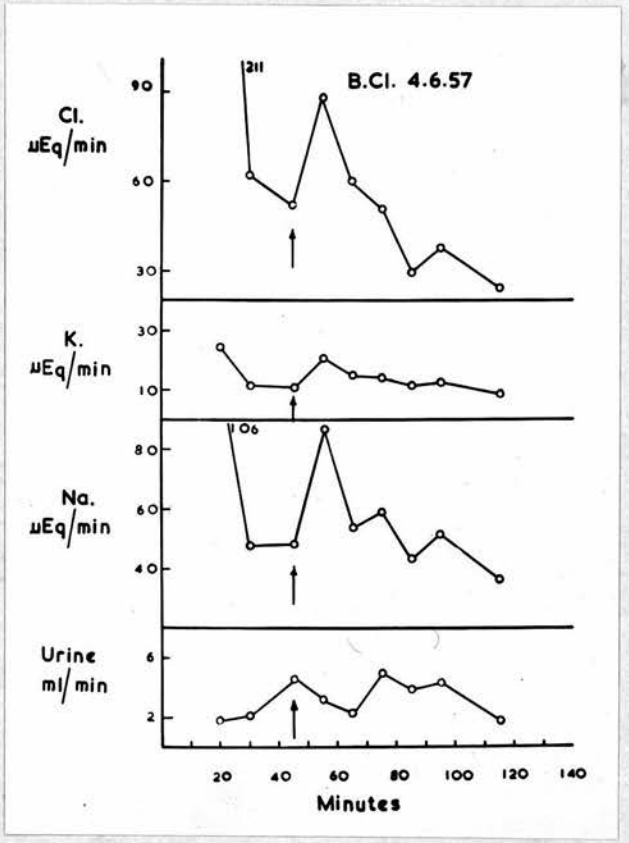

Fig. 13. B.Cl 4.6.57 Water diuresis in a normal dog. 300 ml water at 0 time. 150mU Pitocin administered i.v. at 45 min (arrow).

Table XIII

EFFECT OF POSTERIOR PITUITARY PREPARATIONS ON ELECTROLYTE EXCRETION DURING WATER DIURESIS

	Na			K			Cl			Total
	+	0	−	+	0	−	+	0	−	
Arginine vasopressin	5	0	1	3	1	2	6	0	0	6
Pitressin	16	0	6	16	2	4	16	2	4	22
Oxytocin	8	1	3	5	0	7	6	2	4	12
Pitocin	2	0	0	2	0	0	2	0	0	2
Oxytocin ± Vasopressin	1	1	2	1	0	3	1	0	3	4

\+ Increased excretion.

0 No change.

− Decreased excretion.

The increase in electrolyte excretion was never greater than 20% and did not persist for more than 40 min (Fig 14).

(II) Experiments conducted during the nondiuretic state.

(a) Effect of oxytocin. In 14 experiments oxytocin was administered during the nondiuretic rate of urine flow. There was no consistent significant effect on urine flow. Tables XIV and XVI list the frequency and type of response of the individual electrolytes.

Table XIV

Na			K			Cl		
+	0	--	+	0	--	+	0	--
7	3	4	6	3	5	7	4	3

\+ Increased excretion. 0 No change.

-- Decreased excretion.

These increases in electrolyte excretion were sustained for quite long periods. In two experiments in which the observations continued for a sufficiently time the increases were seen to be still present after 70 min. In eight other experiments the excretion of either Na or Cl or both was elevated for more than 50 min after the injection. (Fig 15).

The alteration in electrolyte excretion could not be correlated with changes in the rate of urine flow. Of the eight experiments in which there was an obvious increase in the rate of electrolyte excretion, there was an increased urine flow in only one, a decreased urine

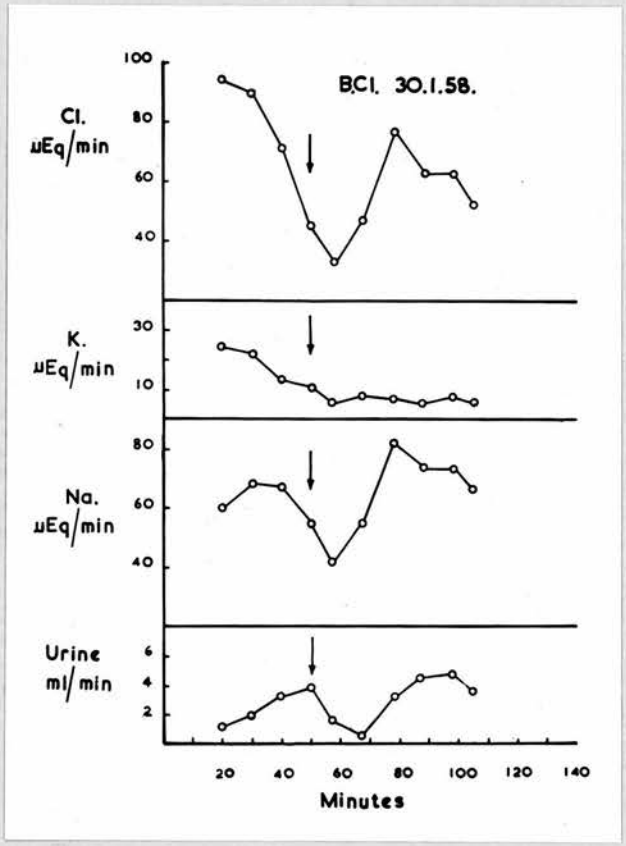

Fig. 14. B.Cl 30.1.58 Water diuresis in a normal dog. 300ml water at 0 time. 80mU oxytocin plus 0.6mU vasopressin administered i.v. at 51 min (arrow).

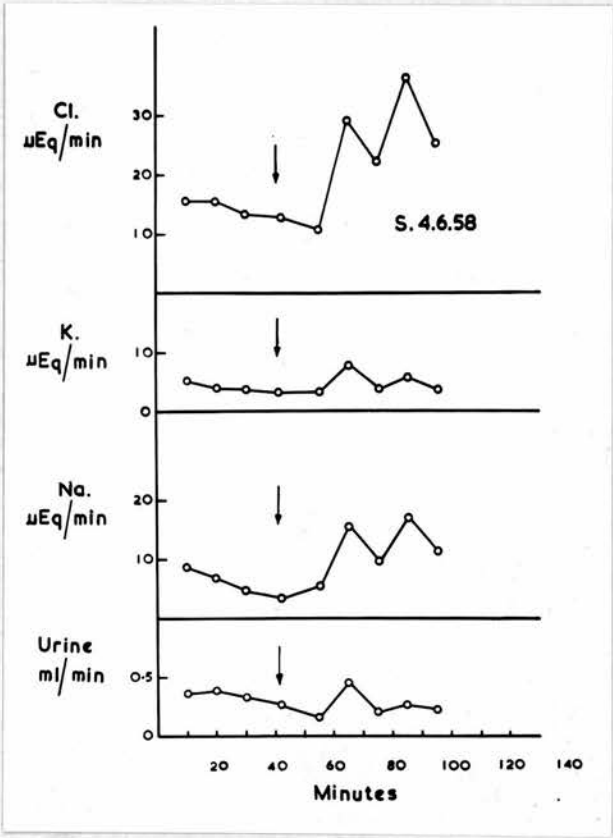

Fig. 15. S 4.6.58 Non diuretic urine flow in a normal dog. 150mU oxytocin administered i.v. at 42 min (arrow).

flow in two, and no alteration in urine volume in the others.

The tendency for oxytocin to cause an increased electrolyte excretion did not appear to be related to the initial level of electrolyte excretion. In eight experiments the excretion rate of Na began at less than 15 uEq/min. In three of these oxytocin caused an increased electrolyte excretion, in four no change, and in one a decrease. All the increases recorded were greater than 50%. In six experiments the excretion rate of Na began at levels ranging from 20 uEq/min to 90uEq/min. In four of these cases oxytocin caused an increase in excretion of Na, which in two cases was greater than 50%. In one experiment the Na excretion was unchanged, and in one experiment the Na excretion was decreased.

The initial level of K excretion also did not appear to influence the response of either K, Na or Cl. The initial excretion of K ranged from 1.5uEq/min to 40uEq/min and the negative electrolyte responses were distributed in a random fashion throughout this range.

(b) Effect of Pitocin. In two experiments on two different dogs Pitocin was administered during the non-diuretic state. In both experiments the excretion of Na and Cl was increased, while the excretion of K and water remained unchanged. The changes were of the same magnitude and duration as those caused by a similar dose of oxytocin.

(c) Effect of vasopressin. In three experiments on two normal dogs the usual antidiuretic dose of vasopressin was administered during the non diuretic state. In all cases, for one sample only the urine flow was reduced to about 50% of the pre-injection level. Tables XV and XVI list the frequency and type of response of the individual electrolytes.

Table XV

Na			K			Cl		
+	0	--	+	0	--	+	0	--
1	1	1	2	0	1	2	0	1

\+ Increased excretion. 0 No change.

-- Decreased excretion.

The increases in electrolyte excretion when seen were small, the greatest being an increase in Cl excretion from 6.5 uEq/min before the injection to an average of 12.2 uEq/min after the injection (Fig 16). In only one case did the increase in electrolyte excretion persist for 60 min.

(d) Effect of Pitressin. On one occasion the usual antidiuretic dose of Pitressin was administered during the non diuretic state. The urine flow was reduced from 1.35 ml/min to 0.5 ml/min for one sample only. The excretion rate of Na was 69 uEq/min before the injection and 72 uEq/min after the injection. This change in electrolyte excretion is not significant. The excretion rates of K and Cl were not altered.

On one occasion, due to an error of dilution, a dose

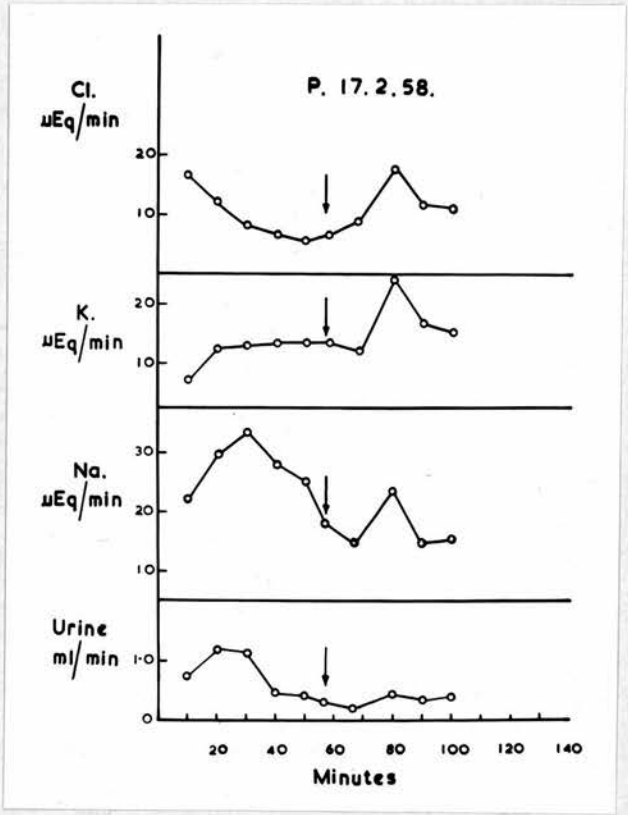

Fig. 16 P 17.2.58 Non diuretic urine flow in a normal dog. 2mU vasopressin administered i.v. at 56 min (arrow). This is the largest increase in electrolyte excretion recorded in any experiment where vasopressin was administered during the non diuretic state.

Table XVI

EFFECT OF POSTERIOR PITUITARY PREPARATIONS

ON ELECTROLYTE EXCRETION DURING THE NON DIURETIC STATE

	Na			K			Cl			Total
	+	0	—	+	0	—	+	0	—	
Oxytocin	7	3	4	6	3	5	7	4	3	14
Pitocin	2	0	0	0	2	0	2	0	0	2
Vasopressin	1	1	1	2	0	1	2	0	1	3
Pitressin	0	1	0	0	1	0	0	1	0	1

\+ Increased excretion.

0 No change.

— Decreased excretion.

of 300mU Pitressin was administered during the non diuretic state. Although the urine flow was inhibited for only 10 min, the excretion rates of all electrolytes increased four to fivefold, and were still elevated 44 min later (Fig 17).

(III) Effect of vasopressin on glomerular filtration rate.

In five experiments on one dog the effect of vasopressin on the excretion of endogenous creatinine was studied during a water diuresis. In three experiments the excretion of endogenous creatinine was not altered by vasopressin, but in two other cases there were increases of 10% and 13% which persisted throughout the antidiuresis.

B. EXPERIMENTS ON HUMANS

(I) Pitressin administered during a water diuresis.

In four experiments in two human subjects doses of 10mU and 15mU Pitressin were administered intravenously during a water diuresis. In all cases an antidiuresis lasting 50 min was produced, but no increase in electrolyte excretion was observed.

(II) Oxytocin administered in the non diuretic state.

In two experiments on one human subject oxytocin was administered during the non diuretic state. On one occasion a single injection of 250mU was made, and on the second occasion a single injection of 150mU was followed by an infusion of 15mU for 15 min. In neither case was the excretion of electrolytes altered by the injection.

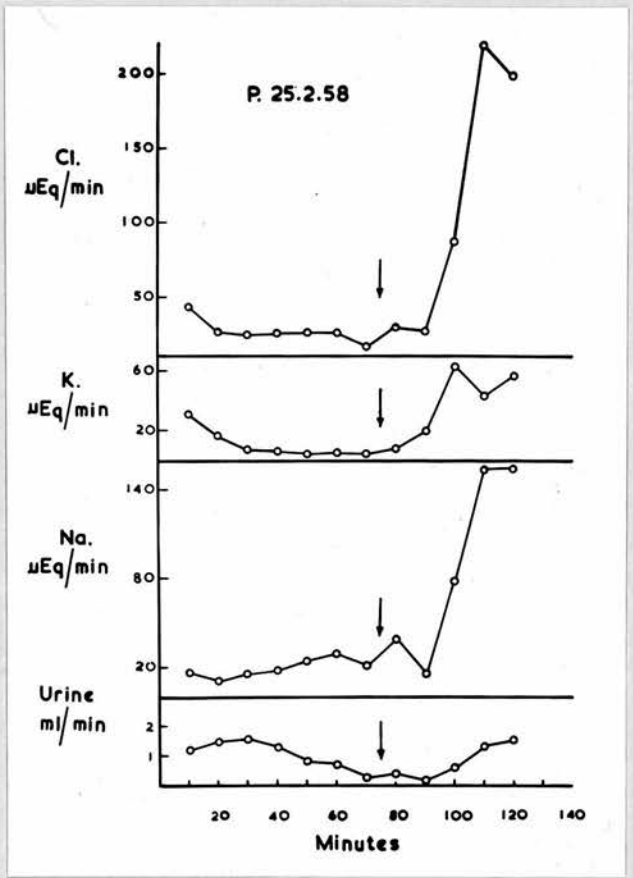

Fig. 17 P 25.2.58 Non diuretic urine flow in a normal dog. 300mU Pitressin administered i.v. at 75 min (arrow).

DISCUSSION

The findings recorded above are presented as evidence that under selected conditions, both vasopressin and oxytocin may increase the renal elimination of electrolytes in the dog. Nevertheless the same data if interpreted using less rigid criteria could be inconclusive.

In interpreting the findings from the water diuresis experiments it is important to be aware of the normal pattern of electrolyte excretion during a water diuresis. In Section I it has been shown that under the conditions of these experiments, the excretion rates of Na, K and Cl could be expected to fall from the onset of the diuresis until the urine flow had returned to resting levels some 80 min later. Similar findings have been reported by Ali, Cross and Pickford (1958). If the test substance is injected near the peak of diuresis, quite large increases in electrolyte excretion will be overlooked if the total excretion during the hour after the injection is merely compared with the excretion during the hour before the injection was made. This method of analysis of the data ignores the underlying downward trend in electrolyte excretion.

In this study the average electrolyte excretion resulting from an injection has been compared with the excretion rate existing during the 10 min period immediately before the injection was made. Even this method of interpretation may fail to reveal some positive results, for in those cases where the electrolyte excretion rates are declining rapidly during the rising

phase of the diuresis, an injection of a posterior pituitary extract may considerably inhibit this decline without causing the actual excretion rate to rise above the pre-injection level. A more accurate analysis of the data would involve the comparison of the actual excretion of electrolytes with the excretion which could have been expected had no injection been made. This refinement has been used in a later section where greater accuracy was required. The crude analysis used here is sufficiently accurate to show the general trend of results, and since the inherent error will cause a consistent underestimation of the magnitude of increases in electrolyte excretion, the significance of the positive findings is enhanced.

In these experiments no difference was detected between the actions of pure arginine vasopressin and Pitressin. Therefore, since arginine vasopressin was in short supply, Pitressin was frequently used in subsequent experiments as the preparation of vasopressin.

These findings are in general in accord with those of Brooks and Pickford (1957). Whereas these workers found it desirable to administer vasopressin by slow intravenous infusion to obtain a clearly demonstrable increase in electrolyte excretion during a water diuresis, in this study a positive response was almost invariably obtained from a single injection. This increase in electrolyte excretion is not accompanied by any significant alteration in the excretion of endogenous creatinine, which was used as a measure of changes in glomerular filtration rate. This finding confirms the

observations of Anslow and Wesson (1955) and Brooks and Pickford (1958). On one occasion, when a massive dose of 300mU of Pitressin was administered during a water diuresis, the electrolyte excretion increased to much higher levels than usual, and remained at these levels for an unusually long time, although the antidiuresis was of normal dimensions. These findings are in direct conflict with those of Little et al (1947), who found in the human that Pitressin caused an increase in electrolyte excretion only when administered in doses too small to produce antidiuresis. The question arises from this experiment whether the electrolyte excreting effect of Pitressin can be dissociated from the antidiuretic effect. Unfortunately no clearance studies were made in this particular experiment, so that it is uncertain whether such massive doses of vasopressin are acting through the same mechanisms as the normal dose, or whether these effects are produced by changes in renal haemodynamics.

The action of vasopressin during the non diuretic state has also been reported by Brooks and Pickford (1957) who found that under these circumstances the normal antidiuretic dose of vasopressin was without effect. The failure of vasopressin to increase electrolyte excretion at low rates of urine flow could be explained by the assumption that during the non diuretic state the dog is already secreting considerable quantities of endogenous vasopressin. Since the antidiuretic effect of vasopressin is proportional to the log of the dose (Dicker, 1953), it is reasonable to assume that a small additional dose

of vasopressin may be without effect on electrolyte excretion. During a water diuresis, when the endogenous vasopressin is very low, the same dose of vasopressin might exert a profound effect. This view is lent support by the increase in electrolyte excretion and decreased urine flow produced when 300mU of Pitressin were administered during the non diuretic state. As discussed above, such massive doses of vasopressin may cause changes in renal haemodynamics (and unfortunately again no clearance studies were made in this case). However, it may well be that large additional doses of vasopressin are required to produce an appreciable response in the presence of endogenous vasopressin.

The action of oxytocin is best seen in the non diuretic state, where it causes the excretion of electrolytes to increase for an hour or more. This increase is said to be accompanied by an increase in renal plasma flow without changes in glomerular filtration (Brooks and Pickford, 1957), although Ali (1958) did produce increases in G.F.R. with slightly larger doses of oxytocin. These changes in renal haemodynamics could be sufficient to explain the action of oxytocin in increasing electrolyte excretion without invoking a direct tubular activity. However, oxytocin produces these changes in renal blood flow even during a water diuresis when the electrolyte effects are very small or absent. The slight increase in electrolyte excretion produced by oxytocin during water diuresis could possibly be explained by altered renal haemodynamics, but they could also be due to the traces

of vasopressin like activity which is present in even pure synthetic oxytocin (Berde, Doepfner and Konzett, 1957). for in all cases where oxytocin increased electrolyte excretion during a diuresis there was some inhibition of urine flow. However, in some of the experiments reported in this section, the increases in electrolyte excretion produced by oxytocin during a water diuresis, though of smaller magnitude than those produced by vasopressin were of longer duration. This finding suggests that even under these circumstances oxytocin may increase electrolyte excretion by a specific action of its own. There is some evidence that such an action may not be directly on the kidney, but may be mediated through a humoral reflex involving the hypothalamus (Brooks and Pickford, 1958). Small doses of oxytocin injected into the carotid artery were found to increase electrolyte excretion in the non diuretic state without altering renal haemodynamics.

Although Pitocin when administered during the non diuretic state had an action identical with that of pure oxytocin, it was found that during a water diuresis Pitocin caused a larger increase in electrolyte excretion than would have been caused by a similar dose of pure oxytocin. This finding is in accord with those of Brooks and Pickford (1957) although the occasional differences between the response to Pitocin and oxytocin reported by these workers were much more obvious than the differences found in this study.

Brooks and Pickford found that Pitocin did not

derive its special activity by virtue of being a mixture of oxytocin contaminated by a small quantity of vasopressin. When such mixtures were artificially prepared from the two pure hormones it was found that they did not cause the large increases in electrolyte excretion seen when Pitocin was administered during a water diuresis. Indeed they found some antagonism between the actions of vasopressin and oxytocin, in that the increases in renal plasma flow produced by oxytocin is abolished if the oxytocin is administered in combination with vasopressin. In this study it was found that the antidiuretic properties of vasopressin and oxytocin were synergistic when administered together. This finding was in conflict with those of Brooks and Pickford (1958) who found the mixtures to be only weakly antidiuretic. Both investigations however showed that mixtures of vasopressin and oxytocin were not particularly potent in increasing electrolyte excretion.

Little significance can be placed upon the few results obtained from human experiments, in this present study. The conditions of these experiments were not standardized and no preliminary studies had been made of the pattern of electrolyte excretion during an unmodified diuresis in these subjects.

SECTION III

EFFECT OF ELECTROLYTE LOADING ON THE RESPONSE TO VASOPRESSIN AND OXYTOCIN

INTRODUCTION

The state of NaCl loading of the test animal has been found by several workers to condition the renal response to posterior pituitary extracts. Anslow and Wesson (1955) found that the increase in electrolyte excretion caused by vasopressin in a water diuresis could be magnified by loading the test dog with NaCl. West, Traegar and Kaplan (1955) found that Pitressin had its greatest effect in increasing osmolar clearance if administered during an osmotic diuresis. Working with rats, Jacobson and Kellogg (1956) found that the chloruretic effect of vasopressin increased with increasing NaCl loading. In Section II it was reported that vasopressin caused the greatest absolute increases in electrolyte excretion in those cases where the electrolyte excretion began at high levels. The greatest percentage increases were, however, seen in those experiments where the initial electrolyte excretion was low.

In the rat the chloruretic action of oxytocin has been found to be increased by NaCl loading (Jacobson and Kellogg, 1956). In the dog, oxytocin increases electrolyte excretion only at low rates of urine flow (Abrahams and Pickford, 1957), but there does not appear to have been any investigation into the effect of NaCl loading on this phenomenon. It was of interest to observe that oxytocin

caused an increase in electrolyte excretion during a water diuresis in a dog which was excreting large amounts of NaCl as a result of a recent meal (Section II). Although Anslow and Wesson (1955) found that NaCl loading did not alter the response to oxytocin during a water diuresis, the following experiments were undertaken in an attempt to explain the large increase in electrolyte excretion caused by oxytocin in this one case where the dog had been accidentally fed.

METHODS

The plan of the experiment was as already described in Section II, except that the morning dose of water contained a measured quantity of the salt under investigation. The dose of NaCl administered in early experiments was set at 1.5 gm, which was thought to be a reasonable estimate of the quantity of NaCl in the usual meal. This dose contains 255 mEq of Na. All subsequent doses of salts were calculated to contain the same number of equivalents of the electrolyte under investigation. In some experiments larger or smaller doses were administered to demonstrate the effect of altering the electrolyte load.

In one experiment when the dog was on a diet of low electrolyte content, the usual dose of $NaHCO_3$ was accompanied by a small dose of $KHCO_3$ (0.5 gm). This K salt was administered to prevent the development of a K deficiency which might alter the renal response to vasopressin by causing degenerative lesions in the tubules. (Hollander, Winters and Williams, 1956; Oliver et al, 1957).

In one series of experiments an acidosis was produced by the inhibition of urinary carbonic anhydrase by a single oral dose of 100 mg Diamox 30 hrs before the induction of water diuresis (Hanley and Platts 1956).

RESULTS

(I) Experiments conducted after NaCl loading.

(a) Vasopressin administered during a water diuresis.

In nine experiments on four dogs the usual antidiuretic dose of vasopressin was administered during a water diuresis in the afternoon following a morning dose of NaCl. The resultant antidiuresis was not as great as that produced by vasopressin during a simple water diuresis. In four of the experiments the reduction in urine flow was slight. In four others the urine flow fell to below 1 ml/min for one sample only (Fig 18). In the remaining experiment the vasopressin was administered by continuous infusion and there was an obvious antidiuresis for the duration of the infusion.

The frequency and type of response of the individual electrolytes is listed in Table XVII.

Table XVII

Na			K			Cl		
+	0	--	+	0	--	+	0	--
4	1	4	7	0	2	5	2	2

\+ Increased excretion. 0 No change.

-- Decreased excretion.

The increase in electrolyte excretion, when seen, was usually of short duration, the downward trend in electrolyte excretion being resumed within 40 min. The magnitude of the increase in Na and K excretion was usually small (about 10%), but the increase in Cl excretion tended to

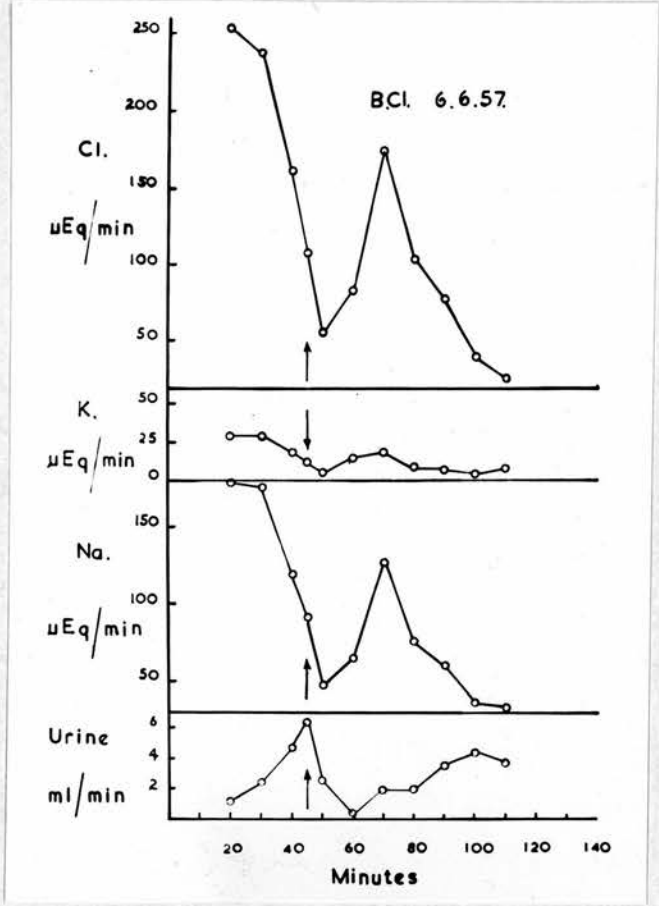

Fig. 18 B.Cl 6.6.57 Water diuresis in a normal dog. 3gm NaCl administered with the morning water load. 300 ml water at 0 time. 2mU Pitressin administered i.v. at 45 min (arrow).

be greater, an increase of 100% being observed on two occasions. However, because of the initial high rates of electrolyte excretion in these experiments, small percentage increases resulted in substantial absolute changes in electrolyte excretion.

The tendency for the electrolyte excretion to increase was not apparently related to the degree of antidiuresis produced, although the largest percentage increase in electrolyte excretion and the greatest antidiuresis were both seen in the experiment where the vasopressin was given by infusion.

(b) Oxytocin administered during a water diuresis.

In 11 experiments on three dogs oxytocin was administered during a water diuresis on the afternoon following a morning dose of NaCl.

There was a variable slight decrease in the urine flow in all experiments. The frequency and kind of response of the individual electrolytes is listed in Table XVIII.

Table XVIII

Na			K			Cl		
+	0	−	+	0	−	+	0	−
7	2	2	4	2	5	5	2	4

The magnitude of the increase in electrolyte excretion (Na, K, and Cl) was extremely variable, but did not exceed 30% (Fig 19). It could not be related to the degree of antidiuresis produced. Neither the absolute nor the relative increase in electrolyte excretion could

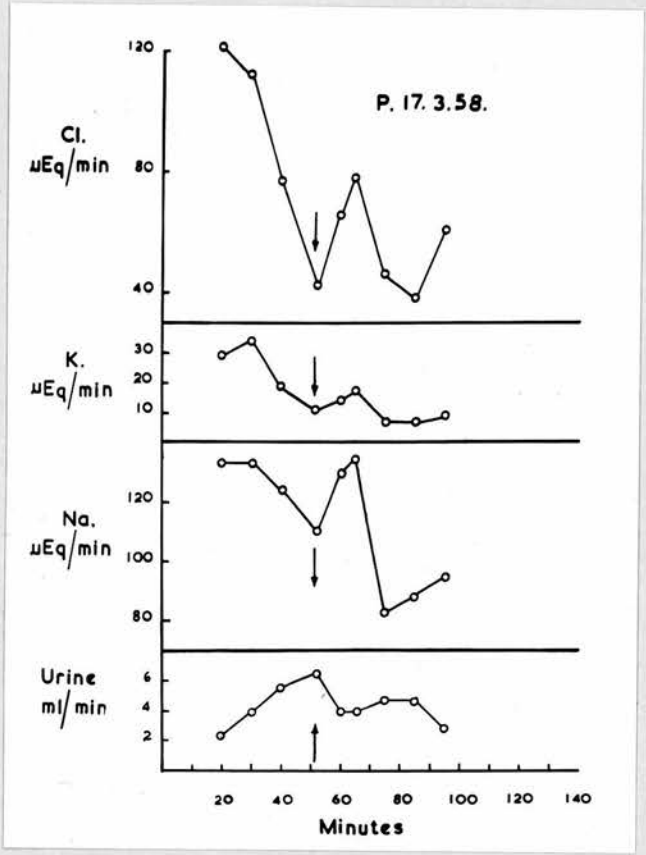

Fig. 19 P 17.3.58 Water diuresis in a normal dog. 2gm NaCl administered with the morning water load. 300 ml water at 0 time. 150mU oxytocin administered i.v. at 51 min (arrow).

be correlated with the levels of electrolyte excretion at the beginning of the experiment.

The duration of the increases was also variable. In two experiments the increase persisted for more than 55 min, but in the remaining experiments the increase had ceased within 30 min.

(c) Oxytocin administered during the non diuretic state. In two experiments on two different dogs oxytocin was administered during the observation of the resting flow on an afternoon following a morning dose of NaCl. On one occasion a single injection of oxytocin caused a transitory decrease in urine flow rate with no net increase in electrolyte excretion.

In the second experiment the oxytocin was administered by continuous slow infusion lasting 20 min. This resulted in an obvious inhibition of urine flow associated with an increase in electrolyte excretion which was maintained for 73 min (Fig 20).

(II) Experiments conducted after $NaHCO_3$ loading.

(a) Vasopressin administered during a water diuresis. In 11 experiments on four dogs the usual antidiuretic dose of arginine vasopressin or Pitressin was administered during a water diuresis on the afternoon following a morning dose of $NaHCO_3$. The usual degree of antidiuresis was produced, but the increase in electrolyte excretion was in most cases quite small, exceeding 25% on only one occasion. In all cases the increased electrolyte excretion

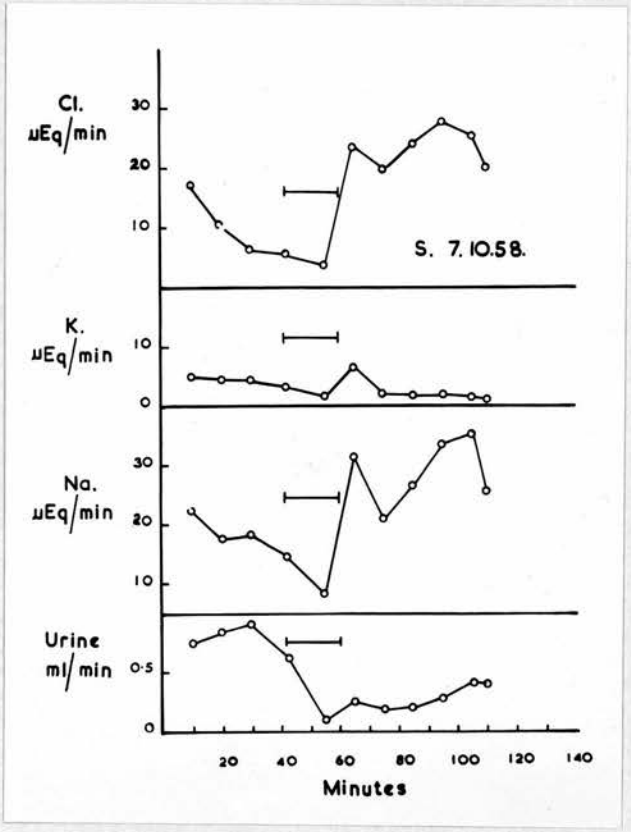

Fig. 20. S 7.10.58 Non diuretic urine flow in a normal dog. 1.5gm NaCl administered with the morning water load. 40mU oxytocin administered i.v. at 40 min, followed by an infusion of 2mU/min for 20 min.

had subsided within 40 min.

Table XIX lists the frequency and type of response of the individual electrolytes.

Table XIX

Na			K			Cl		
+	0	--	+	0	--	+	0	--
7	3	1	6	0	5	11	0	0

(b) Oxytocin administered during a water diuresis.

In four experiments on two dogs oxytocin was administered during a water diuresis on the afternoon following a morning dose of $NaHCO_3$. In every case there was a slight inhibition of urine flow. Table XX lists the frequency and type of response of the individual electrolytes.

Table XX

Na			K			Cl		
+	0	--	+	0	--	+	0	--
2	2	0	4	0	0	2	0	2

In only one case (Fig 21) was the increase much more than a transitory inhibition of the fundamental downward trend in electrolyte excretion.

(III) Experiments conducted after sodium citrate loading.

In one experiment the usual antidiuretic dose of Pitressin was administered during a water diuresis on the afternoon following a morning dose of sodium citrate. There was a normal antidiuresis. The excretion rates of Na and K were unaltered, but the excretion of Cl was

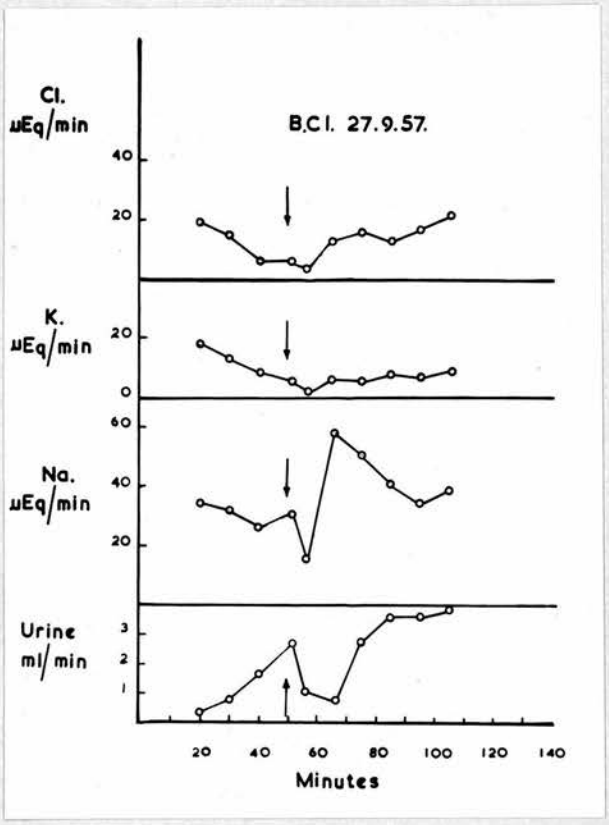

Fig. 21. B.Cl 27.9.57 Water diuresis in a normal dog. 2.15gm $NaHCO_3$ administered with the morning water load. 300 ml water at 0 time. 150mU oxytocin administered i.v. at 50 min (arrow).

increased by 50% for the next 48 min. (Fig 22).

(IV) Experiments conducted after Na_2SO_4 loading.

In one experiment the usual antidiuretic dose of Pitressin was administered during a water diuresis on the afternoon following a morning dose of Na_2SO_4. There was a normal antidiuresis, and the excretion of Na, K, and Cl was elevated for the next 45 min. (Fig 23).

(V) Experiments conducted after KCl loading.

(a) Vasopressin administered during a water diuresis. In two experiments on two dogs arginine vasopressin or Pitressin was administered during a water diuresis on the afternoon following a morning dose of KCl. In both experiments the usual amount of antidiuresis was produced. On both occasions the excretion of Na and Cl was increased, but in only one case was the excretion of K increased. (Fig 24).

(b) Oxytocin administered during a water diuresis. In four experiments on three dogs oxytocin was administered during a water diuresis on the afternoon following a morning dose of KCl. There was a mild inhibition of urine flow in all experiments, but there was no increase in the excretion rate of any electrolyte.

(c) Oxytocin administered during the non diuretic state. In one experiment oxytocin was administered during observation of the resting rate on the afternoon following a morning dose of KCl. In this experiment the

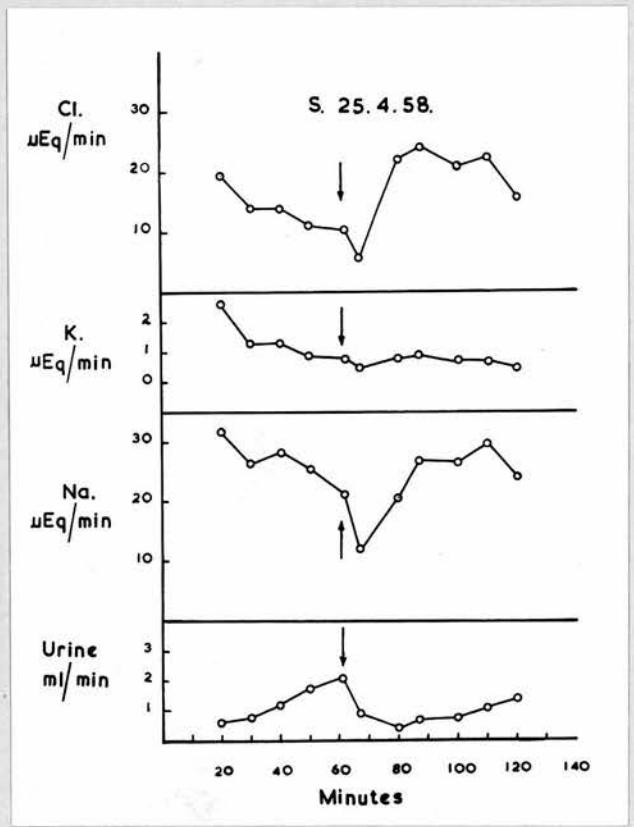

Fig. 22. S 25.4.58 Water diuresis in a normal dog. 2.5gm Na citrate administered with the morning water load. 250ml water at 0 time. 2mU Pitressin administered i.v. at 61 min (arrow).

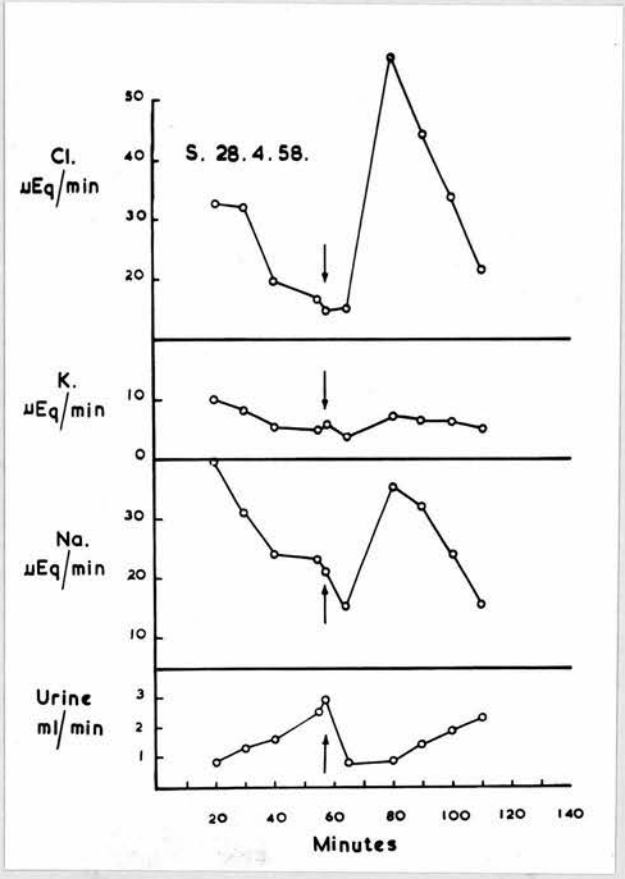

Fig. 23. S 28.4.58 Water diuresis in a normal dog. 1.82gm Na_2SO_4 administered with the morning water load. 250ml water at 0 time. 2mU Pitressin administered i.v. at 57 min (arrow).

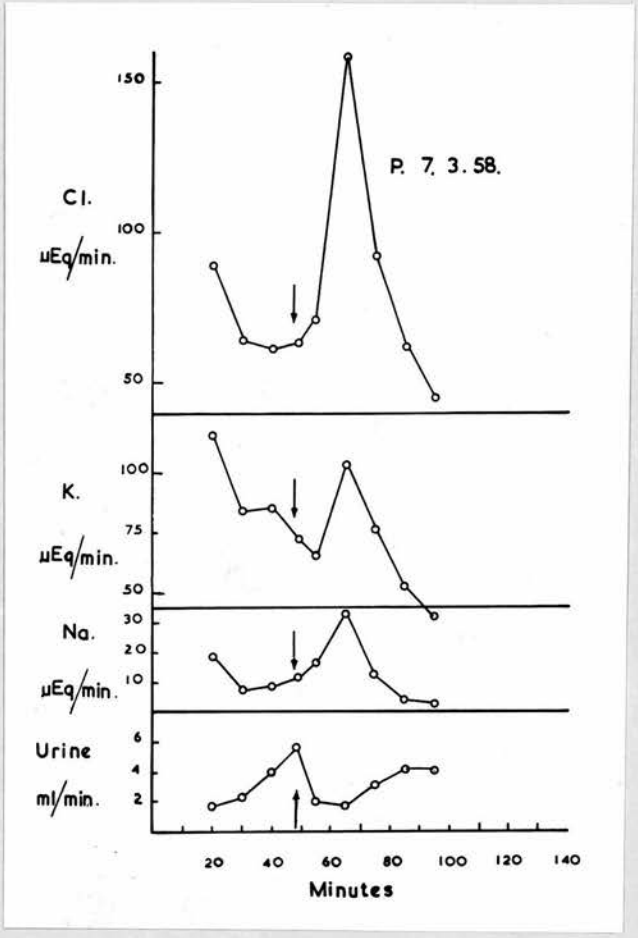

Fig. 24. P 7.3.58 Water diuresis in a normal dog. 2.5gm KCl administered with the morning water load. 300 ml water at 0 time. 4mU Pitressin administered i.v. at 48 min (arrow).

resting flow was somewhat higher than usual. Oxytocin caused a decrease in the urine flow with an increase in the excretion of electrolytes, especially K. (Fig 25).

(VI) Experiments conducted after $KHCO_3$ loading.

(a) Pitressin administered during a water diuresis.

In three experiments on two dogs the usual antidiuretic dose of Pitressin was administered during a water diuresis on the afternoon following a morning dose of $KHCO_3$. In two of the experiments the diuresis reached unusually high levels, and in these cases Pitressin caused only a moderate inhibition of urine flow. In one experiment where the diuresis was no greater than usual Pitressin caused a normal antidiuresis. In all three experiments there was an increase in electrolyte excretion, and in two cases the increase in K excretion was of unusually large proportions (over 50%). (Fig 26). The increase in Na and Cl excretion in all cases was greater than 100%, but this had subsided within 40 min.

(b) Oxytocin administered during a water diuresis.

On one occasion oxytocin was administered during a water diuresis on the afternoon following a morning dose of $KHCO_3$. There was a mild inhibition of urine flow. The excretion of electrolytes was unaltered except that, for about 30 min, the fundamental tendency for Na excretion to diminish was absent.

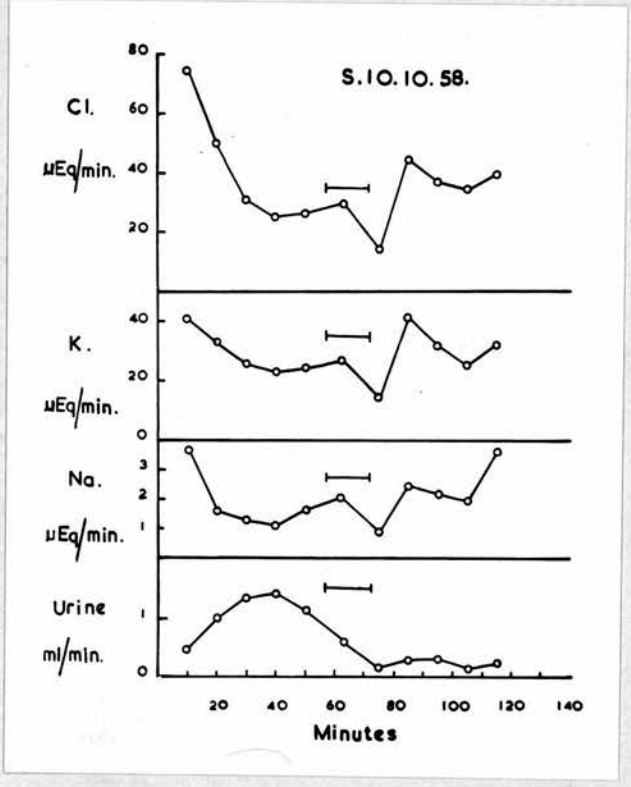

Fig. 25. S 10.10.58 Non diuretic urine flow in a normal dog. 1.9 gm KCl administered with the morning water load. 40mU oxytocin administered i.v. at 57 min, followed by an infusion of 2mU/min for 16 min.

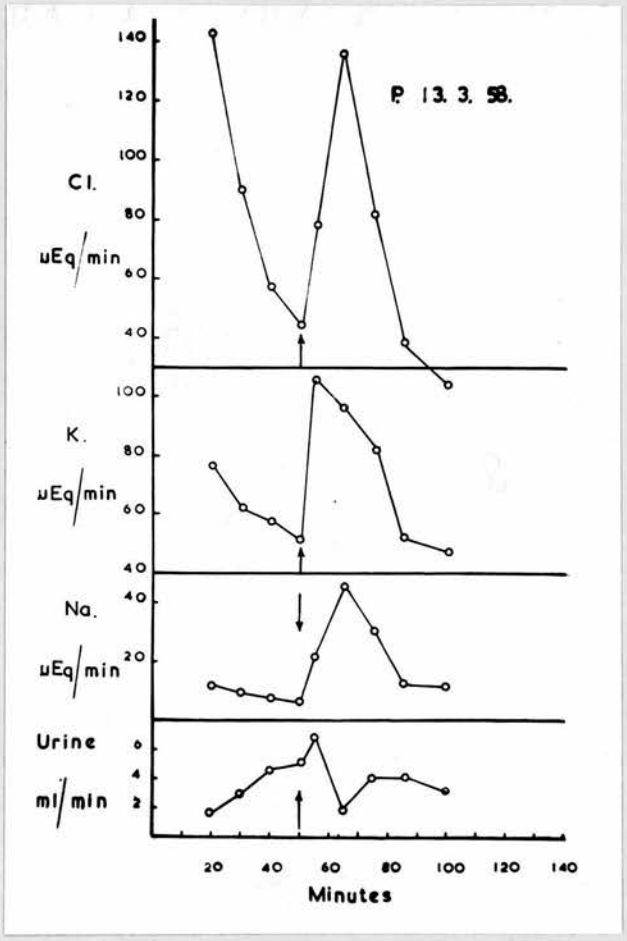

Fig. 26. P 13.3.58 Water diuresis in a normal dog. 3.35gm $KHCO_3$ administered with the morning water load. 300 ml water at 0 time. 4mU Pitressin administered i.v. at 50 min (arrow). Note the large increase in K excretion.

(VII) Experiments conducted after combined $NaHCO_3$ and $KHCO_3$ loading.

In four experiments on one dog the usual antidiuretic dose of Pitressin was administered during a water diuresis on the afternoon following a morning dose of 1.5 gm $NaHCO_3$ and 0.5 gm $KHCO_3$. In all cases there was a normal antidiuresis. The frequency and kind of response of the individual electrolytes is listed in table XXI.

Table XXI

Na			K			Cl		
+	0	--	+	0	--	+	0	--
3	1	0	3	1	0	4	0	0

In all experiments there was an obvious increase in Cl excretion. In three experiments there was an increase in Na excretion of about 30% and a smaller increase in K excretion (Fig 28). The increases lasted about 40 min. During these experiments this dog was on a diet poor in electrolytes.

(VIII) Experiments conducted after K citrate loading.

In two experiments on one dog the usual antidiuretic dose of Pitressin was administered during a water diuresis on the afternoon following a morning dose of K citrate. In both cases a normal antidiuresis was produced. In one experiment there was no alteration in electrolyte excretion. In the other experiment there was an increase in Na and Cl excretion while the excretion of K continued to decrease. (Fig 27).

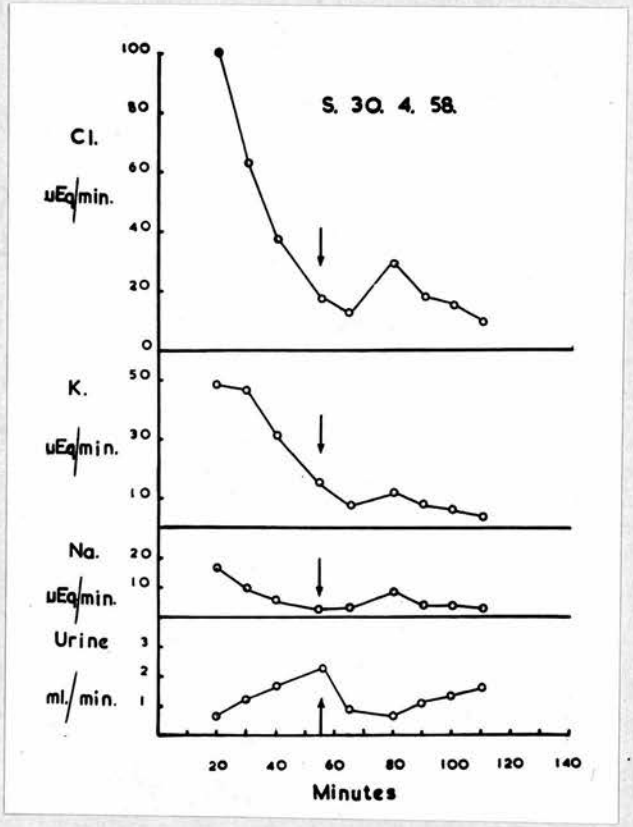

Fig. 27. S 30.4.58 Water diuresis in a normal dog. 2.77gm K citrate administered with the morning water load. 250 ml water at 0 time. 2mU Pitressin administered i.v. at 56 min (arrow).

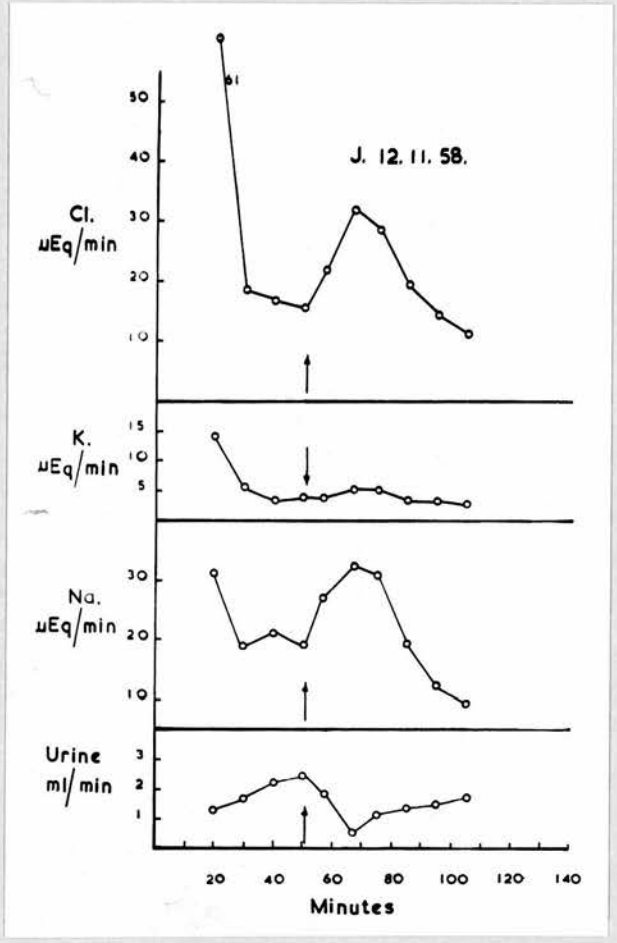

Fig. 28. J 12.11.58 Water diuresis in a normal dog. 1.5 gm $NaHCO_3$ and 0.5 gm $KHCO_3$ administered with the morning water load. 300 ml water at 0 time. 3mU Pitressin administered i.v. at 51 min (arrow).

(IX) Experiments conducted after NH_4Cl loading.

(a) Vasopressin administered during a water diuresis.

In twelve experiments on four dogs the usual antidiuretic dose of arginine vasopressin or Pitressin was administered during a water diuresis on the afternoon following a morning dose of 1.4 gm NH_4Cl. On all occasions there was a brisk antidiuretic response. There was always an increase in Cl excretion which was usually of the magnitude of 50% to 100%. Except in one case where the increase in Cl excretion was small, there was also an increase in Na and K excretion of the order of 50% (Fig 29). These increases lasted about 35 min which corresponded roughly with the duration of antidiuresis.

During six of these experiments the animal (Jill) was on a diet poor in electrolytes. The results were the same as obtained from animals on a normal diet.

(b) Oxytocin administered during a water diuresis.

In six experiments on three dogs oxytocin was administered during a water diuresis on the afternoon following a morning dose of 1.4 gm NH_4Cl. There was a slight inhibition of urine flow in all cases. Table XXII lists the frequency and type of response of the individual electrolytes.

Table XXII

Na			K			Cl		
+	0	−	+	0	−	+	0	−
5	0	1	3	0	3	3	0	3

The percentage increases in electrolytes were small

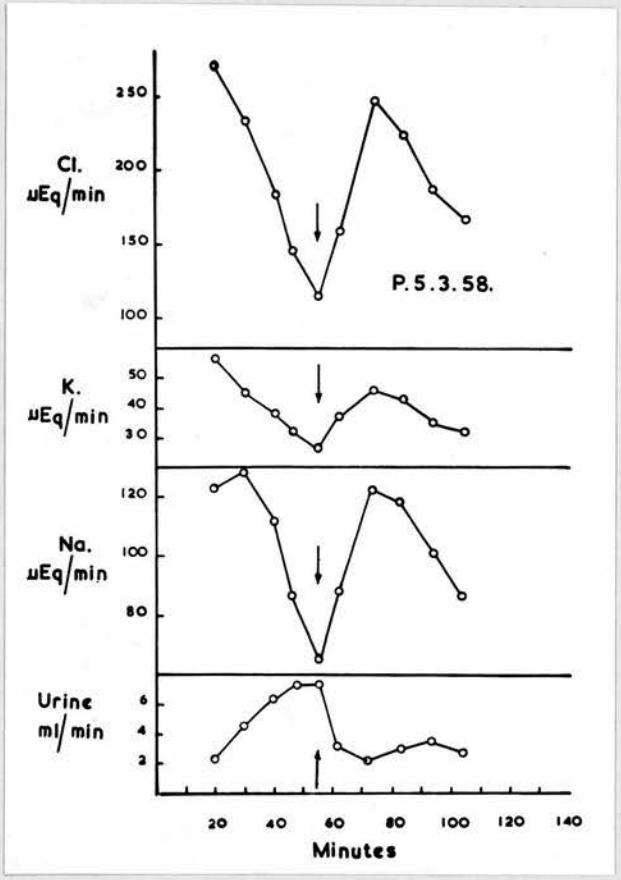

Fig. 29. P 5.3.58 Water diuresis in a normal dog. 1.83 gm NH_4Cl administered with the morning water load. 300 ml water at 0 time. 4mU Pitressin administered i.v. at 55 min (arrow).

(Fig 30) but on one occasion when the excretion began at very low levels the percentage increase in Na excretion was approximately 100%. The increased electrolyte excretion was maintained for about 40 min.

(X) Experiments conducted after $(NH_4)_2SO_4$ loading.

In one experiment the usual antidiuretic dose of Pitressin was administered during a water diuresis on the afternoon following a morning dose of 1.8 gm $(NH_4)_2SO_4$. There was a normal antidiuresis accompanied by a brisk increase in the excretion rates of all three electrolytes. (Fig 31).

(XI) Experiments conducted after NH_4HCO_3 loading.

In one experiment the usual antidiuretic dose of Pitressin was administered during a water diuresis on the afternoon following a morning dose of 1.7 gm NH_4HCO_3. There was a normal antidiuresis. The excretion of Na, K and Cl was increased (Fig 32).

(XII) Experiments conducted during recovery from Diamox.

In four experiments on one dog a water diuresis was induced 30 hours after a dose of Diamox. In all cases the excretion of Cl was elevated and the excretion of Na and K decreased. The urine was of low pH. An injection of vasopressin caused a prompt antidiuresis with an increase in the excretion of Na, K and Cl in all cases. The increases in electrolyte excretion were of the order of 50% to 100% and lasted 30 to 40 min (Fig 33).

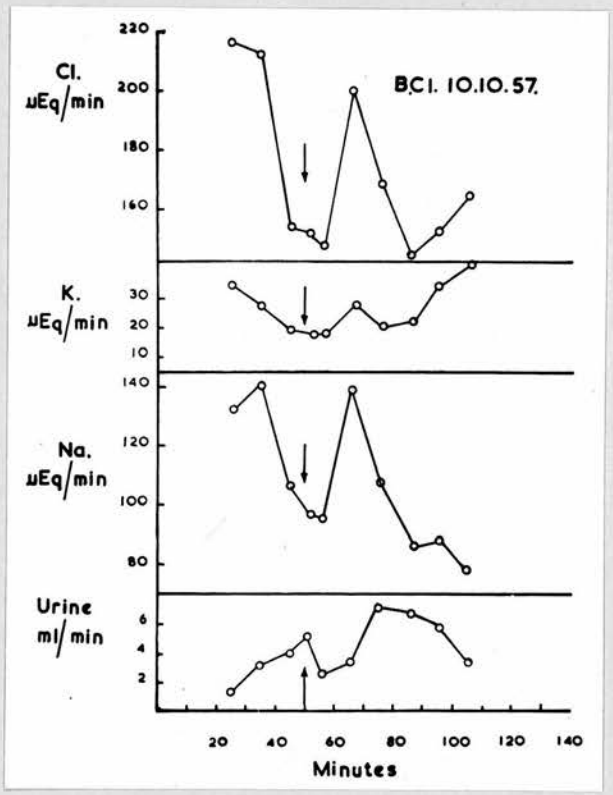

Fig. 30. B.CL 10.10.57 Water diuresis in a normal dog. 2.74 gm NH_4Cl administered with the morning water load. 300 ml water at 0 time. 150mU oxytocin administered i.v. at 50 min (arrow).

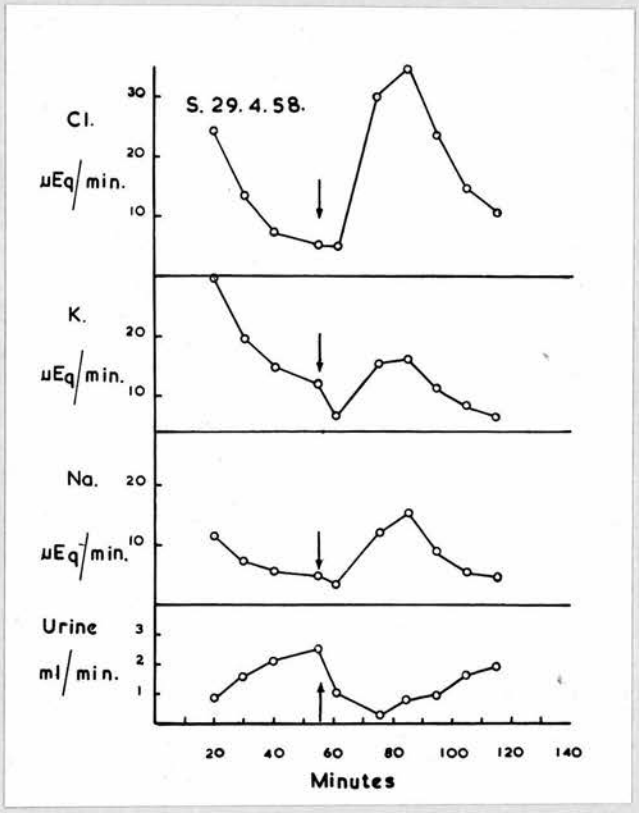

Fig. 31. S 29.4.58 Water diuresis in a normal dog. 1.7 gm $(NH_4)_2SO_4$ administered with the morning water load. 250 ml water at 0 time. 2mU Pitressin administered i.v. at 56 min (arrow).

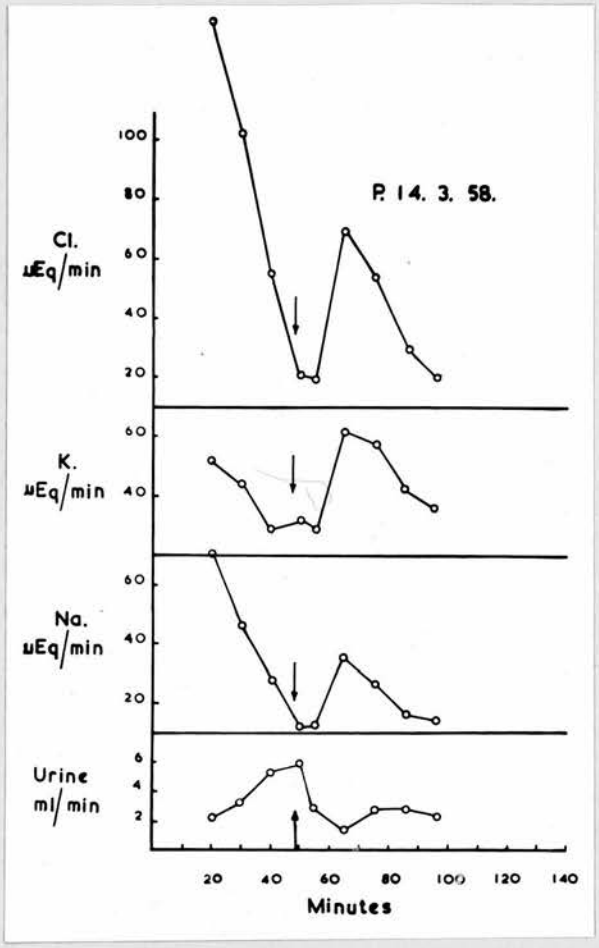

Fig. 32. P 14.3.58 Water diuresis in a normal dog. 2.65 gm $NaHCO_3$ administered with the morning water load. 300 ml water at 0 time. 4mU Pitressin administered i.v. at 49 min (arrow).

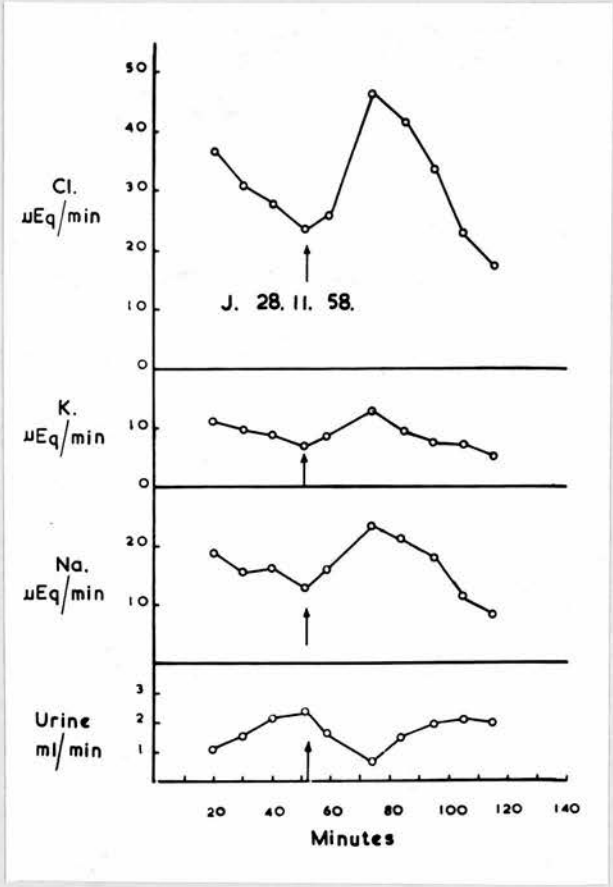

Fig. 33. J 28.11.58 Water diuresis in a normal dog. 100mg Diamox administered 30 hours before the experiment began. 250 ml water at 0 time. 3mU Pitressin administered i.v. at 52 min (arrow).

DISCUSSION

The experiments conducted during a water diuresis superimposed on NaCl loading yielded results which were not in full accord with those of Anslow and Wesson (1955), although the levels of electrolyte excretion in these experiments lay well within the same limits as those investigated by these workers. The electrolyte response to vasopressin was more variable than during a simple water diuresis, and the increases in electrolyte excretion were of much smaller proportions than those produced by vasopressin during the excretion of a simple water load. Another unexpected finding was that after NaCl loading vasopressin caused an increase in K excretion more consistently than it caused an increase in Na excretion.

A possible explanation of the reduced electrolyte response to vasopressin during NaCl loading is that under these circumstances the animal may be undergoing a mild osmotic diuresis. The increased NaCl content of the body fluids could possibly give rise to the liberation of endogenous vasopressin (Verney, 1947), but provided the solute load was large enough, this vasopressin would not cause an obvious decrease in urine flow (Smith, 1956). During a simple water diuresis without prior NaCl loading the endogenous vasopressin could be expected to be minimal. Since the response to vasopressin is proportional to the log of the dose (Dicker, 1953), a much larger dose will be required to produce a measurable effect in the presence of endogenous vasopressin. This argument is supported by the findings that the antidiuretic response

to vasopressin (as well as the electrolyte response) was diminished when NaCl was administered before the water loading.

If the above argument is accepted, it will be agreed that the level of endogenous vasopressin circulating during a water diuresis following NaCl loading will produce a hormonal state similar to that attaining during the observations made on the non diuretic state reported in Section II. It has been claimed that it is this small quantity of circulating endogenous vasopressin which endows oxytocin with its electrolyte excreting properties during the non diuretic state (Brooks and Pickford, 1958). Therefore it is reasonable to expect oxytocin to increase electrolyte excretion during a water diuresis after NaCl loading. This effect did in fact occur more frequently and to a slightly greater degree than during a simple water diuresis, but on no occasion was the increase as great as seen in the one observation made after the accidental morning feeding. The difference between the experimental and accidental situations may be merely a difference in the size of the load of NaCl. However, although the initial excretion of Na and Cl was high in those experiments where oxytocin caused an increase in electrolyte output, no closer correlation could be established between the initial level of electrolyte excretion and the degree of response to oxytocin. It would be of interest to repeat the experiments under conditions of more widely varying NaCl loads.

This altered electrolyte response to the administr-

ation of oxytocin during a water diuresis after NaCl loading is apparently due to some specific effect of NaCl. The same response could not be evoked by loading with $NaHCO_3$, NH_4Cl, KCl, or $KHCO_3$.

Subsequent experiments were designed to show whether the altered response to vasopressin could be produced by loading with either Na or Cl in the absence of the other. A dose of $NaHCO_3$ was administered to provide a Na load without added Cl. When vasopressin was administered during a subsequent water diuresis, the increase in electrolyte excretion was found to be of smaller proportions than that caused by the same dose of vasopressin during a simple water diuresis. (Because of the initial high levels of electrolyte excretion in these experiments however, the absolute increase in electrolyte excretion produced by vasopressin might be larger than the absolute increase produced during a simple water diuresis.) This is in agreement with the findings of Jacobson and Kellogg (1956) who found that vasopressin did not exhibit its naturetic effect after $NaHCO_3$ loading.

An unexpected finding was that even after $NaHCO_3$ loading, when the initial excretion of Na was high and the initial excretion of Cl low, vasopressin more frequently caused a chloruresis than a naturesis. This increase in Cl excretion was frequently of greater magnitude (in absolute quantities) than the increase in Na excretion. The predominance of the Cl response was also seen after loading with sodium citrate and Na_2SO_4. It is probable that only some of the Na_2SO_4 was absorbed

from the gut, but the occurrence of a normal response to vasopressin in this experiment does suggest that the occasional altered responses seen after NaCl or $NaHCO_3$ loading are not due to alteration in distribution of body water as a result of unabsorbed salts in the gut.

When Cl ion was given free of Na in the form of NH_4Cl and vasopressin was administered during a subsequent water diuresis, there resulted a prompt antidiuresis accompanied by an increase in electrolyte excretion of similar proportions to that produced by vasopressin during a simple water diuresis. Because of the higher initial levels of electrolyte excretion after NH_4Cl loading, these increases were greater (in absolute terms) than normal. The absolute increase in Cl excretion always exceeded the absolute increase in Na and K excretions combined.

The above findings suggested that the increase in electrolyte excretion produced by vasopressin could be augmented by loading with free Cl ion, but not as Anslow and Wesson (1955) stated, by loading with NaCl. In this series of experiments the presence of Na appeared to reverse the effect of Cl loading. There is some evidence in the above findings that vasopressin may act to increase Cl excretion specifically, for irrespective of the nature of the loading salt, the absolute increase in Cl excretion was usually more than was necessary to balance the increases in Na and K.

A weakness in the above experiments is that in most cases the extent of the load of the various electrolytes

imposed upon the kidney by the diet was unknown. During simple water diuresis experiments in these dogs, the level of Cl excretion at the beginning of the experiment frequently exceeded the combined excretion of Na and K. The urine was usually acid. These findings suggest that the normal diet may have presented a constant excessive load of Cl unbalanced by Na and K.

It had been found by previous experience that when dogs were fed a diet of "Red Heart" dog food and bread, the urine contained very low concentrations of Na, K and Cl. This diet was therefore fed to a dog during experiments where a low electrolyte intake was desired. When NH_4Cl was administered to this dog, the response to vasopressin during a subsequent water diuresis consisted of a brisk antidiuresis accompanied by an increase in the excretion of Na, K and Cl. The largest percentage increase was of the Cl, which usually increased by 50% to 100%, and because the absolute excretion of Cl exceeded the absolute excretion of Na and K throughout these experiments, the absolute increase in Cl excretion produced by vasopressin exceeded that of Na and K. When the animal on a low electrolyte diet was loaded with $NaHCO_3$ the basal excretion of Na was above the basal excretion of Cl. During a subsequent water diuresis under these conditions vasopressin produced only a small percentage increase in Na, K and Cl excretion, but although there was always an increase in Cl excretion, this increase when expressed as an absolute quantity was less than the combined increase in Na and K.

The above finding that vasopressin causes a larger general increase in electrolyte excretion after NH_4Cl loading than after loading with $NaHCO_3$ suggests that vasopressin may act to increase the excretion of Cl specifically, and that after $NaHCO_3$ loading the response is limited by the availability of Cl. Such a simple explanation is not, however, acceptable for it has been demonstrated that vasopressin can cause an increase in Na and K considerably in excess of what would be required to balance the simultaneous increase in Cl excretion. This last point will be discussed in Section IV, where further evidence is presented that the overall electrolyte response to vasopressin is not determined by the increase produced in Cl excretion.

The occurrence of a brisk antidiuresis and electrolyte response to vasopressin after NH_4Cl loading but not after loading with NaCl or $NaHCO_3$ suggests that loading with the Cl ion has some specific effect in augmenting the response to vasopressin. Caution is however necessary since in all these experiments the Cl was administered as NH_4Cl, and it is possible that the effect was due to the NH_4 ion. Although no studies into the effect of NH_4 loading were undertaken with animals on low electrolyte feeding, the effect of loading with the bicarbonate and sulphate of ammonia had previously been studied in dogs on the normal diet. In these animals vasopressin was found to cause an increase in the excretion of Na, K and Cl which was of the same magnitude as the increase produced by vasopressin in the same animals during a

simple water diuresis. Probably, therefore, the NH_4 ion has no specific effect in augmenting the response to vasopressin.

Then again, changes attributed to Cl loading may be due to altered pH, for NH_4Cl produces a systemic acidosis and an acid urine. In consideration of this, attention was directed to methods whereby an acid urine could be produced without Cl loading. In several experiments the excretion of H was prevented for several hours by the inhibition of carbonic anhydrase. This resulted in a considerable loss of base in the urine, and this in turn led to a state of acidosis, which (when the action of the enzyme inhibitor ceased) was compensated for by the elaboration of an intensely acid urine (Hanley and Platts 1956). This phase lasted for several days after the cessation of carbonic anhydrase inhibition, and during this time the excretion of Na and K was minimal and the excretion of Cl normal. When vasopressin was administered during a water diuresis in this post Diamox state, the electrolyte and antidiuretic responses were again found to be brisk. Increases in the excretion of all electrolytes were between 50% and 100%, and although in the cases of Na and K this represented only small absolute increases (because the initial excretion rate of base was low), the increase in Cl excretion usually amounted to about 20 uEq/min. (Fig 33). This suggests that the brisk response to vasopressin after NH_4Cl loading may be due to the acidosis produced rather than to any specific action of Cl.

The alteration in the excretion of K produced by vasopressin was next considered. Anslow and Wesson (1955) expressed the opinion that vasopressin increased the excretion of K indirectly through its action on Na excretion, i.e. vasopressin caused an increased quantity of Na to be present in the distal tubular fluid and some of this Na is exchanged for K. Ali (1958) adopted the view that vasopressin exerted a direct influence on K excretion, and he presented some evidence that arginine vasopressin was less active towards K than was lysine vasopressin.

In this present study the response of K excretion to vasopressin was extremely variable, whether a commercial extract or pure arginine vasopressin was used. However, in most experiments the excretion of K began at levels less than 20 uEq/min and subsequently decreased as the diuresis proceeded. Under these conditions even a large percentage increase in K excretion would not produce a large absolute increase in K excretion. In several experiments where the K excretion began at higher levels, vasopressin did produce a substantial increase in K output, but just as frequently under similar circumstances vasopressin did not cause any increase in K excretion.

In this context attempts were made to increase K excretion by loading with KCl, $KHCO_3$ and K citrate. Administration of all these salts resulted in an increased K excretion and a depressed Na excretion. During subsequent water diuresis vasopressin was seen to produce large increases in K excretion on several occasions, but

there were just as many cases where vasopressin did not increase K excretion although the excretion of Na and Cl was increased.

In one of two experiments where KCl was administered the increase in K excretion after vasopressin was from 67.5 uEq/min to 81.5 uEq/min, which was a larger absolute increase but a smaller percentage increase than the simultaneous increase in Na output from 11.8 uEq/min to 20.5 uEq/min. In a second experiment after KCl loading vasopressin merely caused an inhibition of the fundamental decline in K excretion, although the output of both Na and Cl increased by 20%. Similar results were obtained in two of the three experiments conducted after $NaHCO_3$ loading. Here a larger absolute (but a smaller percentage) increase was caused in K excretion than in Na excretion. In a third experiment K excretion was not increased although Na and Cl excretion was increased. In this case the K output was decreasing so rapidly during the rising phase of the diuresis that anything other than a large increase in K excretion would have been difficult to demonstrate. This rapid fall in K excretion in the early stages of the diuresis probably explains why no increase in K excretion was detected in the K citrate loading experiments.

The only concrete fact emerging from the above experiments on K is that occasionally, under not very well defined circumstances, vasopressin may cause an increase in K excretion which is larger than the simultaneous increase in Na excretion. The increase in K

excretion was least constant and often of small dimensions after $NaHCO_3$ loading. The most consistent increases in K excretion were seen when vasopressin was administered after NH_4Cl loading and during a post Diamox acidosis. In both situations the increases were usually about 50%. The K excretion in these experiments was, however, very low, and the measurement of urinary K may have lost some of its accuracy at these levels. Therefore not too much significance should be placed upon these percentage increases. These larger percentage increases in K excretion after NH_4Cl may merely be part of the general effect already described wherein both the antidiuretic and electrolyte excreting effects of vasopressin are enhanced by NH_4Cl loading or post Diamox acidosis. However, a specific effect of urinary (or blood) pH may be in operation under these circumstances to alter the excretion of K.

There is good evidence that all urinary K may be the result of distal tubular secretion (Pitts, Gurd, Kessler and Hierholzer, 1958) and that its delivery into the tubule may be in competition with the delivery of H. (Berliner, Kennedy and Orloff, 1954). Thus the excretion of K depends not only on the K load, but also on the activity of the pH regulating mechanisms. It is probable that in the above experiments where vasopressin produced different responses in K excretion under apparently identical conditions of K loading, the variable responses may have been due to factors involved in the regulation of pH.

The results obtained from this section suggest that vasopressin acts to increase the excretion of Na and Cl and that any increase in K excretion is secondary, resulting in an exchange of some of the extra Na passing through the distal convoluted tubule for K. This exchange proceeds only when conditions are favourable for the excretion of K and the conservation of Na.

SECTION IV

EFFECT OF VASOPRESSIN ON ACID BASE EXCRETION

INTRODUCTION

When administered during a water diuresis vasopressin causes an increase in electrolyte excretion (Section II). In Section III the effect of previously loading the test animal with various electrolytes was studied. When $NaHCO_3$ was administered, the initial excretion of Na and K (expressed as µEq/min) could be made to exceed the initial excretion of Cl, but even under these circumstances it was rare for vasopressin to cause an increase in Na and K which exceeded the Cl increase, unless the dog had previously been deprived of Cl.

As has been pointed out, the prominent part usually played by Cl in any increase in electrolyte excretion after a dose of vasopressin, suggests that vasopressin may exert a direct action upon Cl excretion and that changes in the excretion of other electrolytes may be secondary to changes in Cl (Karvonen, Leppanen and Pitkanen, 1953). Experiments were therefore planned to test this hypothesis by attempting to produce increases in Na and K excretion without equivalent increases in Cl excretion and vice versa, and to see whether, and to what extent, the excretion of other electrolytes was increased to maintain ionic equilibrium.

METHODS

The experiments were all based on the general plan described in Section II. Most of the experiments were carried out on one dog which was fed a diet of bread and canned dog food which experience showed was poor in Na, K and Cl.

In those experiments where it was desired to begin with the excretion of Na considerably above the excretion of Cl, 1.5 gm $NaHCO_3$ and 0.5 gm $KHCO_3$ were added to the dog's food daily for at least two days before the experiment. On the day of the experiment 1.5 gm $NaHCO_3$ was administered with the morning dose of water. This procedure always caused the urinary pH to be above 6.5 (and usually above pH 7.0) The dog was assumed to be in a state of metabolic alkalosis.

To produce an acid urine a dose of 1.4 gm NH_4Cl was administered daily with the food for at least two days before the experiment, and again with the morning dose of water on the day of the experiment. The urine under these conditions always had a pH of less than 6.5 and was usually at pH 5.0. The dog was assumed to be in a state of metabolic acidosis. In four cases acidosis was produced by the administration of a dose of 100mg Diamox 30 hrs before the experiment. This lowered the urinary pH to 4.5.

The comparison between the increases in excretion of Cl and base was made by plotting the rates of excretion of the electrolytes (in uEq/min) against the time in minutes from the administration of the water load.

For simplicity the excretion rates of Na and K were added together. In Section I it was stated that during a water diuresis, the rate of excretion of any electrolyte in the 30 min period following the peak of diuresis could confidently be expected to be below the excretion rate at the peak of diuresis. If electrolyte excretion rates were plotted against time after hydration, a line joining the excretion rate at 50 min (the average peak of diuresis) to the rate at 80 min would lie below all intervening points on the graph. (Line AB Fig 1). Thus points on line AB may be used as estimates of the maximum rate of electrolyte excretion to be expected between times 50 min and 80 min after water loading.

When vasopressin is administered, the excretion rate of electrolytes increases above this predicted level, and when the results are plotted graphically the area of the curve above line AB provides a measure (in uEq) of the increase in electrolyte excretion. The increase in Cl excretion may then be readily compared with the increase in excretion of Na and K. For convenience the change in Cl was always expressed as a percentage of the change in Na plus K.

In practice it was found that after a dose of vasopressin the excretion of electrolytes was frequently still elevated at 80 min. At some time later (90 or 100 min), the electrolyte excretion suddenly fell and then proceeded on its gradual downward course. This point at the end of sudden decrease in electrolyte output was taken as point B on line AB (Fig 34 and Fig 35).

The excretion rates of NH_3, titratable acidity, and total PO_4 and changes in urinary pH were all measured using methods described in Section I.

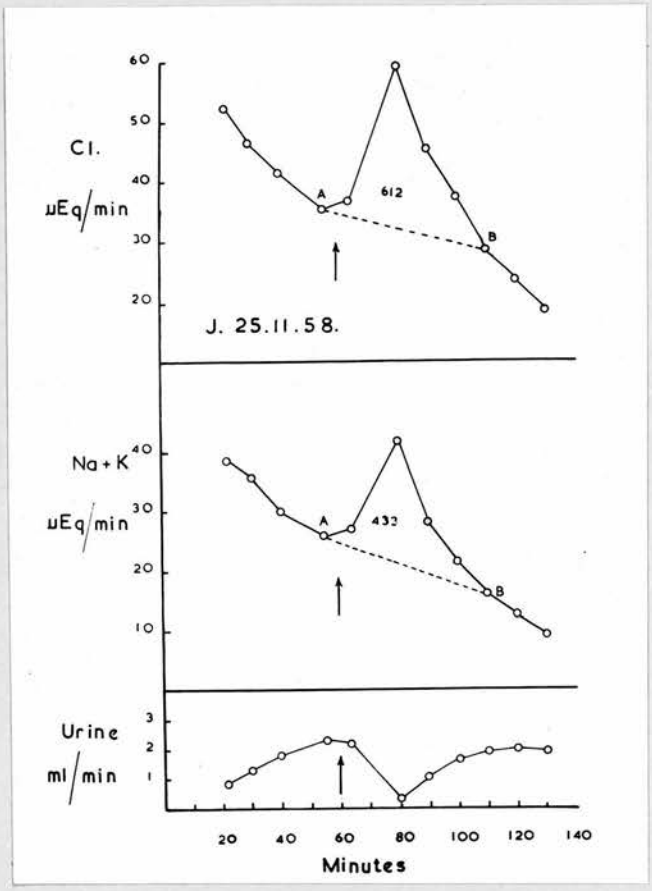

Fig. 34. J 25.11.58 Water diuresis in a normal dog. 1.4 gm NH_4Cl administered with the morning water load. 300 ml water at 0 time. 3mU Pitressin administered i.v. at 60 min (arrow). For Na+K net area above line AB = 433. For Cl net area above AB = 612. Therefore increase in Cl excretion amounts to 144% of increase in Na+K excretion. (See text)

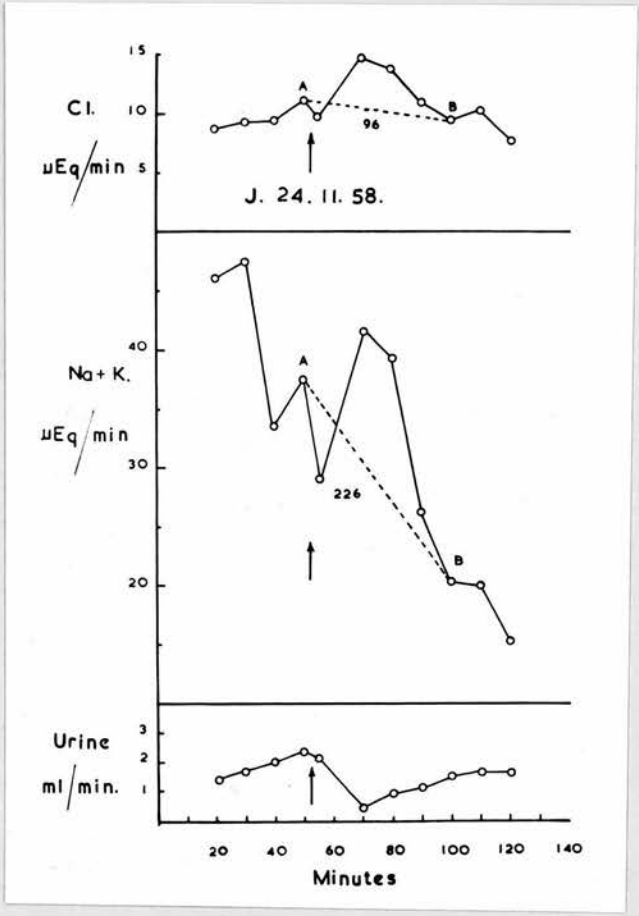

Fig. 35. J 24.11.58 Water diuresis in a normal dog. 1.5 gm $NaHCO_3$ and 0.5 gm $KHCO_3$ administered with the morning water load. 250 ml water at 0 time. 3mU Pitressin administered i.v. at 52 min (arrow). For Na+K net area above line AB = 226. For Cl net area above AB = 96. Therefore increase in Cl excretion amounts to 42.5% of increase in Na+K excretion. (See text).

RESULTS

(I) The effect of urinary pH on the response to vasopressin.

In 14 experiments the usual antidiuretic dose of vasopressin was administered during a water diuresis superimposed on disturbances of acid base balance. A detailed comparison between the increase in Cl excretion and the increase in Na plus K excretion was made. Seven of the experiments were carried out during $NaHCO_3$ alkalosis. Three experiments were during an acidosis resulting from NH_4Cl administration and three were during a post Diamox acidosis.

In all the above experiments an antidiuretic dose of vasopressin caused an increase in the excretion rates of both Cl and base. After $NaHCO_3$ administration the increase in Cl excretion was found to average only 67.2% (± 23.4%) of the corresponding increase in Na plus K excretion. (Fig 35). In the experiments conducted during acidosis the increase in Cl amounted to an average of 156% (±23.8%) of the increase in Na plus K excretion (Fig 34). These findings are highly significant ($t = 6.85$).

Thus vasopressin has been illustrated to be able to increase the excretion of Na plus K without causing an equivalent increase in Cl excretion. An increase in Cl excretion is frequently seen without an equivalent increase in Na plus K excretion. These findings suggest that vasopressin must bring about, though not necessarily be the cause of, an increase in the excretion of other electrolytes in order to maintain ionic equilibrium.

(II) Effect of vasopressin on urinary pH.

In crude experiments where the urine was collected in an open measuring cylinder and the pH measured by indicator at the end of each 10 min collecting period, the findings suggested that vasopressin caused an acid urine to become more acid and an alkaline urine to increase its pH still further. The critical pH at which the direction of the response changed was about pH 6.5.

These findings were confirmed by experiments where the urine was collected under oil, and the pH measured by a glass electrode pH meter. In two cases when the dog had been loaded with NH_4Cl, vasopressin decreased the pH from 5.8 to 5.5 and from 6.3 to 5.8 respectively. In two experiments after $NaHCO_3$ loading vasopressin increased the pH from 6.9 to 7.0 and from 6.5 to 7.1.

(III) Effect of vasopressin on urinary titratable acidity.

The effect of vasopressin administered during a water diuresis on the excretion of titratable acid was measured in eight experiments. In five cases the dog was acidotic and in three experiments the dog was alkalotic. During the acidosis the excretion of acid was about twice that seen during alkalosis.

During the acidosis vasopressin caused acid excretion to be increased by 15% on one occasion, to be unaltered in two experiments, and to decrease by 10% and 19% in two other cases. In all these experiments vasopressin caused an increase in the excretion of Cl.

During alkalosis vasopressin caused acid excretion to decrease by 76%, 74%, and 67%. In all three of these

experiments vasopressin caused an increase in the excretion of Na,K and Cl.

(IV) Effect of vasopressin on the excretion of NH_3.

In five experiments the effect of vasopressin on the excretion of NH_3 was observed during a water diuresis. Three of the experiments were conducted during an acidosis and two during alkalosis.

During an alkalosis the excretion of NH_3 was initially lower (11 to 50 uEq/min) than during an acidosis (40 to 126 uEq/min). The increase produced by vasopressin appeared to bear no relationship to the urinary pH. In two experiments during acidosis there were increases of 30% and 60% in NH_3 excretion, but in one acidosis experiment there was no increase in NH_3 excretion. In the experiments conducted during alkalosis there were increases of 30% and 70% in NH_3 excretion. Because of the lower initial rates of NH_3 excretion in these experiments the increases in NH_3 excretion expressed in absolute values were smaller than the increases produced during the acidotic state. In all these experiments there was a simultaneous increase in the excretion of Na, K and Cl.

(V) Effect of vasopressin on the excretion of total phosphate.

The effect of vasopressin on the excretion of phosphate was studied during water diuresis. In two experiments the dog was alkalotic, and on one occasion it was in a state of acidosis. The initial level of

excretion of phosphate did not appear to be related to the urinary pH.

In one experiment (during an acidosis) vasopressin caused a 50% increase in phosphate excretion. In one experiment during alkalosis vasopressin did not alter phosphate excretion, but in one other experiment after $NaHCO_3$ loading, vasopressin inhibited a rapid downward trend in phosphate excretion for the period of the anti-diuresis. In all these experiments vasopressin also increased the excretion of Na, K and Cl.

DISCUSSION

The above findings illustrate that the increase in electrolyte excretion produced by vasopressin is not merely an increase in NaCl or KCl excretion. Vasopressin causes changes in Cl output quite independently of changes in Na and K. Evidence has also been presented that under certain circumstances vasopressin can alter the excretion of NH_3, H, and PO_4.

These facts make it appear that the action of vasopressin is entirely nonspecific, and that as well as removing water from the urine it causes an increase in the excretion of many urinary solutes. Vasopressin does not, however, increase the excretion of creatinine. In the dog the excretion rate of endogenous creatinine serves as an indirect measure of glomerular filtration rate. Therefore it follows that vasopressin does not alter G.F.R. and that any changes it causes in electrolyte excretion are brought about by altered tubular activity. In this respect the results agree with those of previous workers. We are then left with the hypothesis that vasopressin modifies the tubular handling of Na, K, NH_3, H, Cl and PO_4, and probably alters the excretion of other substances not yet investigated. Thus vasopressin is attributed with a multiplicity of actions at a great number of sites.

A far more compact concept is that vasopressin acts to decrease the proximal resorption of NaCl (Shannon, 1942). This would cause an additional quantity of NaCl to reach the distal convoluted tubule, but there is no

reason to suppose that all this extra NaCl would reach the urine unchanged. All NaCl in the distal tubule will be subjected to the absorbtive, excretory and exchange mechanisms taking place across the tubular wall.

There is evidence that a dilute urine is produced by the reabsorption of solutes from the distal tubular fluid against an osmotic gradient (Smith, 1956). This implies that the distal tubule is capable of absorbing NaCl as such without returning any other electrolyte to the tubular fluid in place of the absorbed electrolytes. Thus, if any of the extra NaCl appearing in the distal tubule as a result of vasopressin activity were subsequently to be reabsorbed by this process, nothing would be left in the urine to show that this extra NaCl had indeed ever been present in the distal tubular fluid. This possibly explains why vasopressin produces only small increases in electrolyte excretion in those experiments where the excretion rates of electrolytes began at low levels. Under such conditions the reabsorption of NaCl would probably be very active, and little of the increased NaCl presenting at the distal tubule would escape into the urine, although what little did escape would probably cause a large percentage increase in the NaCl excretion. If the diet of the animal were rich in NaCl the absorption of NaCl would not be so avid, and more of the increase produced by vasopressin would escape into the urine, although this might not cause a very great percentage increase in total NaCl excretion.

Even after escaping the specific NaCl resorbing

mechanisms the Na and Cl would still be subjected to exchange mechanisms determined by the pH and K requirements prevailing at the time. Pitts, Gurd, Kessler and Hierholzer (1958) have demonstrated that the reabsorption of Na and the excretion of K, NH_4 and H are all related processes that take place in the distal tubule. Tubular Na is thought to be exchanged for cellular H and K. The accumulation of H in the tubular fluid lowers the pH and NH_3 is attracted into the tubule where it is bound as NH_4 (Orloff and Berliner, 1956). There is evidence also that PO_4 is secreted by the distal tubule (Nicholson and Sheperd, 1957), and that over the usual physiological ranges of urinary pH, the pH regulating mechanisms are mainly in the distal tubule (Nicholson, 1957). The site of Cl reabsorption has not been localized but there is evidence that tubular Cl can be replaced by HCO_3 (Hilton, Capeci and Kiss, et al 1956). The reabsorbtion of Cl may be in competition with SO_4 (Berglund and Lotspeich, 1956).

The above hypothesis of vasopressin's action may be summarized by saying that any given dose of vasopressin will always cause an increase in the amount of NaCl reaching the distal tubule. Depending on the electrolyte requirements of the animal, some variable fraction of this NaCl will be reabsorbed as such and the rest will pass to more peripheral segments of the distal tubule, where Na and Cl will be differentially absorbed in exchange for other ions. These last processes are determined by the requirements of pH and Na-K balance prevailing at the time.

During an acidosis vasopressin causes a larger increase in the excretion of Cl than in Na plus K. This difference should be accountable for by an increase in H excretion which may appear in the urine as an increase in titratable acid, or may be bound in an increased amount of urinary NH_4. These changes in urinary NH_4 have been observed in this present study (p 82) although they were not found by Brunner, Kuschinsky and Peters (1956).

During an alkalosis vasopressin causes a larger increase in Na plus K excretion than in Cl excretion (p 80). This difference should be accountable for by an increase in the excretion of some other anion which has been exchanged for tubular Cl. The most probable anion to take on this role is HCO_3, which has been shown to be exchangeable with tubular Cl (Hilton, Capeci and Kiss, 1956). The excretion of HCO_3 has also been shown to be capable of modification to suit the requirements of acid-base balance (Braleau and Gilman, 1953). Unfortunately no observations of HCO_3 excretion have been made in this present study.

In actual fact several serious deviations from the above ideal findings have been observed. The first was the failure to observe an increase in NH_4 excretion in one experiment when the urine was intensely acid. This may have been because the excretion of NH_4 was already maximal before the vasopressin was administered. Although the binding of NH_3 in the urine is determined entirely by pH (Orloff and Berliner, 1956) the supply of NH_3 depends upon the activity of glutaminase. Although the

glutaminase activity can be increased to suit the requirements of acid excretion, such changes develop slowly over a period of days (Rector, Seldin and Copenhaver, 1955). In this case the dog had been acidotic for several days as a result of previous Diamox administration. The glutaminase activity should have been high, but during this experiment the NH_4 excretion was not as high as had been recorded on previous occasions. This suggests that NH_4 excretion was not already maximal, and that it should have been capable of increase.

The increases in NH_4 excretion produced by vasopressin during an acidosis are larger than are required to account for the difference between the increase in Cl excretion and the increase in Na plus K excretion. Similar increases in NH_4 excretion are also seen under conditions of alkalosis where the increase in Na plus K excretion is already larger than the increase in Cl excretion. The excretion of PO_4 is also increased in both acidosis and alkalosis. These findings could be presented as evidence in favour of an entirely nonspecific action of vasopressin on the absorption or excretion of many urinary constituents. If, however, the original assumption that vasopressin acts to increase the amount of NaCl in the distal tubule is valid, these findings suggest that the exchange of Na for H and the exchange of Cl for other anions can proceed concurrently, leading to a nett conservation of NaCl.

The apparently excessive increase in NH_4 excretion in response to vasopressin could possibly be correlated

with changes in the excretion of titratable acid. During alkalosis vasopressin decreases the excretion of titratable acid, which suggests that more of the H which is usually free was bound with extra NH_3. This suggests that vasopressin has s specific action upon the diffusion of NH_3 into the urine. The decrease in excretion of titratable acid may, however, be an artifact. Although the excretion of H_2CO_3 in these experiments is probably not very great, the concentration of H_2CO_3 in the urine will be increased considerably during the period of antidiuresis. This H_2CO_3 is in equilibrium with dissolved CO_2. Thus it is possible that during the antidiuresis the concentration of CO_2 in the urine could rise above the level of plasma CO_2. Brodsky, Miley, Kiam and Shah (1958) have shown that during the excretion of an acid load urinary CO_2 sometimes rises above plasma levels even during a diuresis. When the urine is concentrated five or ten times in antidiuresis, this increased CO_2 level in the urine would appear during the elaboration of less acid urines. Under these conditions CO_2 would tend to diffuse from the urine during its passage through the lower urinary tract. By this means H_2CO_3 would be lost from the urine, leading to a decrease in the excretion of titratable acid. Thus the decrease in the excretion of titratable acid produced by vasopressin may merely be a result of concentrating the urine.

None of the above findings are inconsistent with the theory that the primary action of vasopressin on electrolyte excretion is to increase the amount of NaCl being

presented to the distal tubule, although the origin of this extra NaCl is open to speculation.

SECTION V

EFFECT OF DIAMOX ON THE RESPONSE TO VASOPRESSIN AND OXYTOCIN

INTRODUCTION

There is considerable evidence that the changes in electrolyte excretion produced by posterior pituitary extracts may be modified by the administration of large NaCl loads. This topic has been fully discussed in Section III. The results from these present studies failed to confirm the observations of Anslow and Wesson (1955) that NaCl loading magnified the naturetic effect of vasopressin. It was found however that the largest percentage increases in electrolyte excretion were seen when the excretion rates began at low levels. The greatest absolute increases in electrolyte output were seen when the spontaneous electrolyte excretion rate was high at the beginning of the experiment. Under conditions where the electrolyte excretion was high at the beginning of a diuresis oxytocin occasionally increased the electrolyte excretion still further, although when electrolyte excretion was low oxytocin caused an increased output only during the non diuretic state.

All the methods used to raise electrolyte excretion involved increasing the blood level of the electrolyte under consideration. When these experimental procedures failed to produce results similar to those obtained when electrolyte excretion was spontaneously high, some consideration was given to producing an increased

an increased urinary concentration of electrolyte without increasing the blood level. By this means it was hoped to distinguish between the roles of urinary electrolyte concentration and serum electrolyte concentration in determining the response to vasopressin.

The urinary concentrations of Na and K were increased by the administration of acetazolamide (Diamox). This substance inhibits the activity of carbonic anhydrase, and in the kidney this results in a decreased elaboration of H ion. This H usually plays an important part in the conservation of Na. It is postulated that there is, in the wall of the distal tubule, a H⇌Na exchange system which removes Na from the tubular fluid. In the absence of a rich source of H some of the tubular Na is replaced by K instead of H, and this results in an excessive loss of both Na and K (Pitts and Alexander, 1945; Kaye, 1955). Thus although Diamox increases the urinary concentrations of both Na and K, it is at the same time acting to decrease the serum concentration of these electrolytes.

METHODS

100 mg of Diamox was administered by stomach tube with the usual morning dose of water. The experiment then proceeded as described in Section II.

RESULTS

(I) Vasopressin administered during a water diuresis.

In seven experiments on three dogs vasopressin was administered during a water diuresis. On four occasions the dose of vasopressin was the usual antidiuretic dose (2-4 mU), but in one dog the dose was on one occasion doubled and in two experiments increased sixfold.

In the four experiments where the usual dose of vasopressin was administered normal antidiuresis resulted on three occasions. In the fourth experiment the water diuresis was unusually brisk and 4 mU vasopressin reduced the urine flow from a maximum rate of 7.6 ml/min to a minimum of 2.43 ml/min. There was in all cases an increase in Cl excretion. This increase amounted to 100% on one occasion, but in the other cases it was only about 10% (Fig 36). The excretion of Na and K continued to fall even after the administration of vasopressin, although the rate of this fall was slowed during the antidiuresis. In one experiment there was a slight rise in K excretion of about 10%. This was not the same experiment as that where Cl excretion was increased 100%.

In those experiments where the dose of vasopressin was larger than usual the antidiuresis produced was no greater than usual. The increase in Cl excretion was more obvious than that produced by the smaller doses, and was of the order of 25%. Na excretion remained unaffected by vasopressin, but increases in K excretion of 10%, 25% and 30% were produced.

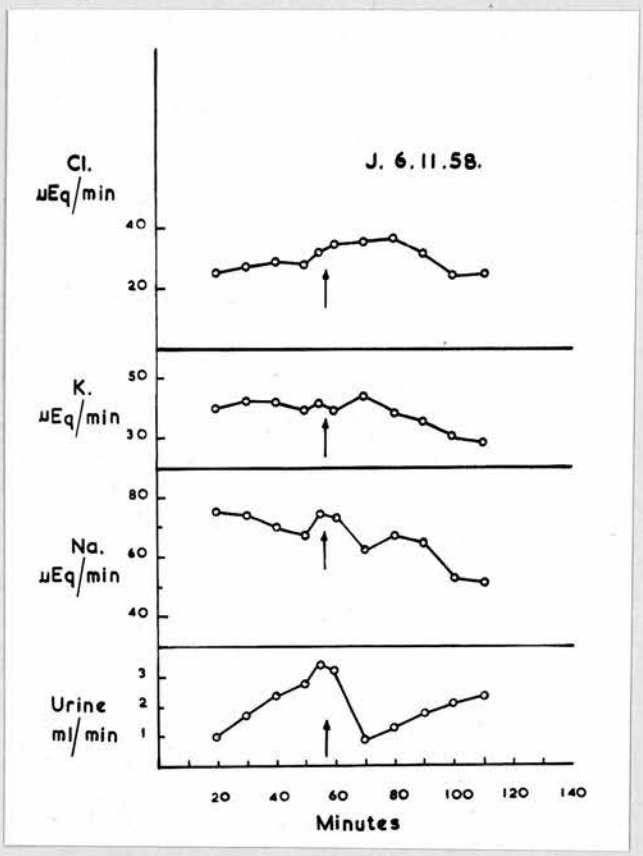

Fig. 36. J 6.11.58 Water diuresis in a dog to which 100mg Diamox had been administered 3 hours previously. 250 ml water at 0 time. 3mU Pitressin administered i.v. at 57 min (arrow).

(II) Oxytocin administered during a water diuresis.

In one experiment oxytocin was administered during a water diuresis. There was a transitory inhibition of urine flow. The levels of Na and K excretion remained unaltered, but there was a small (10%) increase in Cl excretion which lasted only 25 min (Fig 37).

(III) Oxytocin administered during the non diuretic state.

In two experiments oxytocin was administered during the non diuretic state. On both occasions urine flow began at levels no higher than normal, and oxytocin did not alter the flow rate. In both cases there was an increase in Na excretion lasting 40 min. These increases were of 20% and 30%. In one experiment the K excretion was also increased by 15%, but otherwise there were no electrolyte changes. (Fig 38).

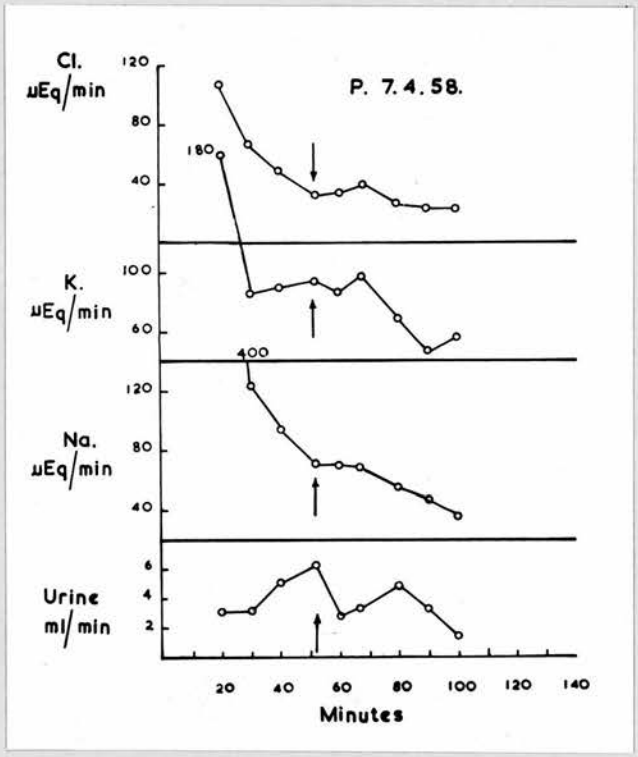

Fig. 37. P 7.4.58 Water diuresis in a dog to which 100mg Diamox had been administered 3 hours previously. 300 ml water at 0 time. 150mU oxytocin administered i.v. at 51 min (arrow).

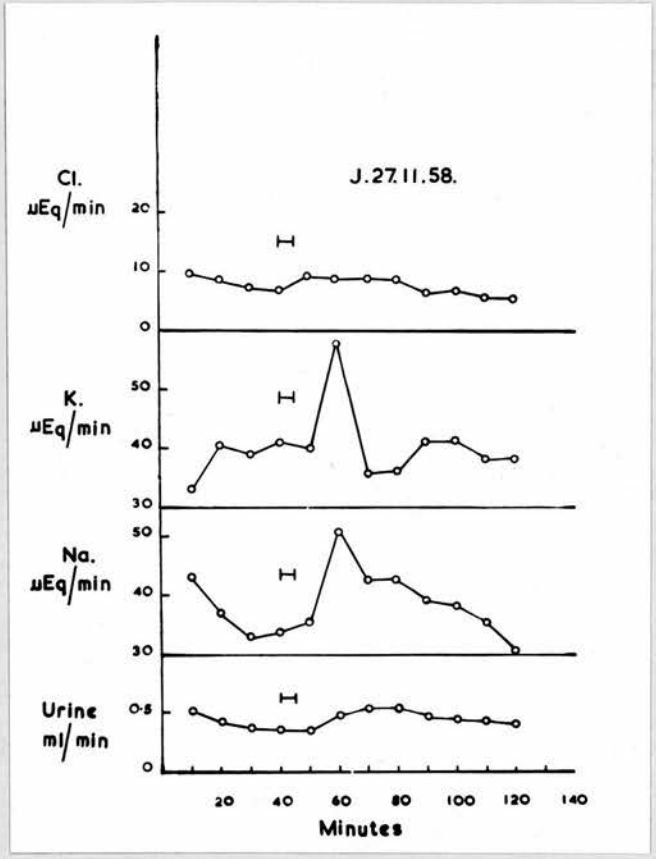

Fig. 38. J 27.11.58 Non diuretic urine flow in a dog to which 100 mg Diamox had been administered 3 hours previously. 150mU oxytocin administered slowly i.v. between 40 - 45 min.

DISCUSSION

These findings show that Diamox diminishes the ability of vasopressin to increase electrolyte excretion. After a dose of Diamox, vasopressin caused only a slight increase in Cl excretion. When the dose of vasopressin was increased to much larger than the usual antidiuretic dose, an increase in K excretion was produced, but in no case did an increase in Na excretion result.

There is a possibility that the increase in electrolyte excretion usually seen during vasopressin antidiuresis may be an artifact resulting from some error in collection or analysis (Anslow and Wesson, 1955). This would explain why the apparent increase in electrolyte excretion was greatest in those experiments where the initial level of electrolyte excretion was high (Section II), for such errors would tend to be proportional to the concentration of electrolytes in the urine. This possibility is, however, excluded by those experiments conducted under the influence of Diamox. Here the initial excretion of Na and K was at very high levels, but vasopressin produced only a very small increase. Thus it would appear that the level of electrolytes in the final urine does not determine the magnitude of the electrolyte response to vasopressin, i.e. the phenomenon is not an experimental artifact.

In view of the fact that the normal action of vasopressin in increasing electrolyte excretion is almost abolished by the previous administration of Diamox, the question arises as to whether this action of vasopressin

is directly or indirectly dependent on carbonic anhydrase. Since at least part of the conservation of Na is undertaken by the H - Na exchange system, it is possible that vasopressin could normally increase Na excretion by inhibiting this exchange. If the system were already fully depressed by Diamox, vasopressin could not produce any further inhibition. This does not explain the changes in Cl excretion caused by vasopressin. Although carbonic anhydrase has been found to be necessary for Cl conservation in the alligator (Coulston and Hernandez, 1957), there is no evidence of a similar mechanism in the dog.

The Na - H exchange mechanism is probably only one of several mechanisms of Na conservation. During the action of Diamox the loss of Na through this defective system must eventually lower the serum Na levels, and this would in turn stimulate the conservation of Na by the remaining intact mechanisms. The increase in Na excretion produced by vasopressin under these circumstances may therefore be no greater than that usually produced by vasopressin during other conditions of Na deficiency. These changes would be quite small and would certainly pass unobserved when mixed with the large quantities of Na and K already in the urine as a result of the action of Diamox.

The usual action of oxytocin in increasing electrolyte excretion during the non diuretic state does not appear to have been modified by the administration of Diamox. This suggests that the actions of vasopressin and oxytocin may be entirely unrelated.

SECTION VI

EFFECT OF RENAL DENERVATION ON THE RESPONSE TO VASOPRESSIN AND OXYTOCIN

INTRODUCTION

Posterior pituitary extracts were found to increase the Cl excretion by a perfused isolated kidney (Starling and Verney, 1925). Obviously nervous mechanisms played no part in the production of this effect. Since that time however, it has been found that both vasopressin and oxytocin exert independent actions upon electrolyte excretion (Section II). It was considered desirable, therefore, to reinvestigate the problem to see whether both these actions persisted after renal denervation.

METHODS

The animal used has been described previously in Section I. Both kidneys were denervated by stripping the renal vessels at a single stage trans abdominal operation. All observations recorded here were made within 6 weeks of the operation. The plan of the experiments was as described in Section II.

On four occasions clearance studies were undertaken. Glomerular filtration rate (G.F.R.) was measured as the clearance of creatinine. The clearance of p-amino hippuric acid (P.A.H.) was used as a measure of renal plasma flow (R.P.F.). Adequate plasma levels of these substances were produced by their oral administration with the afternoon water load. The dose of creatinine was 5.0 gm, and of P.A.H. 2.4 gm. Serum and urinary creatinine was measured by the method described in Section I. P.A.H. was estimated colourimetrically by the method of Smith et al (1945).

RESULTS

(I) Arginine vasopressin or Pitressin administered during a water diuresis.

On three occasions the usual antidiuretic dose of either arginine vasopressin or Pitressin was administered during a water diuresis in a dog with bilateral renal denervation. In all cases there was a normal antidiuresis. There was a definite increase in Cl excretion in all experiments, but the change in the excretion rate of Na and K consisted merely of an inhibition of the fall in electrolyte excretion which was occuring spontaneously. (Fig 39). Such minor changes in Na and K excretion in the presence of large increases in Cl excretion were frequently seen in the normal animal.

In these experiments the transitory decrease in electrolyte excretion which was seen in the early stages of the antidiuresis was of greater magnitude than the decrease seen before the kidneys had been denervated. In two other experiments where clearance studies were undertaken during a diuresis, vasopressin was antidiuretic but had little effect upon the excretion of electrolytes. (Fig 40). The time after the operation at which the observation was made did not appear to determine the nature of the response to vasopressin.

(II) Oxytocin administered during a water diuresis.

On three occasions oxytocin was administered during a water diuresis in the dog with denervated kidneys. On all occasions there was a mild inhibition of urine flow.

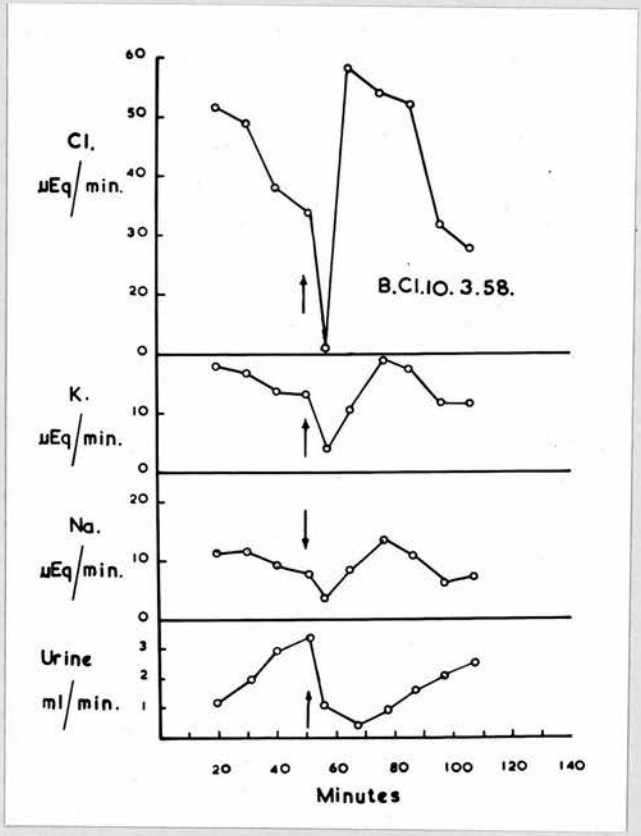

Fig. 39. B.Cl 10.3.58 Water diuresis in a dog with denervated kidneys. 300 ml water at 0 time. 2.5mU vasopressin administered i.v. at 51 min (arrow).

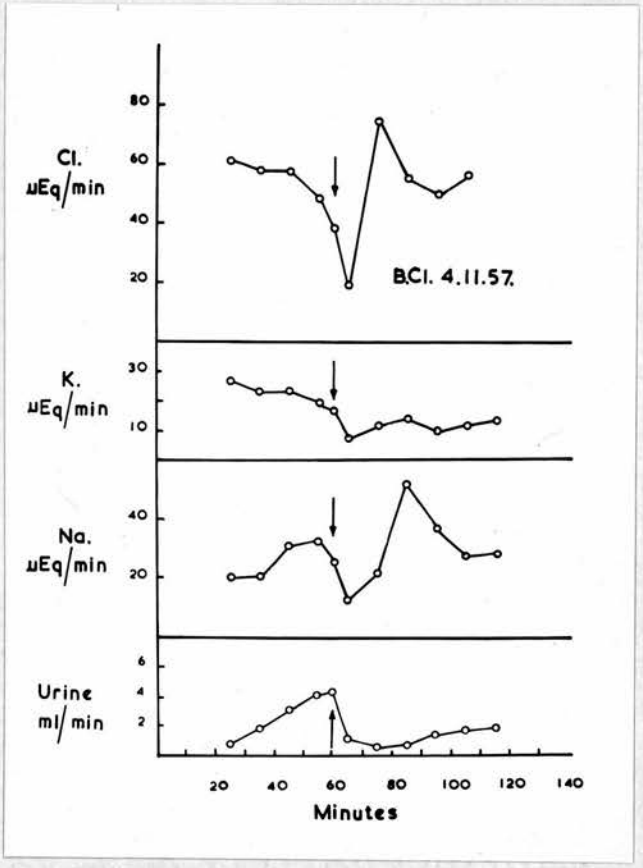

Fig. 40. B.Cl 4.11.57 Water diuresis in a dog with denervated kidneys. 5 gm creatinine and 2.4 gm p-amino hippuric acid in 300 ml water at 0 time. 2mU vasopressin administered i.v. at 59 min (arrow).

Table XXIII lists the frequency and type of response of the individual electrolytes.

Table XXIII

Na			K			Cl		
+	0	--	+	0	--	+	0	--
2	1	0	0	1	2	2	1	0

On the two occasions when the Na excretion was increased, the increase was maintained for more than 50min but the increases were small (Fig 41).

(III) Arginine vasopressin administered during the non diuretic state.

On two occasions the usual antidiuretic dose of vasopressin was administered during the non diuretic state. In one experiment there was no change in urine flow, but the excretion of Na and Cl was elevated by 10% for 40 min. In the other experiment the urine flow was inhibited for 10 min, but there was no change in electrolyte excretion.

(IV) Oxytocin during the non diuretic state.

On two occasions oxytocin was administered to the dog with denervated kidneys during observation of the resting flow. On both occasions the urine flow was inhibited for about 10 min, and an increase in Na and Cl excretion lasting for more than 50 min was produced. The excretion of K remained unaltered (Fig 42)

(V) Clearance studies.

On two occasions the G.F.R. and R.P.F. were measured during a water diuresis immediately before and 12min

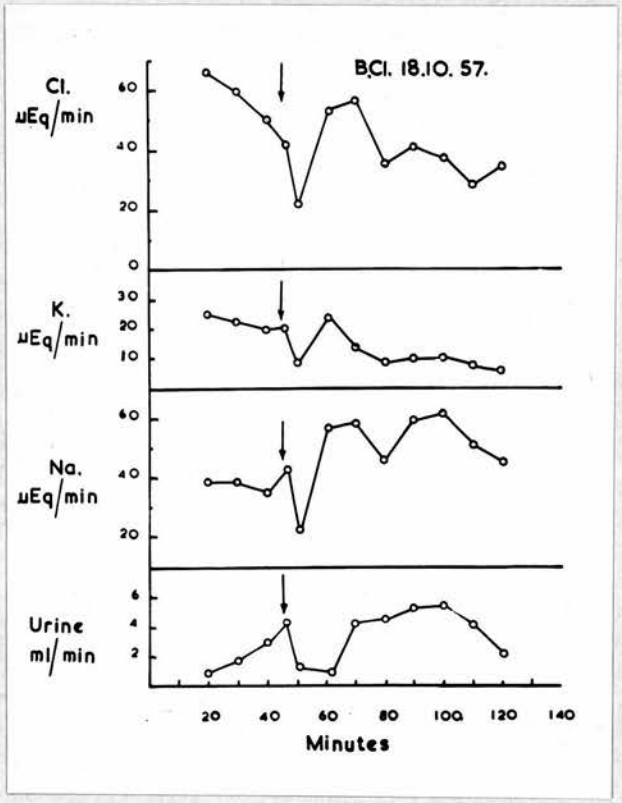

Fig. 41. B.Cl 18.10.57 Water diuresis in a dog with denervated kidneys. 300 ml water at 0 time. 150mU oxytocin administered i.v. at 46 min (arrow).

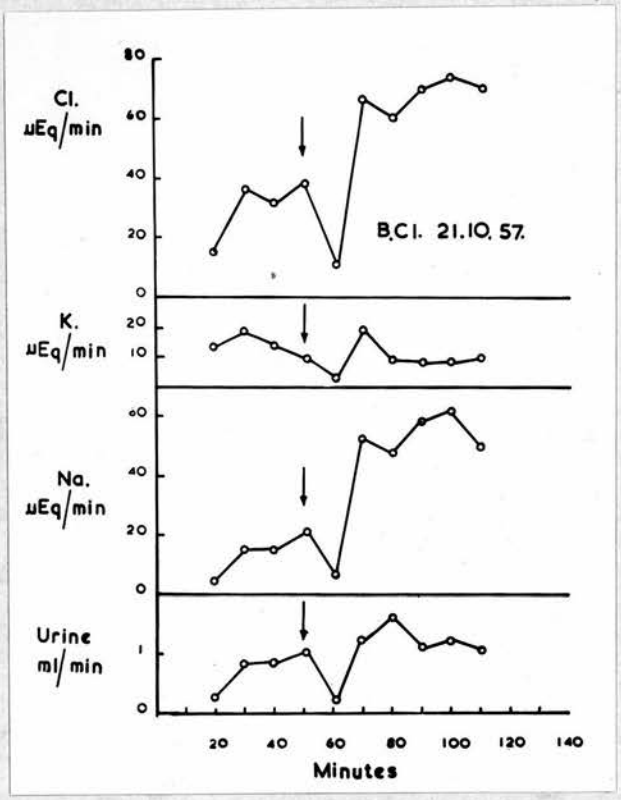

Fig. 42. B.Cl 21.10.57 Non diuretic urine flow in a dog with denervated kidneys. 150mU oxytocin administered i.v. at 50 min (arrow).

after an injection of vasopressin. In one case neither the G.F.R. nor R.P.F. were significantly changed. In the second experiment the G.F.R. increased from a pre-injection level of 68 ml/min to 80 ml/min. The R.P.F. increased from 230 ml/min to 313 ml/min. In neither of these experiments did vasopressin cause an increase in electrolyte excretion.

Clearance studies were also made before and after an injection of oxytocin during a water diuresis. On two occasions G.F.R. was measured, and no significant change noted. On one of these occasions the R.P.F. was also measured, and was found to increase from 194 ml/min 20 min later.

DISCUSSION

The dog with denervated kidneys responded to vasopressin in almost the same manner as it did when normal. Vasopressin was active during a water diuresis, when it inhibited the diuresis and caused an increase in electrolyte excretion. During the non diuretic state vasopressin was without effect. Oxytocin's activity was mainly seen in the non diuretic state when it caused an increase in electrolyte excretion lasting about 60 min, but during a diuresis oxytocin caused only a slight diminution in urine flow with a very small increase in electrolyte excretion.

The greatest deviation from normal in these experiments was the large decrease in electrolyte excretion seen in the early stages of an antidiuresis. This is possibly due to increased dead space. Morales, Crowder, Fishman, Maxwell and Gomez (1950) have found that dead space usually adapts to changes in urine flow. Over a wide range of flow rates the appearance time of dyes from blood to bladder was always approximately 100 secs. It is probable that nervous mechanisms are involved in this change of dead space. Although Morales et al. postulated that the changes were due to alteration in calibre of the renal tubules, the importance of changes in volume of the renal pelvis and ureter must not be overlooked. The nerve supply to these structures would have been interfered with in this animal.

The changes in renal haemodynamics caused by oxytocin are much the same as described by Brooks and Pickford

(1957), namely an elevation of R.P.F. without any significant change in G.F.R. The slight increase in both G.F.R. and R.P.F. caused by vasopressin in one case has not previously been described in normal animals. However, these changes were not confirmed in a second experiment.

SECTION VII

THE EFFECT OF ADRENALECTOMY ON THE RESPONSE TO VASOPRESSIN AND OXYTOCIN

INTRODUCTION

When large water loads are administered in company with injections of a long acting preparation of vasopressin (Pitressin tannate), there is a uniform expansion of all the fluid compartments (Cheek and West, 1956). In the dog this results in an increase in Na excretion with a decrease in K output, which has been related to a decreased production of aldosterone (Bartter, Liddle, Duncan, Barber and Delea, 1956). Beck, Dyrenfurth, Giroud and Venning (1955) reported similar findings in humans except that here no decrease in K excretion was seen.

Cheek and West (1956) reported that these changes in Na excretion did not occur if the Pitressin tannate was administered in the absence of a water load. They therefore postulated that Pitressin could increase Na excretion only through its action in expanding the body fluids and decreasing aldosterone production.

These changes in Na excretion produced by Pitressin tannate do not appear until 6 or 12 hours after the Pitressin tannate injection and water loading (Wrong, 1956). In the present study intravenous injections of vasopressin have been found to cause an immediate increase in electrolyte excretion. This is obviously an entirely different phenomenon from that produced by Pitressin tannate. Investigations were therefore undertaken to see if this

acute effect of vasopressin was dependent upon any alteration in adrenal cortical function.

METHODS

The dog used was subjected to a two stage trans abdominal adrenalectomy, and was maintained by daily injections of 8 mg hydrocortisone and 0.7 mg desoxy-corticosterone acetate (D.C.A.). The diet was a standard stew prepared from bread and canned dog food (Red Heart). Half a pint of milk was allowed each morning together with three small uniform dog biscuits. An additional 1 gm of NaCl was provided in the daily food bowl.

The maintainance therapy was judged to be adequate by the continued well being of the dog, its maintenance of constant weight, and the maintenance of serum electrolyte values within normal limits. The adrenalectomy was found to have been complete at subsequent postmortem examination.

The experiments conformed to the pattern described in Section II, all the details of those experiments being applicable here. The maintenance dose of steroids was usually administered one hour before the morning water load.

Control observations were made before the adrenalectomy. This particular dog was found to be comparatively resistant to the action of posterior pituitary hormones. A dose of at least 4mU vasopressin was required to increase electrolyte excretion during a water diuresis. No dose of oxytocin was found which could elevate electrolyte excretion during the non diuretic state. The kidneys displayed no microscopic lesions at post mortem examination.

RESULTS

(I) Vasopressin during a water diuresis.

In all, vasopressin was administered to the adrenal-ectomised dog on 15 occasions during a water diuresis. In some of these experiments the steroid dosage was more or less than the daily maintenance dose. These experiments will be discussed again in greater detail below. In three experiments where vasopressin was administered as a single injection of 4mU, there was a normal antidiuresis but little effect on electrolyte excretion other than a transitory inhibition of the general downward trend in electrolyte excretion. In the other 12 experiments the initial dose of 4mU vasopressin was followed by an infusion of 0.2mU/min for from 8 to 15 min. In all these experiments there was a prompt antidiuresis outlasting the infusion by at least 15 min (Fig 43). There was always some increase in electrolyte excretion during the antidiuresis. Table XXIV lists the frequency and type of response of the individual electrolytes.

Table XXIV

Na			K			Cl		
+	0	--	+	0	--	+	0	--
5	2	5	10	2	0	10	0	2

\+ Increased excretion 0 No change -- Decreased excretion

The increases in Na excretion were never greater than 25%. The Cl excretion never increased by more than 50%. On two occasions there were 100% increases in K

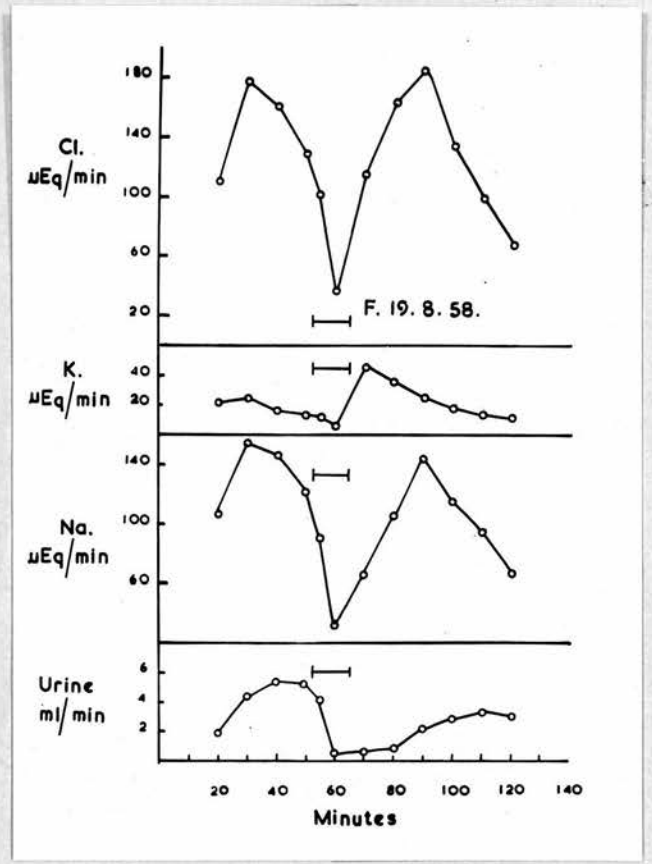

Fig. 43. F 19.8.58 Water diuresis in an adrenalectomized dog. No steroids administered on morning of experiment. 250 ml water at 0 time. 5mU vasopressin administered i.v. at 52 min, followed by an infusion of 0.2mU/min for 13 min.

excretion, and on three other occasioms the increase in K excretion exceeded 70%. These K changes were larger than those produced by a similar dose of vasopressin when the animal was normal.

(a) Effect of low steroid dosage. In four of the 15 experiments recorded above the usual dose of D.C.A. was witheld until after the experiment was concluded. In one of these cases half the usual dose of hydrocortisone was administered at the usual time, but in the other cases this was also witheld. In each of these four cases vasopressin caused a prompt antidiuresis and an increase in electrolyte excretion (Fig 43). Tables XXV and XXVII list the frequency and type of response of the individual electrolytes.

Table XXV

Na			K			Cl		
+	0	--	+	0	--	+	0	--
0	3	1	4	0	0	4	0	0

\+ Increased excretion 0 No change

-- Decreased excretion

The increase in K excretion ranged from 30% to 100% and the increases in Cl excretion were from 10% to 50%.

(b) Effect of high steroid dosage. In five of the 15 experiments described above the morning dose of steroid had been greater than required for normal maintenance. In three experiments the usual dose of hydrocortisone was administered, with twice or three times the usual

dose of D.C.A. In two experiments twice the usual dose of hydrocortisone was administered with either twice or three times the usual dose of D.C.A. In all these cases there was a prompt antidiuresis and an increase in electrolyte excretion (Fig 44). The frequency and type of response of the individual electrolytes is listed in Tables XXVI and XXVII.

Table XXVI

Na			K			Cl		
+	0	--	+	0	--	+	0	--
4	1	0	5	0	0	4	0	1

+ Increased excretion. 0 No change

-- Decreased excretion

The increase in Na excretion was about 10%. The increase in K ranged from 10% to 100% with an average of 70%. The increase in Cl excretion averaged 55% with a range from 20% to 70%.

(c) Effect on Na/K ratio. In the above 9 experiments where the dose of steroid was varied, the effect of vasopressin on the ratio of Na/K was studied. In all the experiments the excretion of Na plus K at the beginning of the experiment ranged from 83 uEq/min to 210 uEq/min. There was no correlation between the original level of Na plus K excretion and the dose of the steroids which had been administered.

In those experiments where the dog had been deprived of steroids K accounted for an average of 16.5% (± 3.36%) of the total Na plus K excretion at the beginning of the

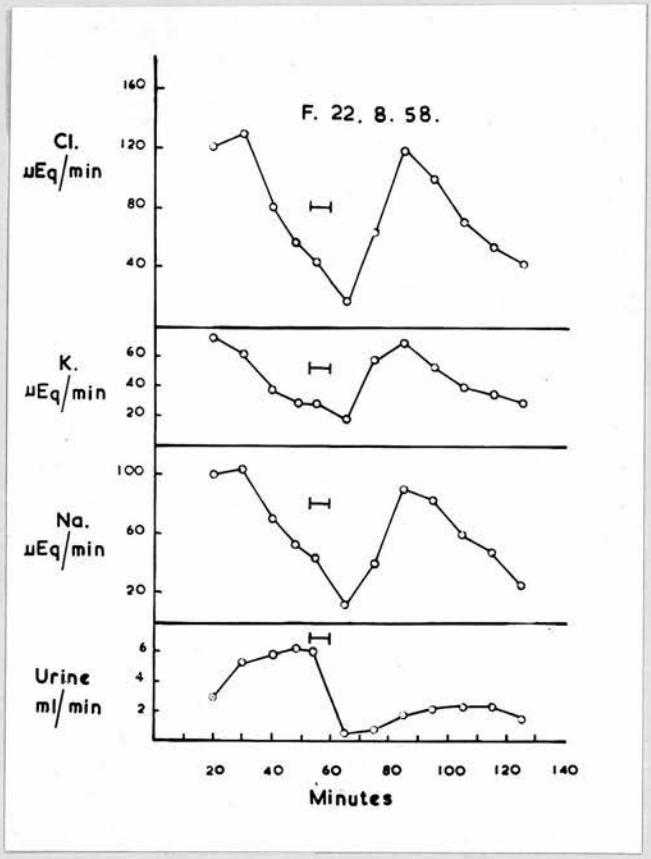

Fig. 44. F 22.8.58 Water diuresis in an adrenalectomized dog. 2.0mg D.C.A. and 0.4mg hydrocortisone on morning of experiment. 250 ml water at 0 time. 5mU vasopressin administered i.v. at 53 min, followed by an infusion of 0.2mU/min for 7 min.

Table XXVII

THE EFFECT OF VASOPRESSIN ON ELECTROLYTE EXCRETION DURING WATER DIURESIS IN THE ADRENALECTOMIZED DOG

	Na			K			Cl			Totals
	+	0	--	+	0	--	+	0	--	
Low steroid dosage	0	3	1	4	0	0	4	0	0	4
High steroid dosage	4	1	0	5	0	0	4	0	1	5

\+ Increased excretion

0 No change

-- Decreased excretion

diuresis. When electrolyte excretion increased during the antidiuresis produced by vasopressin, K was found to account for 55% (± 9.8%) of the total Na plus K excretion during this period of increased electrolyte excretion.

In those experiments where steroid administration was excessive, K accounted for 40.5% (± 9.25%) of the total Na plus K excretion at the beginning of the diuresis. During the vasopressin antidiuresis K excretion amounted to 59.8% (± 3.42%) of the total Na plus K excretion.

Thus although the total increase in Na plus K excretion produced by vasopressin was much the same under conditions of high and low steroid maintenance, the increase in K was greatest during low steroid dosing, and the increase in Na excretion was greatest during high steroid maintenance. Under both conditions the Na/K ratio was near unity during the peak electrolyte excretion after vasopressin.

(II) Oxytocin during the non diuretic state.

In five experiments during which the dog was on normal steroid maintenance oxytocin was administered during the non diuretic state. The doses of oxytocin were increased from a single injection of 150mU to infusions of 2mU/min after priming doses of 40mU amd 80mU. Eventually doses of 80mU and 160mU were followed by infusions of 4mU/min. The infusions were maintained for from 10 to 17 min. In all cases the urine flow diminished for one sample when the infusion was started, but there was no significant change in electrolyte excretion.

DISCUSSION

The high levels of urinary K, and the ease with which a water load was excreted suggest that in this animal, even on the days of steroid deprivation, there was an adequate, even a more than sufficient, level of circulating steroids. This was probably due to delayed absorption of part of the previous day's maintenance dose, and was certainly not due to the presence of any residual adrenal tissue (as was proved by post mortem examination). Thus although the findings illustrate that the acute action of vasopressin in increasing electrolyte excretion can proceed in the absence of an adrenal gland, no attempt has been made to demonstrate this activity in the complete absence of adrenal steroids.

Throughout each of these experiments the dog had a fairly steady tissue level of exogenous hydrocortisone and D.C.A. over which there was no internal control. These levels may have increased or decreased a little depending upon the relative rates of steroid absorption and utilization. During a water diuresis there may have been a temporary decrease in the tissue concentrations of steroids due to dilution of the tissue fluids by the ingested water. However unless some control over steroid utilization is postulated it is difficult to see how the level of steroids could, in this dog, be varied to suit any particular requirement.

The persistence of the action of vasopressin in increasing electrolyte excretion even after adrenalectomy does not conflict with the findings of Beck et al (1955)

and Bartter et al (1956). Although these workers found that Pitressin tannate increased electrolyte excretion by decreasing aldosterone production, it is obvious from the time course of the changes they were observing that they were dealing with a phenomenon different from that at present under consideration. It is unfortunate that they and other workers (Wrong, 1956; Cheek and West, 1956) wrote of this delayed action of Pitressin tannate in a manner which suggests that the electrolyte changes they observed are the only electrolyte changes produced by vasopressin.

The present findings are not unexpected. The early observations of Starling and Verney (1925), that pituitary extracts caused a chloruresis in the isolated perfused kidney, clearly illustrated that this action was a direct renal action not mediated through any alteration in steroid secretion. In that study, however, the posterior pituitary extract used was relatively crude by modern standards and may have possessed properties other than those exhibited by pure vasopressin.

The speed with which vasopressin caused an increase in electrolyte excretion also suggested a direct renal action. In several cases the increase in electrolyte excretion was seen within 5 min of the injection, and in all cases the increase was present within 15 min. These times include the time required for the urine to traverse the urinary dead space. Although it may be possible in the normal dog for aldosterone secretion to be halted within 5 min of a stimulus it seems improbable that in

such a short period of time aldosterone degradation would have proceeded far enough to cause an appreciable lowering of the levels of aldosterone already in circulation.

Another theoretical objection to the hypothesis that vasopressin acts on electrolyte excretion by expanding the extracellular fluid compartment and decreasing aldosterone secretion, is the small changes in body water content which could be expected under these circumstances. In these experiments vasopressin was usually administered when the urine flow was running at about 5 ml/min. There was an immediate antidiuresis during which the urine flow decreased to about 0.5 ml/min. Thus in the first 5 min of an antidiuresis not more than 25 ml of water which would otherwise have been excreted is retained. Since the increase in electrolyte excretion is frequently seen within 5 min of a dose of vasopressin, it follows that the electrolyte changes must have been initiated when considerably smaller volumes than 25 ml of water had been retained. Such small changes in body water seem very small to produce large changes in aldosterone secretion in a dog of about 13 kg.

There is a possibility that vasopressin may act to redistribute the existing body water within the fluid compartments, and that this redistribution may lead to altered aldosterone production. Friedman, Friedman and Nakashima (1955) working with rats, found that large doses of Pitressin caused water and Na to move into the cells. In humans suffering from diabetes insipidus, Pasqualini and Codevilla (1959) found that Pitressin was able to

reduce thirst even if no water was taken. This suggested a redistribution of existing body water. Even these arguements are without much weight for they suggest that Pitressin causes water to move into the cells, whereas a decreased aldosterone secretion would appear to depend upon an expanded extracellular volume (Bartter et al 1956)

Perhaps the most serious theoretical objection to the adrenal playing an intermediate role in vasopressin's increase of electrolyte excretion is the observation that vasopressin frequently causes a simultaneous increase in both Na and K excretion. If the increase in Na excretion were a result of decreased aldosterone activity, a simultaneous decrease in K excretion would be expected.

The electrolyte response to vasopressin in this animal was not quite normal in that an increase in K excretion was always a dominant feature of the response, whereas when the animal was normal the increase in K excretion was frequently insignificant. The basal K excretion in this animal after operation was unusually high, perhaps due to the presence of a rich source of dietary K in the milk provided. As already mentioned, the levels of D.C.A. administered were high enough to enable this K load to be excreted even on the days when the steroids were witheld, probably due to delayed absorption of the previous day's dose.

Shannon (1942) has presented evidence that the increase in Na excretion due to vasopressin is due to decreased proximal reabsorption of filtered Na. Anslow and Wesson (1955) further suggest that any increase in K

excretion during a vasopressin induced antidiuresis is due to the exchange in the distal tubule of some of this extra Na for K. These theories are supported by the present study where the increase in K excretion in response to vasopressin always overshadowed the increase in Na excretion. In these experiments the initial K excretion was always high, and it is a reasonable assumption that the Na - K exchange system was highly active.

It is not easy to explain why in the vasopressin experiments undertaken during excessive D.C.A. administration, the increase in Na excretion was relatively larger than in experiments during lower steroid administration. In both sets of circumstances the administration of vasopressin caused the K/Na ratio to increase, but the increase in both cases was to a value just above unity. In this state, when the concentrations of Na and K in the urine were equal, the dynamics of the Na - K exchange system would be different from when the concentration of Na in the urine was in considerable excess of K. It is possible that the exchange would be slowed as the concentration of urinary K increased.

During those experiments where the D.C.A. dosage was relatively low, the initial urinary K/Na ratio was low. Thus if vasopressin caused an additional quantity of Na to present in the distal tubule from higher in the nephron all this extra Na could be exchanged for K before the K/Na ratio exceeded unity and the exchange was slowed. During those experiments where the D.C.A. dosage was high,

the K/Na ratio began at high levels (just below unity). Only part of any extra quantity of Na appearing in the distal tubule would under these circumstances be exchanged for K before the exchange was slowed by the K/Na ratio approaching unity. Thus some of the extra Na would appear in the urine.

Although the above reasoning fits the experimental findings, it is extremely speculative, and depends on the rather tenuous hypothesis that adrenal steroids act by setting the Na - K exchange system to resorb Na until the concentration of Na in the distal tubular fluid reaches a predetermined minimal level. Since the site of final concentration of the urine is probably distal to the site of Na - K exchange (Wirz 1957), studies based on the final composition of the urine are unlikely to confirm or deny this hypothesis. More complete understanding of the Na - K exchange system would appear to await studies made on distal tubular fluid obtained by micropuncture.

The results obtained from administering oxytocin during the non diuretic state are inconclusive. No increase in electrolyte excretion was observed, but this may not have been due to the absence of the adrenal gland. This particular dog, even before adrenalectomy, did not respond to oxytocin by increasing electrolyte excretion. Further studies are therefore required on other dogs.

SECTION VIII

EFFECT OF INTERMEDIATE LOBE HORMONE ON EXCRETION OF WATER AND ELECTROLYTES

INTRODUCTION

Commercial preparations of oxytocin have been observed to have an action on renal function which is not exhibited by highly purified natural oxytocin. Working with the conscious dog, Brooks and Pickford (1957) found that during water diuresis highly purified natural oxytocin exerted no effect other than to cause a moderate decrease in urine flow. A similar dose of commercial oxytocin (Pitocin) in about 60% of cases interrupted the diuresis and caused a simultaneous increase in the excretion of Na, K and Cl. This electrolyte response was similar to, but sometimes of greater magnitude than that caused by an antidiuretic dose of vasopressin. Brooks and Pickford were unable to reproduce this action of commercial oxytocin by contaminating pure natural oxytocin with highly purified vasopressin. This suggested the existence of a third substance, present as a contaminant in commercial oxytocin which either increased electrolyte excretion itself, or modified the action of one or both of the known posterior lobe hormones.

The melanophore expanding hormone of the intermediate lobe (M.S.H.) is a possible contaminant of all pituitary extracts. When it was first isolated in a moderately pure form this hormone was thought to possess potent antidiuretic activity. (Sulzberger, 1933). This property

was, however later attributed to the presence of residual posterior pituitary hormones, and purified extracts of M.S.H. were declared to be without antidiuretic activity (Fraser, 1937; Landgrebe and Waring, 1941). As there do not appear to have been any extensive investigations into the actions of M.S.H. on electrolyte excretion, this present study was undertaken to determine whether M.S.H. was the active substance which occasionally contaminated commercial oxytocin.

METHODS

The experiments were all based on the same plan as has already been described in Section II. The dog used in this study had already been used in previous studies so that its usual response to the various hormones was known.

The M.S.H. used was from a sample prepared by Dr J. Porath using the method of Porath, Roos, Landgrebe and Mitchell (1955). Subdivisions of this sample were made by dissolving in distilled water, dividing by volume, and as rapidly as possible freeze drying and sealing in a vaccuum. Each batch of subdivisions was tested for activity on an isolated frog skin, and at the end of the series of experiments a sample was submitted to Dr. B. Hobson for accurate assay of the residual potency by the method of Landgrebe and Waring (1944). The oxytocin used was the synthetic oxytocin described previously, and the vasopressin was the highly purified arginine vasopressin supplied by Dr. V. du Vigneaud.

RESULTS

(I) M.S.H. administered during a water diuresis.

In six experiments doses of M.S.H. were administered during a water diuresis. In one experiment where the dose used was 100 I.U, M.S.H. caused an antidiuresis and an increase in electrolyte excretion of a similar magnitude and time course as that produced by a dose of 2mU vasopressin (Fig 45).

In the remaining experiments the doses of M.S.H. were of 10mU, 70mU, 140mU and 2.3 I.U. On one occasion an injection of 70mU was followed by a slow infusion of 100mU during the subsequent 15 min. In all these experiments the normal course of the water diuresis and of the electrolyte excretion proceeded unmodified by the injection (Fig 46).

(II) M.S.H. administered during the non diuretic state.

In five experiments on one dog M.S.H. was administered during the non diuretic state. The doses used were 70mU and 2.3 I.U. On no occasion was any significant change in electrolyte excretion observed.

(III) M.S.H. and vasopressin administered during a water diuresis.

On one occasion a dose of 70mU M.S.H. was administered with 2mU vasopressin during a water diuresis. There was a prompt antidiuresis and a simultaneous inhibition of the downward trend in electrolyte excretion. This response was similar to the response produced by a dose of 2mU vasopressin under similar circumstances on the

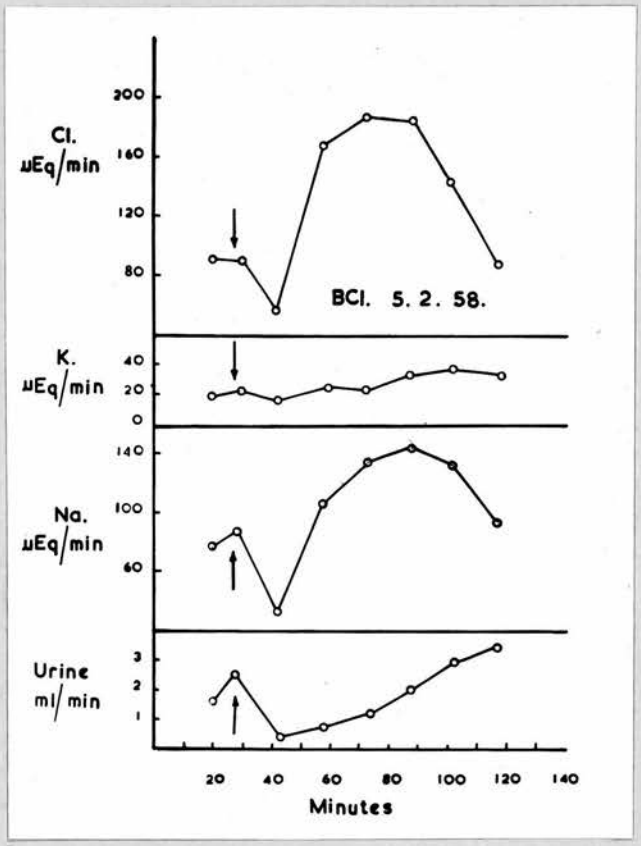

Fig. 45. B.Cl 5.2.58 Water diuresis in a dog. 300 ml water at 0 time. 100 IU pure M.S.H. administered i.v. at 28 min (arrow).

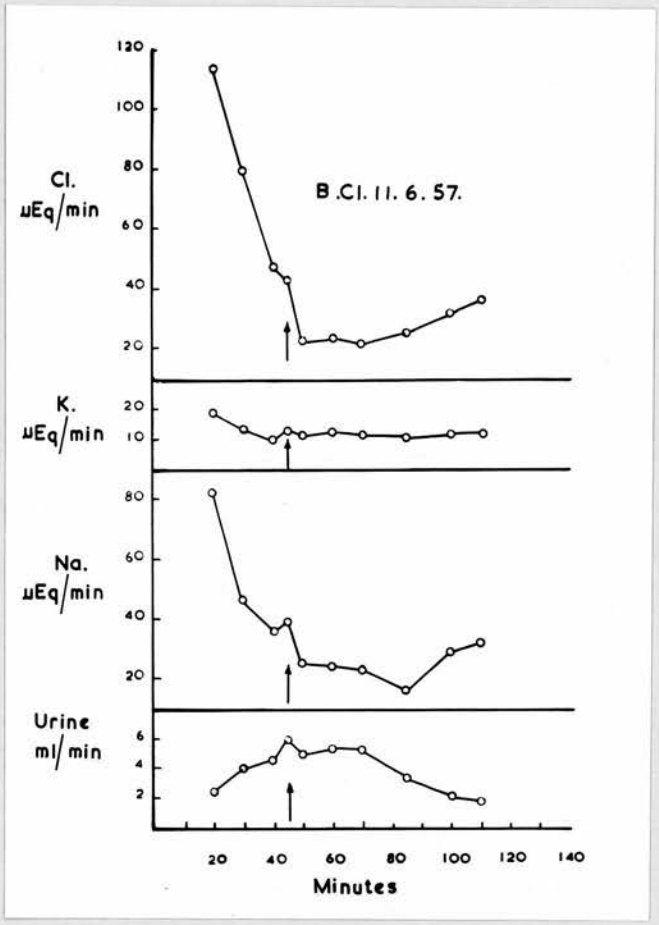

Fig. 46. B.Cl 11.6.57 Water diuresis in a dog. 300 ml water at 0 time. 2.3 IU pure M.S.H. administered i.v. at 45 min (arrow).

following day.

(IV) M.S.H. and vasopressin administered during the non diuretic state.

In one experiment during the non diuretic state, 2mU vasopressin was administered with 2.3 I.U. M.S.H. There was a slight inhibition of urine flow for 10 min, but there was no net increase in electrolyte excretion.

(V) M.S.H. and oxytocin administered during a water diuresis.

In three experiments 150mU oxytocin was administered with 70mU M.S.H. during a water diuresis. In all cases there was a mild inhibition of urine flow and a small increase in the excretion of electrolytes. These increases were less than 30% and were no larger than those occasionally produced by oxytocin alone under similar circumstances. (Fig 47).

(VI) M.S.H. and oxytocin administered during the non diuretic state.

In one experiment a dose of 70mU M.S.H. was administered with 150mU oxytocin during the non diuretic state. There was a slight increase in urine flow accompanied by an increase of about 100% in the excretion rates of Na, K and Cl. These increases lasted about one hour., and were of the same magnitude and time course as those frequently produced by oxytocin alone.

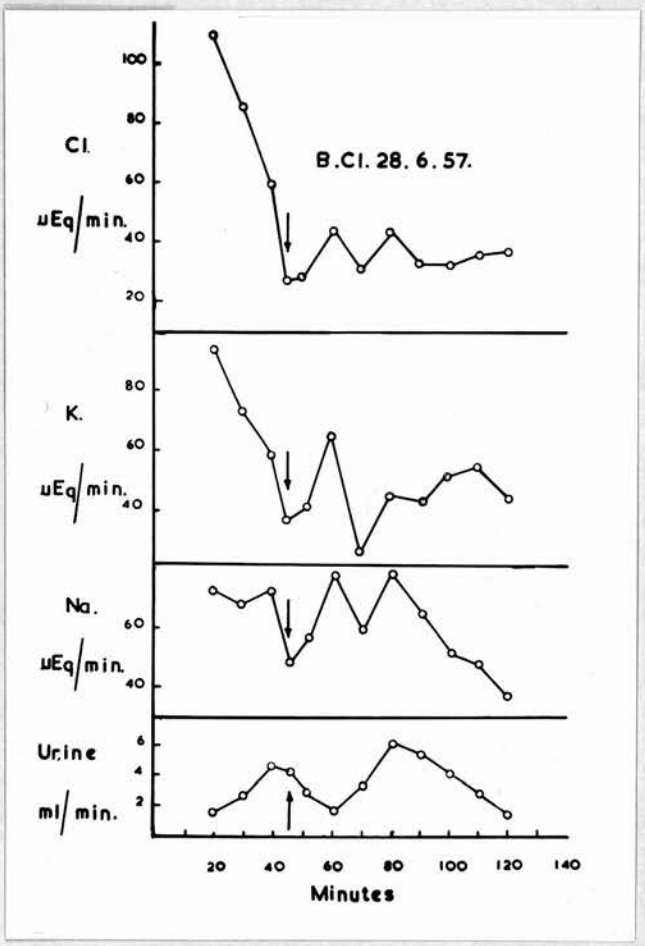

Fig. 47. B.Cl 28.6.57 Water diuresis in a dog. 300 ml water at 0 time. 150mU oxytocin plus 70mU pure M.S.H. administered i.v. at 46 min (arrow).

(VII) M.S.H., oxytocin and vasopressin administered during a water diuresis.

In one animal a dose of 80mU oxytocin and 0.6mU vasopressin was found to be antidiuretic without causing any significant increase in electrolyte excretion. (Fig 14 Section II). On three occasions this mixture was combined with a dose of 2.3 I.U M.S.H. The same degree of antidiuresis resulted, and the increase in electrolyte excretion was either minute or absent (Fig 48).

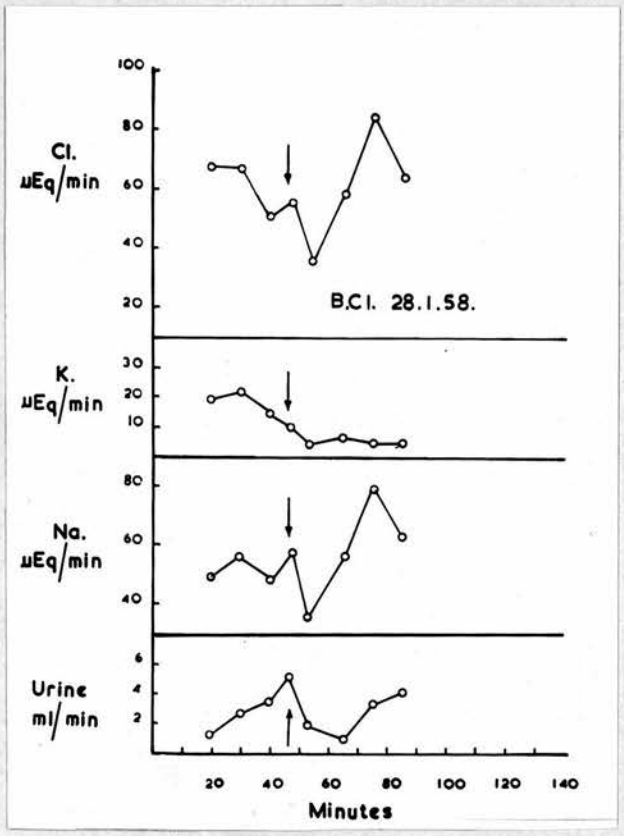

Fig. 48. B.Cl 28.1.58 Water diuresis in a dog. 300 ml water at 0 time. 80mU oxytocin plus 0.6mU vasopressin and 2.3 IU pure M.S.H. administered i.v. at 47 min (arrow)

DISCUSSION

The above findings would appear to dispose of the suspicion that M.S.H. was responsible for the unexpected actions of commercial oxytocin reported by Brooks and Pickford (1957). The doses of M.S.H. used in this study varied from small ones to those grossly in excess of any expected level of contamination in commercial extracts. Indeed the preparation used by Brooks and Pickford (Pitocin) was reported by Landgrebe and Waring (1950) to be free of M.S.H. A more recent assay using a method sensitive to 1mU failed to detect any M.S.H. in a dose of Pitocin containing 300mU oxytocin (Hobson, personal communication).

There is also no evidence that M.S.H. exerts any direct influence on the excretion of water and electrolytes. The one occasion on which a massive dose of 100 I.U M.S.H. inhibited a diuresis and increased the output of electrolytes can probably be attributed to the presence of contaminating vasopressin. This dose was administered during studies into the excretion of M.S.H. and when compared with the dose levels required for pigment changes in amphibia appears to be well beyond physiological limits. Although the extract was stated to contain less than 0.001% vasopressin (Porath, personal communication), such a level of contamination might explain the findings in this case.

It may be that some action of M.S.H. on renal function would have appeared had the observations been continued beyond the 60 to 90 min periods used in this

study, but short as this time is, it represents at least three times the circulating life of an injected dose of M.S.H. (Landgrebe and Waring, 1941). Lerner, Shizume and Bunding (1954) administered massive doses of M.S.H. to human subjects daily for 36 days. Unfortunately they did not investigate urinary excretion of electrolytes, but their studies of serum electrolytes did not reveal a significant deviation from normal. Since any gross change in the urinary excretion of electrolytes if maintained for 36 days might be expected to cause some secondary alteration in serum electrolyte levels, the failure to observe these changes suggests that prolonged administration of M.S.H. does not alter renal function to any significant degree.

CONCLUSIONS

The foregoing experiments, although considered for the sake of clarity under separate sections, are all closely related and were conceived as a series of logical steps towards the elucidation of the site and nature of the renal action of posterior pituitary hormones. Thus Section I is merely an investigation of the unmodified pattern of water and electrolyte excretion in all the various circumstances under which the actions of vasopressin and oxytocin were later studied. These control studies were very useful in that they provided a clearly defined baseline against which the changes produced by vasopressin and oxytocin could be observed. It was established that during a water diuresis the absolute excretion of electrolytes decreased as the urine flow increased, and that the rate of electrolyte excretion did not increase again until at least 30 min after the peak of the diuresis. This phenomenon was not altered by an increased electrolyte intake or by denervation of the kidneys or adrenalectomy. The experiments did not, however, yield any information as to how these changes in electrolyte excretion were produced. During the non diuretic state electrolyte excretion tended to remain steady despite minor fluctuations in the rate of urine flow.

Although a large number of observations were made on the action of oxytocin, for reasons stated below, only a few broad generalized conclusions could be drawn from the results. The main renal action of oxytocin was seen

during the non diuretic state where an increase in electrolyte excretion occurred in the absence of any alteration in the rate of urine flow. Although these changes occurred consistently, the experimental errors due to poor drainage of the urinary dead space are potentially large under these conditions of low urinary flow rates. Therefore it is difficult to obtain data of sufficent reliability to permit any more detailed investigation of the phenomenon.

Oxytocin was also demonstrated to have a weak action in increasing electrolyte excretion when administered during a water diuresis. This action could occasionally be enhanced by previously loading the test animal with an oral dose of NaCl, but as this effect could not always be reproduced, it proved an unsatisfactory phenomenon for investigation. The problem of these variable responses to oxytocin was further considered in Section VIII. When the oxytocic substance used is a commercial extract of pituitary glands it is possible that some of the variability of response may be due to occasional contamination of the extract with other active substances. Since the intermediate lobe hormone is a possible contaminant of all pituitary extracts, this substance was investigated for renal activity, either alone or in company with vasopressin or oxytocin. The findings were completely negative. Since the renal response to pure synthetic oxytocin is just as unpredictable as the response to commercial extracts (Section II), it seems probable that the cause of the variation lies in the internal

environment of the test animal rather than in contamination of the oxytocin.

The action of vasopressin on electrolyte excretion proved a much more satisfactory field of study. During a water diuresis vasopressin was found to reduce the urine flow and to increase electrolyte excretion (Section II). This action of vasopressin was not modified by renal denervation (Section VI). Vasopressin was also demonstrated to increase electrolyte excretion during a water diuresis in the adrenalectomized dog (Section VII), thus proving that the effect was not mediated through alteration in adrenal steroid output. These phenomena are produced by doses of vasopressin which have no demonstrable effect upon renal plasma flow or glomerular filtration rate (Sellwood and Verney, 1955 and Section II). These findings therefore suggest that vasopressin may exert a direct action upon the renal tubular epithelium.

Section III resulted from the observation that the actual increase in electrolyte excretion produced by vasopressin was greatest in those experiments where the electrolyte excretion was already high at the beginning of the experiment. This condition was reproduced by administering a dose of NaCl to the test animal several hours before the water diuresis was induced. Subsequently it was found that the augmented electrolyte response to vasopressin could be produced by previously loading the test animal with $NaHCO_3$, $KHCO_3$, or NH_4Cl. This suggested that the response to vasopressin could be increased by

loading with Na^+, K^+, or Cl^- independently.

In Section IV further analyses of the above experiments revealed that, depending on the pH of the urine, vasopressin could cause an increase in Na and K excretion independently of an increase in Cl output, or alternatively an increase in Cl excretion independently of an increase in Na and K. In the latter case it was shown that ionic equilibrium in the urine was maintained by an increased excretion of ammonium. Unless it is postulated that vasopressin exerts a direct action on the excretion of many ions, these findings are probably best explained by the hypothesis that vasopressin causes an increased amount of NaCl to appear in the distal tubular fluid from somewhere higher in the nephron. This extra NaCl is then either reabsorbed as such or exchanged for other ions in the distal tubule, depending on the requirements of homeostasis prevailing at the time.

Vasopressin would appear, therefore, to have at least two distinct renal actions. Firstly it promotes the reabsorption of water from the tubular fluid. There is good evidence that this action occurs near the end of the nephron in the collecting ducts (Wirz, 1957). The second action of vasopressin is to increase electrolyte excretion, which as stated above, appears to be due to an action located somewhere proximal to the distal convoluted tubule. These two actions may not, however, be as distinct as would at first appear. In the following paragraphs an attempt will be made to present a hypothesis wherein these two activities are brought together

and attributed to a single action occurring at one specific site.

Micropuncture techniques have demonstrated that fluid reaching the distal tubules is usually hypotonic to plasma (Wirz, 1957). The final concentrating mechanism must therefore lie still more distally, probably in the collecting ducts. Wirz does not however favour the concept that water is actively reabsorbed from the collecting ducts against an increasing osmotic gradient. He presents good evidence that during antidiuresis the concentration of all the constituents of the entire renal pyramid increases progressively towards the tip. Thus blood in the arterial and venous loops of the medulla, and tubular fluid in the loops of Henle are progressively concentrated as they descend towards the tip of the pyramid, and rediluted as they ascend again. The extracellular fluid of the pyramid shares in this process of progressive concentration. The collecting ducts pass straight through the renal pyramid from the cortex to the papilla. The urine in these ducts can therefore be concentrated by the loss of water to the hypertonic surroundings, and since this urine never ascends again through the pyramid, the concentration is permanent. Thus, although the final urine may become highly concentrated as it passes through the pyramid, the osmotic gradient across any particular portion of the duct wall will be quite small.

Wirz supports his hypothesis with a working model of the medullary loop, in which the adjacent walls of the descending and ascending limbs of the loop of Henle

are represented as a single semipermeable membrane. He is able to show that when an osmotic gradient is set up between the contents of the two limbs, the progressive concentration of the contents of both limbs in the region of the hairpin bend will proceed spontaneously. He postulates that in life this osmotic gradient is achieved by the active reabsorption of Na from the ascending limb. This reabsorption is thought to proceed at a constant rate irrespective of the conditions prevailing at the time.

During the elaboration of a dilute urine, the descending limb is said to be impermeable to water and Na, and the ascending limb only slightly permeable to water. Thus the countercurrent concentrating mechanism cannot be initiated. The tubular fluid is diluted by the removal of more Na than water from the ascending limb and after further ionic exchange and absorption this dilute fluid is delivered to the ureter as dilute urine. Under the influence of vasopressin however, the descending limb is thought to become permeable to water. This sets the counter current concentrating mechanism in operation, the region of the tip of the pyramid becomes highly concentrated, and as a result water is removed from the final urine.

The fundamental point of this hypothesis is that the removal of Na from the ascending limb of the loop renders the extracellular fluid of the pyramid slightly hypertonic. Under the influence of vasopressin water is able to pass out of the descending limb into this hyper-

tonic region, resulting in an increased osmotic pressure of the tubular fluid. This sets the progressive concentrating mechanism in motion. However this hypothesis would be just as well served if it were postulated that the concentration of the contents of the descending limb were achieved by the passage of Na into the tubule, as well as water out of it. Wirz has suggested this as a possibility. Most of the Na passing into the descending limb would be reabsorbed from the ascending limb, but it is probable that some would escape to the distal tubule. This would explain why, under the influence of vasopressin, an increased amount of NaCl appears to reach the distal tubule.

If this theory is correct, it is unnecessary to postulate that vasopressin acts at two different sites in the kidney, decreasing Na reabsorption proximally and increasing water reabsorption from the collecting ducts. It is now postulated that vasopressin acts only on the descending limb of the loop of Henle, causing Na to pass into the tubular fluid. This enables the counter current concentrating mechanism to be initiated, leading to the removal of fluid from the final urine.

SUMMARY

1. The effect of posterior pituitary hormones on the excretion of water and electrolytes was studied in dogs.

2. During an unmodified water diuresis, the rate of excretion of electrolytes decreased as the urine flow increased and continued to fall for about 30 min after the peak of diuresis. In the non diuretic state electrolyte excretion was steady and independent of minor fluctuations in urine flow. These patterns of water and electrolyte excretion were not modified by adrenalectomy, renal denervation or by previously loading the dog with various salts.

3. When administered during a water diuresis, vasopressin decreased the urine flow and increased the rate of electrolyte excretion. Vasopressin was without apparent effect during the non-diuretic state. During a water diuresis pure oxytocin was without much effect, although commercial oxytocin occasionally exhibited an action similar to that of vasopressin. In the non-diuretic state oxytocin increased electrolyte output without any consistent effect on urine flow.

4. In some experiments the basal excretion of electrolytes was elevated by loading the dog with various salts three hours before a water diuresis was induced. During the subsequent diuresis vasopressin caused an increase in electrolyte excretion which was greater in absolute

amounts than that produced when the basal excretion of electrolytes was low. However, when the changes were expressed as percentages the reverse was the case. The electrolyte response to vasopressin was greatest when the loading salt contained Cl. Oxytocin occasionally caused an increase in electrolyte excretion during a water diuresis when the basal electrolyte excretion was high.

5. During a water diuresis vasopressin caused increases in Na, K, Cl, NH_3 and phosphate output which were independent of each other and which were determined by the urinary pH. Vasopressin did not alter the glomerular filtration rate.

6. The action of vasopressin on electrolyte excretion was almost absent when the animal was under the influence of acetazoleamide (Diamox). Acetazoleamide did not inhibit the action of oxytocin.

7. The actions of vasopressin and oxytocin were not modified by renal denervation.

8. An adrenalectomized dog on adequate steroid maintenance exhibited the same electrolyte response to vasopressin as a normal dog.

9. Pure M.S.H. was found to have no effect upon electrolyte excretion.

10. It is postulated that the primary action of vasopressin is to alter the permeability of the thin

segment of the medullary loop to NaCl and that this results in the initiation of the concentrating mechanism of the renal tubules.

REFERENCES

ABRAHAMS, V.C. & PICKFORD, M. (1954) J. Physiol. 126 329

ABRAHAMS, V.C. & PICKFORD, M. (1956) Brit. J. Pharmacol. 11 44

ADOLPH, E.F. & ERICSON, G. (1926) Amer. J. Physiol. 79 377

ALI, M.N. (1958) Brit. J. Pharmacol. 13 131

ALI, M.N., CROSS, R.B. & PICKFORD, M. (1958) J. Physiol. 141 177

ANSLOW, W.P. & WESSON, L.G. (1955) Amer. J. Physiol. 182 561

BAIRD, S. & PICKFORD, M. (1958) J. Physiol. 144 80

BARTTER, F.C., LIDDLE, G.W., DUNCAN, L.E., BARBER, J.R. & DELEA, C. (1956) J. clin. Invest. 35 1306

BECK, J.C., DYRENFURTH, I., GIROUD, C. & VENNING, E.H. (1955) Arch. intern. Med. 96 463

BERDE, B., DOEPFNER, W. & KONZETT, H. (1957) Brit. J. Pharmacol. 12 209

BERGLUND, F., & LOTSPEICH, W.D. (1956) Amer. J. Physiol 185 533

BERKHIN, E.B. (1956) Biul. eksper. Biol. Med. 42 7 (cited International Abstracts 1958. 11 511, no. 5645

BERLINER, R. W., KENNEDY, T.J. & ORLOFF, J. (1954) The Kidney p.147 Ed. Lewis, A.A.G. & Wolstenholme, G.E.W. London: Churchill

BRALEAU, P. & GILMAN, A. (1953) Amer. J. Physiol 175 33.

BRODSKY, W. A. & RAPOPORT, S. (1951) J. clin. Invest. 30 282

BRODSKY, W.A., MILEY, J.F., KAIM, J.T., & SHAH, N.P. (1958) Amer. J. Physiol. 193 108

BROOKS, F.P.& PICKFORD, M. (1957) The Neurohypophysis, ed Heller, H. London: Butterworths, p.141

BROOKS, F.P. & PICKFORD, M. (1958) J. Physiol. 142 468

BRUNNER, H., KUSCHINSKY, G., MUNCHOW, O. & PETERS, G. (1957) Arch. exp. Path. Pharmak. 230 80

BRUNNER, H., KUSCHINSKY, G. & PETERS, G. (1956) Arch. exp. Path. Pharmak. 228 434

CHALMERS, T.M., LEWIS, A.A.G. & PAWAN, G.L.S. (1951) J Physiol. 112 238

CHEEK, D.B. & WEST, C.D. (1956) Amer.J. Physiol. 184 69

COLE, S.W. (1919) Practical Physiological Chemistry. Cambridge: Heffer

COULSTON, R.A. & HERNANDEZ, T. (1957) Amer. J. Physiol. 188 485

CROXATTO, H., ROSAS, R. & BARNAFI, L. (1956) Acta Physiol. Latinoamer. 6 147

DeWARDENER , H. & HERXHEIMER, A. (1957) J. Physiol. 139 42

DICKER, S.E. (1953) J. Physiol. 122 149

DICKER, S.E. (1957) J. Physiol. 139 108

EGGLETON, M.G. (1943) J. Physiol. 102 150

FRASER, A.M. (1937a) J. Pharmacol. 60 82

FRASER, A.M. (1937b) J. Pharmacol. 60 89

FRIBERG, O., KARVONEN, M.J., & LEPPANEN, V. (1950) Ann. Med. Exp. Biol. Fenn. 28 144

FRIEDMAN, S., FRIEDMAN, C. & NAKASHIMA, M. (1956) Nature 177 1079

FRIEDMAN, S., NAKASHIMA, M.& FRIEDMAN, C. (1955) Amer. J. Physiol. 184 97

GAUNT, R. (1951) Recent Progr.Hormone Res. 6 247

HANLEY, T. & PLATTS, (1956) J. clin Invest. 35(1) 20

HILTON, J.G., CAPECI, N.E., KISS, G.T., KRUESI, O.R., GLAVIANO, V.V. & WEGRIA, R. (1956) J. Clin. Invest. 35 481

HOLLAND, B. & STEAD, E.A. (1951) Arch.intern.Med. 88 571

HOLLANDER, W., WINTERS, R.W., WILLIAMS, T.F.,HOLLIDAY, M., OLIVER, J. & WELT, L.G. (1956) J. clin. Invest. 35 713

JACOBSON, H.N. & KELLOGG, R.H. (1956) Amer. J. Physiol. 184 376

KARVONEN, M.J., LEPPANEN, V. & PITKANEN, M.E. (1953) Ann. Med. Exp. Biol. Fenn. 31 117

KAPLAN, S.A., WEST, C.D. & FOMON, S.J. (1953) Amer. J. Physiol. 175 363

KAYE, M. (1955) J. clin. Invest. 34(1) 277

KING,E.J. & WOOTTON, I.D.P. (1956) Micro-analysis in Medical Biochemistry. London: Churchill

KLEEMAN, C.R., EPSTEIN, F.H., & WHITE, C. (1956) J. clin. Invest. 35(1) 746

KLISIECKI, A., PICKFORD, M., ROTHSCHILD, P. & VERNEY, E.B. (1932a) Proc. Roy. Soc. B. 112 496

KLISIECKI, A., PICKFORD, M., ROTHSCHILD, P. & VERNEY, E.B. (1932b) Proc. Roy. Soc. B. 112 521

KUSCHINSKY, G. & BUNDSCHUH, H.E. (1939) Arch. exp. Path. Pharmak. 192 683

LANDGREBE, F. W. & WARING, H. (1941) Quart. J. exp. Physiol. 31 31

LANDGREBE, F.W. & WARING, H. (1944) Quart. J. exp. Physiol. 33 1

LANDGREBE, F.W. & WARING, H. (1950) The Hormones Vol. 11 ed. Pincus,G. & Thimann, K.V. p.506. New York: Academic Press

LERNER, A.B., SHIZUME, K. & BUNDING, I. (1954) J. clin. Endocr. 14 1463

LITTLE, J.M., WALLACE, S.L., WHATLEY, E.C. & ANDERSON, G.A. (1947) Amer. J. Physiol. 151 174

MARSHALL, E.K. (1920) J. Pharmacol. 16 141

MORALES, P.A., CROWDER, C.H., FISHMAN, A.P., MAXWELL, M.H., GOMEZ, D.M. (1950) Amer. J. Physiol. 163 454

NICHOLSON, T.F. (1957) Canad. J. Biochem. & Physiol. 35 419

NICHOLSON, T.F. & SHEPHERD, G.W. (1957) Canad. J. Biochem. & Physiol. 37 103

O'CONNOR,W.J. (1948) Proc. R. Soc. Med. 41 666

OLIVER, J., Mac.DOWELL, M., WELT, L.G., HOLLIDAY, M.A., HOLLANDER, W., WINTERS, R.W., WILLIAMS, T.F. & SEGAR, W.E. (1957) J.exp. med. 106 563

ORLOFF, J. & BERLINER, R.W. (1956) J. Clin. Invest. 35 234

PASQUALINI, R.Q. & CODEVILLA, A. (1959) Acta endocrinol. Copenhagen. 30 37

PITTS, R.F. & ALEXANDER, R. S. (1945) Amer. J. Physiol. 144 239

PITTS, R.F., GURD, R.S., KESSLER, R.H. & HIERHOLZER, K. (1958) Amer.J. Physiol. 194 125

PORATH, J., ROOS, P., LANDGREBE, F.W. & MITCHELL, G.M. (1955) Biochim. Biophys. Acta. 17 598

PRIESTLEY, J.G. (1921) J. Physiol. 55 305

RALLI, E.P., RAISZ, W.G., LESLIE, S.H., DUMM, M.E. & LAKEN , B. (1950) Amer. J. Physiol. 163 141

RECTOR, F.C., SELDIN, D.W. & COPENHAVER, J.H. (1955) J. clin. Invest. 34 20

RIOCH, D. McK. (1930) J. Physiol. 70 48

ROBINSON, F.J., POWER, M.H. & KEPLER, E.T. (1941) Proc. Mayo Clin. 16 577

ROSCOE, M.H. (1956) Acta med. Scand. 156 277

SARTORIUS, O.W. & BURLINGTON, H. (1956) Amer. J. Physiol. 185 407

SARTORIUS, O.W. & ROBERTS, K. (1949) Endocrinology 45 273

SCHLEGEL, J.U. & STONE, H. (1957) Amer. J. Physiol. 190 287

SELLWOOD, R.V. & VERNEY, E.B. (1954) Philos. Trans. 238B 361

SHANNON, J.A. (1942) J. exp. Med. 76 371 & 387

SMITH, H.W. (1956) Principles of Renal Physiology. New York: Oxford University Press

SMITH, H.W., FINKELSTEIN, N., ALIMINOSA, L., CRAWFORD, B. & GRABER, M. (1945) J. clin. Invest. 24 388

STARLING, E.H. & VERNEY, E.B. (1925) Proc. Roy. Soc. B. 97 321

SULZBERGER, M.B. (1933) J. Amer. med. Ass. 100(2) 1928

THOMSON, W.B. (1959) J. Physiol. 145 12p

THORN, N.A. (1958) Physiol. Rev. 38 169

UNNA, K. & WALTERSKIRCHEN, L. (1936) Arch. exp. Path. Pharmak. 181 681

VERNEY, E.B. (1947) Proc. Roy. Soc. B. 135 25

VISSCHER, M.B., FETCHER, E.S., CARR, C.W. GREGOR, H.P., BUSHEY, M.S. & BARKER, D.E. (1944) Amer.J. Physiol. 142 550

WEST, C.D., TRAEGER, J. T. & KAPLAN, S.A. (1955) J. clin. Invest. 34 887

WIRZ, H. (1957) The Neurohypophysis, ed Heller, H., London: Butterworth, p157

WRONG, O. (1956) Clin. Sci. 15 401