THESIS

ON

AN INVESTIGATION INTO THE MODE OF ACTION OF CERTAIN HORMONAL AND CHEMICAL FACTORS WHICH INFLUENCE THE SECRETION OF URINE BY THE KIDNEY

SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

by

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CONTENTS

INTRODUCTION

This thesis consists of two parts, both concerned with the study of substances which can reduce the volume output of the kidney. The substance investigated in part one is 5-hydroxytryptamine. This substance, isolated only recently is of widespread occurrence not only among the various tissues of the individual, but amongst individual species throughout the animal kingdom.

A great deal is known of its effects upon the living organism, and they are many. Suggestions as to its true physiological role have been diverse, ranging from a possible haemostatic, a factor in brain metabolism, and a specific regulating hormone of the kidney. Part one is concerned with the latter aspect. Since the anti-diuretic activity of 5-hydroxytryptamine has only recently been discovered, the experiments are concerned with roughly outlining its properties and seeing whether its action on the kidney can be regarded as specific.

Part two is concerned with the anti-diuretic activity of morphine. In this part an attempt has been made to localise further the anti-diuretic action of morphine. This substance exerts its effect through the hypothalamico-hypophyseal system, causing the release of the anti-diuretic hormone. The experiments have been done to find out, if possible, more about the workings of this system.

I am very grateful for Professor Whitteridge

making my stay in his department possible, and it is a pleasure to acknowledge my debt of gratitude to Dr. Mary Pickford for all that she has done for me in this period.

Technical assistance during most of these experiments has been admirably given by Miss Dorothy Craig, and Mr. W. T. Hunt has ensured that the animals have received the best of attention. The photomicrographs were done by the Photomicrography Unit of the Pathology Department under the guidance of Mr. T. C. Dodds. My grateful thanks to Miss Pamela Dance, B.Sc., for doing so many jobs so willingly and so well.

Some of the work described in this thesis has been communicated to the Pharmacological Society (Oxford, July, 1953) and to the Physiological Society (Sheffield, April, 1955.

PART I

5-Hydroxytryptamine Anti-diuresis

Introduction

The presence within the body of a substance with anti-diuretic properties arouses curiosity as to its possible function and importance. This is particularly so when an existing theory can adequately explain the mechanism of anti-diuresis. Hence our interest in 5-hydroxytryptamine. This substance, isolated recently from blood (Rapport, 1949) was not first thought to be of any particular importance in controlling renal function. The workers responsible for the initiation of the researches which led up to its isolation (Rapport, Green and Page, 1948 a & b) were more concerned with the vasoconstrictor properties of the substance, and its possible importance in hypertension.

T. G. Brodie (1900) first showed that if blood is allowed to clot, then the serum has a number of effects upon the vasculature, including constriction of the kidney. Such experiments as he did gave results which were the sum of the action of a number of different substances, both vasodilator, and vasoconstrictor. Stewart and Harvey (1912) showed that serum caused vasoconstriction of the vessels of perfused kidneys, and identified the activity as being related to a thermo-stable alcohol-soluble material.

The presence of a substance or substances,

called "vasotonins" in blood which was allowed to clot was noted several times in the 1920's and 1930's. Eicholtz and Verney (1924) could not get any blood to flow through a kidney perfused with defibrinated blood by means of a pump, the vasoconstriction being so pronounced. This vasoconstriction they attributed to vasotonins appearing spontaneously in the blood. They pointed out that early workers had ridiculously low flow figures through perfused kidneys. Jacoby (1890) could only force 100-200 mls of blood through a perfused pig kidney in one minute. Pfaff and Tyrode (1903) perfusing dog kidneys had flow rates of only 3 to 7 mls/min.

More was learnt about the properties of vasotonins by the researches of Hemingway (1931) and Bayliss and Ogden (1932). Hemingway showed that by passing defibrinated blood through lungs for five or ten minutes the vasoconstrictor activity is lost. Bayliss and Ogden showed that vasotonins are formed rapidly if blood is allowed to stand, the process apparently being complete in ninety minutes. Like Hemingway they showed that passage through the lungs of blood which had stood and acquired vasoconstrictor activity caused a loss of such activity. Once passed through the lungs and bereft of its vasoconstrictor properties, the blood does not reacquire such properties after standing for a further period. They showed that it was not the process

of oxygenation of the blood which caused it to lose its vasoconstrictor properties.

The general subject of vasoactive substances in blood benefited greatly from the publishing, in 1936 of a book by Gaddum "Gefässerweiternde Stoffer der Gewebe", in which the activity of both vasodilators and constrictors was distinguished. He attributed the vasoconstrictor action on kidneys of blood which has been allowed to stand, or which has suffered mechanical damage to vasotonins, ruling out other possibilities.

The list of organs capable of destroying vasotonins was extended by the work of Bing (1941) who showed that both the liver and the spleen could remove circulating vasotonins. In this decade (1940-1950), work on vasotonins was accelerated. Reid and Bick were concerned with the problems of the large scale storage of blood as a result of the great wartime needs. In 1942 they published the results of investigations on reactions to the administration of stored serum. They showed that a substance was released during the clotting process, a substance which was alcohol soluble, and which was highly vasoactive. They added the important fact to our existing knowledge that this substance was released from the platelets during the clotting process. This findings was confirmed by Zucker in 1944 and again by Reid and Rand in 1951 (a & b).

In Cleveland, Rapport, Green and Page were investigating a serum vasoconstrictor which they thought might be of importance in hypertension (Rapport, Green and Page, 1948a). They were soon able to isolate the material, using the perfused rabbit ear as a test for its presence (Rapport, Green and Page, 1948 b & c). In 1949 Rapport established the structure of the material as the indole-alkylamine, 5-hydroxytryptamine. The synthesis was accomplished by Hamlin and Fisher in 1951, and it soon became clear that the substance referred to as vasotonin in this country and serotonin in the United States was 5-hydroxytryptamine (Reid and Rand, 1951; Page, 1952; Freyburger, Graham, Rapport, Seay, Govier, Swoap and Vander Brook, 1952).

In the general appraisal of its properties, the effects on the kidney were neglected until the announcement by Erspamer and Asero in 1952, that the substance which they had been investigating for many years under the name of enteramine was in fact 5-hydroxytryptamine. Erspamer and his colleagues had succeeded in isolating enteramine from a host of tissues, but came across its anti-diuretic properties accidentally.

In 1948, Erspamer and Perosa were investigating the hypotensive activity of extracts of octopus salivary gland. They found that the administration of some of their extract to a man with diabetes

insipidus reduced his daily urine output of 9 litres. They showed their extract to be anti-diuretic when injected into rats, to be thermostable and alcohol soluble, but not to be the anti-diuretic hormone. Extracts of all other parts of the octopus failed to show any anti-diuretic action.

Erspamer and Ottolenghi (1950) showed the active principle in the octopus salivary gland to be enteramine like, and extended the range of animals in which it could be shown to produce anti-diuresis to include frogs, toads, guinea-pigs and dogs. After sub-cutaneous injection, the onset of anti-diuresis was rapid, occurring in a few minutes, and the effect prolonged, lasting up to six hours. Diuresis whether produced by xanthine, mercurials, salts or urea was inhibited by the extract. In 1951, Erspamer and Ottolenghi extended their investigations, and tried to localise the site of the action of their extract. Clearance measurements were made of the renal plasma flow, and glomerular filtration rate in dogs, both conscious and anaesthetised with sodium pentobarbital.

It is somewhat unfortunate that the only figures in this paper show that no reliance can be placed in their clearance determinations. The dose of extract (given sub-cutaneously) was so enormous as to either shut down the urine flow completely, or to reduce it to such a low figure as to render clearance estimations of doubtful value.

Nevertheless, Erspamer and Ottolenghi assert that the profound anti-diuresis observed was "due to a reduction or inhibition of glomerular filtration" and that "increased tubular re-absorption, supposing it exists, could, at the best, be only of secondary importance". In their anaesthetised dogs, administration of the extracts caused a rise of 60-100 mmHg. in the systemic blood pressure.

Extending their clearance observations to rats, Erspamer and Ottolenghi (1952a) showed that sub-cutaneous administration of their extracts reduced both glomerular filtration rate and renal plasma flow. This they suggest, shows that the extract exerts a specific effect upon the vessels of the afferent glomerular bed of the kidney. These experiments were repeated with similar results using a sample of 5-hydroxytryptamine (Erspamer and Ottolenghi, 1952b). The effective dosage was small, as little as 4·1 µg/kg of 5-hydroxytryptamine base producing an anti-diuresis after sub-cutaneous injection. The conclusion was reached that "Enteraimine represents a physiological hormonal regulator of the tonus of the intra-renal vascular system and, as a consequence, of the blood flow through the kidney".

This was the situation when the researches reported upon here were first contemplated, the contention from a relatively isolated European school that 5-hydroxytryptamine was involved in the

control of kidney excretion, and the knowledge that substances which were almost certainly 5-hydroxytryptamine had long been shown to cause kidney vasoconstriction.

Statement of problem

The problem was to investigate further the mode of action and the importance of the antidiuretic activity of 5-hydroxytryptamine.

Experimental

(The abbreviation 5HT will now be used for 5-hydroxytryptamine.)

The first part of the work was to confirm that 5HT was anti-diuretic. Having confirmed that this was so, an attempt was made to investigate how the anti-diuresis was brought about. Experiments were done to see whether the anti-diuresis was accompanied by any changes in renal haemodynamics, and to see whether the changes actually observed were mediated through the renal nerves. Experiments were also done to find out if the anti-diuretic dosages were sufficient to cause general vasoconstriction and contraction of smooth muscle. Each section of the investigation required different techniques which are described at the heading of each section.

A large number of experiments were done on a group of trained dogs. Their histories, together with the description of operative procedures used on them and salient post mortem findings are given in the appendix.

The Effect of Intravenously Administered 5HT on the Course of Water Diuresis and Renal Clearances in the Conscious Dog

Method

These experiments were performed on the group of trained bitches whose histories are described in the appendix. The experiments were done in a room which was soundproofed, but not perfectly so.

On the day of an experiment, food was withheld from the animal, and at 11·30 a.m. it was given 250 mls of tepid water by stomach tube. Its drinking water was then removed. At 2 p.m. the animal was given a further dose of tepid water by stomach tube. The amount given was the same for any one dog, and depended on its size: the range was 200-400 mls. The dogs were then lain on their sides with the minimum of physical restraint, and the bladder catheterised. After the bladder contents had been allowed to drain, the urine was collected into a graduated measuring cylinder by connecting a short piece of glass on to the catheter.

The 5HT for injection was diluted in normal saline just before the injection from a stock solution containing 1 mg/ml of 5-hydroxytryptamine creatinine sulphate in acidified 0·9% saline. The dilution was such as to give a final volume for injection of 1-2 mls. Injections were made into the hind leg vein. In those experiments in which clearance values were to be measured, 6-9 mls of a

35% w/v solution of diodone (the diethanolamine salt of 3:5 di-iodo-4-pyridone-N acetic acid) was injected sub-cutaneously 30 minutes before the second dose of water. 3-4 gms of creatinine were added to this water. The creatinine clearance gave the glomerular filtration rate (Smith, 1951), the diodone clearance the renal plasma flow (Smith, Golding and Chasis, 1938; Corcoran, Page and Smith, 1941). The actual dosage of diodone and creatinine was based upon the animal's size. Samples of blood for analysis were removed from the hind or fore limb vein at the mid point of the relevant urine collection. In investigating the effects of injected 5HT an allowance was made for the dead space of the collecting system. Estimations of diodone were carried out by the method of Alpert (1941), and of creatinine by the alkaline method as described by Hawk,, Oser and Summerson (1947).

All doses of 5HT are referred to as the base, although the injection was of the creatinine sulphate complex.

Results

52 observations have been made upon the effects on urine flow of administering 5HT intravenously during the course of water diuresis, the range of doses employed being from 0·72 µg/kg to 31·0 µg/kg. These results are summarised in Table 1.

5HT, when injected in a sufficient dose causes

anti-diuresis. The duration of the anti-diuresis, and the degree of inhibition of flow rate is increased when the dose is increased (Fig. 1). The effect is not, however, consistent (Fig. 2). Doses below 1 µg/kg do not appear to be anti-diuretic, doses from 1-4 µg/kg are uncertain in their effects. Doses of from 4 to 10 µg/kg, are more certain in their effects, but are not invariably anti-diuretic. In the 26 experiments where doses greater than 10 µg/kg have been injected an anti-diuresis did not result in three experiments. In these three experiments there was an increase in the rate of urine flow, (Fig. 3), twice followed by anti-diuresis (Fig. 2). The anti-diuresis in response to these larger doses was often quite marked, lasting on occasions for longer than one hour (Figs. 4 and 5).

In these experiments, attempts were made to see if the injection of 5HT produced renal vascular changes by measuring renal clearances before and after the injection. Two of these experiments were unsuccessful because of mistakes in the chemical analysis. If there is an anti-diuresis following injection of 5HT it is invariably accompanied by a decrease in both diodone and creatinine clearances. The mean value for this fall was 51·5% for creatinine clearance, and 53·5% for diodone clearance. Figs. 5, 6, 7, 8 and 9 show that there is an abrupt fall in the clearance values immediately following the injection. The clearance values

then rise, sometimes independently of the urine flow, and may come back to the pre-injection figure during the anti-diuresis (Figs. 5 and 9). Sometimes there is partial recovery during the anti-diuresis (Figs. 6 and 7) and on occasions, the clearance figures closely follow the urine flow (Fig. 8). There is some correlation between the dosage of 5HT and the clearance changes (Fig. 9). On a few occasions clearance values following upon the anti-diuresis rise to above the pre-injection level (Figs. 8 and 9).

Clearances were measured in one of the three experiments where a rise in urine flow followed the injection of 5HT, causing a rise in diodone and creatinine clearance.

Table 1

EFFECT OF INTRAVENOUSLY ADMINISTERED 5HT ON URINE FLOW

Dog No.	Date	Dose HT µg/kg	Effect	
96	24:11:52	0·72	None	
96	26:11:52	1·19	Just perceptible	
96	27:11:52	1·38	Just perceptible	
110	24: 4:53	1·45	None	
110	24: 4:53	1·45	None	
102	28:11:52	1·74	Just perceptible	
96	27: 1:53	3·90	Just perceptible	
96	27: 1:53	3·90	Just perceptible	
102	28: 1:53	4·35	Anti-diuresis	
96	28: 1:53	4·75	Just perceptible	
96	27: 1:53	5·95	None	
109	4:12:52	6·2	Anti-diuresis	
112	9: 6:53	6·45	Anti-diuresis	
111	23: 2:53	7·25	Anti-diuresis	
96	26: 1:53	7·9	Anti-diuresis	
96	28: 1:53	7·9	Anti-diuresis	
117	23:10:54	8·1	Anti-diuresis	
102	21: 1:53	8·7	Anti-diuresis	
108	8: 1:53	8·7	Anti-diuresis	
108	5: 1:53	8·7	Anti-diuresis	
108	5: 1:53	8·7	Anti-diuresis	
108	7: 1:53	8·7	Anti-diuresis	c
109	29: 1:53	9·3	None	
119	6:12:54	9·5	Anti-diuresis	
119	8:12:54	9·5	Anti-diuresis	c
119	19:11:54	10	Anti-diuresis	c
119	26:11:54	10	Anti-diuresis	c
96	22:10:54	10	Anti-diuresis	c
117	25:10:54	10	Diuresis	c
96	17:12:54	10·7	Anti-diuresis	c
119	3:12:54	11	Anti-diuresis	c
117	22:11:54	11	Anti-diuresis	c
117	3:11:54	12	Anti-diuresis	c
117	17:11:54	12	Anti-diuresis	c
109	29: 1:53	12·4	Doubtful	
96	8:11:54	13·0	Anti-diuresis	c
108	12: 1:53	13·0	Anti-diuresis	
108	9: 1:53	13·0	Anti-diuresis	
117	10:12:54	14·4	Anti-diuresis	c
111	23: 2:53	14·5	Anti-diuresis	
96	29:10:54	15·2	Anti-diuresis	c
109	14: 1:53	15·5	Anti-diuresis	
109	4:12:53	15·5	None	
102	31:12:52	17·4	Anti-diuresis	c
108	12: 1:53	17·4	Anti-diuresis	
108	22: 1:53	21·7	Anti-diuresis	
108	23: 1:53	21·7	Anti-diuresis	
102	17:12:52	21·8	Anti-diuresis	
102	20:15:52	21·8	Anti-diuresis	
109	13: 1:53	23·6	Anti-diuresis	
109	30:12:52	31·0	Anti-diuresis	c

c denotes clearance measurements made.

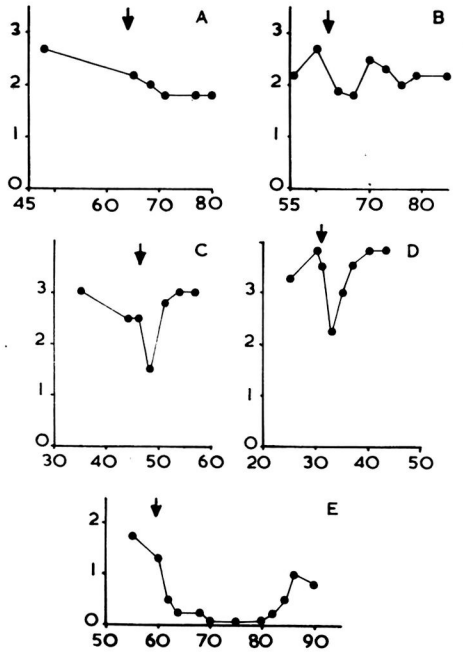

Fig. 1. Frisk (96). Effect of i.v. injection of 5HT on urine flow.

Abcissa:- Time in minutes after administration of water.

Ordinate:- Urine flow in mls/min.

Time of injection indicated by arrow.

A 0·72 µg/kg.

B 1·19 µg/kg.

C 1·38 µg/kg.

D 3·90 µg/kg.

E 7·90 µg/kg.

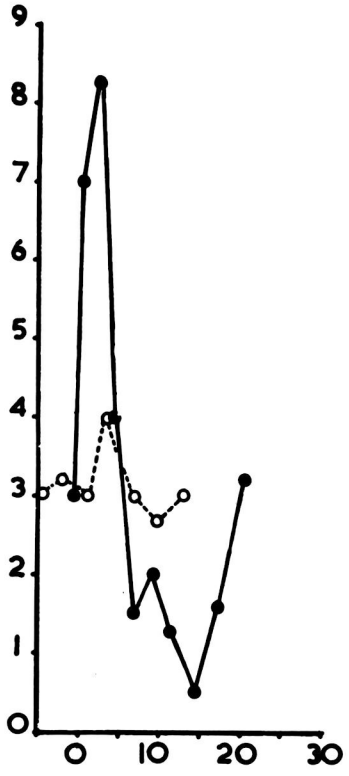

Fig. 2. Angela (109). Effect of same dose of 5HT on urine flow on two different occasions.

●——● 14:1:53

o-----o 4:12:52

15·5 µg/kg i.v. at time = 0.

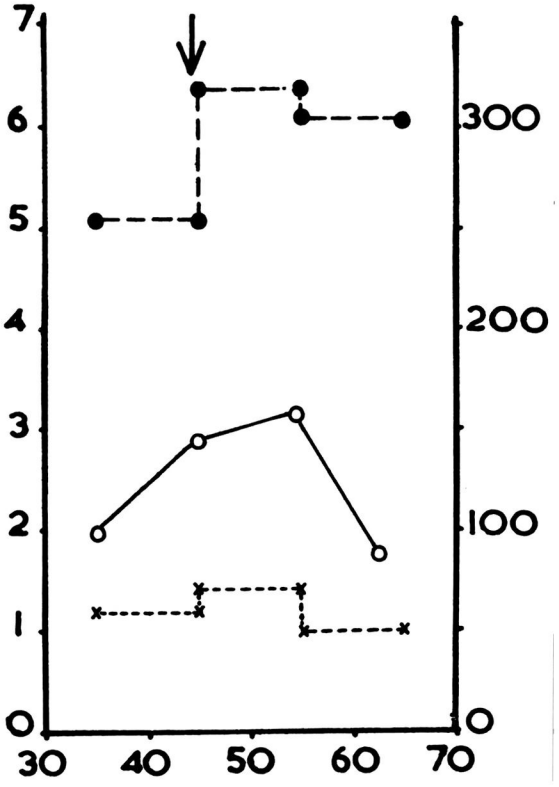

Fig. 3. Bouncer (117). Effect of i.v. 5HT on urine flow and clearances.

Abcissa:-	Time in minutes after administration of water.
Left ordinate:-	Urine flow in mls/min.
Right ordinate:-	Clearances in mls/min.
O———O	Urine flow.
●-----●	Diodone clearance.
x-----x	Creatinine clearance.

At arrow 10 µg/kg 5HT.

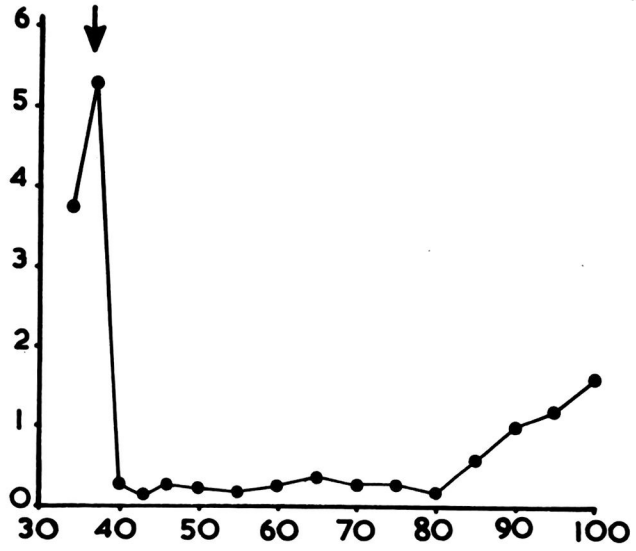

Fig. 4. Angela (109). The effect of 5HT on urine flow.

Abcissa:- Time in minutes from administration of water.

Ordinate:- Urine flow in mls/min.

At arrow 23·6 µg/kg i.v.

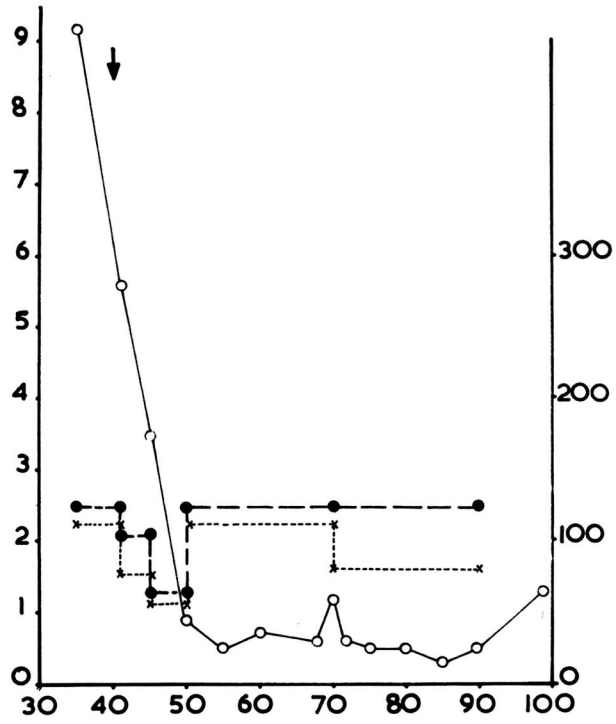

Fig. 5. Angela (109). The effect of 5HT on urine flow and renal clearances.

Abcissa:-	Time in minutes after administration of water.
Left ordinate:-	Urine flow in mls/min.
Right ordinate:-	Clearances in mls/min.
O———O	Urine flow.
●-----●	Diodone clearance.
x-----x	Creatinine clearance.

At arrow 31 µg/kg i.v.

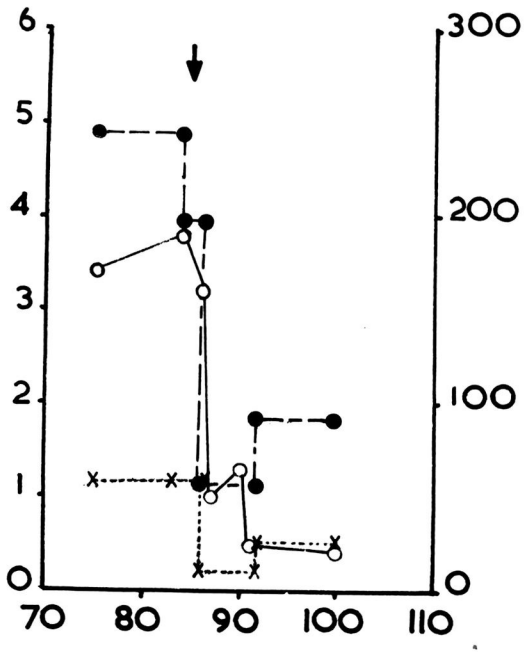

Fig. 6. Kit (119). The effect of 5HT on urine flow and renal clearances.

Abcissa:-	Time in minutes from administration of water.
Left ordinate:-	Urine flow in mls/min.
Right ordinate:-	Clearances in mls/min.
O———O	Urine flow.
●-----●	Diodone clearance.
x-----x	Creatinine clearance.

At arrow 11 ug/kg i.v.

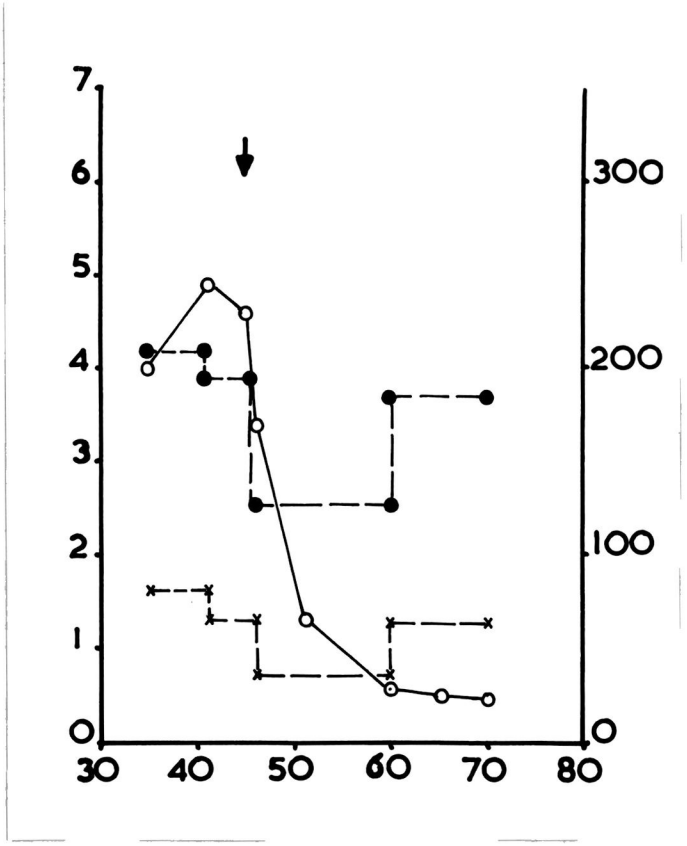

Fig. 7. Kit (119). The effect of 5HT on urine flow and renal clearances.

Abcissa:-	Time in minutes from the administration of water.
Left ordinate:-	Urine flow in mls/min.
Right ordinate:-	Clearances in mls/min.
O———O	Urine flow.
●-----●	Diodone clearance.
x-----x	Creatinine clearance.

At arrow 10 µg/kg i.v.

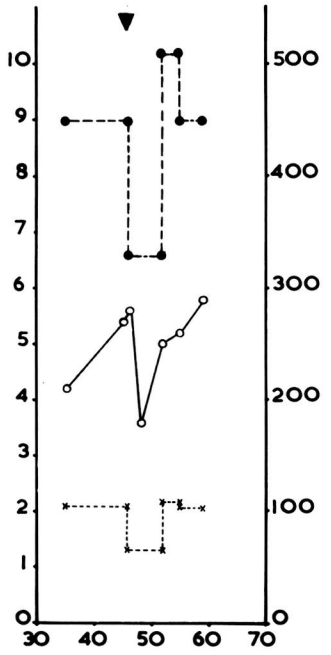

Fig. 8. Bouncer (117). The effect of 5HT on urine flow and renal clearances.

Abcissa:-	Time in minutes after administration of water.
Left ordinate:-	Urine flow in mls/min.
Right ordinate:-	Clearances in mls/min.
O———O	Urine flow.
●-----●	Diodone clearance.
x-----x	Creatinine clearance.

At arrow 11 µg/kg i.v.

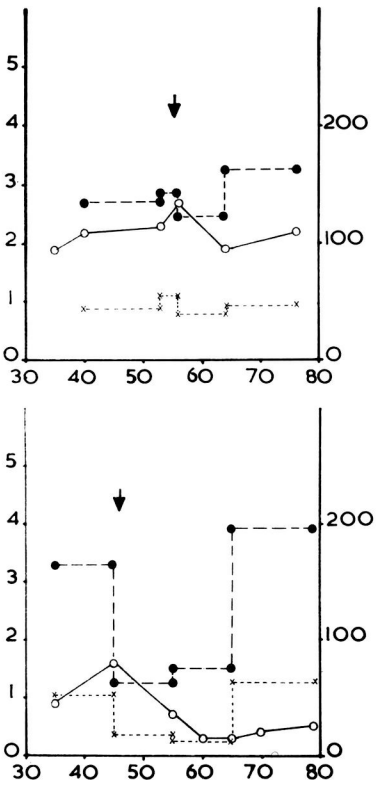

Fig. 9. Frisk (96). The effect of 5HT on urine flow and renal clearances.

Abcissa:-	Time in minutes after administration of water.
Left ordinate:-	Urine flow in mls/min.
Right ordinate:-	Clearances in mls/min.
O———O	Urine flow.
●-----●	Diodone clearance.
x-----x	Creatinine clearance.

Above 10 µg/kg i.v. at arrow.

Below 15·2 µg/kg i.v. at arrow.

The Effect of Intra-Carotid Injections of 5HT on Water Diuresis in the Conscious Dog

It has been shown (Verney, 1947; Pickford and Watt, 1951) that substances which cause an anti-diuresis by releasing the anti-diuretic hormone are effective in smaller doses when injected into the carotid artery, than when injected into a vein. Experiments were thus made to see whether doses of 5HT were any more effective when injected into the carotid artery, than into a vein.

Method

The experiments were carried out on conscious dogs with carotid loops. The routine followed was exactly the same as that used to observe the effects of intravenously administered 5HT, except that the injections were given into the carotid artery. The only precaution taken was to use a new hypodermic needle for every injection.

Results

Four experiments were carried out, the doses of 5HT ranging from 8·1 µg/kg to 15·5 µg/kg (see Table 2). In no case did 5HT injected into the carotid artery produce a greater effect than the same amount injected intravenously (Figs. 10 and 11). It produced a considerably smaller effect in one experiment (Fig. 11), but this may have been due to its variability.

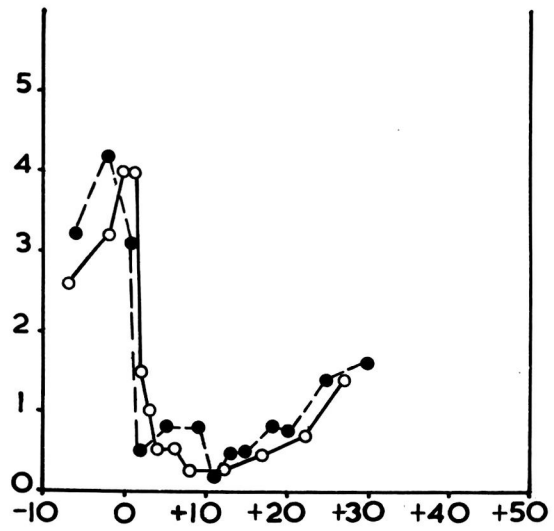

Fig. 10. Smoky (102). A comparison of the effect of intra-carotid and intravenous 5HT.

Abcissa:-	Time in minutes.
Ordinate:-	Urine flow in mls/min.
O———O	9:12:53, 8·7 µg/kg i.c.
●-----●	21:1:53, 8·7 µg/kg i.v.

Both injections at time = 0.

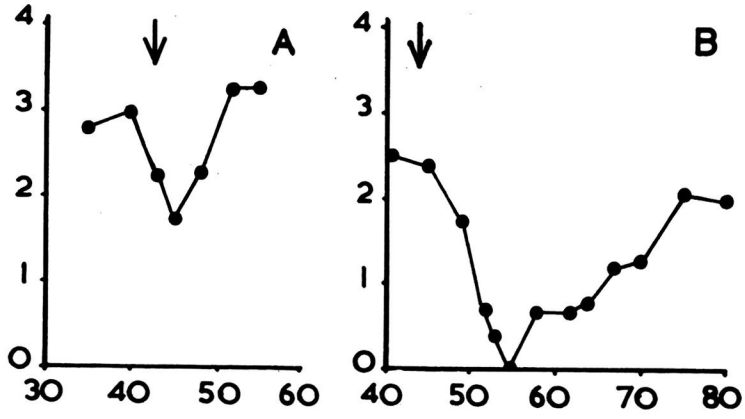

Fig. 11. Judy (108). A comparison between the effect of intra-carotid and intravenous injections of 5HT on urine flow.

Abcissae:- Time in minutes after the administration of water.

Ordinates:- Urine flow in mls/min.

A 8·7 µg/kg 5HT i.c. at arrow.

B 8·7 µg/kg 5HT i.v. at arrow.

The Effect of the Sub-cutaneous Injection of 5HT on Urine Flow in the Conscious Dog

In their experiments upon rats, Erspamer and Ottolenghi (1952b) showed the sub-cutaneous administration of doses of 5HT as low as 4·1 µg/kg to be anti-diuretic. It was decided to see if this sensitivity to sub-cutaneous 5HT was characteristic of the dog also.

Method

The routine for inducing water diuresis was the same as used previously. The sub-cutaneous dose was given either just after the administration of water, or just before the peak of diuresis.

Results

Three experiments, using dosages ranging from 18·6 µg/kg to 74 µg/kg, were performed. The sub-cutaneous injection of 5HT in these doses has no effect upon water diuresis (Fig. 12). Since the supply of 5HT was limited, larger doses could not be spared for further experiments. Results are summarised in Table 3.

Table 2

EFFECT OF INTRA-CAROTID INJECTION OF 5HT ON URINE FLOW

Dog No.	Date	H.T. Dose (µg/kg)	Effect
109	11:12:52	8·1	Anti-diuresis
102	9:12:52	8·1	Anti-diuresis
108	9:1 :53	8·7	Anti-diuresis
109	11:12:52	15·5	Anti-diuresis

Table 3

EFFECT OF SUBCUTANEOUSLY ADMINISTERED 5HT ON URINE FLOW

Dog No.	Date	H.T. Dose (µg/kg)	Effect
109	5:12:52	18·6	No effect
112	9: 6:53	32·0	No effect
112	11: 6:53	74·0	No effect

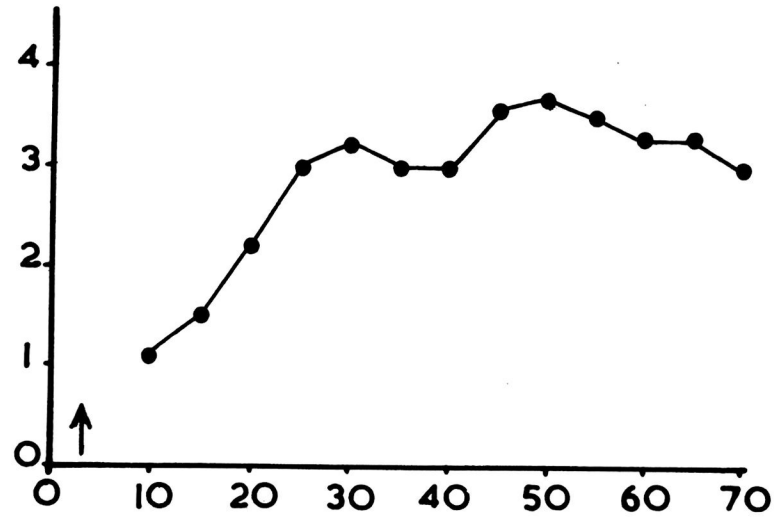

Fig. 12. Darkie (112). The effect of the subcutaneous injection of 5HT on urine flow.

Abcissa:- Time in minutes after the administration of water.

Ordinate:- Urine flow in mls/min.

At arrow 74 µg/kg 5HT subcutaneously.

The Effect of Experimental Diabetes Insipidus upon 5HT Anti-Diuresis

It has been established (Verney, 1926; Fisher, Ingram and Ranson, 1938; Verney, 1946 and 1947) that the water volume output by the kidneys is dependent upon the amount of circulating anti-diuretic hormone and that the release of this hormone is governed by the hypothalamic-neurohypophyseal system. Drugs can bring about an anti-diuresis by causing the release of ADH (De Bodo, 1944). Such anti-diureses are characterised by little or no change in clearance values, and so this mechanism seemed unlikely to be involved in the production of anti-diuresis by 5HT. However, the opportunity occurred of investigating the effect of 5HT on water diuresis of a dog in which the hypothalamic-neurohypophyseal system has been destroyed by a suitable lesion. The animal had a daily water intake of 30-50% of its body weight, and had reached a state of permanent polyuria.

Method

The method was exactly as employed for investigating the effect of intravenous 5HT on normal dogs.

Results

As yet, only two observations have been made, and it is realised that no strong conclusion may be drawn from so few experiments. Further observations are at present being made. The injection of 14·5

µg/kg caused a very brief anti-diuresis (Fig. 13) which was somewhat surprising in view of the size of the dose. The second injection of 29·0 µg/kg was given at the end of diuresis, and produced an anti-diuresis with no recovery to pre-injection level over a prolonged period. It is characteristic that the injection of an anti-diuretic during the falling phase of diuresis is liable to produce an anti-diuresis with no period of recovery. Hence doubts exist as to the validity of the results of the second injection.

It is hoped to clear this point up when further work is done.

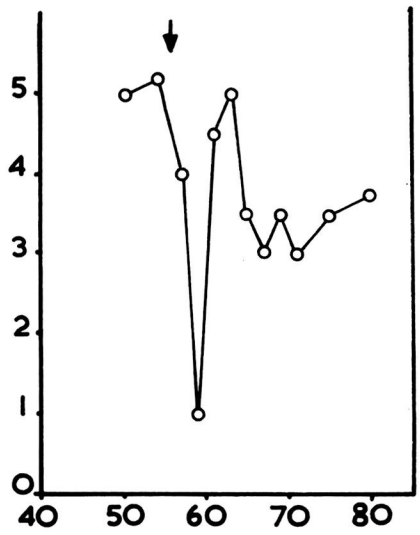

Fig. 13. Lady (110). Effect of the i.v. injection of 5HT on the rate of urine flow of a dog with experimental diabetes insipidus.

Abcissa:- Time in minutes after the administration of water.

Ordinate:- Urine flow in mls/min.

At arrow 14·5 µg/kg 5HT i.v.

The Effect of 5HT on the Excretion of Urine by the Denervated Kidney

Although the kidney receives a rich nerve supply, very little is known about its function, and it seems to be of little or no importance in controlling the minute to minute urine output (Bykow and Alexejew-Berkmann, 1931; Klisiecki, Pickford Rothschild and Verney, 1933; Surtshin, Meuller and White, 1952). However, that the nerves have some effect is shown under anaesthesia, when a denervated kidney excretes at a more rapid rate than a normally innervated one. The denervation diuresis is generally attributed to a purely vascular effect causing an increase in the glomerular filtration rate of the denervated kidney, and not to any effect upon the tubules (Surtshin, Meuller and White, 1952). 5HT might mediate its effect through these nerves, and in order to investigate this, experiments were done on anaesthetised dogs to see whether kidney denervation affected 5HT anti-diuresis.

Method

In outline, the method was to record urine flow from each ureter of an anaesthetised dog, one kidney being denervated. The effect of 5HT on the denervated kidney could then be matched against the normal kidney.

Dogs were anaesthetised with chloralose, and the abdomen opened along the mid-line. The left kidney was freed of its connections and the nerves

surrounding the renal vessels stripped off. Both ureters were then cannulated and the urine from each collected separately. Since the urine flow was very low, even when the animals were hydrated before being anaesthetised, diuresis was produced by the constant intravenous infusion of 0·9% saline.

Diodone and creatinine clearances were measured. Diodone was injected sub-cutaneously and creatinine given by mouth before inducing anaesthesia. If necessary these substances were added to the saline infusion to maintain the blood concentration.

Of the five experiments done, three were carried out as planned. In one experiment the dog (Smoky, 112) turned out to have only one kidney, and observations were made on this after it had been denervated. The fifth experiment was abandoned when it was found impossible to induce a diuresis.

In all experiments blood pressure was recorded from a cannula in a femoral artery.

Results

5HT caused anti-diuresis in the denervated kidney. The effect is transitory (Fig. 14), and the doses required are very high (Fig. 15). This may be related to the initial low rates of flow. 5HT has little effect upon the innervated kidney of the anaesthetised dog, presumably due to the intense vasoconstriction already existing. The

large doses of 5HT necessary to produce anti-diuresis in the denervated kidney occasionally produce a transient diuresis in the innervated kidney (Fig. 15). This is probably due to the vasopressor effect of 5HT causing a slight increase in blood flow, since diodone clearance values increased slightly.

The clearance measurements lacked accuracy because of the low rates of urine flow, but the anti-diureses seen were accompanied by falls in diodone and creatinine clearances.

The Effect of Anti-diuretic Doses of 5HT upon Systemic Blood Pressure

In describing 5HT as a specific kidney regulator, Erspamer (1954 a & b) states that the doses he used were below those which influence systemic blood pressure. Experiments were then done to see whether this applied to the doses which are known to be anti-diuretic when administered intravenously to dogs.

Method

The initial attempts at measuring changes in

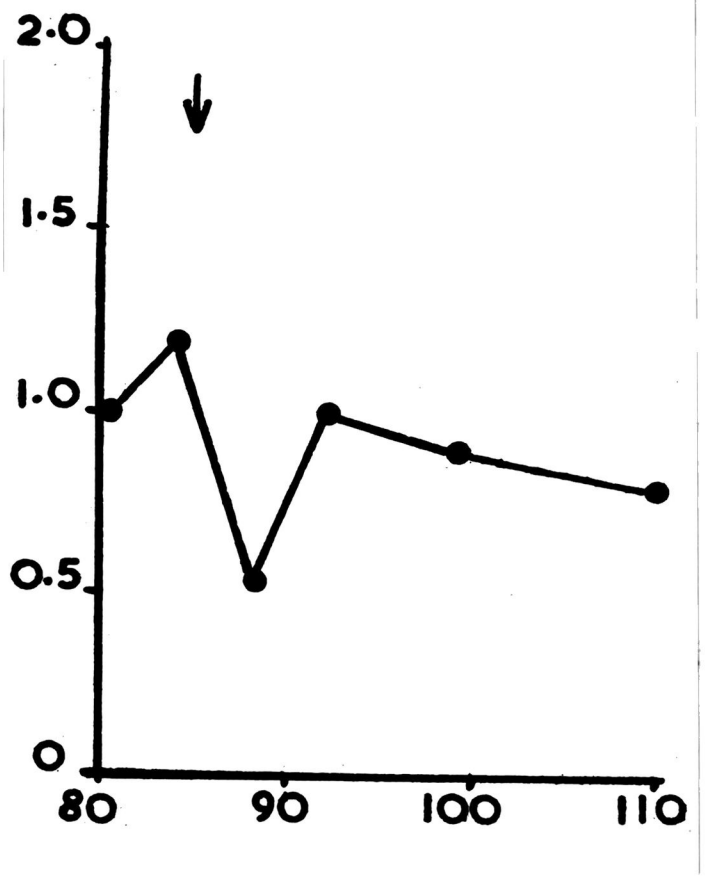

Fig. 14. Smoky (102). The effect of the intravenous administration of 5HT on the urine flow of the denervated kidney of the anaesthetised dog.

This observation was made in the course of the acute experiment. The diuresis resulted from the intravenous infusion of 0·9% saline.

Abcissa:- Time in minutes from beginning of urine collection from the ureter.

Ordinate:- Urine flow in mls/min.

At arrow 17·4 µg/kg 5HT i.v.

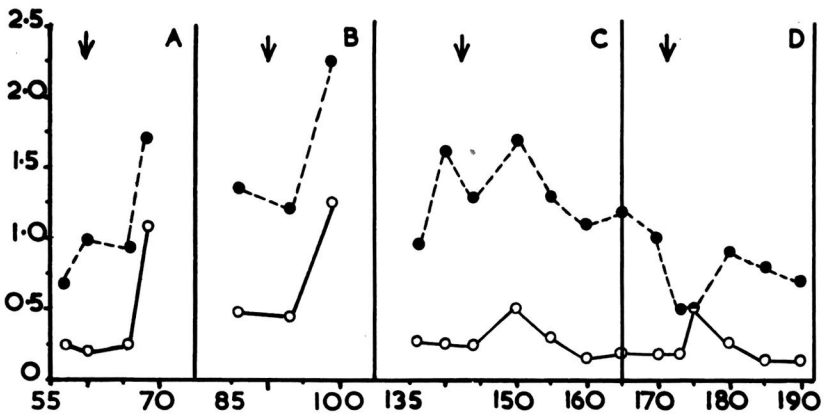

Fig. 15. Dog HT3. The effect of the intravenous injection of 5HT on the urine output of the denervated and innervated kidney of a chloralose anaesthetised dog.

Abcissa:- Time in minutes after commencement of urine flow collection.

Ordinate:- Urine flow in mls/min.

O———O Innervated kidney.

●- - - -● Denervated kidney.

A at arrow 17·4 µg/kg.

B at arrow 31·0 µg/kg.

C at arrow 34·8 µg/kg.

D at arrow 65·5 µg/kg.

Table 4

THE EFFECT OF INTRAVENOUS 5HT UPON THE MEAN BLOOD PRESSURE OF THE PENTOBARBITONE ANAESTHETISED DOG

Part I. PRESSURE RISE PRECEDED BY A BRIEF FALL

Dose 5HT as µg base/kg.	Extent of Fall MM Hg	Extent of Rise MM Hg	Duration of Effect in secs.
2·20	10	10	70
2·55	10	10	30
2·55	40	40	30
3·60	34	80	35
4·40	14	20	80
5·10	10	70	23
7·25	40	80	110
7·25	40	60	340

Part 2. PRESSURE RISE ONLY

2·55	50	20
2·55	130	15
2·55	66	18
3·60	100	40
3·52	170	44
4·80	50	20
5·10	110	20
5·10	120	68
5·10	140	50
5·10	190	50
5·10	180	60
6·60	30	70
7·20	40	37
7·22	70	330
7·25	80	120
9·60	70	260
10·20	120	82
~~10·80~~	~~36~~	~~118~~
~~10·80~~	54	140
10·80	40	118
10·80	60	153
10·85	140	235
11·00	40	75
11·00	44	105
11·00	44	85
11·00	40	80
14·50	40	42
15·40	46	95
16·30	60	115
18·20	126	300
21·60	50	250
24·30	50	84
25·40	70	180
48·25	64	310

blood pressure were made on conscious dogs equipped with carotid loops, using a conventional sphymomanometer and a specially made narrow cuff. The narrow cuff was placed on the caudal end of the loop and the artery palpated at the cephalic end. The method proved too inaccurate. The effect upon blood pressure was then investigated in a series of experiments on 15 anaesthetised dogs. The series included Darkie (112).

The anaesthetics employed were either chloralose or sodium pentobarbitone (Nembutal, Abbott). Blood pressure was recorded from either a femoral or carotid artery using a mercury manometer.

Results

Doses of 5HT as low as 2 µg/kg have an effect on blood pressure of the anaesthetised dog when given intravenously, causing a rise of 80-120 mmHg. The first rise is followed by a period during which the blood pressure is maintained at 10-15 mmHg above the preinjection level (Table 4). Fig. 18 shows the effect on blood pressure of graded doses of 5HT.

The Effect of 5HT on Flow through the Ureter

Since all the records of urine flow in conscious dogs have been made in dogs with the bladder catheterised, then if the ureter contracts in response to the dose of 5HT false results may be obtained. In a high proportion of experiments, the response to the injection of 5HT was an abrupt fall

in the rate of urine flow followed by a slight recovery, and then a sustained anti-diuresis (Fig. 15a). This could be explained by the ureter contracting and damming back some of the urine in the renal pelvis, and then relaxing, and causing an apparent increase in the rate of flow. Experiments were done to investigate this possibility.

Methods

Dogs of both sexes were used. Anaesthesia was induced either by intravenous sodium pentobarbitone (Nembutal, Abbott) or by intravenous chloralose. The abdomen was opened along a mid-line incision and one ureter mobilised. A cannula was then tied into the upper end of the ureter. By passing the thread used to tie in the cannula between the body of the ureter and the artery which runs along its length, interference with the blood supply was kept at a minimum. A cannula was inserted in the lower end of the ureter in a like manner, in all except two experiments. In these two experiments, the lower cannula was put in through the opening of the ureter into the bladder. This procedure allowed of the perfusion of about two inches of ureteral lumen. Normal saline was introduced through the top cannula at constant pressure using a Marriott bottle. The outflow was recorded using an electronic drop recorder arranged to actuate a signal marker writing on a kymograph. Blood pressure was recorded from the carotid artery using a

mercury manometer. A femoral vein was cannulated for the injection of drugs.

Results

Ten experiments were performed. Perfusion by this method produces a rhythmical flow through the ureter. Below pressures of about 20 cms of water there is no flow. Pressure above this produces an intermittent flow. The higher the perfusion pressure, the larger each period of flow, and the faster the flow during each period (Fig. 16). The intravenous injection of 5HT in doses which bring about anti-diuresis in the unanaesthetised animal cause an interruption of flow through the ureter. There is a latency of a few seconds after the injection of 5HT, and as the blood pressure of the animal begins to rise, so flow through the ureter stops (Fig. 17). The duration of the stoppage is proportioned to the dose (Fig. 18). After a dose of 10 µg/kg, the stoppage lasted 30-60 seconds (Fig. 19). It could be overcome by raising the perfusion pressure above 55 cms of water. The only times when 5HT did not have an effect on flow through the ureter, the blood supply had been impaired during the preparation of the perfusion.

Summary of Results

5HT is anti-diuretic when administered intravenously in relatively small doses. The initial effect, at least, appears to be due to an effect upon the renal vasculature which decreases renal

plasma flow and glomerular filtration rate. The effect cannot be regarded as specific, since the doses which are required to bring about an anti-diuresis are capable of causing a considerable rise in systemic blood pressure and causing contraction of the smooth muscle of the ureter.

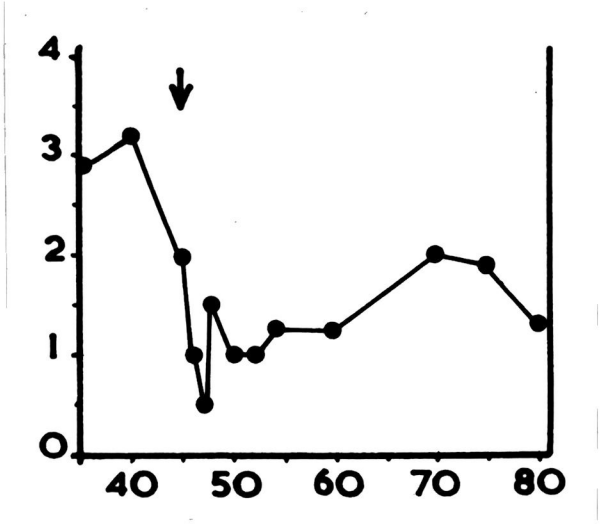

Fig. 15a. Smoky (102). The effect of 5HT on urine flow.

Abcissa:- Time in minutes after the administration of water.

Ordinate:- Urine flow in mls/min.

At arrow 4·35 µg/kg i.v.

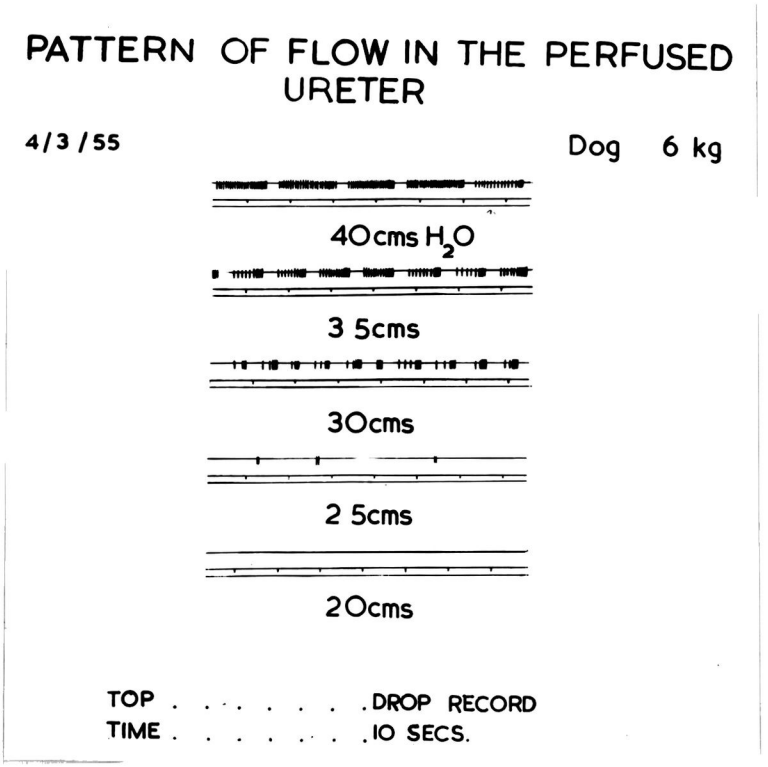

Fig. 16. The flow through the perfused ureter of an anesthetised dog showing the effect of varying the perfusion pressure.

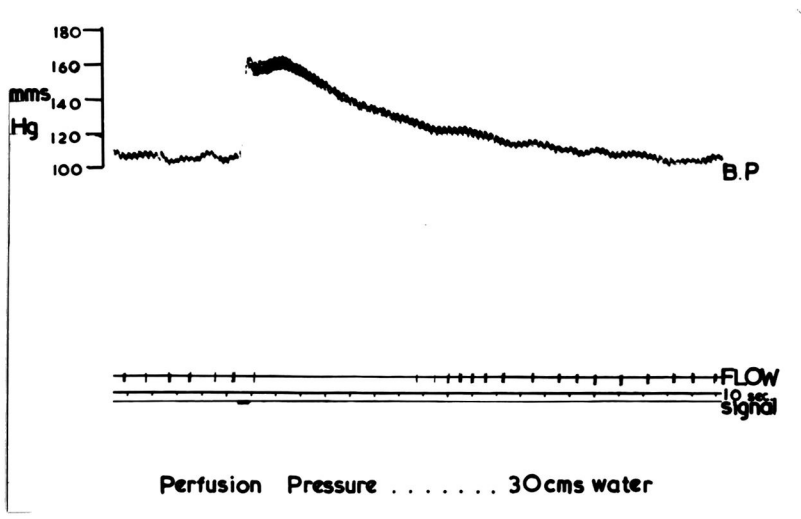

Fig. 17. The effect of an intravenous injection of 5HT on the blood pressure and the flow through a perfused ureter of an anaesthetised dog.

Top Record:- Blood pressure recorded from the left carotid artery.

Injection of 5HT at signal.

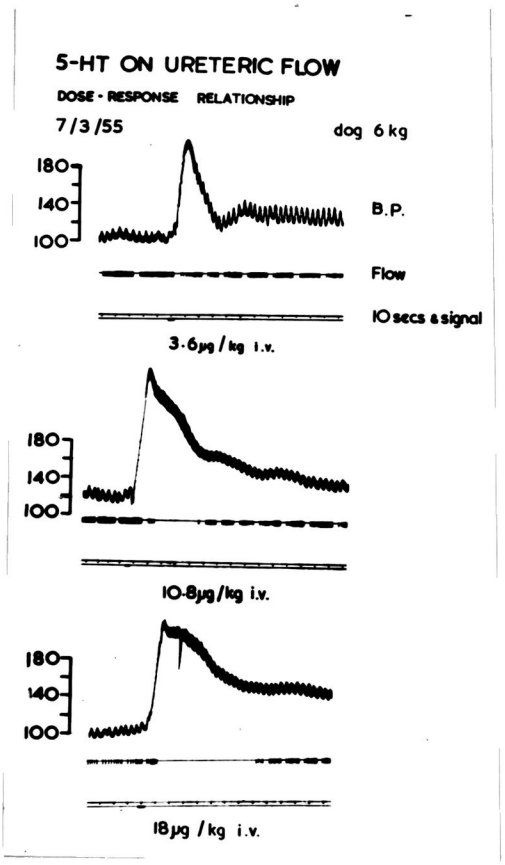

Fig. 18. The effect of various doses of 5HT on the blood pressure and the flow through a perfused ureter in the chloralose anaesthetised dog.

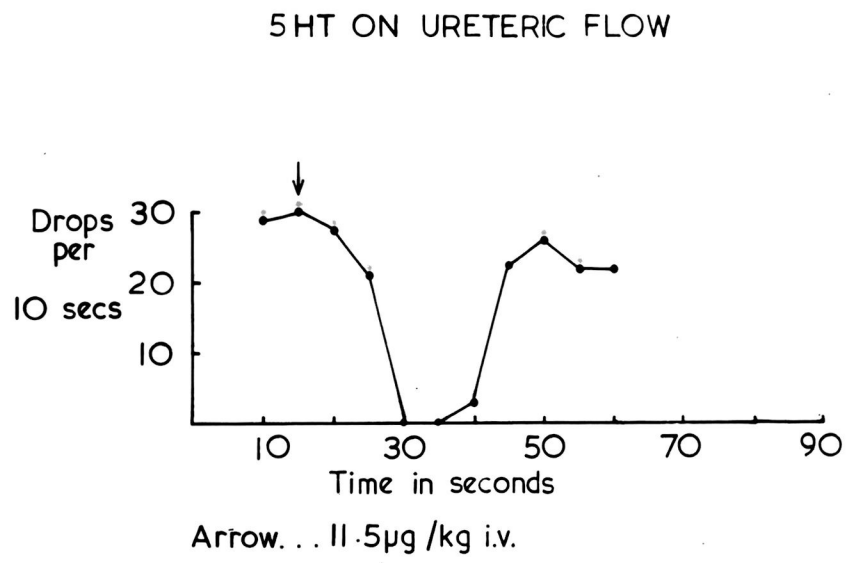

Fig. 19. Graph of the effect of 5HT on the flow through the perfused ureter of an anaesthetised dog.

40 drops = 1 ml.

Discussion

Techniques

The techniques employed are not, for the most part original, but not all their limitations may be generally appreciated. The use of the conscious dog is essential for investigating anti-diuretics, as anaesthetics themselves influence the secretion of urine by the kidney, furthermore, the repeated use of the same animal enables the response to be compared from day to day. There are, however, disadvantages in the use of a conscious animal, since they are sensitive to changes in their environment which the experimenter may not perceive. This is particularly important when studying diuresis, because of the wide range of stimuli which can cause the release of the anti-diuretic hormone.

O'Connor and Verney (1945) showed that one could not invariably produce an anti-diuresis by applying an unpleasant stimulus. This was due to the effect of adrenaline which can, it seems, block the release of anti-diuretic hormone. If the kidneys are denervated, the splanchnics cut, and the anterior part of the sympathetic chain removed, then the application of the unpleasant stimulus invariably produces an anti-diuresis through the neurohypophyseal mechanism. The effects of adrenaline can seriously modify emotional responses. It can cause polyuria or anti-diuresis (Pickford, 1952). Eränkö and Karvonen (1952) believe that adrenaline

can provoke the release of the anti-diuretic hormone. External stimuli can thus be responsible for considerable interference in the rate of urine flow.

This can only be overcome by providing a constant undisturbed environment, both physical and emotional. The former is relatively easy to provide, but not the latter. In order to reduce the possibility of external stimuli affecting the course of water diuresis, experiments were done in a small windowless room, which was partially sound-proofed. The experimenter had to move to prepare for the injection. This preparation itself may cause emotional stimulus (Abrahams and Pickford, 1954). The routine was standardised as far as possible to reduce the likelihood of unknown factors influencing the diuresis, but on occasions there were delayed diureses and unexpected anti-diureses.

One factor which has not been stressed in this type of work is that the material injected may be subjectively unpleasant and thus produce an emotional anti-diuresis. Whether or not this might have been a property of 5HT will be discussed later, but it is a factor which must be considered.

Renal clearances as a measure of kidney blood flow and glomerular filtration rate

The accurate measurement of blood flow through an organ of a conscious animal is a difficult procedure. For the kidney, two methods are commonly

used, one, the thermostromuhr, necessitates the implantation of a heating coil and thermocouple at operation. It has a number of disadvantages. The method relies on calibration after death, and such calibrations are unreliable. The presence of the apparatus round the renal artery can lead to partial occlusion. The measurement of flow through a vessel under such conditions is liable not to give a true picture, particularly when the substance which is under investigation has a direct action on the renal artery itself.

The second method, the measurement of renal clearances does not involve any surgical interference at all. As a development of the Fick principle, it should be capable of a high degree of accuracy. In practice this is not so, but since the method has such widespread uses, the errors involved are well known. Furthermore, since the method has been used in this laboratory, there was a fund of knowledge and experience to draw upon.

In theory, the technique of clearances is an ideal method for obtaining data on the dynamics of the kidneys of intact animals. Providing the urine flow and kidney blood flow are relatively constant, and the blood level of clearance substances maintained, then renal clearances will, in practice, give useful information. As a technique for measuring rapid kidney blood flow fluctuations, particularly when they are accompanied by an

alteration in urine flow, the technique has severe limitations. If the substance used for measuring renal plasma flow (RPF) is diodone, then it is almost entirely extracted from the plasma in one passage through the kidney. Should the anti-diuretic employed reduce blood flow by one half, and urine flow by 80% (a common occurrence), then, if the plasma is still cleared in one passage the concentration of urinary diodone must rise $2\frac{1}{2}$ times. If the blood flow is maintained during the anti-diuresis, as it might be, then the urinary concentration may be raised ten times.

The sudden rise in urinary diodone can lead to high concentrations of diodone in the tubule and may interfere with tubular transport. Any small error in the urinary volume estimations will lead to a very high error in estimated RPF. If the urine flow resumes its pre-injection value, then it has to wash out a very strong solution from the ureters, renal pelvis, and even from the bladder. This can lead to an apparent overshoot in the RPF value.

Homer Smith (1951) has described a further shortcoming of using clearances for measuring RPF and GFR changes concommittant with urine flow changes. Abrupt falls in the rate of urine flow lead to abrupt false drops in clearance values, and an abrupt rise leads to a false rise.

The theoretical values got for diodone and

creatinine clearances do not represent the RPF and GFR. Certain errors inherent in the method, such as the absorption by the erythrocytes of a proportion of the diodone, mean that the figures got by simple analysis require further correction. In most experiments, the correction factors used are arbitrary, and may themselves be inaccurate. Here, in preference, the diodone and creatinine clearance values have been given, and they are regarded as a guide to RPF and GFR, and not as absolute figures. Since the changes which were expected were large, no attempt was made to include all the precautions necessary when changes of a few per cent are looked for (Sellwood and Verney, 1955).

As has been mentioned earlier, clearances are most reliable when the blood concentration of the substance whose clearance is being studied is maintained at a steady level. Normally this is done by constant intravenous infusion of the material at a rate approximating to the excretion rate. There are disadvantages in this method when clearances are used for estimating rapidly changing renal blood flows together with the course of diuresis. It will be very difficult to ensure that the rate of infusion matches the excretion rate, and the infusion itself may influence the course of diuresis.

The method chosen to overcome this difficulty was to inject the diodone subcutaneously some thirty minutes before the water was given, and to give the creatinine with the water.

This method ensures the minimal interference with the course of diuresis, but unfortunately adequate and relatively constant concentrations are then only maintained at the peak of diuresis. Since it was at this period when clearance measurements were made, this method proved highly satisfactory. On occasions after a prolonged antidiuresis the blood levels of creatinine and diodone were still high enough to ensure accurate measurements.

One unforeseen error is that the dog may resent repeated venipuncture and its urine flow may be inhibited as a result, thus introducing unsuspected error.

The use of anaesthesia

In two types of experiments, those in which the effect of renal denervation was assessed, and those upon the effect of the renal nerves, general anaesthesia was unavoidable. This is not necessary for the measurement of blood pressure, but as has been stated, conventional methods were unsatisfactory. However, a method for continuous recording of blood pressure changes has been evolved, and the failure to do this has only been due to the misfortunes which so far have accompanied the fashioning of femoral loops. Although the injection of 5HT into anaesthetised animals is followed by marked blood pressure

rises, it is not certain that these are so marked in the unanaesthetised animal, or that they occur at all. If anything, anaesthesia seems to increase the animals' susceptibility to 5HT. Intravenous 5HT is lethal to conscious rats when given in doses greater than 50 mg/kg (Freyburger, Graham, Rapport, Seay, Govier, Swoap and Vander Brook, 1952), but if the animals are anaesthetised, then the lethal dose is as low as 0·1 mg of the creatinine sulphate complex (Correll, Lyth, Long and Vanderpoel, 1952).

Any extrapolations which are made must be somewhat guarded.

The mode of action of 5HT anti-diuresis

The rapid fall in the rate of urine flow which occurs after the intravenous injection of 5HT has been shown to be accompanied by a large decrease in diodone and creatinine clearances. The maintained anti-diuresis is more variable, and may be accompanied with recovery in flow rates. It seems, therefore, likely that, initially, the anti-diuresis is purely due to the vascular changes. These changes must involve the afferent renal vessels since the fall in creatinine clearance matches the

fall in diodone clearances, but it does not mean that the efferent vascular bed does not constrict as well.

It is possible that 5HT decreases the number of glomeruli through which there is an active circulation, presumably by a vascular constriction. All that this would mean is that some glomerular vessels are more sensitive to 5HT than others. Any more precise localisation of the effect is not possible, since so little is known about the factors controlling renal blood flow.

The kidney receives a rich sympathetic innervation from the spinal segments T_4 to L_4 by way of the splanchnics and the coeliac ganglia. These nerves have been thought to have a vasomotor role since Claude Bernard (1859) showed that cutting them increased the rate of urine production. Although we now know that increased urine production does not necessarily imply increased renal blood flow, Bernard was correct in his findings. However, as has been mentioned, no difference can be found in the behaviour of a kidney with the nerves severed, providing that the animal is not anaesthetised (Bykow and Alexejew-Berkmann, 1930; Klisiecki, Pickford, Rothschild and Verney, 1933).

Yet, experimenters have always been impressed by the ability of the kidney to keep its blood flow at a steady rate in the face of systemic blood pressure changes (Unna, 1935; Hartmann, Ørsov and Rein,

1936; Opitz and Smyth, 1937; Sringorum and Centenara, 1937; Sringorum, 1938) and surprisingly, this ability is maintained even after severing or anaesthetising the nerves to the kidney (Opitz and Smyth, 1937; Smith, Rovenstine, Goldring, Chasis and Ranges, 1939). Extreme vasomotor changes, and moderately large doses of adrenaline and nor-adrenaline can nevertheless influence kidney blood flow (Richards and Plant, 1922; Springorum, 1938; Pickford and Watt, 1951) and Unna's remark "Es kann die Nierendurchblutung unabhängig von arteriellen Druck reguliert werden" should not be taken literally. If the vasomotor change is large the kidney blood flow will be altered.

The only conclusion as to the mode of 5HT action is that it must act directly upon the smooth muscle of the renal vessels. There is no evidence that it acts through the nerves. If 5HT anti-diuresis is a specific effect of 5HT upon the renal vasculature, then we should not expect the doses of 5HT which are needed to produce an anti-diuresis to exert an effect upon any other part of the vascular system. This is not so. The injection of 5HT is invariably accompanied by a few deep laboured breaths. This might well be due to bronchoconstriction which is associated with pulmonary vasoconstriction (Gaddum, Hebb, Swan and Silver, 1953). The doses of 5HT which they used (on isolated perfused cat lungs) were 10-20 µg. Our experience with anaes-

thetised animals indicates that anti-diuretic doses exert a considerable effect on blood pressure, and certainly the work on the ureter indicates the doses used to be effective on smooth muscle.

The initial action of 5HT upon the blood vessels of the kidney which produces the sharp fall in the rate of urine flow cannot be regarded as a specific action of 5HT, but part of a more general smooth muscle stimulating effect. It is more difficult to explain the prolonged anti-diuresis caused by a single large intravenous injection of 5HT. Amine oxidase has been shown to destroy 5HT very rapidly (Blaschko, 1952), and at circulation rates of 200 mls/min through a cat's lung, half of the 5HT is destroyed in 4 minutes (Gaddum et al, 1953). It would seem unlikely that a circulating level of 5HT sufficient to maintain intense vasoconstriction could be maintained for more than a few minutes. In acute experiments, doses of 5HT of the order of 5-10 µg/kg have, after the preliminary large increase in mean blood pressure, maintained the pressure at about 10 mmHg above the pre-injection level for 8 minutes. This effect, which will be discussed later, does suggest though that 5HT can have a more prolonged effect than is commonly thought (Erspamer, 1954 a & b, Page, 1954).

However, the prolonged anti-diuresis is not always accompanied by a decrease in kidney blood flow. It may be so, but it is also often accompanied

by a return of the clearance values to pre-injection, or slightly above pre-injection levels. It is possible that the prolonged anti-diuresis may be an indirect effect of the injection of 5HT. Verney and Vogt (1943) showed that occlusion of the renal artery for a period of 2 minutes could lead to the suppression of urine formation for as long as one hour. If the initial vasoconstriction produced by 5HT is extreme, it might bring about this ischaemic anti-diuresis. The effect of this manoeuvre on renal blood flow is unknown. The prolonged anti-diuresis with renal flow and filtration rates returning to normal levels suggests the possibility of the release of anti-diuretic hormone. Emotional anti-diuresis in response to unpleasant stimuli have been mentioned earlier. It behoves us to pay attention to the account by Page (1954) of the sensations got by intravenous injection of 5HT into human subjects "The symptoms provoked are variable in the extreme, ranging from none at all to feelings of dissolution, fullness in the chest, tingling and pricking all over, itchy nostrils, difficulty in breathing, desire to empty bladder and bowels, pain in stomach and bladder, weakness, nausea, desire to sneeze, generalised numbness and/or ache, burning in throat, mouth and hands".

From this account, it is impossible to exclude the possibility of the neurohypophyseal release of anti-diuretic hormone. The experiments on the

effect of intra-carotid injections rule out the possibility of 5HT acting specifically to release anti-diuretic hormone. It should be regarded as a side effect. Erspamer and Coreale (1955) preclude this from having any effect upon the anti-diuresis seen in the rat after sub-cutaneous 5HT since, they say, 5HT anti-diuresis is characterised by a decrease in chloride excretion, and the injection of anti-diuretic hormone produces an increase in chloride excretion. This does not necessarily apply to the effect got after intravenous injection in the dog.

It seems of considerable importance that in anaesthetised animals, the effects of 5HT are transient, the long component never having been seen.

Barac (1953) has hypophysectomised a dog under chloralose anaesthesia and shown 5HT still to produce an anti-diuresis. Technically this is a poor experiment, as the operation does not necessarily destroy the hypothalamico-neurohypophyseal system and sources of ADH may still be available, but it emphasises the vascular nature of the effect. The limited experiments upon an animal with diabetes insipidus do show that 5HT can produce an anti-diuresis in such an animal, but a considerable volume of work should be undertaken on animals with diabetes insipidus to elucidate further the possibility that 5HT can, on intravenous injection into the conscious dog, liberate anti-diuretic hormone. As

yet, there is no evidence that the prolonged anti-diuresis produced by 5HT is a specific action of this substance. It may be significant that when other vasoactive substances have had prolonged kidney effects, these have been shown to be due to the release of anti-diuretic hormone (Dearborn and Lasagna, 1952).

Other workers investigating the anti-diuretic effect of 5HT on dogs, have had different results from those reported here. Sala and Castegnaro (1953) have published the results of clearance experiments on dogs given large amounts of 5HT sub-cutaneously and by intravenous infusion. In the three experiments for which they give any data 5HT was given sub-cutaneously in doses ranging from 0•1 to 3 mg/kg. This produced a mild anti-diuresis, but the effect upon clearance values is highly variable, the most constant effect being an increase in the RPF, sometimes to double its resting level. Unable to explain the anti-diuresis on the basis of alterations in clearance values they suggest that it is due to a specific effect of 5HT producing tubular reabsorption. Since the anti-diuretic hormone acts by causing tubular reabsorption of water and since the possibility of its release was not excluded in their experiments, their contention cannot be accepted without further experimental proof.

Corcoran, Masson, Greco and Page (1954) have

investigated 5HT anti-diuresis on rats and dogs. They infused low doses of 5HT intravenously into conscious dogs and could find no effect on urine flow or clearance values at rates of 2-3 µg/kg/min. Infusion at 6 µg/kg/min caused vasodilatation in their dogs but no oliguria. Larger doses (11 and 14 µg/kg/min) caused a fall in both creatinine and PAH clearances in a dog anaesthetised with pentobarbitone sodium. They did not proceed with higher doses "because of the obvious discomfort they elicited". Since our single injection technique meant that the whole dose was given rapidly (2 or 3 seconds) a very high local concentration was built up. To obtain such a high local concentration with constant infusion techniques, the dose per minute would have to be built up to very high levels. This is probably the reason for the inability of Corcoran et al to get an anti-diuresis in their experiments, their dosage has been too low. In their experiments on rats, Corcoran et al find that the effective anti-diuretic dosage of 5HT is high, 4·2 mg/kg, and at this dosage there are vasomotor changes, the rats flushing at the extremities. It is noteworthy that although Erspamer and Ottolenghi (1952b) claim the effective anti-diuretic dose of 5HT on rats to be as low as 4 µg/kg, yet in experiments where the anti-diuresis has been studied (Erspamer, 1953), the dose range used is much higher (200-1000 µg/kg)

Erspamer has described the effect of 5HT upon blood pressure in the dog as moderate (1954a). This is not confirmed by the papers which he cites. Douglas and Toh (1953) say that "larger doses as a rule give pressor effects which were often considerable". As a large dose they gave 200 µg to a 12·7 kg dog. Heymans and Heuvel-Heymans (1953) got a pressure rise of 120 mmHg after the injection of 100 µg 5HT into a whole animal. Schneider and Yonkman (1954) found that doses of 50-100 µg/kg produced rises of up to 50 mmHg. In our experience doses of 5HT as low as 2·25 µg/kg given intravenously have produced blood pressure rises as high as 130 mmHg. After the initial blood pressure rise (which in lower doses usually follows a fall of very brief duration) there is often a small sustained rise of blood pressure of a minute or two's duration. To illustrate this, Table 4 shows the duration and highest blood pressure resulting from 42 intravenous injections of 5HT into pentobarbitone anaesthetised dogs. Erspamer (1954a) has said that the release of the entire platelet content of 5HT (7·3 µg/kg) into a dog "would cause nothing but an insignificant and brief pressure change". The effect would be brief, but not insignificant.

One of Erspamer's arguments in favour of 5HT having a specific effect upon the kidney, is that the early difficulties in perfusing isolated kidneys with defibrinated blood were due to such small

amounts of 5HT, that they would not be active on the systemic circulation (1954 a & b). The amount of 5HT which, he says, can be obtained from blood varies from 0·04-0·28 µg/ml. On anaesthetised dogs we find that amounts of 5HT which give final blood concentrations of about 0·025 µg/ml are sufficiently vasoconstrictor to raise the blood pressure by more than 100 mmHg. Doses of 5HT into conscious human beings, which are unlikely to give final blood 5HT concentrations above 0·03 µg/kg have been shown to be vasopressor (Spiers and Stone, 1952).

These experiments lead, then to the conclusions that 5HT in sufficiently high dosage is anti-diuretic. The initial steep fall in urine flow appears to be due to two components, the contraction of the ureter and a non-specific vascular effect causing vasoconstriction. The mechanism of the prolonged anti-diuresis seen with the largest doses of 5HT is not known, but may be due to the release of endogenous anti-diuretic hormone, or result from the renal ischaemia produced by intense vasoconstriction. In the dog the evidence does not point to the role of 5HT as a specific hormone regulating kidney function.

ADDENDUM

Since this account was written an experiment has been done which adds further to the belief that 5HT anti-diuresis is a non-specific effect. A

dose of 15 µg/kg of 5HT has been introduced into the aorta of a conscious dog above the renal arteries, using a cardiac catheter. This dose caused only a fleeting anti-diuresis, associated with a fall of 6 mmHg in mean arterial pressure.

APPENDIX

Dogs used in Experiments on Conscious Animals

Dog No. 96 (Frisk)

Brown and white short haired terrier bitch. 8·2 kg.

8:10:49 Perineotomy.

23:8:51 20 µg DFP injected into each supra-optic nucleus.

24:12:54 Killed by i.v. Numbutal. Cyst in neurohypophysis, but plenty of functioning tissue left.

Dog No. 102 (Smoky)

Grey-black long haired terrier-type mongrel bitch. 7·9 kg.

25:1:51 Perineotomy.

8:2:51 Left-sided carotid loop with sinus denervation.

19:6:51 At operation one uterine horn opened, and balloon inserted with polythene tube leading out per vaginum.

21:6:51 Polythene removed by animal. Balloon removed from uterus via abdominal incision.

6:11:51 Balloon placed in uterus at operation.

9:11:51 Balloon removed at operation.

30:1:53 Used for acute experiment. P.M. showed the presence of only one slightly fibrosed kidney.

Dog. No. 108 (Judy)

Black and white fox terrier bitch. 10·1 kg.

15:8:52 Carotid loop with sinus denervation. Perineotomy.

6:12:52 At operation, balloon inserted in left uterine horn with polythene tube leading out per vaginum.

12:12:52 At operation, balloon transferred to other uterine horn.

19:12:52 Operation to remove balloon.

5:2:53 Animal unwell. Uterine infection. Killed by i.v. nembutal. Post mortem showed pyelosalpinx. Other tissues normal.

Dog No. 109 (Angela)

Black and tan terrier-type mongrel bitch. 11·8 kg.)

22:10:52 Carotid loop with sinus denervation. Perineotomy.

24:2:53 Bilateral ovariectomy. Balloon in one uterine horn with polythene tube leading to exterior per vaginum.

27:2:53 Right uterine horn exteriorised.

15:4:53 Uterine infection successfully treated with penicillin.

15:6:53 Left uterine horn exteriorised.

10:7:54 Died of peritonitis resulting from intussusception while we were absent.

Dog No. 110 (Lady)

Brown collie bitch. 14 kg.

12:1:53 Carotid loop with denervated sinus. Perineotomy.

15:4:53 Bilateral ovariectomy. Left uterine horn exteriorised.

19:4:53 Mild infection controlled with penicillin.

28:5:53 Diabetes insipidus brought about by section of supraoptico-hypophyseal tracts.

10:7:54 Killed. All normal except hypothalamo-neurohypophyseal system. Few supra-cells all loaded with Gomori positive materal. Atrophic neurohypophysis.

Dog No. 111 (Topsy)

Small fox terrier bitch. 7 kg.

13:1:52 Carotid loop with sinus denervation. Perineotomy.

19:1:54 Bilateral ovariectomy. Left uterine horn exteriorised.

1:2:54 Attempt at section of supra-optico-hypophyseal tracts.

3:3:54 Attempt at section of supra-optico-hypophyseal tracts.

13:4:54 Dog becoming obese.

30:4:54 Killed by i.v. nembutal. Wt. 8•3 kg. All normal except hypothalamic-neurohypophyseal system. Reduction in number of supra-optic cells. 3rd ventricle swollen, particularly at its posterior

end. Neurohypophysis shrunken and cellular.

Dog No. 112 (Darkie)

Black terrier type mongrel bitch. 13·5 kg.

11:2:53 Carotid loop with sinus denervation.

19:2:53 No pulsation in carotid loop.

22:6:53 Left uterine horn exteriorised.

5:2:54 Bilateral ovariectomy.

17:2:54 Attempt to cut supra-optico hypophyseal tracts.

12:3:54 Killed by i.v. nembutal. All normal except hypothalamic-neurohypophyseal system. Lesion has damaged optic chiasma. 3rd ventricle greatly enlarged. Supra-optic cells reduced on right side. Para-ventricular cells reduced. Neurohypophysis shrunken and cellular with large numbers of colloid inclusions.

Dog No. 117 (Bouncer)

White bull terrier bitch. 17 kg.

1:6:54 Removed left ovary and exteriorised left uterine horn. Perineotomy.

19:1:55 Attempt at cutting supra-optico hypophyseal tracts.

20:1:55 Dog appears to be permanently drowsy.

24:1:55 Used for acute experiment. All normal at P.M. except hypothalamic-hypophyseal system. One extensive lesion extending into anterior hypothalamus on right side,

and one at level of supra-optic nucleus on the left side. Large amount of colloid (degenerating cells?) in supra-optic and paraventricular areas. Both pairs of nuclei reduced in size.

Dog No. 119 (Kit)

Young Alsatian bitch. 15 kg.

12:11:54 Perineotomy.

24:1:55 Perineotomy incision extended.

2:2:55 Left carotid loop with sinus denervation. Right femoral loop.

5:2:55 Dog died of haemorrhage after biting femoral artery.

Operative Procedures

Perineotomy

The urethra in the bitch empties into the vagina. If an animal is to be subjected to repeated catheterisation, then it is convenient to slit the perineum from the vaginal orifice for a sufficient length so as to render the urethra accessible. Animals were anaesthetised usually with pentobarbitone sodium (Nembutal, Abbott), 0·05 gm/kg intravenously. Latterly, anaesthesia has been induced with thiopentone sodium (Pentothal, Abbott) and maintained by ether. The perineum was shaved, and the animals placed on their backs with the hind legs drawn up towards the head. The vaginal orifice was held open and the perineum cut along the mid-line. The incision should be just long enough to enable the urethral meatus to be seen. If the incision is too long, the uterus is liable to prolapse. The cut edge of the vaginal wall was then stitched to the cut edge of the perineal skin. The wound was then dressed with hot vaseline.

Formation of carotid loop

To allow of injections directly into the carotid artery a length of the vessel was exteriorised and enclosed in a loop of skin. The method followed was similar to that of Van Leersum (1911) except that the nerve from the carotid sinus was cut before fashioning the loop.

Section of the supra-optico-hypopseal tracts

This was done by the diasphenoid route by the method of Pickford (1939). The only modification was that the tracheal cannula was dispensed with. In outline, the method is to remove the bone at the base of the brain and expose the optic chiasma and the anterior margin of the pituitary. A stab-wound is placed so as to interrupt the supra-optico-hypophyseal tracts.

Uterine operations

The dogs used in the 5HT investigation were also being used for experiments upon uterine activity. As a result a number of dogs were subjected to operations to create uterine fistulae, and to ovariectomy. As far as is known, none of these procedures are likely to interfere with kidney function.

BIBLIOGRAPHY

ABRAHAMS, V. C. & PICKFORD, MARY (1954). SIMULTANEOUS OBSERVATIONS ON THE RATE OF URINE FLOW AND SPONTANEOUS UTERINE MOVEMENTS IN THE DOG AND THEIR RELATIONSHIP TO POSTERIOR LOBE ACTIVITY. J. Physiol. 126, 329-346.

ALPERT, L. K. (1941). A RAPID METHOD FOR THE DETERMINATION OF DIODRAST-IODINE IN BLOOD AND URINE. Johns Hopk. Hosp. Bull. 68, 522-537.

BARAC, G. (1953). RECHERCHES SUR LA BRÛLURE. SUR L'EFFET ANTIDIURÉTIQUE DE LA 5-HYDROXYTRYPTAMINE CHEZ LE CHIEN. Arch. int. Physiol. 61, 403-406.

BAYLISS, L. E. & OGDEN, E. (1933). 'VASO-TONINS' AND THE PUMP OXYGENATOR KIDNEY PREPARATION. J. Physiol. 77, 34P-35P.

BERNARD, C. (1859). LEÇONS SUR LES PROPRIÉTÉS PHYSIOLOGIQUES ET LES ALTERATIONS PATHOLOGIQUES DES LIQUIDES DE L'ORGANISME. 1st Ed. Vol. 2, 146-172, Paris, Baillière et Fils.

BING, R. J. (1941). THE EFFECT OF VASOCONSTRICTOR SUBSTANCES IN SHED BLOOD ON PERFUSED ORGANS. Amer. J. Physiol. 133, 21-28.

BLASCHKO, H. (1952). ENZYMIC OXIDATION OF 5-HYDROXYTRYPTAMINE IN MAMMALIAN AND CEPHALOPOD TISSUE. Biochem. J. 52, X.

BODO, R. C. DE (1944). THE ANTIDIURETIC ACTION OF MORPHINE AND ITS MECHANISM. J. Pharmacol. 82, 74-85.

BRODIE, T. G. (1900). THE IMMEDIATE EFFECT OF AN INTRAVENOUS INJECTION OF BLOOD SERUM. J. Physiol. 26, 48-71.

BYKOW, K. M. & ALEXEJEW-BERKMANN, I. A. (1930). DIE AUSBILDUNG BEDINGTER REFLEXE AUF HARNAUSSCHEIDUNG. Pflüg. Arch. ges. Physiol. 224, 710-721.

CORCORAN, A. C., SMITH, H. W. & PAGE, I. H. (1941). THE REMOVAL OF DIODRAST FROM BLOOD BY THE DOG'S EXPLANTED KIDNEY. Amer. J. Physiol. 134, 333-337.

CORCORAN, A. C., MASSON, G. M. C., GRECO, F. DEL. & PAGE, I. H. (1954). 5-HYDROXYTRYPTAMINE (SEROTONIN): ITS LACK OF SPECIFIC RENAL ACTION. Arch. int. Pharmacodyn. 97, 483-491.

CORRELL, J. T., LYTH, L. F., LONG, S. & VANDERPOEL, J. C. (1952). SOME PHYSIOLOGICAL RESPONSES TO 5-HYDROXYTRYPTAMINE CREATININE SULPHATE. Amer. J. Physiol. 169, 537-544.

DEARBORN, E. H. & LASAGNA, L. (1952). THE ANTIDIURETIC ACTION OF EPINEPHRINE AND NOR-EPINEPHRINE. J. Pharmacol. 106, 122-128.

DOUGLAS, W. W. & TOH, C. C. (1953). THE RESPIRATORY STIMULANT ACTION OF 5-HYDROXYTRYPTAMINE (SEROTONIN) IN THE DOG. J. Physiol. 120, 311-318.

EICHHOLTZ, F. & VERNEY, E. B. (1924). ON SOME CONDITIONS AFFECTING THE PERFUSION OF ISOLATED MAMMALIAN ORGANS. J. Physiol. 59, 340-344.

ERÄNKÖ, O. & KARVONEN, M. J. (1952). ADRENALINE ANTIDIURESIS IN THE DOG. Nature, Lond. 170, 331.

ERSPAMER, V. & PEROSA, L. (1948). PRESENZA DI UN PRINCIPIO ANTIDIURETICO NELLE GHIANDOLE SALIVARI POSTERIORI DEGLI OCTOPODI. Experientia 4, 486-487.

ERSPAMER, V. & OTTOLENGHI, A. (1950). ANTIDIURETIC ACTION OF ENTERAMINE. Experientia 6, 428.

ERSPAMER, V. & OTTOLENGHI, A. (1951). PRELIMINARY RESEARCHES ON THE MECHANISM OF THE ANTIDIURETIC ACTION OF ENTERAMINE. Experientia 7, 191-193.

ERSPAMER, V. & ASERO, B. (1952). IDENTIFICATION OF ENTERAMINE, SPECIFIC HORMONE OF ENTEROCHROMAFFIN CELL SYSTEM, AS 5-HYDROXYTRYPTAMINE. Nature, Lond. 169, 800.

ERSPAMER, V. & OTTOLENGHI, A. (1952a). ANTIDIURETIC ACTION OF SMALL DOSES OF ENTERAMINE EXTRACTS IN THE RAT. EXTRACTS OF POSTERIOR SALIVARY GLANDS OF OCTOPUS VULGARIS. Experientia 8, 31-33.

ERSPAMER, V. & OTTOLENGHI, A. (1952b). ANTIDIURETIC ACTION OF PURE SYNTHETIC ENTERAMINE IN HYDRATED RATS. Experientia 8, 232-233.

ERSPAMER, V. & OTTOLENGHI, A. (1953). PHARMACOLOGICAL STUDIES OF ENTERAMINE. 8. ACTION OF ENTERAMINE ON THE DIURESIS AND THE RENAL CIRCULATION OF THE RAT. Arch. int. Pharmacodyn. 93, 177-201.

ERSPAMER, V. (1953). PHARMACOLOGICAL STUDIES ON ENTERAMINE (5-HYDROXYTRYPTAMINE). 9. INFLUENCE OF SYMPATHOMIMETIC AND SYMPATHOLYTIC DRUGS ON THE PHYSIOLOGICAL AND PHARMACOLOGICAL ACTIONS OF ENTERAMINE. Arch. int. Pharmacodyn. 93, 293-316.

ERSPAMER, V. (1954a). PHARMACOLOGY OF INDOLE-ALKYLAMINES. Pharmacol. Rev. 6, 425-487.

ERSPAMER, V. (1954b). IL SISTEMA CELLULARE ENTEROCROMAFFINE E L'ENTERAMINA (5-IDROSSITRIPTAMINA). Rendiconti Scientifica Farmitalia 1, 1-193.

ERSPAMER, V. & COREALE, P. (1955). FURTHER OBSERVATIONS ON THE ACTION OF 5-HYDROXYTRYPTAMINE (5-HT) ON THE URINE FLOW AND CHLORIDE EXCRETION IN THE RAT. Arch. int. Pharmacodyn. 101, 99-112.

FISHER, C., INGRAM, W. R. & RANSON, S. W. (1938). DIABETES INSIPIDUS AND THE NEURO-HORMONAL CONTROL OF WATER BALANCE. A CONTRIBUTION TO THE STRUCTURE AND FUNCTION OF THE HYPOTHALAMICO-HYPOPHYSEAL SYSTEM. Ann Arbor, Michigan: Edward Bros. Inc.

FREYBURGER, W. A., GRAHAM, B. E., RAPPORT, M. M., SEAY, P. H., GOVIER, W. M., SWOAP, O. F. & VAN DER BROOK, M. J. (1952). THE PHARMACOLOGY OF 5-HYDROXYTRYPTAMINE (SEROTONIN). J. Pharmacol. 105, 80-86.

GADDUM, J. H. (1936). GEFÄSSERWEITERNDE STOFFE DER GEWEBE. Leipzig: G. Theime.

GADDUM, J. H., HEBB, C. O., SILVER, ANN, & SWAN, A. A. B. (1953). 5-HYDROXYTRYPTAMINE. PHARMACOLOGICAL ACTION AND ITS DESTRUCTION IN PERFUSED LUNGS. Quart. J. exp. Physiol. 38, 255-262.

HAMLIN, K. E. & FISCHER, K. E. (1951). THE SYNTHESIS OF 5-HYDROXYTRYPTAMINE. J. Amer. chem. Soc. 73, 5007-5008.

HARTMANN, H., ØRSKOV, S. L. & REIN, H. (1936). DIE GEFÄSSREAKTIONEN DER NIERE IM VERLAUFE ALLGEMEINER KREISLAUFREGULATIONSVORGÄNGE. Pflüg. Arch. ges. Physiol. 238, 239-250.

HAWK, P. B., OSER, B. L. & SUMMERSON, W. H. (1947). PRACTICAL PHYSIOLOGICAL CHEMISTRY. 12th ed. London: Churchill.

HEMINGWAY, A. (1931). SOME OBSERVATIONS ON THE PERFUSION OF THE ISOLATED KIDNEY BY A PUMP. J. Physiol. 71, 201-213.

HEYMANS, C. & HEUVEL-HEYMANS, G. VAN DEN. (1953). SUR LA PHARMACOLOGIE DE L'HYDROXYTRYPTAMINE (SÉROTONINE) ET D'UNE SUBSTANCE ANALOGUE. Arch. int. Pharmacodyn. 93, 95-104.

JACOBY, C. (1890). APPARAT ZUR DURCHBLUTUNG ISOLIERTER ÜBERLEBENDER ORGANE. Arch. exp. Path. Pharmak. 26, 388-400.

KLISIECKI, A., PICKFORD, MARY, ROTHSCHILD, P. & VERNEY, E. B. (1933). THE ABSORPTION AND EXCRETION OF WATER BY THE MAMMAL. Part 1. Proc. Roy. Soc. B. 112, 496-521.

O'CONNOR, W. J. & VERNEY, E. B. (1945). THE EFFECT OF INCREASED ACTIVITY OF THE SYMPATHETIC SYSTEM IN THE INHIBITION OF WATER DIURESIS BY EMOTIONAL STRESS. Quart. J. exp. Physiol. 33, 77-90.

OPITZ, E. & SMYTH, D. H. (1937). NIEREN DURCHBLUTUNG BEI REIZUNG DES CAROTIS-SINUS. Pflüg. Arch. ges. Physiol. 238, 633-637.

PAGE, I. H. (1952). THE VASCULAR ACTION OF NATURAL SEROTONIN, 5 AND 7-HYDROXYTRYPTAMINE AND TRYPTAMINE. J. Pharmacol. 105, 58-73.

PAGE, I. H. (1954). SEROTONIN (5-HYDROXYTRYPTAMINE). Physiol. Rev. 34, 563-588.

PICKFORD, MARY (1939). THE INHIBITORY EFFECT OF ACETYLCHOLINE ON WATER DIURESIS IN THE DOG, AND ITS PITUITARY TRANSMISSION. J. Physiol. 95, 226-238.

PICKFORD, MARY & WATT, J. A. (1951). A COMPARISON OF THE EFFECTS OF INTRAVENOUS AND INTRACAROTID INJECTIONS OF ACETYLCHOLINE IN THE DOG. J. Physiol. 114, 333-335.

PICKFORD, MARY (1952). ANTIDIURETIC SUBSTANCES. Pharmacol. Rev. 4, 254-283.

PFAFF, F, & VEJNX-TYRODE, M. (1903). UEBER DURCHBLUTUNG ISOLIERTEN NIEREN UND DEN EINFLUSS DEFIBRINIRTEN BLUTES AUF DIE SECRETION DER NIEREN. Arch. exp. Path. Pharmak. 49, 324-341.

RAND, M. & REID, G. (1951). SOURCE OF SEROTONIN IN SERUM. Nature, Lond. 168, 385.

RAPPORT, M. M., GREEN, A. A. & PAGE, I. H. (1948a). PARTIAL PURIFICATION OF THE VASOCONSTRICTOR IN BEEF SERUM. J. Biol. Chem. 174, 735-741.

RAPPORT, M. M. et al. (1948b). ENZYMATIC INACTIATION OF SERUM VASOCONSTRICTOR. Proc. Soc. exp. Biol., N.Y. 68, 582-584.

RAPPORT, M. M. et al. (1948c). SERUM VASOCONSTRICTOR (SEROTONIN). IV. ISOLATION AND CHARACTERISATION. J. biol. Chem. 176, 1243-1251.

RAPPORT, M. M. (1949). SERUM VASOCONSTRICTOR (SEROTONIN). V. PRESENCE OF CREATININE IN THE COMPLEX. A PROPOSED STRUCTURE OF THE VASOCONSTRICTOR PRINCIPLE. J. biol. Chem. 180, 961-969.

REID, G. & BICK, M. (1942). PHARMACOLOGICALLY ACTIVE SUBSTANCES IN SERUM. Aust. J. exp. Biol. med. Sci. 20, 33-46.

REID, G. & RAND, M. (1951). PHYSIOLOGICAL ACTIONS OF THE PARTIALLY PURIFIED SERUM VASOCONSTRICTOR (SEROTONIN). Aust. J. exp. Biol. med. Sci. 29, 403-415.

RICHARDS, A. N. & PLANT, O. H. (1922). THE ACTION OF MINUTE DOSES OF ADRENALINE AND PITUITRIN ON THE KIDNEY. Amer. J. Physiol. 59, 191-202.

SALA, G. & CASTEGNARO, E. (1953). INFLUENCE OF ENTERAMINE (5-HYDROXYTRYPTAMINE) ON RENAL FUNCTION OF THE DOG. Proc. Soc. exp. Biol., N.Y. 82, 621-623.

SCHNEIDER, J. A. & YONKMAN, F. F. (1954). SPECIES DIFFERENCE IN THE RESPIRATORY AND CARDIOVASCULAR RESPONSE TO SEROTONIN (5-HYDROXYTRYPTAMINE). J. Pharmacol. 111, (84-98).

SELLWOOD, R. V. & VERNEY, E. B. (1955). THE EFFECT OF WATER AND OF ISOTONIC SALINE ADMINISTRATION ON THE RENAL PLASMA AND GLOMERULAR FILTRATE FLOWS IN THE DOG, WITH INCIDENTAL OBSERVATIONS OF THE EFFECTS ON THESE FLOWS OF COMPRESSION OF THE CAROTID AND RENAL ARTERIES. Philos. Trans. B. 238, 361-396.

SMITH, H. W., GOLDRING, W. & CHASIS, H. (1938). THE MEASUREMENT OF THE TUBULAR EXCRETORY MASS, EFFECTIVE BLOOD FLOW AND FILTRATION RATE IN THE NORMAL HUMAN KIDNEY. J. clin. Invest. 17, 263-278.

SMITH, H. W., ROVENSTINE, E. A., GOLDRING, W., CHASIS, H. & RANGES, H. A. (1939). THE EFFECTS OF SPINAL ANAESTHESIA ON THE CIRCULATION IN NORMAL UNOPERATED MAN, WITH REFERENCE TO THE AUTONOMY OF THE ARTERIOLES, AND ESPECIALLY THOSE OF THE RENAL CIRCULATION. J. clin. Invest. 18, 319-341.

SMITH, H. W. (1951). THE KIDNEY. STRUCTURE AND FUNCTION IN HEALTH AND DISEASE. New York: Oxford University Press. 2nd Ed.

SPIERS, T. D. & STONE, R. E. (1952). EFFECT OF SEROTONIN ON BLOOD PRESSURE AND LACK OF EFFECT OF ANTI METABOLITE. J. Amer. med. Ass. 150, 1599-1600.

SPRINGORUM, P. W., CENTENARO, D. (1937). DIE VERSCHIEDENE BEITEILIGUNG BEIDER NIEREN AN DIURESE-ÄNDERUNGEN UND VASOMOTORISHEN REAKTIONEN. Pflüg. Arch. ges. Physiol. 239, 440-450.

SPRINGORUM, P. W. (1938). ÜBER DIE UNABHÄNGIGKEIT HORMONALER UND ZENTRALNERVÖSER DIURESEHEMMUNG VON DER NIERENGESAMTDURCHBLUTUNG UND DEM ARTERIELLEN DRUCK. Pflüg. Arch. ges. Physiol. 240, 342-347.

STEWART, H. A. & HARVEY, S. C. (1912). THE VASO-DILATOR AND VASOCONSTRICTOR PROPERTIES OF BLOOD SERUM AND PLASMA. J. exp. Med. 16, 103-125.

SURTSHIN, A., MUELLER, C. BARBER & WHITE, H. C. (1952). EFFECT OF ACUTE CHANGES IN GLOMERULAR FILTRATION RATE ON WATER AND ELECTROLYTE EXCRETION: MECHANISM OF DENERVATION DIURESIS. Amer. J. Physiol. 169, 159-173.

UNNA, K. (1935). ARTERIELLER DRUCK UND NIEREN-DURCHBLUTUNG. Pflüg. Arch. ges. Physiol. 235, 515-519.

VAN LEERSUM, E. C. (1911). EINE METHODE ZUR ERLEICHTERUNG DER BLUTDRUCKMESSUNG BEI TIEREN. Pflüg. Arch. ges. Physiol. 142, 377-395.

VERNEY, E. B. (1926). THE SECRETION OF PITUITRIN IN MAMMALS, AS SHOWN BY PERFUSION OF THE ISOLATED KIDNEY OF THE DOG. Proc. Roy. Soc. B. 99, (487-517).

VERNEY, E. B. & VOGT, M. (1943). OBSERVATION ON THE EFFECTS OF RENAL ISCHAEMIA UPON ARTERIAL

PRESSURE AND URINE FLOW IN THE DOG. Quart. J. exp. Physiol. 32, 35-65.

VERNEY, E. B. (1946). ABSORPTION AND EXCRETION OF WATER. THE ANTIDIURETIC HORMONE. Lancet. 2, 739-744 and 781-783.

VERNEY, E. B. (1947). THE ANTIDIURETIC HORMONE AND THE FACTORS WHICH DETERMINE ITS RELEASE. Proc. Roy. Soc. B. 135, 25-106.

ZUCKER, MARJORIE B. (1944). A STUDY OF THE SUBSTANCES IN BLOOD SERUM AND PLATELETS WHICH STIMULATE SMOOTH MUSCLE. Amer. J. Physiol. 142, 12-26.

PART II

Morphine Anti-diuresis and the Hypothalamus

Frey was the first to report the anti-diuretic action of morphine. In 1907 he published the results of experiments on the effects of narcosis on diuresis. Amongst the narcotics investigated was morphine. He showed that it was anti-diuretic, the effect not being due to any absorptive delay. Surprisingly morphine would not inhibit a saline diuresis. This led Frey correctly to surmise a difference in the mechanism of saline and water diuresis. The anti-diuretic effect of morphine was confirmed by Schwartz and Wiechowski (1914). They had invented a metal cannula for the collection of urine from conscious dogs. In the short note describing their apparatus they mention that 5-10 mg/kg of morphine injected sub-cutaneously would diminish the rate of urine secretion of their dogs. Stehle and Bourne (1928) also observed this anti-diuresis in conscious dogs, confirming Frey's observation that it was not due to any absorptive delay. Measurements of urea clearances suggested the anti-diuresis to be due to increased tubular re-absorption. Fee (1928) conducted an experiment on himself showing that sub-cutaneous morphine inhibited water diuresis. Since the morphine caused gastro-intestinal disturbances, the effect

may have been partially due to delayed absorption. In 1929 Fee published work showing that the inability of decerebrate animals to show a water diuresis was in fact due to the morphine which was given as preoperative medication. If morphine was omitted, the decerebrate animals secreted urine normally.

Bonsmann (1930) again confirmed morphine's anti-diuretic effect on conscious dogs using doses of 0·5-2 mg sub-cutaneously. Dsikowski (1936) showed that the effect could not be attributed to water being retained in the stomach.

Bodo and Sweet (1938) attempted an analysis of morphine's anti-diuretic action. Having shown morphine to cause the release of adrenaline from the adrenal medulla (Bodo, Cotui and Benaglia, 1937), they proceeded to eliminate the possibility of this adrenaline causing an anti-diuresis. They reported that total hypophysectomy, destroying as they thought, the hypothalamic-neurohypophyseal mechanism controlling water diuresis, did not affect the morphine anti-diuresis. The obvious implication was that morphine did not exert its effect through any hypothalamic-neurohypophyseal mechanism.

However, they were obliged to modify their conclusions (De Bodo and Sweet, 1940) as a result of observations by Gersh (1939) that structurally, the infundibulum was related to the neurohypophysis

and could probably maintain function in the absence of the neurohypophysis in total hypophysectomy. Hence their 1938 experiments could not be considered as excluding the possibility of morphine acting through the neurohypophyseal mechanism. In their 1940 paper De Bodo and Sweet reported the effects of morphine on dogs where the hypophyseal stalk and the supra-optic tracts were severed. These animals had a permanent diabetes insipidus as a result of the operation, and could produce little or no anti-diuretic hormone. Administering the same dose of morphine as in their totally hypophysectomised animals, De Bodo and Sweet could not demonstrate any anti-diuresis. Surprisingly, after this excellent demonstration of the necessity of the hypothalamic-neurohypophyseal system for producing morphine anti-diuresis De Bodo and Sweet state that "the neurohypophysis is not essential for the anti-diuretic action of morphine".

De Bodo published in 1944 a long paper on the anti-diuretic action of morphine. The previous observations that total hypophysectomy did not diminish morphine's action were confirmed as were the observations that morphine would not produce anti-diuresis in dogs with diabetes insipidus. The adrenaline-producing action of morphine was again shown not to be responsible for the anti-diuresis. Fresh work included a study of the

intestinal absorption time for water which confirmed the observations of Klisiecki, Pickford, Rothschild and Verney (1933) that water was absorbed within forty minutes of administration. Using this information De Bodo was able to give the morphine at such a time as to exclude it having any effect upon water absorption. Other fresh work included showing that morphine did not potentiate the action of the anti-diuretic hormone. Its lack of activity in the animals with diabetes insipidus precluded it having any effect upon the urinary tract. De Bodo was able to confirm Frey's (1907) observation that morphine does not inhibit a saline diuresis, this being a characteristic of the anti-diuretic hormone.

These experiments led De Bodo to the conclusion that "the anti-diuretic hormone of the neurohypophysis is necessary for the anti-diuretic effect of morphine". He suggested that since Pickford (1939) had shown a cholinergic mechanism to be involved in the release of anti-diuretic hormone, morphine's anti-cholinesterase properties might be responsible for its anti-diuretic activity. Handley and Kellar (1950) measured PAH and creatinine clearances on both normal and diabetes insipidus dogs, and showed in both cases that the intravenous injection of 2 mg/kg of morphine caused anti-diuresis, there being a decrease in both PAH and creatinine clearance at the same time. Since a smaller dose

produced anti-diuresis without a fall in clearance values they concluded that the release of anti-diuretic hormone by morphine might be supplemented by a second action, a decrease in the number of active glomeruli.

Duke, Pickford and Watt (1951) investigated De Bodo's (1944) suggestion that morphine anti-diuresis might be due to its anti-cholinesterase action. They used dogs which had diabetes insipidus produced by the injection of the anti-cholinesterase, DFP into the supra-optic nuclei. These animals do not show anti-diuresis as a result of acetyl choline injections as do normal dogs (Duke, Pickford and Watt, 1950), presumably through the inhibition of the cholinergic pathway.

Nevertheless minute intravenous doses of morphine produce an anti-diuresis in these animals. Morphine seems not to be anti-diuretic by virtue of its anti-cholinesterase properties. These workers also showed that morphine is anti-diuretic in far smaller doses than hitherto suspected (0·08 mg/kg). Anti-diuresis produced by these small doses does not produce the great fall in clearances noted by Handley and Keller (1950). Minute doses of morphine injected directly into the supra-optic nuclei of chloralosed dogs was profoundly anti-diuretic. Duke, Pickford and Watt conclude, like De Bodo, that morphine exerts its anti-diuretic action via the neurohypophysis.

Morphine would thus seem to be a substance, which in small doses causes the release of the anti-diuretic hormone. This raises a number of interesting possibilities, as morphine might be a useful tool in investigating the exact mode of release of the anti-diuretic hormone. The system which performs this function, the hypothalamic-neurohypophyseal system, is most unusual, being functionally part of the brain. The recognition that the neurohypophysis produced a hormone was due to its close association with the obviously glandular anterior pituitary. Oliver and Schäfer (1894), encouraged by finding a substance of great pressor activity in the supra-renals proceeded to test watery extracts of a number of other organs including the thyroid, spleen and pituitary. They communicated their results to the Physiological Society in 1895, noting that pituitary extracts had pressor activity. This pressor activity was shown by Howell (1898) to be due to extracts of the neurohypophysis, and not of the pituitary as a whole.

This pressor activity which led to the discovery of the endocrine function of the neurohypophysis has not been shown to have any physiological role. Of the many other activities which extracts of the neurohypophysis have, three appear of some importance. These are the property, in very small quantities, of causing contraction of the uterus

(first described by Dale in 1906), the property of the same fraction in causing "let-down" during suckling (Schäfer, 1915), and the property of reducing the output of urine by the kidney (van der Velden, 1913). It has proved possible to isolate two polypeptides (one oxytocic/let down and one anti-diuretic) and even to synthesise them (Pierce and du Vigneaud, 1950; Turner, Pierce and du Vigneaud; du Vigneaud, Ressler and Trippett, 1953; du Vigneaud, Ressler, Swan, Roberts and Katsoyannis, 1954).

The physiological role of the anti-diuretic hormone has been most fully worked out, largely as a result of the work of Verney and his co-workers over the last thirty years. With Starling (1925) he showed that a kidney isolated from the body, and perfused by means of a heart-lung preparation, secreted a urine which was very dilute. The addition of neurohypophyseal extract to the perfusion checked the rate of secretion. Verney showed in 1926 that another way of checking the profuse secretion of the isolated kidney was to include the head of an animal into the circuit. With Klisiecki, Pickford and Rothschild (1933) he was able to relate the pattern of diuresis to the absorption of water from the gut. At the same time they showed the renal nerves not to be involved in mediating anti-diuresis. The anti-diuretic hormones of the neurohypophysis is regarded as the mechanism for controlling the output of water.

However, there is still considerable doubt as to where these hormones are formed. The neurohypophysis itself consists of tissue most unlike that of any other gland. Early workers (Müller, 1871; Schwalbe, 1881; Toldt, 1884) considered the adult organ to consist of connective tissue. Later workers (Berkley, 1894; Retzius, 1894; Herring, 1908) came to the conclusion that the organ consisted largely of ependymal and glial cells interspersed with nerve fibres and the occasional nerve cell (Shanklin, 1943). All these investigators commented on the unusual appearance of the glial cells. Bucy (1930) studying them in the bovine neurohypophysis was sufficiently impressed by their individuality to give them the name of "pituicytes". These cells were later called "parenchymatous cells" by Gersh (1939).

This lack of secretory-type cells caused some people to regard the pars intermedia as the site of formation of the hormones (Herring, 1908; Cushing, 1933). Cushing stated that "It is scarcely conceivable that the neural lobe is capable independently of elaborating a hormone". He thought that the hormones migrated across the neurohypophysis ultimately to be released into the ventricle. This theory suffered from two deficiencies, an inability to find hormones other than intermedin within the pars intermedia, and the fact that so-called oxytocin within the cerebro-spinal

fluid turned out to be calcium (Van Dyke, Bailey and Buey, 1929).

Cajal (1902) showed that nerve fibres entered the neurohypophysis from an area posterior to the optic chiasma. These observations were extended by later workers, and it is now considered that fibres enter the neurohypophysis from the supra-optic and paraventricular nuclei, as well as from the tuberal region (Fisher, Ingram, Hare and Ranson, 1935; Fisher, Ingram and Ranson, 1938; Harris, 1948a). Fisher, Ingram and Ranson (1938) showed that these hypothalamic nuclei, together with the neurohypophysis, acted as a unit in controlling water balance. Destruction of the supra-optic nuclei led to atrophy of the neurohypophysis as did section of the supra-optico-hypophyseal tracts. Both of these procedures led to diabetes insipidus as would removal of the neurohypophysis together with the medium eminence. It was this work, together with the work of Verney and his collaborators which fully proved the role of this system in controlling water balance. The general theory which emerged was of the cells of the anterior hypothalamic nuclei modulating the secretory activity of pituicytes.

The removal of the neurohypophysis does not always lead to diabetes insipidus. Gersh (1939) noted cells within the medium eminence of a type similar to those in the neurohypophysis. These

are spared in the operation for removal of the neurohypophysis and Gersh suggested that they could hypertrophy and adequately maintain function. His findings were not substantiated by Hickey, Hare and Hare (1941). The fact that there were not secretory-type cells within the neurohypophysis still worried observers, as did the inability of this theory to account for the presence of neurohypophyseal hormones within the hypothalamus (Abel, 1925; Sato, 1928; Trendelenburg, 1928; Melville and Hare, 1945; Bargmann, 1949 a&b. Zetler, 1952; Vogt, 1953).

The only theory which fits in with Fisher, Ingram and Ranson's (1938) findings and which satisfies the other requirements is that of neurosecretion. This theory suggests that the cells of the supra-optic and paraventricular nuclei make the neurohypophyseal hormones which are than transported down their axons to the neurohypophysis for ultimate release. The theory originated principally as a result of the histological work of B. and E. Scharrer (1940). They noticed in brain nuclei of fish, homologous to the supra-optic and paraventricular nuclei of the mammals, cells which although neural in their gross appearance, have inclusion bodies typical of secretory cells. Demonstrated with ease in fish, they were difficult to find in mammals.

As so often happens in physiology, the situation can be rapidly altered by an advance in technique. In this case, it was the finding of Bargmann (1949a) that a haematoxylin stain, developed by Gomori (1941) for the staining of the β cells of the pancreatic islets, selectively stained the cells of the supra-optic and paraventricular nuclei, together with their axons and the neurohypophysis. These findings were soon confirmed and extended to show that the mammillo-infundibular nucleus also took up the stain (Smith, 1951).

It was quickly shown that the depth of staining could be related to the amount of extractable hormone (Zetler, 1953; Hild and Zetler, 1953).

Animals were thirsted for long periods, a procedure calculated to bring about the release of large quantities of anti-diuretic hormone. As the period of thirst proceeded, so the amount of stainable material declined. When the animals are allowed access to water again, the material reappears.

The material can be traced along the axons, into the neurohypophysis. Since it is suggested that material is normally transported along nerve axons (Weiss, 1944; Thomsen, 1954), it is not unreasonable to suppose that the material in the axon derives from the cell body. Further evidence for this has been provided by Hild (1951), and Scharrer and Wittenstein (1952) who have shown

that after section of the supraoptico-hypophyseal tracts material accummulates proximal to the site of section. The exact nature of the material is open to some doubt. Pre-treatment of the fresh tissue with organic solvents removes the stainable material, but the organic solvent does not contain the hormones (Scharrer and Scharrer, 1954). Nevertheless the stainable material is rich in cystine, one of the amino-acids which is present in the anti-diuretic and oxytocic polypeptides, (Barrnet, 1954; Sloper, 1954; Adams and Sloper, 1955).

The detailed morphological arrangement of the neurosecretory system may be that proposed by Bodian (1951). The neurohypophysis in many young animals is lobulated, (Fig. 1). In the opossum this lobulation is very marked, and persists into adult life. Detailed examination shows that fibres from the hypothalamus enter each lobule, and terminate at the periphery in close association with a vascular septum. It is suggested that the material is liberated into the vessels of this septum. This implies the function of the neurohypophysis to be largely physical, consisting of a device to bring the nerve termination in close association with blood vessels.

The neurosecretory theory is not so unrealistic when one considers that many nerve cells are continually engaged in synthesising and releasing acetyl choline, adrenaline and nor-adrenaline.

Fig. 1. Lobulation in neurohypophysis of 6 week old pig.

Gomori Chrome-alum haematoxylin phloxin stain:

8μ. x 300.

It is logical to expect them to be capable of synthesising other materials.

Statement of the Problem

It seemed that, since secretory activity on the part of the hypothalamic-hypophyseal system was accompanied by well defined cytological changes, then there was a possibility of confirming and further localising the site of morphine's action. The work of Bodo and Sweet (1938) and De Bodo (1944) suggested that the neurohypophysis proper was not necessary for the release of the anti-diuretic hormone, it could be liberated from the hypothalamic nuclei. Hence, after the administration of large doses of morphine we might expect to see those cytological changes within the cells associated with hyper-activity, e.g. degranulation. It was decided to see if this was so.

METHOD

Most of the work on morphine anti-diureis having been done on dogs, it would have been preferable to have used this species in the current investigation. This would have involved the slaughter of a considerable number of animals, and because of the size of the hypothalamus would have involved the examination of at least 150 sections per animal. It seemed as though the use of a smaller animal would be more economical.

Morphine was known to produce anti-diuresis in rats, but the mechanism appeared to be through the renal nerves, (Lipschitz and Stokey, 1947). The evidence which led to this conclusion was based upon assays of what was thought to be anti-diuretic hormone appearing in the urine after the injection of morphine. Since the assay values which were regarded as normal were extremely high, the work was suspect, and as a preliminary to the main investigation it was decided to re-investigate morphine anti-diuresis in the rat.

Using the method of Burn (1931) morphine was shown to be anti-diuretic in doses rather less than those used by Lipschitz and Stokey (1947). Doses of morphine sulphate of 400 µg/kg were strongly anti-diuretic. That this was not due to a spasm of the urethra or associated sphincters was shown by repeating the observations on narcotised rats by the method of Ames and Van Dyke (1952). Finally

the dosage of morphine needed to cause anti-diuresis was found in a number of rats which were then subjected to an operation for the production of diabetes insipidus by the method of Richter (1930). Those animals which had a high rate of water turnover after the operation were almost insusceptible to a dose of morphine which had previously produced anti-diuresis. It appeared that although in the large doses employed by Lipschitz and Stokey (1947), the renal nerves may influence the course of diuresis, with smaller doses of morphine, the neurohypophyseal mechanism is involved.

For the actual investigations, animals were chosen which had been used for assays by the method of Burn (1931), and which should be used to handling and injections, since they normally excreted rapidly even after the gagging and forced feeding associated with the assay procedure.

The conduct of the individual experiments was as follows - two animals were selected, being of the same sex and weight. They were allowed free access to water and food up to and during the time of the experiment. The morphine was given as the sulphate, the injection being given sub-cutaneously. Immediately the experimental animal had been injected, the other animal (the control) received an injection of normal saline, the volume being the same as that of the morphine injection. Thirty to sixty minutes after the last injection both

animals were decapitated. The brains were dissected immediately and fixed in Bouin's fluid for 24-48 hours. The tissues were dehydrated in either Dioxan or iso-propyl alcohol and embedded in paraffin wax. Serial sections were cut at 8μ, every 10th section being retained and stained by chrome-alum-haematoxylin and phloxin, according to the method of Gomori (1941). To avoid the grosser errors due to temperature fluctuations and ageing of solutions, all sections from the same experiment were processed together.

In three experiments, an additional control was employed to see whether the handling and saline injections influenced the appearance of the hypothalamico-hypophyseal system. This control animal was removed from its cage and immediately decapitated.

The doses of morphine injected ranged from 260 μg to 80 mg. The animals used averaged 250 gms.

In some of the experiments, the neurohypophysis separated from the pituitary during the preparation of the tissues and was lost. Unfortunately, this was not seen till the section had been cut.

Cell counts, when done, were done on every section, that is, at 80μ intervals. A cell described as "loaded" has an unusually large amount of basophilic material in its cytoplasm (See Figs. 2, 3 and 4).

The areas on the sections which were examined were the paraventricular and supra-optic nuclei,

the median eminence and the neurohypophysis.

Photographs were taken using a pale yellow filter and Ilford Panchromatic Plates.

In the description of the hypothalamico-hypophyseal system, the nomenclature of Rioch, Wislocki and O'Leary (1940) has been used with one exception. They include the median eminence in the neurohypophysis. In examining the tissue, this has not been found to be a logical inclusion and for the purpose of this investigation, the neurohypophysis is taken to mean the neural lobe plus the infundibulum, the median eminence being described separately.

RESULTS

Experiment 1 — 260 µg morphine sulphate s/c.

In this experiment one animal (R.15) was given a single injection of morphine sulphate which was five times the anti-diuretic dosage. When stained, the cells of the control animal (R.12) showed a remarkable variation. Fig. 2 shows an area of supra-optic nucleus in which some cells exhibit an intensely basophilic cytoplasm, whereas other cells show hardly any basophilia. By contrast, the supra-optic cells of R.15 were more uniform (Fig. 3), there being very few of the intensely stained cells. There were no obvious differences in the paraventricular nuclei or median eminence. Unfortunately, at some stage the neurohypophysis separated out from the pituitary, and was lost.

Experiment 2 — 2 mg morphine sulphate s/c.

Again, a feature of the control animal (R.10) was the variability of the appearance of the individual cells of the supra-optic and paraventricular nuclei (Fig. 4). The cytoplasm of some cells was only just visible, and in others it was densely stained. This was also evident in the supra-optic cells of R.16, the injected animal (Fig. 5). No difference could be observed in the paraventricular nuclei of the two animals. However, the median eminence of R.16 had an accummulation of stained material, none being present in the median eminence of the control animal (Figs. 6 and 7). No

difference could be observed in the neurohypophyses of the two animals.

Experiment 3 — 3 mg morphine sulphate s/c.

The control animal (R.44) showed depletion of basophilic material, densely stained supra-optic cells being rare (Fig. 8). The cells of the supra-optic nucleus of the experimental animal (R.45) showed no obvious difference from those of the control, but a number of deeply stained axons were visible (Fig. 9). No difference was visible in the paraventricular nuclei. In the median eminence of R.45, basophilic material was present (Fig. 10). The neurohypophysis of the morphine injected animal had more basophilic material present than that of the control animal (Figs. 11 and 12).

Experiment 4 — 5 mg morphine sulphate s/c.

Cell counts showed that whereas 98% of the paraventricular cells and 53% of the supra-optic cells of the control animal (R.48) had considerable amounts of basophilic material in the cytoplasm, the percentage fell to 33 and 2 in the experimental animal (R.49). Both animals showed an accummulation of basophilic material ventral and medial to the supra-optic nuclei (Fig. 13), and there were a number of heavily loaded axons amongst the depleted cells of the supra-optic nucleus of R.49 (Fig. 14). The neurohypophysis of R.49 was pale in the ventral area, and dense in the dorsal area (Fig. 15).

Experiment 5 7 mg morphine sulphate s/c.

In this experiment a further control was employed. An animal (R.55) was decapitated after removal from its cage with the minimum of handling. Its hypothalamus and pituitary were dissected out and fixed, dehydrated, embedded and stained together with the hypothalamus and pituitary of the experimental animal (R.38) and the saline injected control (R.54). There was no difference apparent between the cells of the paraventricular nuclei of any of the animals. Cell counts showed R.55 to have 33% of its supra-optic cells loaded with basophilic material. The injected control (R.54) showed considerable depletion (Fig. 16), only 7·5% of its supra-optic cells being loaded. R.38 showed a depletion of the same order, 3·5% of its cells being loaded with basophilic material. There was no apparent difference between the neurohypophysis of R.38 and R.54, but in comparison, the neurohypophysis of R.55 was densely stained (Figs. 17 and 18). Basophilic material was present in the median eminence of R.54.

Experiment 6 30 mg morphine sulphate s/c.

In this experiment three animals were again used, following the procedure of experiment 5. Both animals which received injections (R.58, morphine and R.59, saline) showed depletion of basophilic material in supra-optic cells, the figures being similar for both animals. 27% of cells were

loaded with basophilic material in R.58, and 33% in R.59. In contrast, R.60 which received no injection had basophilic material in most of its cells. R.59 had more basophilic material in its hypothalamus than R.58. The paraventricular cells looked similar in all animals. R.59 had more basophilic material in its neurohypophysis than R.58, but the difference was only slight. Basophilic material was present in small quantities in the median eminence of both R.58 and R.59.

Experiment 7 60 mg morphine sulphate s/c.

This experiment followed the same plan as experiments 5 and 6. No difference was discernible between cells of the paraventricular nuclei. The animal which was not injected (R.63) had appreciable amounts of basophilic material in 79% of its supraoptic cells, and the animal which was injected with saline (R.62) had basophilic material in 67% of its cells. In contrast, R.61, which had received 60 mg of morphine had basophilic material in only 15% of its cells. The only other point of difference is in the amount of basophilic material in the neuro-hypophyses R.62 shows almost total depletion of basophilic material, unlike R.63 and R.61 (Figs. 19 and 20).

Experiment 8 60 mg morphine sulphate s/c.

In this experiment only two animals were used, a saline injected control (R.77) and the experimental animal (R.78). Unfortunately both neurohypo-

physes were lost, and the only difference observed was in the supra-optic nucleus. The cells of R.77 were unusually densely stained, 91% showing accummulation of basophilic material. In R.78 this figure fell to 40%.

Experiment 9 — 80 mg morphine sulphate s/c.

The morphine injected animal, R.81, had few loaded cells in its supra-optic nucleus (5%). The figure was low in the saline injected animal (20%). Both showed unusually little material in their paraventricular cells (40% and 50% loaded cells respectively). No basophilic material was present in either median eminence. The neurohypophysis had come away from the pituitary in both animals and was lost.

Experiment 10 — 80 mg morphine sulphate s/c.

No difference could be observed in any of the structures examined in either the morphine injected animal (R.93) or the saline injected animal (R.94). Both paraventricular and supra-optic nuclei contained a high proportion of loaded cells (63% and 64% respectively). Both neurohypophyses contain moderate amounts of basophilic material, and there was little basophilic material in either hypothalamus of median eminence.

Table 1

SUMMARY OF MORPHINE EXPERIMENTS

DOSAGE AND ANIMALS USED

Exp. No.	Animal No.	Total Dose Morphine Sulphate	Methods of Administration	Control Animal A	Control Animal B
1	R.15	260µg	single dose s/c	R.12	-
2	R.16	2mg	1mg/hour s/c	R.10	-
3	R.45	3mg	1mg/hour s/c	R.44	-
4	R.49	5mg	1mg first hour thereafter 2mg/hour s/c	R.48	-
5	R.38	7mg	1mg/hour s/c	R.54	R.55
6	R.58	30mg	10mg/hour s/c	R.59	R.60
7	R.61	60mg	30mg/hour s/c	R.62	R.63
8	R.78	60mg	20mg/hour s/c	R.77	-
9	R.81	80mg	20mg/hour s/c	R.82	-
10	R.93	80mg	20mg/2 hour s/c	R.94	-

Control Animal A - received saline when experimental animal received morphine.

Control Animal B - decapitated with minimum handling.

Table 2

SUMMARY OF RESULTS

Exp. No.	Paraventricular Nuclei	Supra-optic nuclei	Median Eminence	Neurohypophysis
1	No difference.	Fewer loaded cells in morpine injected animal.	No difference	Lost
2	No difference.	No difference.	Colloid present in morphine injected animal.	No difference.
3	No difference.	Axons visible in morphine injected animal.	Colloid present in morphine injected animal.	Depletion in morphine injected animal.
4	Few loaded cells in morphine injected animal.	Fewer loaded cells in morphine injected animal. Colloid present in both animals. Stained axons in morphine injected animals.	No difference.	Ventral area depleted in morphine injected animal.
5	No difference.	Saline and morphine injected animals show depletion.	Colloid in saline injected animal.	Depletion in both injected animals.
6	No difference.	Depletion in both injected animals.	Colloid in both injected animals.	Depletion in both injected animals.
7	No difference	Depletion in morphine injected animals.	No difference.	Great depletion in saline injected animal.
8	No difference.	Depletion in morphine injected animals.	No difference	Lost
9	No difference	Depletion in morphine injected animals.	No difference.	Lost
10	No difference	No difference	No difference	No difference

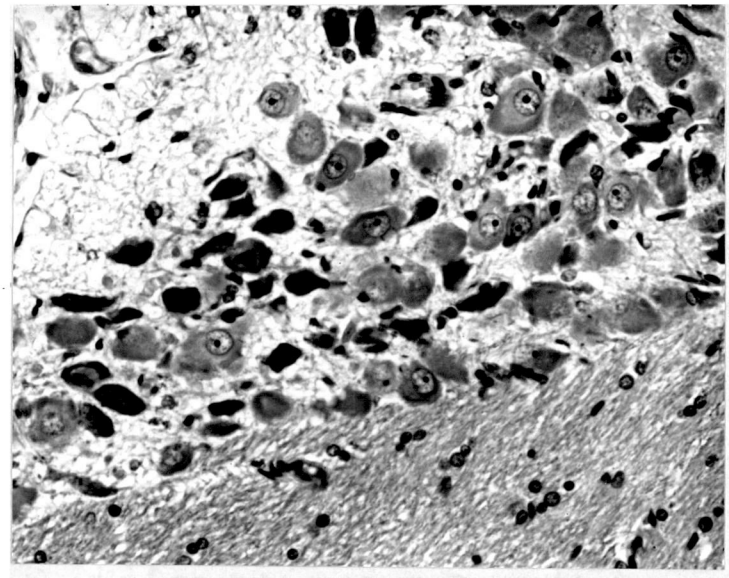

Fig. 2. R.12. Supra-optic cells. To show range of basophilia in a normal nucleus. Chrome-alum haematoxylin phloxin. 8μ. x 300.

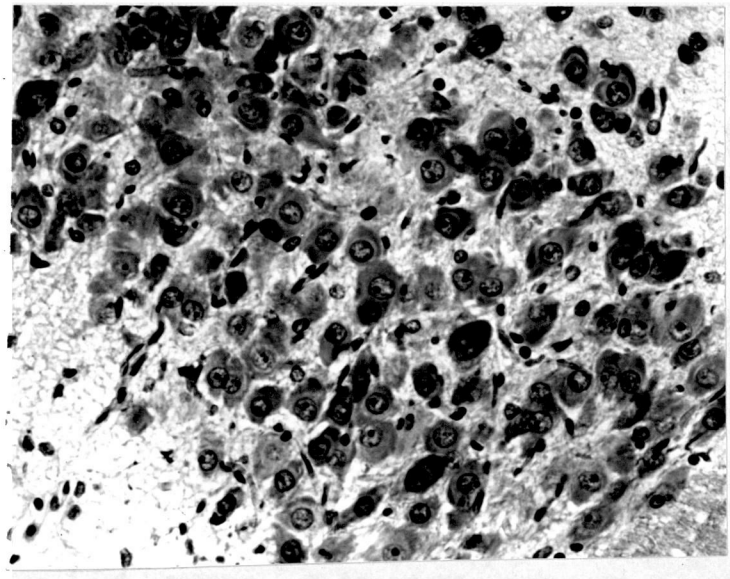

Fig. 3. R.15. Supra-optic cells. Morphine injected animal (260µg s/c). Note the uniformity of staining and the absence of extremely densely stained cells.

Chrome-alum haematoxylin phloxin. 8µ. x 300.

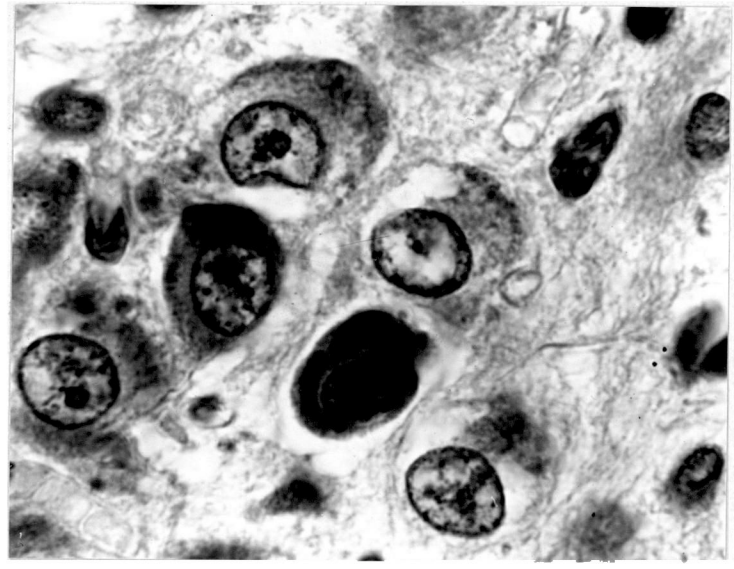

Fig. 4. R.10. Supra-optic cells of control animal. Note the extreme variation in the degree of basophilia. The cytoplasm is hardly visible in the bottom right hand cell. Chrome-alum haematoxylin phloxin. 8μ.x 1150.

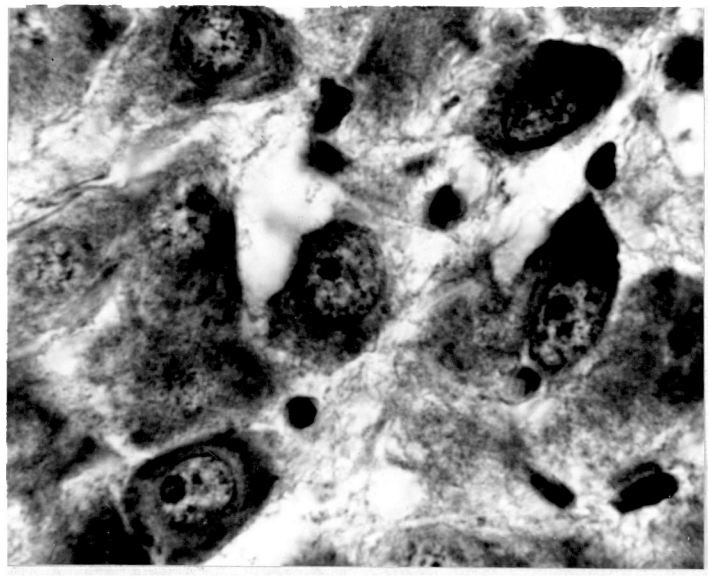

Fig. 5. R.16. Supra-optic cells of injected animal (2mg s/c). Variation in basophilia. Chrome-alum haematoxylin phloxin. 8μ.x 1100.

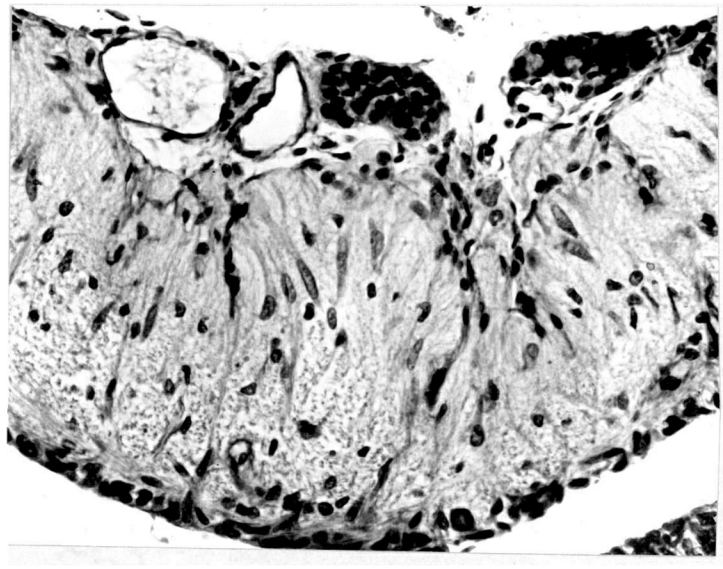

Fig. 6. R.10. Median eminence of control animal. No droplets of stainable material. Chrome-alum haematoxylin phloxin. 8μ. x 300.

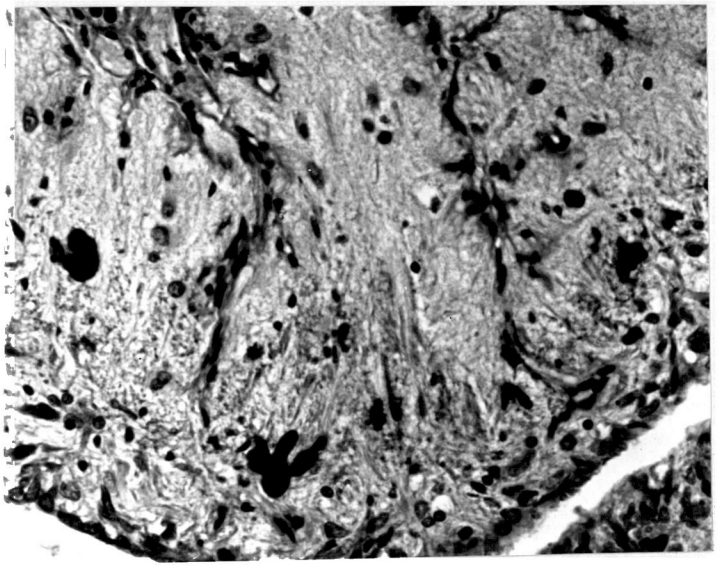

Fig. 7. R.16. Median eminence of injected animal. Note the presence of several collections of colloid.
Chrome-alum haematoxylin phloxin. 8μ. x 300.

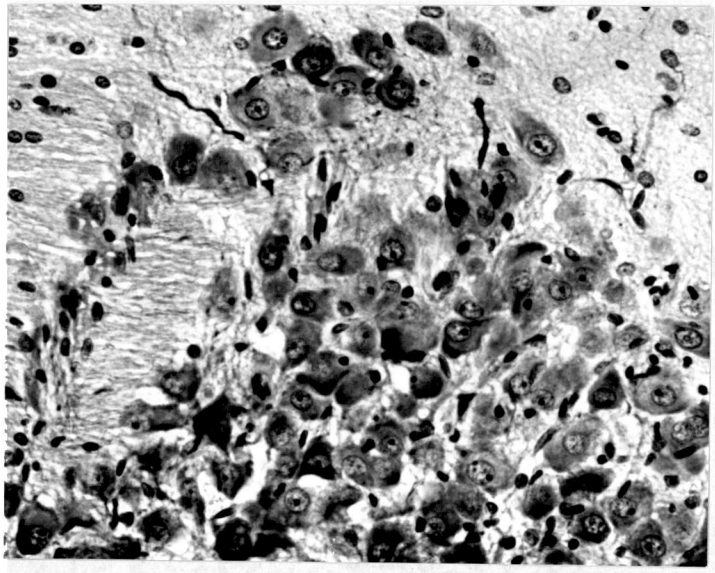

Fig. 8. R.44. Supra-optic nucleus of control animal. Fewer densely stained cells than in previous control animals.
Chrome-alum haematoxylin phloxin. 8μ. x 300.

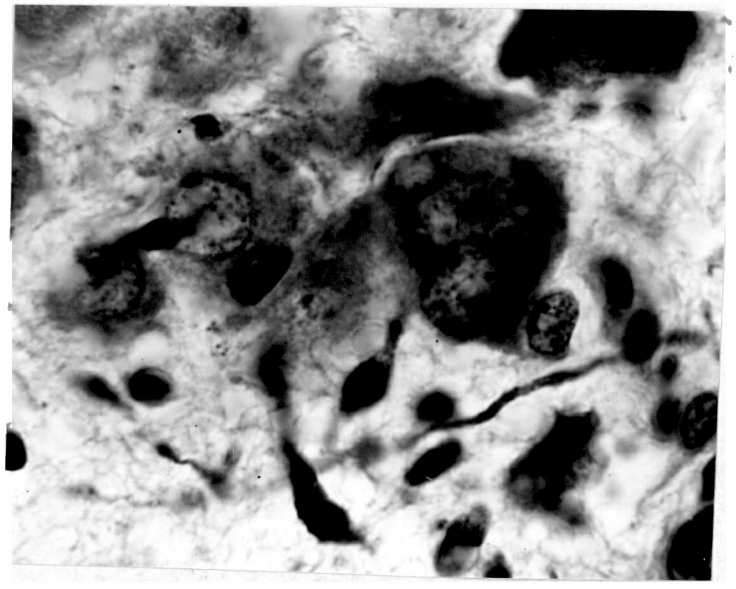

Fig. 9. R.45. Cells of the supra-optic nucleus. Note the deeply stained fibre tracking from right to left.

Chrome-alum haematoxylin phloxin. 8μ. x 1150.

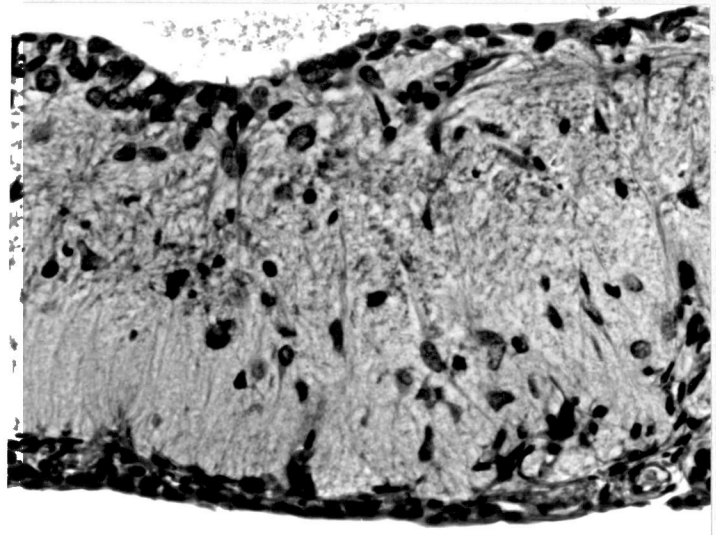

Fig. 10. R.45. Median eminence. Groups of small accumulations of stained material. Chrome-alum haematoxylin. 8μ. x 375.

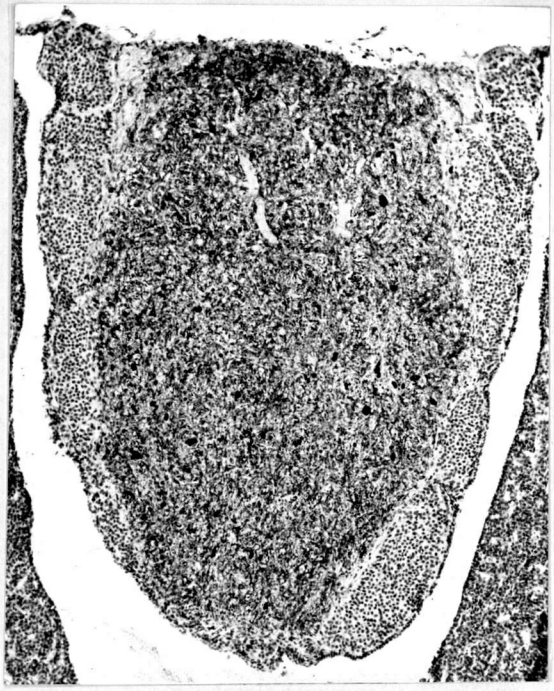

Fig. 11. R.44. Neurohypophysis. Moderate amount of stainable material.
Chrome-alum haematoxylin. 8μ. x 65.

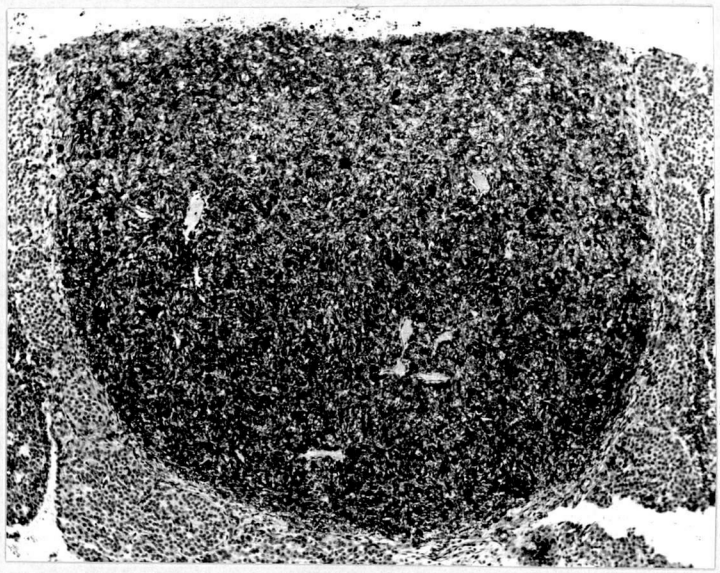

Fig. 12. R.45. Neurohypophysis. Accumulation of material in ventral part.
Chrome-alum haematoxylin. 8μ. x 65.

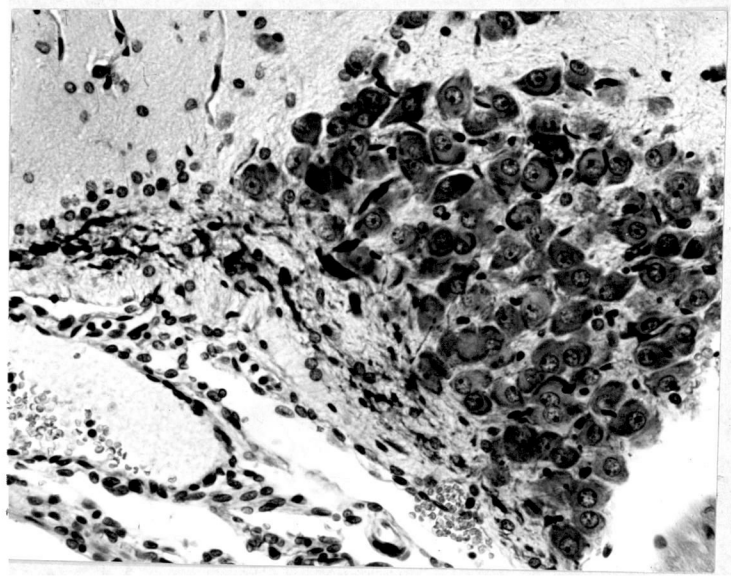

Fig. 13. R.48. Supra-optic nucleus. Note the colloid on the left hand side of the photograph.

Chrome-alum haematoxylin phloxin. 8μ. x 225.

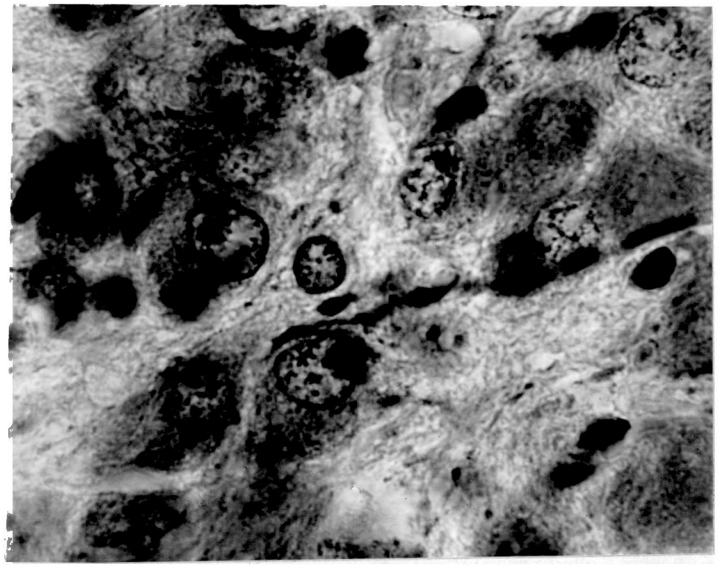

Fig. 14. R.49. Supra-optic nucleus. Axon going from right to left between depleted cells of injected animal.
Chrome-alum haematoxylin phloxin. 8μ. x 1100.

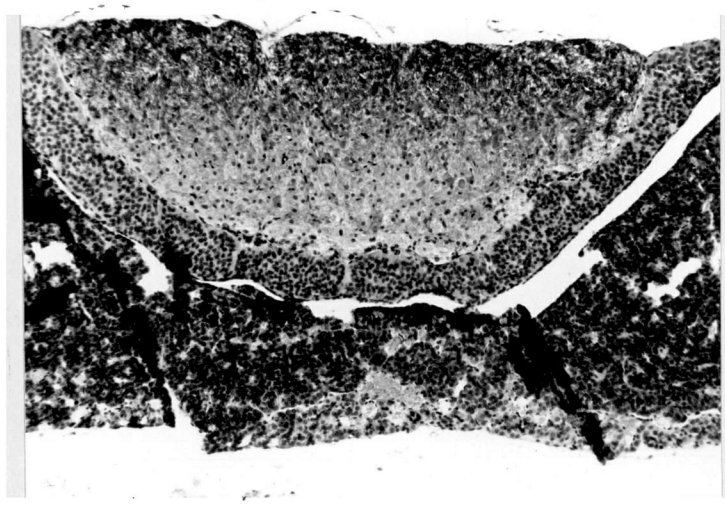

Fig. 15. R.49. Neurohypophysis. Unusual distribution of stainable material, all being in dorsal area.

Chrome-alum haematoxylin phloxin. 8μ. x 80.

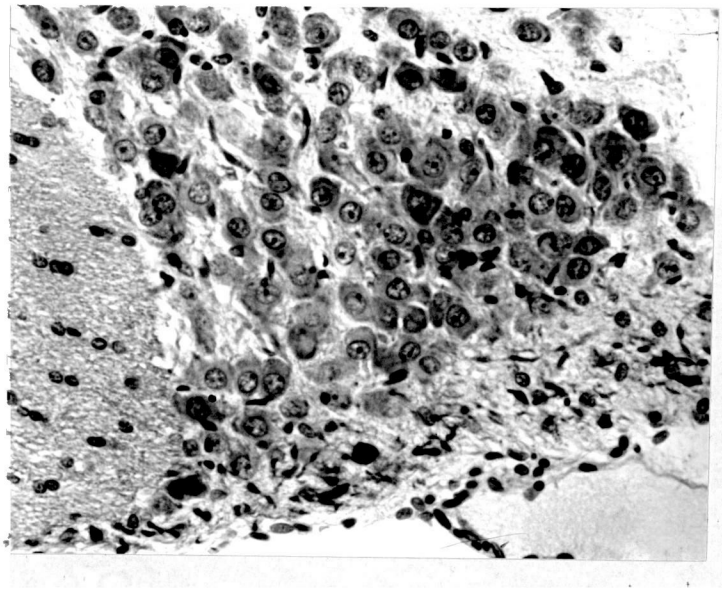

Fig. 16. R. 54. Supra-optic nucleus. Considerable depletion in injected control. Chrome-alum haematoxylin phloxin. 8μ. x 300.

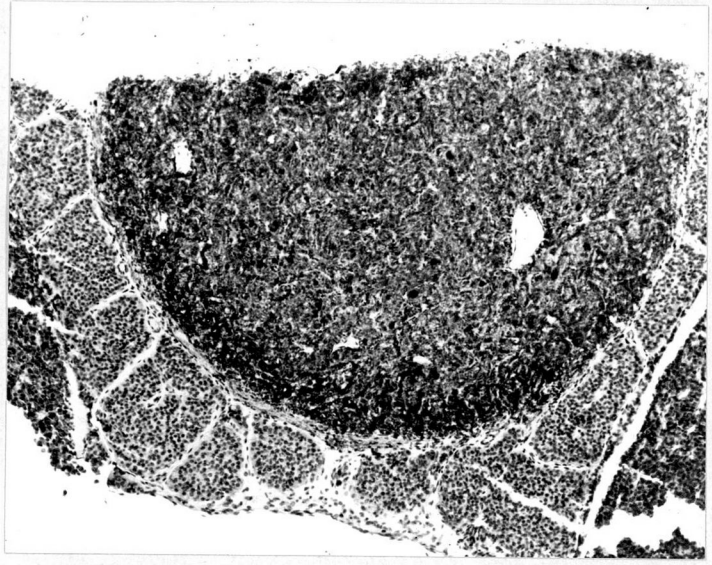

Fig. 17. R.55. Neurohypophysis. Non-injected control. Dense accummulation of material at periphery.

Chrome-alum haematoxylin phloxin. 8μ. x80.

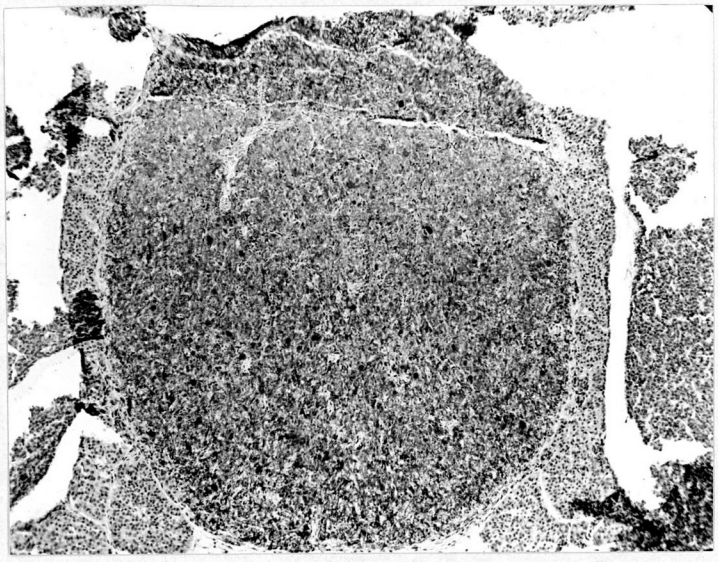

Fig. 18. R.54. Neurohypophysis. Injected control. Relatively small accummulation of material.

Chrome-alum haematoxylin phloxin. 8μ. x 65.

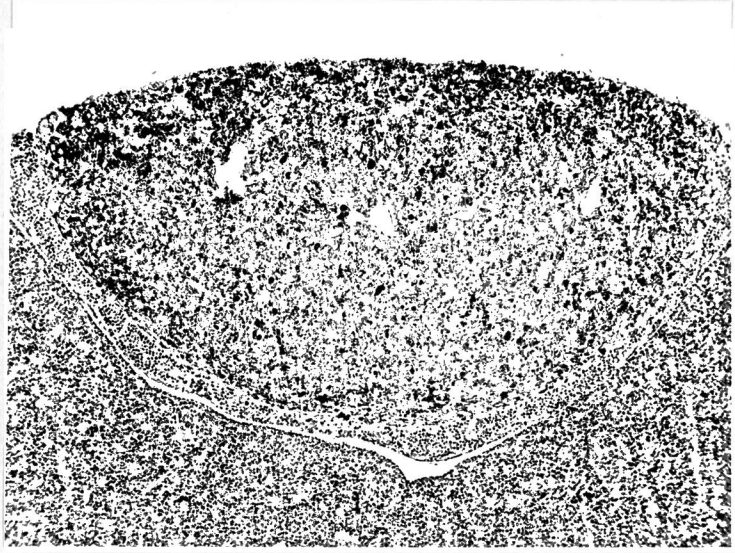

Fig. 19. R.63. Neurohypophysis. Non-injected control.

Chrome-alum haematoxylin phloxin. 8μ. x 65.

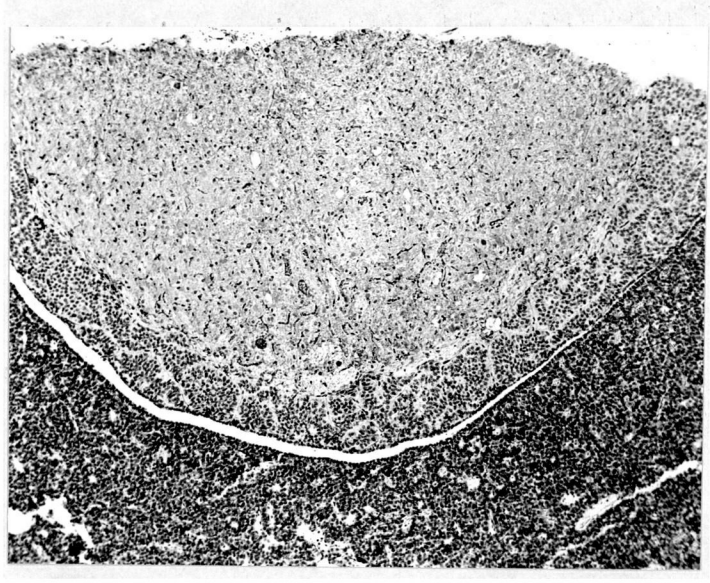

Fig. 20. R.62. Neurohypophysis. Saline injected control.

Chrome-alum haematoxylin phloxin. 8μ x 65.

Summary of Results

The cells of the supra-optic nucleus in control animals revealed a wide variation in basophlia. In contrast, the paraventricular nucleus was remarkably uniform. In ten experiments, 23 pairs of paraventricular nuclei were examined, and only in 3 animals was there any departure from the customery high degree of basophilia. The median eminence varied greatly in the amount of an apparently structureless material, conveniently called colloid. The appearance of the neural lobe was highly variable, at times it was intensely stained, but at other times there appeared to be little or no basophilic material present.

Correlation of the histological picture with the morphine injections was difficult particularly in view of the appearance of the controls. It seemed that handling the animals and giving them injections was sufficient to cause the loss of basophilic material both from the supra-optic nuclei and also from the neurohypophysis, but the action of morphine can only be interpreted against the background of behaviour of the controls.

With the low dose (Exp. 1) the intensely stained cells were not present after the injection of morphine, but there were no extremes of depletion. When the dose was raised (Exp. 2), colloid appeared in the median eminence. This was seen too in the morphine injected animal in experiment 3. In this

experiment, where injections were given over a prolonged period the control animal had few of the intensely stained cells.

In experiment 4 there was a considerable difference between the control and the experimental animal, the supra-optic nucleus of the injected animal being almost devoid of basophilic material in the cytoplasm although there were numbers of densely stained axons tracking through the nucleus. Its neurohypophysis also showed depletion of basophilic material.

The employment of the extra control animal in experiment 5 brought out the fact that handling could influence the degree of basophilia, there being no significant difference in appearance between the saline and morphine injected animals, although a considerable difference existed between them and the non-injected animal. This was emphasised again in experiment 6 where there was little difference in the appearance of the two injected animals, yet both showed a depletion of basophilic material compared with the animal which had received no injections.

Experiment 7 produced a different result. The cells of the supra-optic nucleus of the morphine injected animal were considerably depleted, but the saline injected animal had only a few less deeply stained cells than the morphine-injected animal. In this experiment the neurohypophysis of the saline injected animal had almost no basophilic material

present. In experiments 8 and 9, the only difference was in the number of densely stained cells in the supra-optic nuclei. The numbers were reduced in both morphine injected animals. The last experiment gave an unusual result, not because of the lack of difference between the saline and morphine injected animal, but because of the relatively high proportion of loaded cells in both animals.

From these experiments it was clear that the appearance of the cells of the supra-optic nucleus of the normal animal was highly variable. The basophilic material in the supra-optico-hypophyseal system appeared to be labile. Handling the animals appeared to be sufficient to cause a loss of the basophilic material in some animals, but the injection of morphine seemed a more certain way of causing the loss of basophilic material. In the experiments with relatively low doses of morphine it was almost as though one could see the material being mobilised, with the appearance of axons and basophilic material in the median eminence. It was, in fact, only in these animals that stained axons can be seen.

The neurohypophyseal staining also showed this remarkable lability, the variations in basophilia being very striking. It was unfortunate that some neurohypophyses were lost, but from those remaining it was only possible to say that, whereas the injection of saline was sufficient to cause total loss

of material, the effect was more unpredictable than the effect of morphine, although both could be equally potent.

DISCUSSION

Normally the cells of the paraventricular nuclei were most constant in their appearance, whereas those of the supra-optic nuclei showed great variability (Figs. 2 and 4). This variability was largely in the amount of cytoplasmic basophilic material. The best known cytoplasmic basophilic material in nerve cells in the Nissls substance, and early workers showed that the amounts of this material present in the nerve cell varied very widely (see Barker, 1899). Attempts were made early on to try and relate the appearance of this substance to the activity of nerve cells (Mann, 1894). It is now known that in most cells, the cytoplasmic basophilic material is ribonucleic acid, and that this substance is involved in protein metabolism (Wilson, 1954). Using very refined methods, including micro-incineration and ultra-violet absorption, basophilia in cells generally and in nerve cells in particular has been studied from the viewpoint of cellular activity (Hyden, 1943; Caspersson, 1950). This work indicates that in many nerve cells the Nissl substance (nucleic acids) is lost as a result of periods of prolonged activity. Einarson (1933, 1935) and Einarson and Krogh (1955) have confirmed this using a staining technique which is relatively specific for nucleic acids.

This raises the question of whether what has

been seen within the supra-optic nucleus using the chrome-alum haematoxylin stain is an exaggerated picture of what is seen elsewhere, or whether something other than nucleic acids is stained. There are reasons for thinking that the chrome-alum haematoxylin may stain some other material, not the least of which is the fact that alternate serial sections stained with a "classical" stain such as toluidine blue show a different distribution of cytoplasmic basophilic material.

In thechrome-alum haematoxylin staining procedures, the first step is to oxidise the tissue for one minute in acidified potassium permanganate. This oxidation is known to increase tissue basophilia in sulphur containing tissues (Dempsey, Singer and Wislocki (1950). It is thought that the oxidation converts sulphide and sulphydryl groups to sulphonic acid, and it is the presence of this substance which gives the enhanced basophilia. Since the anti-diuretic and oxytocic hormones contain a sulphur-rich amino-acid (cystine) and are present within the hypothalamus it is not without the bounds of possibility that the intense staining of the supra-optic and paraventricular cells is due to the presence of these hormones. Since the staining bath is at a pH of 2·3-3·0 nucleic acids may also be stained.

This is in conflict with the evidence cited earlier that solvents which do not remove the

neurohypophyseal hormones from the hypothalamus can remove the material which the chrome-alum haematoxylin stains. Because a tissue after pre-treatment with a substance does not take up a given stain, it does not necessarily mean that the material has been removed, it may have been altered physically or chemically. It is relevant that after Bouin or Zenker fixation it is impossible to extract the basophilic material by organic solvents (Scharrer and Scharrer, 1954).

Eränkö (1951a) has reported the same variations in basophilia within the supra-optic and paraventricular nuclei of rats as are reported here, and he suggests that this represents cells in all phases of activity cycles. His studies on the distribution of acid and alkaline phosphatases within these nuclei (Eränkö, 1951b) show that these cells are likely to be very active metabolically, and this might account for their varying levels of basophilia being more obvious than in other nerve nuclei.

Irrespective of whether the basophilic material which has been stained is nucleic acid or the neurohypophyseal hormones themselves, the loss of material indicates that the cells have passed through a period of intense activity. On this basis it is proper to conclude that morphine can cause considerable activity of the cells of the supra-optic nucleus, presumably with the associated

release of the anti-diuretic hormone. Morphine is a more effective and more certain stimulus to release of basophilic material than handling.

The finding that merely handling the animal can (although not always) cause a depletion of basophilic material is confirmatory evidence for the theory that the activity of the adenohypophysis may be influenced by the hypothalamus. The mediator of this influence may be the antidiuretic and/or the oxytocic hormone. Harris (1948 a & b) has produced a considerable amount of evidence which argues for such a humoral linkage. The evidence that such a link is mediated by neurohypophyseal hormones comes from a variety of sources. Many workers have shown that the release of hormones from the adenohypophysis can be brought about by the injection of neurohypophyseal hormones (Libretti and Martini, 1953; Fraja and Martini, 1953; Martini and Morpurgo, 1955). Animals subjected to procedures which will cause the relase of adrenocorticotrophic hormone lose anti-diuretic hormone from their hypothalamus (Kovacs and Bachrach, 1951), and such aniamsl have an increased serum anti-diuretic activity (Mirsky, Stein and Paulisch, 1954). Brobeck and McCann (1954) have shown that the paraventricular nucleus is unlikely to be involved in this response, but that hypothalamic lesions which block the release of adrenocorticotrophic hormones also cause diabetes insipidus with

associated atrophy of the supra-optico-hypophyseal system.

All this evidence suggests strongly that the depletion which has been seen in the supra-optic nucleus of those animals which have received saline injections is correlated with this "stress" reaction. The suggestion that the paraventricular nucleus is not associated with this reaction would explain the relative constancy of its appearance.

According to the theory of neurosection, it would be expected that the material formed in the hypothalamus would have to travel to the neurohypophysis to be released into the blood stream. When low doses of morphine were used, or when the animal received only one injection the appearance of axons which are readily stained and colloid within the median eminence suggests that this may be happening. In higher doses, there is no obvious morphological evidence of a pathway between the hypothalamus and neurohypophysis such as has been described in the dog and cat. This may be due to the animal being killed after such prolonged stimulus. Had the animal been killed earlier such a pathway might have been seen. The neurohypophysis was depleted when the cells of the supra-optic nucleus were, and it may be that its depletion preceded the depltion of the cells, but the possibility of hormones going directly from the cells to the circulation cannot be excluded. This is particularly so

since in experiment 7 depletion of the cells was seen in a morphine injected animal which nevertheless had a remarkable amount of basophilic material within its neurohypophysis.

The lability of basophilic material within the neurohypophysis has been stressed in these experiments. Several injections of saline over a period of several hours can deplete the neurohypophysis entirely. This lability has been commented upon by Rothballer (1953), who has shown a similar depletion after pricking rats' tails. These very quick responses to handling suggest that the hypothalamico-hypophyseal system may be involved in generalised "stress" responses.

The emotional anti-diuresis may thus be regarded not as a primary event, but as a side effect due to the anti-diuretic hormone being involved in this system.

These experiments indicate that morphine can exert an effect upon the hypothalamico-hypophyseal system, but they do not show whether morphine does exert its effect directly upon the cells of the supra-optic and paraventricular nuclei, or on the neurohypophysis. It does however cause the release of basophilic material from the supra-optic nucleus and the neurohypophysis. Morphine does not appear to cause the release of the basophilic material from the paraventricular nucleus. The release of basophilic material from the hypothalamico-

hypophyseal system can be induced by other non-specific disturbances, and this may be due to the system having a dual function. As has been suggested, as well as controlling the volume of urine excreted, it may also be involved in a humoral mechanism controlling the adenohypophysis.

Epilogue

The study of anti-diuretics has shown how artificial are the boundaries of the systems of the body. The reduction in the volume output of the kidney may be brought about by the activity of one of a number of systems, and the study of anti-diuresis can lead one into as diverse topics as those described here. It increases the realisation of how closely integrated are the workings of the body, and the wonderment that the razor edge which we call its internal environment is so perfectly maintained.

BIBLIOGRAPHY

ABEL, J. J. (1924). PHYSIOLOGICAL, CHEMICAL AND CLINICAL STUDIES ON PITUITARY PRINCIPLES. Johns Hopk. Hosp. Bull. 35, 305-328.

ADAMS, C. W. M. & SLOPER, J. C. (1955). TECHNIQUE FOR DEMONSTRATING NEUROSECRETORY MATERIAL IN THE HUMAN HYPOTHALAMUS. Lancet 1, 651-652.

AMES, ROSE G. & VAN DYKE, H. B. (1952). ANTIDIURETIC HORMONE IN THE SERUM OR PLASMA OF RATS. Endocrinology 50, 350-360.

BARGMANN, W. (1949a). ÜBER DIE NEUROSEKRETORISCHE VERKNUPFUNG VON HYPOTHALAMUS UND HYPOPHYSE. Klin. Wschr. 27, 617-622.

BARGMANN, W. (1949b). ÜBER DIE NEUROSEKRETORISCHE VERKNÜPFUNG VON HYPOTHALAMUS UND NEUROHYPOPHYSE. Z. Zellforsch. 34, 610-634.

BARKER, L. F. (1899). THE NERVOUS SYSTEM AND ITS CONSTITUENT NEURONES. 1st ed. New York: Appleton & Co.

BARRNET, R. J. (1954). HISTOCHEMICAL DEMONSTRATION OF DISULFIDE GROUPS IN THE NEUROHYPOPHYSIS UNDER NORMAL AND EXPERIMENTAL CONDITIONS. Endocrinology 55, 484-501.

BERKLEY, H. J. (1894). THE FINER ANATOMY OF THE INFUNDIBULAR REGION OF THE CEREBRUM INCLUDING THE PITUITARY GLAND. Brain 17, 515-547.

BODIAN, D. (1951). NERVE ENDINGS, NEUROSECRETORY SUBSTANCE AND LOBULAR ORGANISATION OF THE NEUROHYPOPHYSIS. Johns Hopk. Hosp. Bull. 89, 354.

BODO, R. C. DE. (1944). THE ANTIDIURETIC ACTION OF MORPHINE AND ITS MECHANISM. J. Pharmacol. 82, 74-85.

BODO, R. C. DE., COTUI, F. W. & BANAGLIA, A. E. (1937). STUDIES ON THE MECHANISM OF MORPHINE HYPERGLYCAEMIA. J. Pharmacol. 61, 48-57.

BODO, R. C. & SWEET, J. E. (1938). THE ROLE OF THE PITUITARY GLAND AND THE ADRENAL MEDULLA IN THE ANTIDIURETIC EFFECT OF MORPHINE AND PHENOBARBITOL. J. Pharmacol. 63, 3.

BODO, R. C. DE & SWEET, J. E. (1940). THE ANTIDIURETIC ACTION OF MORPHINE IN DIABETES INSIPIDUS. J. Pharmacol. 69, 276-277.

BONSMANN, M. R. (1930). ÜBER EINWIRKUNG VON OPIUMDERIVATIVEN AUF DIE DIURESE DES HUNDES SOWIE BEOBACHTUNG ÜBER GEWOHNUNGEN AN DIESE. Arch. exp. Path. Pharmak. 156, 145-159.

BROBECK, J. R. & McCANN, S. M. (1954). EVIDENCE FOR A ROLE OF THE SUPRAOPTICOHYPOPHYSEAL SYSTEM IN REGULATION OF ADRENOCORTICOTROPHIN SECRETIONS. Proc. Soc. exp. Biol., N.Y. 87, 318-324.

BUCY, P. C. (1930). THE PARS NERVOSA OF THE BOVINE HYPOPHYSIS. J. comp. Neurol. 50, 505-519.

BURN, J. H. (1931). ESTIMATION OF THE ANTIDIURETIC POTENCY OF PITUITARY (POST. LOBE) EXTRACT. Quart. J. Pharm. 4, 517-529.

CAJAL, S. RAMON (1894). ALGUNOS CONTRIBUCIONES AL CONOCIMIENTO DEL CEREBRO. 3. HYPOPHYSIS. An. Soc. esp. Hist. nat. 3. Cited by CAJAL, S. R. in HISTOLOGIE DU SYSTÈME NERVEUX DE L'HOMME ET DES VERTÉBRÉS. 1955, 489. Instituto Ramon Y Cajal, Madrid.

CASPERSSON, T. O. (1950). CELL GROWTH AND CELL FUNCTION. A CYTOCHEMICAL STUDY. 126-134. New York: W. W. Norton & Co. Inc.

CUSHING, H. (1933). POSTERIOR PITUITARY ACTIVITY FROM AN ANATOMICAL STANDPOINT. Amer. J. Path. 9, 539-547.

DALE, H. H. (1906). ON SOME PHYSIOLOGICAL ACTIONS OF ERGOT. J. Physiol. 34, 163-206.

DEMPSEY, E. W., SINGER, M. & WISLOCKI, G. B. (1950). THE INCREASED BASOPHILIA OF TISSUE PROTEINS AFTER OXIDATION WITH PERIODIC ACID. Stain Tech. 25, 73-80.

DSIKOWSKI, W. (1936). EINFLUSS DER OPIUMALKALOIDE AUF DEN WASSERHAUSHALT DES ORGANISMS. Arch. int. Pharmacodyn. 53, 457-475.

DUKE, HELEN, PICKFORD, MARY & WATT, J. A. (1950). THE IMMEDIATE AND DELAYED EFFECTS OF DIISOPROPYLFLUOROPHOSPHATE INJECTED INTO THE SUPRAOPTIC NUCLEI OF DOGS. J. Physiol. 111, 81-88.

DUKE, HELEN, PICKFORD, MARY & WATT, J. A. (1951). THE ANTIDIURETIC ACTION OF MORPHINE: ITS SITE AND MODE OF ACTION IN THE HYPOTHALAMUS OF THE DOG. Quart. J. exp. Physiol. 36, 149-158.

EIRNARSON, L. (1933). NOTES ON THE MORPHOLOGY OF THE CHROMOPHIL MATERIAL OF NERVE CELLS AND ITS RELATION TO NUCLEAR SUBSTANCES. Amer. J. Anat. 53, 141-189.

EINARSON, L. (1935). HISTOLOGICAL ANALYSIS OF THE NISSL-PATTERNS AND -SUBSTANCES OF NERVE CELLS. J. Comp. Neurol. 61, 101-133.

EINARSON, L. & KROGH, E. (1955). VARIATIONS IN THE BASOPHILIA OF NERVE CELLS ASSOCIATED WITH INCREASED ACTIVITY AND FUNCTIONAL STRESS. J. Neurol. 18, 1-12.

ERÄNKÖ, O. (1951a). THE CYTOLOGY OF THE NUCLEUS SUPRAOPTICUS OF THE RAT. Ann. Med. exp. Fenn. 29, 158-173.

ERÄNKÖ, O. (1951b). HISTOCHEMICAL EVIDENCE OF INTENSE PHOSPHATASE ACTIVITY IN THE HYPOTHALAMIC MAGNOCELLULAR NUCLEI OF THE RAT. Acta. physiol. scand. 24, 1-6.

FEE, A. R. (1928). THE RENAL EXCRETION OF CHLORIDES AND WATER. J. Pharmacol. 34, 305-316.

FEE, A. R. (1929). STUDIES ON WATER DIURESIS. (PT. 1). J. Physiol. 68, 39-44.

FISHER, C., INGRAM, W. R., HARE, W. K. & RANSON, S. W. (1935). THE DEGENERATION OF THE SUPRAOPTICO-HYPOPHYSEAL SYSTEM IN DIABETES INSIPIDUS. Anat. Rec. 63, 29-52.

FISHER, C., INGRAM, W. R. & RANSON, S. W. (1938). DIABETES INSIPIDUS AND THE NEURO-HORMONAL CONTROL OF WATER BALANCE: A CONTRIBUTION TO THE STUDY OF THE HYPOTHALAMIC-HYPOPHYSEAL SYSTEM. Ann Arbor, Michigan: Edwards Bros. Inc.

FRAJA, A. & MARTINI, L. (1953). ALCUNE OBSERVAZIONI SUL PASSAGIO IN CIRCOLO DELL'ORMONE TREOTROPO. Arch. int. Pharmacodyn. 93, 167-176.

FREY, E. (1907). DIE HINDERUNG DER WASSERDIURESE DURCH DIE NARKOSE. Pflüg. Arch. ges. Physiol. 120, 66-92.

GERSH, I. (1939). THE STRUCTURE AND FUNCTION OF THE PARENCHYMATOUS GLANDULAR CELLS IN THE NEUROHYPOPHYSIS OF THE RAT. Amer. J. Anat. 64, 407-429.

GOMORI, G. (1941). OBSERVATIONS WITH DIFFERENTIAL STAINS ON HUMAN ISLETS OF LANGERHANS. Amer. J. Path. 17, 395-406.

HANDLEY, CARROL A. & KELLER, A. D. (1950). CHANGES IN RENAL FUNCTION PRODUCED BY MORPHINE IN NORMAL DOGS AND DOGS WITH DIABETES INSIPIDUS. J. Pharmacol. 99, 33-37.

HARRIS, G. W. (1948a). NEURAL CONTROL OF THE PITUITARY GLAND. Physiol. Rev. 28, 139-179.

HARRIS, G. W. (1948b). HYPOTHALAMIC CONTROL OF THE ANTERIOR PITUITARY GLAND. Ciba Foundation: Colloquia on Endocrinology. IV. 106-114.

HERRING, P. T. (1908). THE HISTOLOGICAL APPEARANCES OF THE MAMMALIAN PITUITARY BODY. Quart. J. exp. Physiol. 1, 121-159.

HICKEY, R. C., HARE, K. & HARE, RUTH (1941). SOME CYTOLOGICAL AND HORMONAL CHANGES IN THE POSTERIOR LOBE OF THE RAT'S PITUITARY AFTER WATER DEPRIVATION AND STALK SECTION. Anat. Rec. 81, 319-332.

HILD, W. (1951). EXPERIMENTELL-MORPHOLOGISCHE UNTERSUCHUNGEN ÜBER DAS VERHALTEN DER NEUROSEKRETORISCHEN "BAHN" NACH HYPOPHYSENSTIELDURCHTRENNUNGEN, EINGRIFFEN IN DEN WASSERHAUSHALT UND BELASTUNG DER OSMOREGULATION. Virchows Arch. 319, 526-546.

HILD, W. & ZETLER, G. (1953). EXPERIMENTELLER BEIWEIS FÜR DIE ENTSTEHUNG DER SOG. HYPOPHYSENHINTERLAPPENWIRKSTOFFE IM HYPOTHALAMUS. Pflüg. Arch. ges. Physiol. 257, 169-201.

HOWELL, W. H. (1898). THE PHYSIOLOGICAL EFFECTS OF EXTRACTS OF THE HYPOPHYSIS CEREBRI AND INFUNDIBULAR BODY. J. exp. Med. 3, 245-258.

HYDEN, H. (1943). PROTEIN METABOLISM IN THE NERVE CELL DURING GROWTH AND FUNCTION. Acta physiol. scand. 6, Supp. 17.

KLISIECKI, A., PICKFORD, MARY, ROTHSCHILD, P. & VERNEY, E. B. (1933). THE ABSORPTION AND

EXCRETION OF WATER BY THE MAMMAL. (PT. 1). Proc. Roy. Soc. B. 112, 496-521.

KOVACS, K. & BACHRACH, D. (1951). HYPOTHALAMUS AND WATER METABOLISM STUDIES ON THE ANTIDIURETIC SUBSTANCE OF THE HYPOTHALAMUS AND HYPOPHYSIS. Acta med. scand. 141, 137-152.

LIBRETTI, A. & MARTINI, L. (1953). STUDI SUI RAPPORTI TRA IPOFISI POSTERIORE E IPOFISI ANTERIORE -NOTA 2. Arch. int. Pharmacodyn. 93, 163-166.

LIPSCHITZ, W. I. & STOKEY, E. (1947). MECHANISMS OF ANTIDIURESIS IN THE DOG AND IN THE RAT. Amer. J. Physiol. 148, 259-268.

MANN, G. (1894). HISTOLOGICAL CHANGES INDUCED IN SYMPATHETIC, MOTOR AND SENSORY NERVE CELLS BY FUNCTIONAL ACTIVITY. J. Anat., Lond. 29, 100-108.

MARTINI, L. & MORPURGO, C. (1955). NEUROHORMONAL CONTROL OF THE RELEASE OF ADRENOCORTICOTROPHIC HORMONE. Nature, Lond. 175, 1127-1128.

MELVILLE, ELEANOR, V. & HARE, K. (1945). ANTIDIURETIC MATERIAL IN THE SUPRAOPTIC NUCLEUS. Endocrinology 36, 332-339.

MIRSKY, I. A., STEIN, M. & PAULISCH, G. (1954). THE SECRETION OF AN ANTIDIURETIC SUBSTANCE INTO THE CIRCULATION OF ADRENALECTOMISED AND HYPOPHYSECTOMISED RATS EXPOSED TO NOXIOUS STIMULI. Endocrinology 55, 28-39.

MULLER, W. (1871). UEBER DEN BAU DER CHORDA DORSALIS UND DES PROCESSUS INFUNDIBULI CEREBRI. Jena. Z. Naturw. 6, 354. Cited by Herring, P. T. (1908). Quart. J. exp. Physiol. 1, 121-159.

OLIVER, G. & SCHÄFER, E. A. (1894). ON THE PHYSIOLOGICAL ACTION OF EXTRACT OF THE SUPRARENAL CAPSULES. J. Physiol. 16, i-iv.

OLIVER, G. & SCHÄFER, E. A. (1895). ON THE PHYSIOLOGICAL ACTION OF EXTRACTS OF THE PITUITARY BODY AND CERTAIN OTHER GLANDULAR ORGANS. J. Physiol. 18, 277-279.

PICKFORD, MARY (1939). THE INHIBITORY EFFECT OF ACETYL CHOLINE ON WATER DIURESIS IN THE DOG, AND ITS PITUITARY TRANSMISSION. J. Physiol. 95, 226-238.

PIERCE, J. G. & DU VIGNEAUD, V. (1950). PRELIMINARY STUDIES ON THE AMINO-ACID CONTENT OF A HIGH POTENCY PREPARATION OF THE OXYTOCIC HORMONE OF THE PITUITARY GLAND. J. biol. Chem. 182, 359-366.

RETZIUS, G. (1894). DIE NEUROGLIA DER NEUROHYPOPHYSE DER SÜNGETHIERE. Biol. Untersuch. 6, 21-24.

RICHTER, C. P. (1930). EXPERIMENTAL DIABETES INSIPIDUS. Brain, 53, 76-85.

RIOCH, D. McK., WISLOCKI, G. M. & O'LEARY, J. L. (1940). A PRÉCIS OF PREOPTIC, HYPOTHALAMIC AND HYPOPHYSIAL TERMINOLOGY WITH ATLAS. Res.

Publ. Ass. nerv. ment. Dis. 20, 3-30.

ROTHBALLER, A. B. (1953). CHANGES IN THE RAT NEUROHYPOPHYSIS INDUCED BY PAINFUL STIMULI WITH PARTICULAR REFERENCE TO NEUROSECRETORY MATERIAL. Anat. Rec. 115, 20-41.

SATO, G. (1928). UBER DIE BEZIEHUNGEN DES DIABETES INSIPIDUS ZUM HYPOPHYSENHINTERLAPPEN UND ZUM TUBER CINEREUM. Arch. exp. Path. Pharmak. 131, 45-69.

SCHÄFER, E. A. (1915). NOTE ON PRECEDING PAPER BY SIMPSON AND HILL: "THE MODE OF ACTION OF PITUITARY EXTRACT ON THE MAMMARY GLAND". Quart. J. exp. Physiol. 8, 379-383.

SCHARRER, E. & SCHARRER, B. (1940). SECRETORY CELLS WITHIN THE HYPOTHALAMUS. Res. Publ. Ass. nerv. ment. Dis. xx, 170-194.

SCHARRER, E. A. & WITTENSTEIN, G. J. (1952). THE EFFECT OF THE INTERRUPTION OF THE HYPOTHALAMO-HYPOPHYSEAL NEUROSECRETORY PATHWAY IN THE DOG. Anat. Rec. 112, 387.

SCHARRER, E. & SCHARRER, B. (1954). HORMONES PRODUCED BY NEUROSECRETORY CELLS. Recent Progr. Hormone Res. 10, 183-240.

SCHWALBE, G. (1881). LEHRBUCH DER NEUROLOGIE, 476-479. Erlangen: Eduard Besold.

SCHWARZ, C. & WIECHOWSKI, W. (1914). METHODE ZUR ANLEGUNG EINER PERMANENTEN BLASENFISTEL. Z bl. Physiol. 28, 439-446.

SHANKLIN, W. M. (1943). ON THE PRESENCE OF NERVE CELLS IN THE NEUROHYPOPHYSIS OF THE DOG. J. Anat., Lond. 77, 241-242.

SLOPER, J. C. (1954). HISTOCHEMICAL OBSERVATIONS ON THE NEUROHYPOPHYSIS IN DOG AND CAT WITH REFERENCE TO THE RELATIONSHIP BETWEEN NEUROSECRETORY MATERIAL AND POSTERIOR LOBE HORMONE. J. Anat., Lond. 88, 576-577.

SMITH, S. W. (1951). THE CORRESPONDENCE BETWEEN HYPOTHALAMIC NEUROSECRETORY MATERIAL AND NEUROHYPOPHYSIAL MATERIAL IN VERTEBRATES. Amer. J. Anat. 84, 195-231.

STARLING, E. H. & VERNEY, E. B. (1925). THE SECRETION OF URINE AS STUDIED ON THE ISOLATED KIDNEY. Proc. Roy. Soc. B. 97, 321-363.

STEHLE, R. L. & BOURNE, W. (1928). THE EFFECTS OF MORPHINE AND ETHER ON THE FUNCTION OF THE KIDNEYS. Arch. intern. Med. 42, 248-255.

THOMPSEN, ELLEN (1954). EXPERIMENTAL EVIDENCE FOR THE TRANSPORT OF SECRETORY MATERIAL IN THE AXONS OF THE NEUROSECRETORY CELLS OF CALLIPHORA ERYTHROCEPHALIA MEIG. Pubb. Staz. zool. Napoli. 24, Supp. Convegno sulla neurosecrezione. 48-49.

TOLDT, C. (1884). LEHRBUCH DER GEWEBELEHRE. 2er. Auflage. 290-291. Stuttgart: Ferdinand Enke.

TRENDELENBURG, P. (1928). ANTEIL DER HYPOPHYSE UND DES HYPOTHALAMUS AM EXPERIMENTELLEN DIABETES INSIPIDUS. Klin. Wschr. 7, 1679-1680.

TURNER, R. A., PIERCE, J. G. & DU VIGNEAUD, V. (1951). THE PURIFICATION AND THE AMINO-ACID CONTENT OF VASOPRESSIN PREPARATIONS. J. biol. Chem. 191, 21-28.

VAN DYKE, H. B., BAILEY, P. & BUCY, P. C. (1929). THE OXYTOCIC SUBSTANCE OF CEREBROSPINAL FLUID. J. Pharmacol. 36, 545-610.

VAN DEN VELDEN, R. (1913). DIE NIERENWIRKUNG VON HYPOPHYSENEXTRAKTEN. BEIM MENSCHEN. Klin. Wschr. 50, (2), 2083-2086.

VERNEY, E. B. (1926). THE SECRETION OF PITUITRIN IN MAMMALS, AS SHOWN BY PERFUSION OF THE ISOLATED KIDNEY OF THE DOG. Proc. Roy. Soc. B. 99, 487-517.

VIGNEAUD, V. DU., RESSLER, CHARLOTTE & TRIPPETT, S. (1953). THE SEQUENCE OF AMINO-ACIDS IN OXYTOCIN WITH A PROPOSAL FOR THE STRUCTURE OF OXYTOCIN. J. biol. Chem. 205, 949-957.

VIGNEAUD, V. DU., RESSLER, CHARLOTTE, SWAN, J. M., ROBERTS, C. W. & KATSOYANNIS, P. G. (1954). THE SYNTHESIS OF OXYTOCIN. J. Amer. chem. Soc. 76, 3115-3121.

VOGT, M. (1953). VASOPRESSOR, ANTIDIURETIC AND OXYTOCIC ACTIVITIES OF EXTRACTS OF THE DOGS HYPOTHALAMUS. Brit. J. Pharmacol. 8, 193-196.

WEISS, P. (1944). EVIDENCE OF PERPETUAL PROXIMO-DISTAL GROWTH OF NERVE FIBRES. Biol. Bull., Wood's Hole 87, 160.

WILSON, J. WALTER. (1954). BASOPHILIC COMPONENTS OF THE CYTOPLASM. Journal of Histochemistry and Cytochemistry. 2, 317-321.

ZETLER, G. (1952). ÜBER DEN HORMONGEHALT VON HYPOPHYSENHINTERLAPPEN UND VORDEREN HYPOTHALAMUS DURSTEN DER HUNDE. Arch. exp. Path. Pharmak. 216, 193-195.

ZETLER, G. (1953). SIND ADIURETIN, VASOPRESSIN UND OXYTOCIN DRIE VERSCHIEDENE STOFFE, ODER NUR DIE WIRKUNGSKOMPONENTEN EINES EINZIGEN HORMON-MOLEKULE? Arch. exp. Path. Pharmak. 218, 239-250.