SOME EFFECTS OF OVARIAN HORMONES ON THE VASCULAR SYSTEM AND OTHER TISSUES

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by

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CONTENTS.

INTRODUCTION.

Although it is still less than half a century since the hormonal activity of ovarian extracts was first demonstrated, much has since been learned of the methods by which these hormones control the reproductive function of the female. In addition to this control, other effects have also been discovered. Among these are their vasodilator actions, and their water and electrolyte retaining effects, and it is with these two aspects that this thesis is concerned. As these topics are, in the present state of knowledge, so divergent, the thesis is presented in two parts; the first being concerned with the vascular effects of oestrogens and progesterone, and the second with their effects on water and electrolyte excretion.

The work reported was carried out during the tenure of a Muirhead research scholarship, and a communication on some of the results has been given to the Physiological Society. (St. Bartholemew's hospital, February, 1958)

I acknowledge with great pleasure my gratitude to Dr. Mary Pickford for her invaluable advice, and unfailing encouragement while the work was in progress.

PART 1.

THE VASCULAR EFFECTS OF OVARIAN HORMONES.

INTRODUCTION.

The earliest clinical observation on the vasodilator action of ovarian hormones was made by Maurice Raynaud in 1862. In his original description of the disease which bears his name, he wrote of one patient:-

"Menstruation does not appear to have any influence upon the appearance of the phenomenon, but it is a remarkable fact that the complete disappearance of attacks of local syncope has always been noted by this lady as the first index of a commencing pregnancy."

More direct evidence of this action was not produced until over half a century later, when preparations of the ovarian hormones became available. Although very impure by modern standards these extracts made possible direct experimental observation on the effects of their administration, and progress in this field expanded rapidly. McGrath (1935) found that ergot-induced gangrene in female rats could be prevented by simultaneous treatment with oestrogens, and shortly afterwards it was discovered that castrate male rats could be similarly protected. (Suzman, Freed, and Prag 1938). McGrath had been investigating such presenile vascular disturbances as thrombo-angiitis

obliterans, and his experiments on the effects of oestrogens were prompted by his observation that the disease had an apparent sex-linkage, being much less common among women. His later publication (McGrath and Hermann, 1944) on the use of oestrogens in the treatment of vascular diseases indicated that a number of such conditions could be relieved in this way, and he concluded that oestrogens were of considerable use in the treatment of vascular disturbances where there was a significant component of vaso-motor imbalance.

Experimental evidence has confirmed the vasodilator action of oestrogens in both animals and humans. Most of the observations on humans have been made by Reynolds, though his earliest work was done on animals. He found that injections of oestrogens into ovariectomised rabbits caused a dilatation of the pre-capillary arterioles and small venules in the ear, with a steady decrease in skin temperature, but without a change in blood pressure. (Reynolds and Foster, 1939a). In 12 out of 20 human males tested, similar injections caused an increase in the volume of the finger, without any marked effect on the finger skin temperature. The lack of response in the remaining cases was unexplained. The character of the response suggested that it was due to a dilatation of the minute vessels of the skin, although there was no measurable increase in the rate of blood

flow through the skin. His observations were thereafter primarily concerned with the dermo-vascular actions of the hormones, and a comparative study was reported (Reynolds, Hamilton, di Palma, Hubert, and Foster, 1942) on castrate, eunuchoid, and normal men. The results were largely negative, though there was some indication that there was a higher degree of fluctuation in the exitability of the skin vessels in the castrates than in the other groups. In women, Abramson, Zaleela and Schkloven (1941) confirmed the dilator action of oestrogens on the hand, but not on the foot, leg, or forearm.

Although oestrogens have been shown to have no acute effect on the blood pressure of rabbits, there is some evidence that long term administration may have such an effect. Grollman, Harrison and Williams (1940) found that daily injections of oestrogen or progesterone to rats caused in most a gradual elevation of the blood pressure to hypertensive levels, and attributed this to renal injury, since the effect could be abolished by the administration of renal extracts. This observation was confirmed by Leathem and Dill (1943) who also investigated the response to such treatment in hypophysectomised rats. In these animals, the blood pressure of untreated hypophysectomised controls decreased to reach a steady low level. Oestrogen administration caused a greater fall in

the blood pressure, below that of the operated controls.

Clinically, oestrogens have been used for some time in the relief of various vascular disorders. The rationale of their use in conditions of vascular constriction is obvious, due to their dilator action. But in conditions such as the menopause, though presumably correcting an insufficiency, the actual mechanism is less obvious, in terms of direct vascular actions, since the flushes are obviously of the nature of a transient vasodilatation. The possibility suggests itself that, as well as a direct effect on the vascular system, oestrogens may modify the effects of other vasoactive substances. There is a certain amount of evidence to support this hypothesis. Byrom, (1938) used the degree of renal damage in rats in response to a single subcutaneous dose of vasopressin as a test response, and found that it was increased by oestrogen administration to ovariectomised animals. The effect of progesterone appeared to depend on the oestrogen/progesterone ratio, and this was not measurable. The pressor response to adrenaline in dogs was used by Boxill and Brown (1955) in tests on animals with different hormone backgrounds; among the different groups tested, only the dogs with intact ovaries, in oestrus from oestrogen administration, showed any increase in pressor response over the other groups,

in response to a standard dose of adrenaline. They considered therefore that the presence of both oestrogen and progesterone was important for this increased response. Contrary to these results are those of Vick, Ederstrom, and Verger (1956) who found in rats that the sensitivity of isolated strips of blood vessels to adrenaline was increased by castration in females, though this had little effect in males.

The present position of our knowledge on the effects of ovarian hormones on the circulation therefore appears to be far from complete. The problems heavily outweigh the known facts. All that has really been established is that oestrogens exert a dilator effect on the minute vessels, for example those of the uterus, skin, and possibly muscle. In this we are hardly more advanced than at the time of Raynaud's original discovery. Progesterone may or may not enhance this action. The ovarian hormones may sensitise tissues to the effect of other substances. Some of the problems which remain as yet unsolved are the basic mechanism of these effects, and the etiology of the symptoms which may accompany imbalance of the ovarian hormones, such as the menopausal flushes. A further complication in the evaluation of the experimental work on the subject is in the wide variety of oestrogenic substances which have been used by different workers, and the differences

which may arise from the use of different experimental animals. How far the results are comparable is as yet unknown. One can only assume that they may be so. The work reported here was undertaken to try to elucidate some of the outstanding problems in this field of research.

METHOD OF APPROACH TO PROBLEM.

The problem was approached by making use of substances other than oestrogens, and which were known to be vaso-active. It was possible that changes in their vascular effects might occur if the ovarian hormones were removed or administered, and that such changes might throw some light on the action of the ovarian hormones. The vaso-active substances used were mainly vasopressin and oxytocin, though some observations were also made using nor-adrenaline and acetyl choline.

The hormones of the posterior lobe of the pituitary gland have been known for some time to have actions on the circulatory system. In 1911 Campbell tested whole pituitary extracts on perfused isolated blood vessels of cats, and concluded that the gland contained at least two substances with vascular actions; one causing contraction, and one relaxation of the vessels. He thought that each could exert its effect on all the arteries, except those of the kidney, where relaxation nearly always occurred. Although he was using an extract of the whole pituitary, it would appear likely that the effects he observed were in fact due to the hormones of the posterior lobe, as no vascular actions have ascribed to those of the anterior lobe of the gland. In the following year it was found that in the bird whole posterior lobe extracts caused a fall in blood pressure in the majority, due to dilatation of

the peripheral arteries. (Paton and Watson, 1912) In the cases where there was no depressor action, they considered the increase in ventricular contraction sufficient to counteract the arterial dilatation. In other species the effects differ. Gaddum (1928) using the separated hormones, vasopressin and oxytocin, found that a depressor action could be obtained with oxytocin on some occasions, in cats, if the tone of the vessels was high after repeated injections of vasopressin. Some animals failed to give this response, for reasons which could not be elucidated. A pressor response was invariably obtained with vasopressin. Woodbury and Abreu (1944) in a comparative study of the vascular effects of oxytocin, found species differences to exist. Thus in the rabbit and cat a dilator and depressor action was found, but in the dog no depressor action could be obtained. On the other hand, using the dog, Brooks and Pickford (1957,1958) have found that although oxytocin caused no change in systemic blood pressure, it regularly dilated the renal vessels. In a survey of the results which had been obtained in different animals, Sawyer and Sawyer (1952) stated that oxytocin was depressor in the frog, toad, alligator and bird, but had no effect on the blood pressure of the rat, while vasopressin was pressor in all except the toad.

It therefore seemed of interest to compare

the actions of these two hormones on the blood vessels, especially in the light of the known actions of oxytocin on the vascular system of the female genital organs, and in addition to test the responses to other pressor substances such as nor-adrenaline. Difficulty existed in finding pure naturally produced vasodilators, and since oxytocin has this property, this was another reason for its use in the present studies.

EXPERIMENTAL METHODS.

Most experiments were made on female albino rats, and so far only 5 males have been used. All the animals were anaesthetised with 5mg/100g body weight intraperitoneal sodium pentobarbitone. Rats of approximately 200g body weight were used throughout, to obviate the necessity of calculating comparable dose levels of the test substances for each animal. All results given are based on 200g animals. Two experimental methods were used to study the vascular reactions to the test drugs. Firstly, the arterial blood pressure was recorded kymographically by means of a polythene cannula tied into the carotid artery, and connected to a mercury manometer with a float recorder. The femoral vein was also cannulated, and all injections made by this route. In the second method, the blood vessels of the mesentery were viewed under a low power microscope, so that direct observation could be made of the changes occurring in the calibre of the vessels on either intravenous or topical administration of drugs. Zweifach and Chambers, who devised the mesenteric method for the observation of small vessels in the rat, found that where an intravenously administered agent produces no visible constriction or dilatation, nevertheless changes in tone can be detected by the fact that there is a change in the threshold constrictor dose of topically applied adrenaline.

In some cases blood pressure was recorded at the same time as the mesenteric vessels were observed, but this presented many technical difficulties. For instance, the heparin which must be injected for blood pressure recording sometimes caused localised oozing, which obscured the blood vessels. Moreover, it is difficult for a single observer to watch the size of the mesenteric vessels, and to observe the pressure recording simultaneously. Thus separate observations proved more satisfactory once the relation of vessel response to pressure change had been established.

The vasopressin used was du Vigneaud's highly purified natural substance, except where it is stated that Parke Davies' Pitressin was employed. The oxytocin was the synthetic brand Syntocinon (Sandoz). The ovarian hormone preparations were Stilboestrol diproprionate and Progestin, both obtained from British Drug Houses.

In order to study as many different natural and induced hormone levels as possible, the following groups of animals were tested:

1. Normal rats.
2. Ovariectomised rats.
3. Normal, with administered ovarian hormones.
4. Ovariectomised, with administered hormones.
5. Pregnant and lactating rats.

Experiments were also conducted to determine the effects of such procedures as vagotomy and decerebration on the responses.

RESULTS.

NORMAL RATS.

The results in this group were intended to provide the 'normal' responses, with which those of other groups could be compared. However, a considerable variation was found, in both the blood pressure responses to the posterior lobe hormones, and in the reactivity of the mesenteric vessels after their injection or topical application. As expected, after intravenous injection, vasopressin always caused a rise in blood pressure in all animals, though the least dose to produce this effect was variable. (Table 1) With the mesenteric preparation, constriction of the arterioles and capillaries invariably occurred after either intravenous injection or topical application of vasopressin. (Table 2). With large doses of vasopressin the change in calibre of the vessels was obvious, but with small doses the constriction was more easily detected by the increased sensitivity to a following topical application of adrenaline. In the 6 experiments where blood pressure was recorded simultaneously with observation of the vessels, it was found that the constriction lasted the same length of time as the elevation of blood pressure. This time varied with the size of dose, ranging from approximately 30 secs for threshold doses to as much as 7 or 8 minutes for very large, unphysiological doses. The

Test drug	No. of rats	Blood pressure response		
		Rise	Fall	No effect
Vasopressin	15	15 (0.05-0.5mU)	0	0
Oxytocin	17	3 (10-20mU)	0	14 (2-100mU)

Table 1. The effects of posterior pituitary hormones on the carotid blood pressure of normal female rats without reference to the phase of the oestrous cycle.
Threshold doses for effect induced given in brackets.

Test drug	No. of rats	Mesoappendix vessels	
		Constriction	Dilatation
Vasopressin	14	14 (0.05-0.5mU)	0
Oxytocin	16	3 (15-20mU)	13 (20-30mU)

Table 2. The effects of posterior pituitary hormones on the mesoappendix vessels of normal female rats, without reference to the oestrous cycle.
Threshold doses for effect induced given in brackets.

The threshold dose was the same for both observed effects. With oxytocin the effects were less constant. In 14/17 animals, i.e. 83%, the intravenous injection of from 2 to 100 mU had no effect on the blood pressure, while in the remaining animals there was a pressor response to doses of above 10 to 20 mU. (Table 1). The mesenteric preparation showed a similarly variable response. (Table 2). In the majority (13/16, i.e. 81%-) there was a dilatation of the arterioles and capillaries, after either intravenous or topical administration, and in the remaining 19% a constrictor response was obtained by either route. Both methods showed that the constrictor response occurred when the pressor response was obtained, and the dilator response when there was no effect on the blood pressure. In the 5 males tested, oxytocin had no effect on the blood pressure, and the threshold dose of vasopressin was in the higher part of the range found in females. (Table 1) In all experiments on the normal females and males, the hormones were injected at least 8 times in each rat, and the results obtained in any one animal were constant.

No notice had been taken of the stage of the oestrous cycle when these observations were made, and it was later found that this had an important bearing on the type of response obtained. The experiments were therefore repeated, taking vaginal

smears just prior to observations, and it was found in 9 experiments that at all times during the cycle, except on the day of full oestrus, oxytocin was dilator, with no effect on the blood pressure. (Table 3). On the day of oestrus, however, oxytocin was pressor and constrictor. The type of response to oxytocin on any one day was not altered by increasing the dose given over a range of 2 to 100mU. Vasopressin was always pressor and constrictor, but on the day of oestrus the threshold dose was lower than at other times. Figures 1 and 2 show typical blood pressure records during oestrus and dioestrus. In the experiments from which the records are taken, control injections of 0.9% saline had no pressor effect, though this was found in some experiments. All injections were made in a standard volume of 0.3ml.

Stage of cycle	No. of rats	Blood Pressure		Mesoappendix Vessels	
		Rise	No effect	Constriction	Dilatation
Oestrus:	3				
Oxytocin		3	0	3	0
Vasopressin		3	0	3	0
All other stages:	6				
Oxytocin		0	6	0	6
Vasopressin		6	0	6	0

Table 3. The vascular effects of posterior lobe hormones in female rats, with reference to the phase of the oestrous cycle.

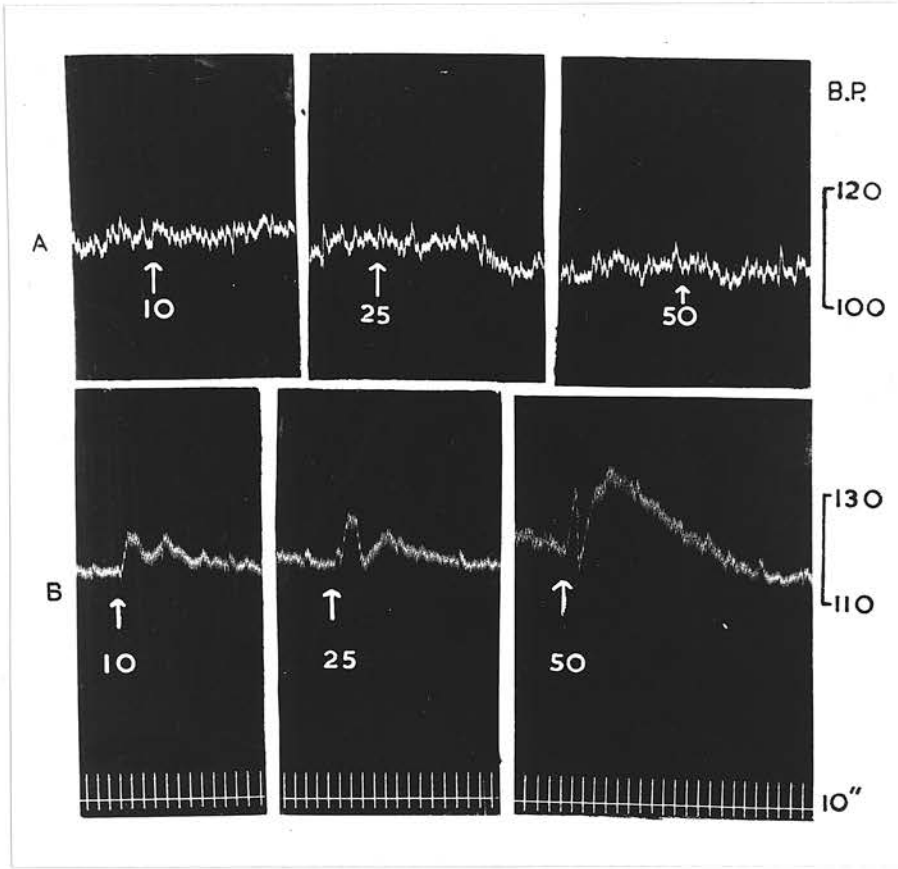

Figure 1. The effect of oxytocin on the blood pressure of the rat.

A. Dioestrus.

B. Oestrus.

The arrow indicates time of intravenous injection, and the number by the arrow the milliunits of oxytocin injected.

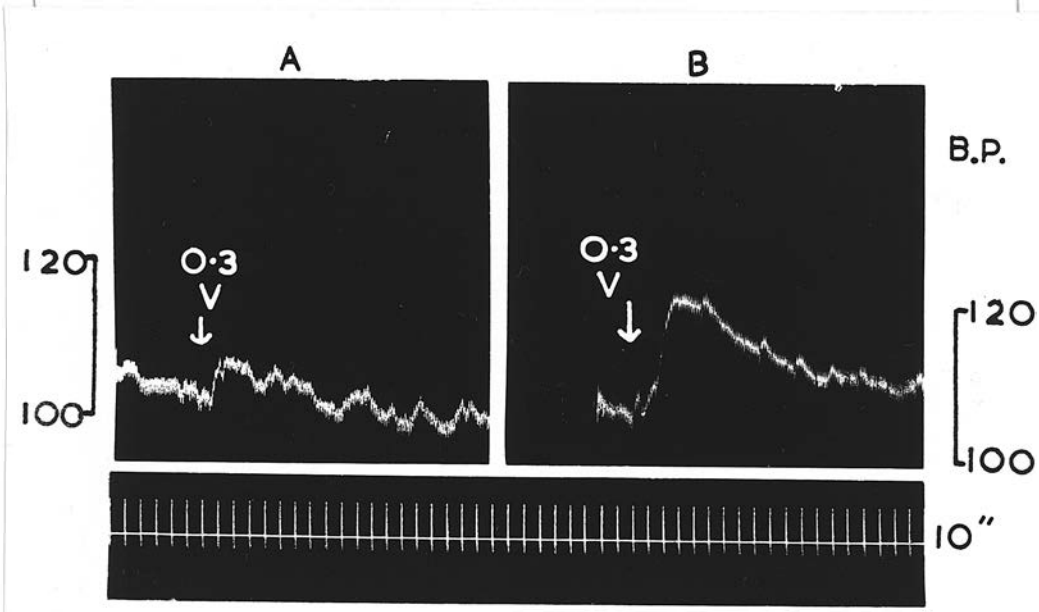

Figure 2. The effect of intravenous injection of 0.3 mU vasopressin on the blood pressure of the rat.

A. Dioestrus.

B. Oestrus.

OVARIECTOMISED RATS.

Bilateral ovariectomy was performed under ether anaesthesia in two series of 8 rats, and the animals taken serially in order to observe the vascular responses to oxytocin and vasopressin. In these animals the endogenous ovarian hormones were prevented from affecting the responses obtained. Although the results were consistent in that oxytocin now gave no pressor response, while vasopressin always did so, another fact emerged; this was the variation with time of the threshold doses required to give the responses. Figure 3 shows the threshold dose of oxytocin to give a dilatation of the mesenteric vessels (though as usual, no change in blood pressure), and of vasopressin to give a pressor response, plotted against days after ovariectomy. For the first two days there was no change in the threshold values; then from the 3rd until the 7th or 8th days the threshold dose of oxytocin was decreased by a factor of approximately five, while that of vasopressin was increased by a similar amount. The values then returned to normal dioestrous levels, and remained there for at least twenty days. The later values are not shown on the graph. Thus, above threshold levels, the sensitivity to oxytocin was increased for a few days, while that to vasopressin was decreased over a coterminous period. The dose levels shown are for 200g body weight, and are the average of the two series.

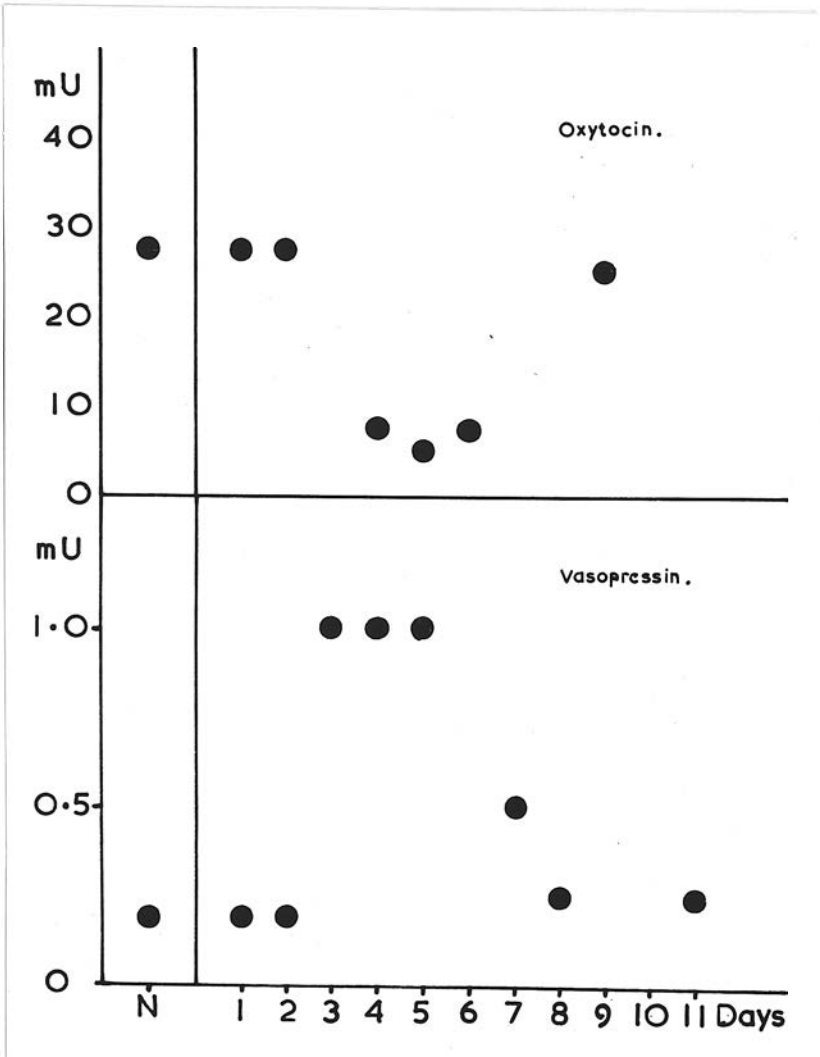

Figure 3. The threshold doses of posterior lobe hormones affecting the blood vessels, plotted against days after ovariectomy.

NORMAL RATS, WITH ADMINISTERED OVARIAN HORMONES.

The hormones were administered sub-cutaneously in oil, 24 or more hours before observations were made. The response of 15 female rats to posterior lobe hormones after such treatment are summarised in table 4. Animals were treated with various dose levels of the ovarian hormones, but in order to obtain a definite and clear cut response, stilboestrol at a dose of 3.5µg or more per 100g, and progesterone at or above 90µg per 100g were necessary, and in the results given these doses were always used. As on all occasions each animal was tested with a number of doses of both posterior lobe hormones. A pressor and constrictor response to oxytocin was obtained whenever stilboestrol or progesterone had been given on the previous day. On the second and subsequent days after ovarian hormone administration this type of response was lost, and the pattern reverted to that seen in dioestrous animals. As in the normal oestrous animal, the pressor response to vasopressin was enhanced whenever a pressor response to oxytocin was obtained. In the four cases where stilboestrol and progesterone were given together, the oestrous type of response was obtained in two, and the dioestrous type in two. Figure 4 shows typical examples of the blood pressure responses in this series.

Evaluation of these results was complicated

Days after hormone administration	No. of rats	Response to Oxytocin	
		Pressor	No effect
Stilboestrol			
Day 1	3	3	0
Day 2	2	0	2
Day 3	1	0	1
Progesterone			
Day 1	3	3	0
Day 2	2	0	2
Stilboestrol + Progesterone			
Day 1	4	2	2

Table 4. The effect of ovarian hormone administration on the blood pressure responses to oxytocin in normal rats.

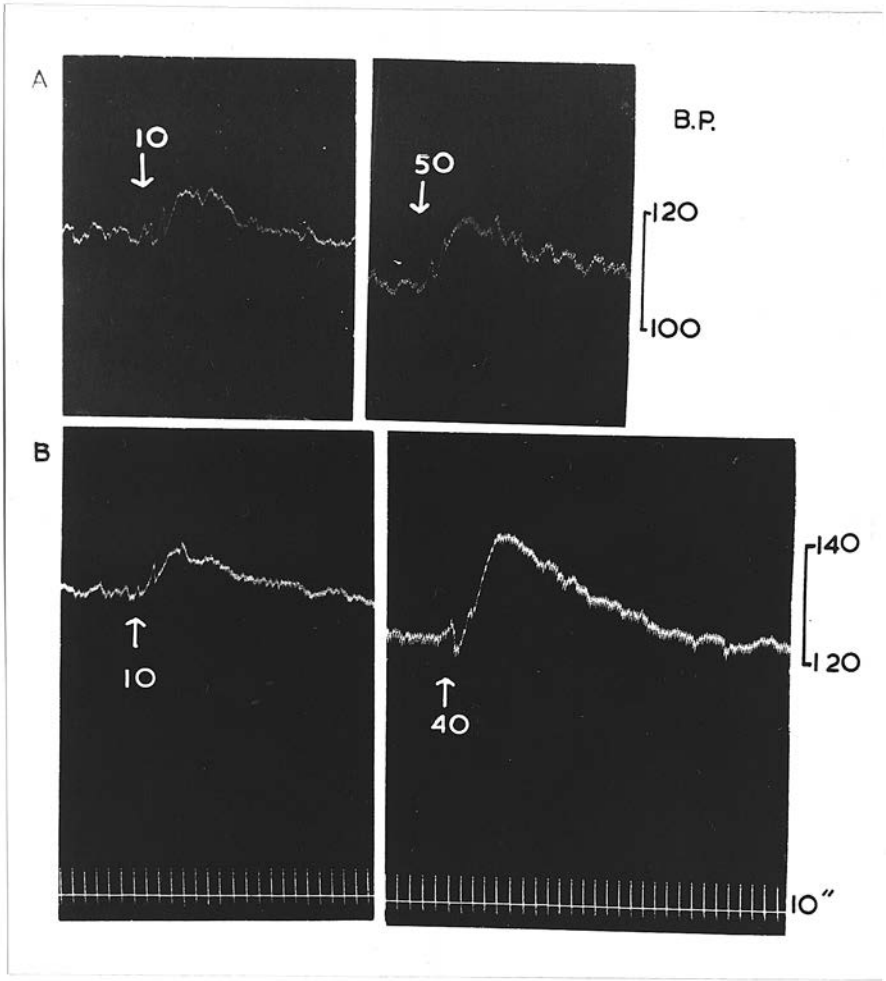

Figure 4. The effect of oxytocin on the carotid blood pressure of normal female rats, pretreated with oestrogen and progesterone.

A. Stilboestrol, 3.5µg per 100g.

B. Progesterone, 90µg per 100g.

Oxytocin given intravenously at arrows, and dose in mU shown by figures beside arrows.

by the unknown background of the animals' own natural hormones, and as the effects of simultaneous administration of stilboestrol and progesterone had produced variable results, it was thought advisable to repeat the series on ovariectomised rats, to determine the effects of purely exogenous hormones, and to try and determine whether a synergistic or antagonistic relationship existed between oestrogen and progesterone in this respect.

OVARIECTOMISED RATS, WITH ADMINISTERED HORMONES.

The results in 6 animals differed from those of normal rats. Table 5 summarises the results, and figure 5 shows responses typical of those obtained in this series. Neither stilboestrol (2 experiments), nor progesterone (2 experiments) alone caused the appearance of a pressor response to oxytocin, but when given together (4 experiments) in the proportions of one part of stilboestrol and up to forty parts of progesterone, the pressor response appeared on the day following the administration of the ovarian hormones. Again, where the pressor response to oxytocin was given, there was also an enhanced pressor response to vasopressin.

Ovarian Hormones Given	No. of rats	Blood Pressure Response to O	
		Rise	No Effect
Stilboestrol	2	0	2
Progesterone	2	0	2
Stilboestrol + Progesterone	4	4	0

Table 5. The effect of ovarian hormone treatment on the blood pressure responses to oxytocin in ovariectomised rats.

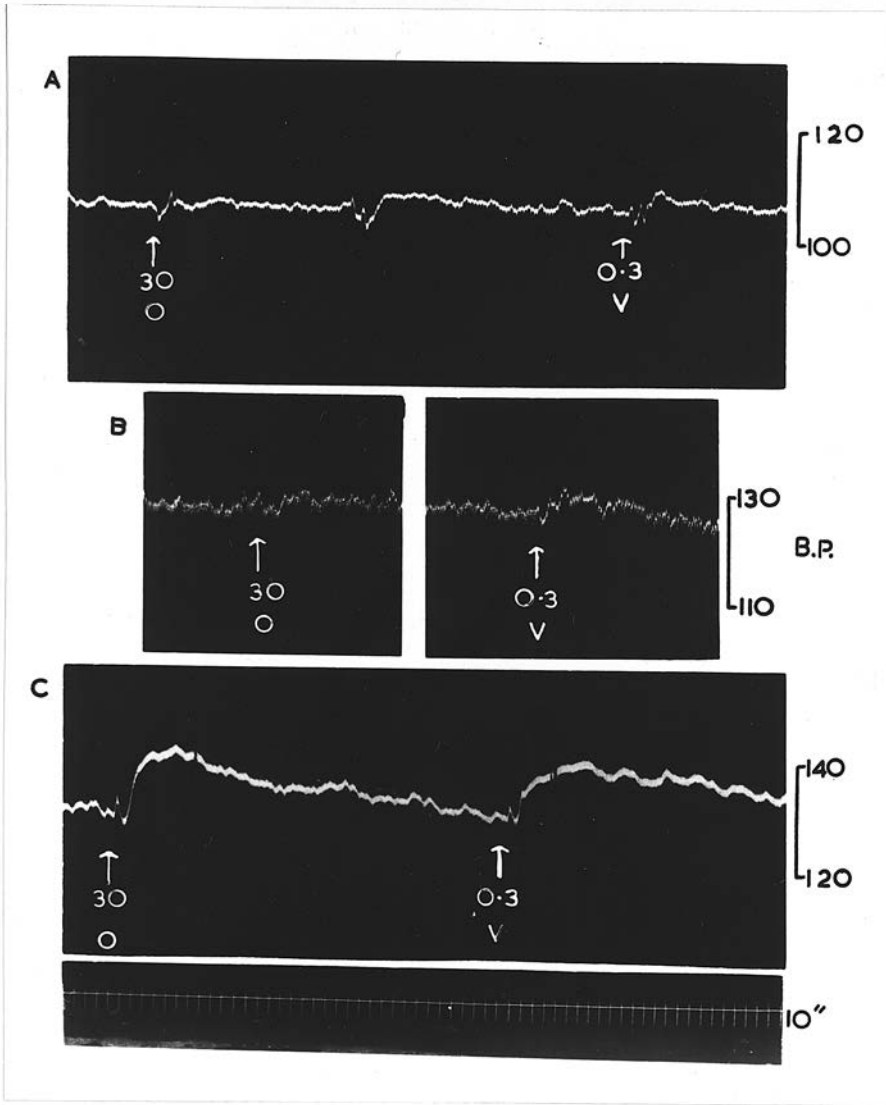

Figure 5. The effect of posterior lobe hormones on the carotid blood pressure of ovariectomised rats pretreated with ovarian hormones.

A. Stilboestrol, 3.5µg per 100g.

B. Progesterone, 90µg per 100g.

C. Stilboestrol + progesterone, doses as A and B.

Oxytocin and vasopressin by intravenous injection in doses indicated by figures beside arrows. Arrows show moment of injection.

PREGNANCY AND LACTATION.

A series of observations was made on the blood pressure responses of 11 rats through pregnancy and lactation. During the first half of pregnancy (until the tenth day), oxytocin had no effect on the blood pressure, while vasopressin had a pressor action comparable to that seen during dioestrus. After the tenth day of pregnancy, however, the responses were similsr to those of the oestrous rat. (Table 6 and figure 6) That is, vasopressin had a more marked pressor action, and oxytocin was also pressor. The magnitude of the pressor response to a given dose of either hormone increased as pregnancy progressed beyond the tenth day. The change in response at the tenth day was abrupt, i.e. on the ninth day the responses were of the dioestrous type, and on the tenth day of the oestrous type. However, only one series of observations has so far been made, and it is thus not possible to state definitely that the tenth day is invariably that on which the type of response obtained changes. By the twentieth day, presumably shortly before parturition, as other rats under observation all had twenty one day pregnancies, as little as 4mU of oxytocin was pressor, as compared with 10-20mU in normal oestrus. The threshold dose of vasopressin was not markedly decreased, being about 0.1mU, as compared with approximately 0.3mU during dioestrus, and 0.05-0.1mU during oestrus.

Day of pregnancy	No. of rats	Blood Pressure Responses			
		Vasopressin		Oxytocin	
		Rise	No effect	Rise	No effect
5	1	1	0	0	1
8	1	1	0	0	1
9	1	1	0	0	1
10	1	1	0	1	0
11	1	1	0	1	0
14	1	1	0	1	0
20	1	1	0	1	0
Parturition	1	1	0	0	1
Post partum 1	1	1	0	0	1
2	1	1	0	0	1
3	1	1	0	0	1

Table 6. The blood pressure responses to posterior pituitary hormones during pregnancy and lactation.

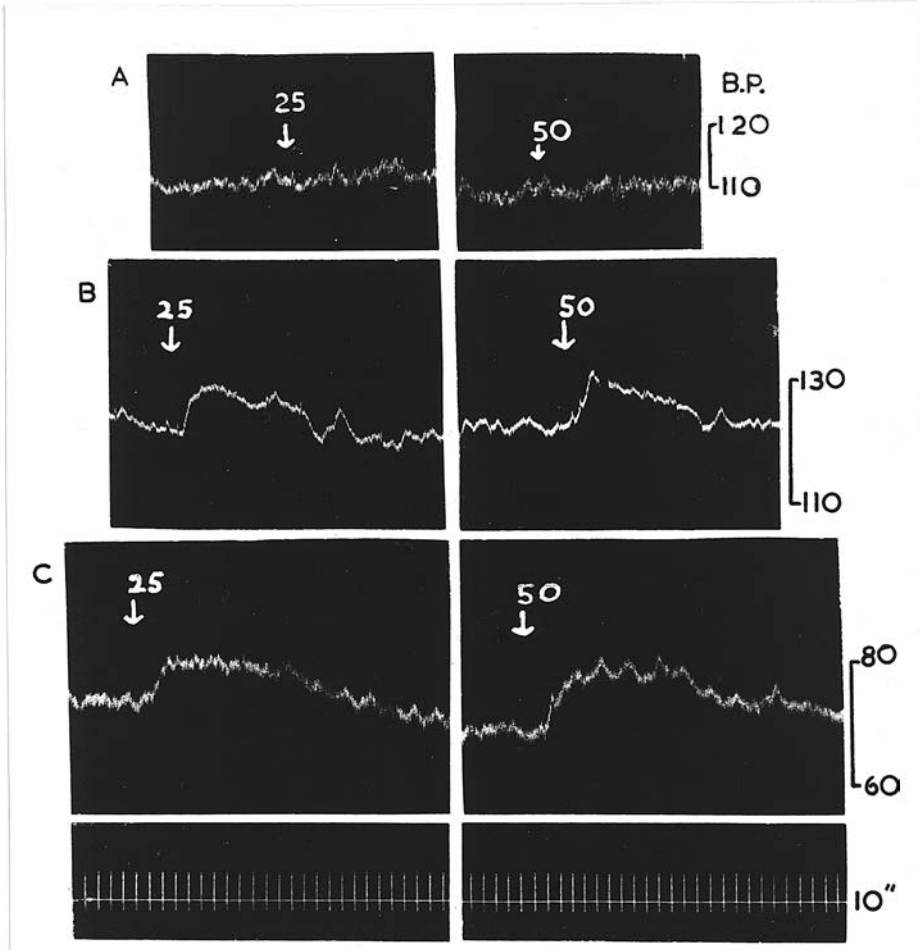

Figure 6. The effect of oxytocin on the blood pressure of the pregnant rat.

A. 9 days pregnant.

B. 14 days pregnant.

C. 20 days pregnant.

Arrows indicate the time of intravenous injection, and the numbers beside arrows the mU of oxytocin injected.

One rat was tested during parturition itself. Two pups had been born at the time of observation, and the uterus was found to contain four more. In this animal the dioestrous type of response was obtained. (Figure 7). During lactation in three animals, the response was also of the dioestrous type. (Figure 7). A considerable amount of milk ejection was observed in these animals on oxytocin injection, though no attempt was made to find the minimal dose which would produce this. No significant change was observed in general blood pressure levels in this series, with the exception of the one rat studied during parturition. In this animal the blood pressure was only 30mm Hg, but as only one of this type of animal was used, little importance can be attached to this.

No observations were made on the meso-appendix preparation in this series, but, from those made in other experiments, it may be assumed that where there was no action of oxytocin on the blood pressure, there would be a dilatation of the minute vessels, and a constrictor effect where a pressor response was obtained. Whether such observations would have shown a decreasing dilator response to oxytocin during the first half of pregnancy, until the day on which a constrictor response appeared, or whether such a change would be sudden, is not known.

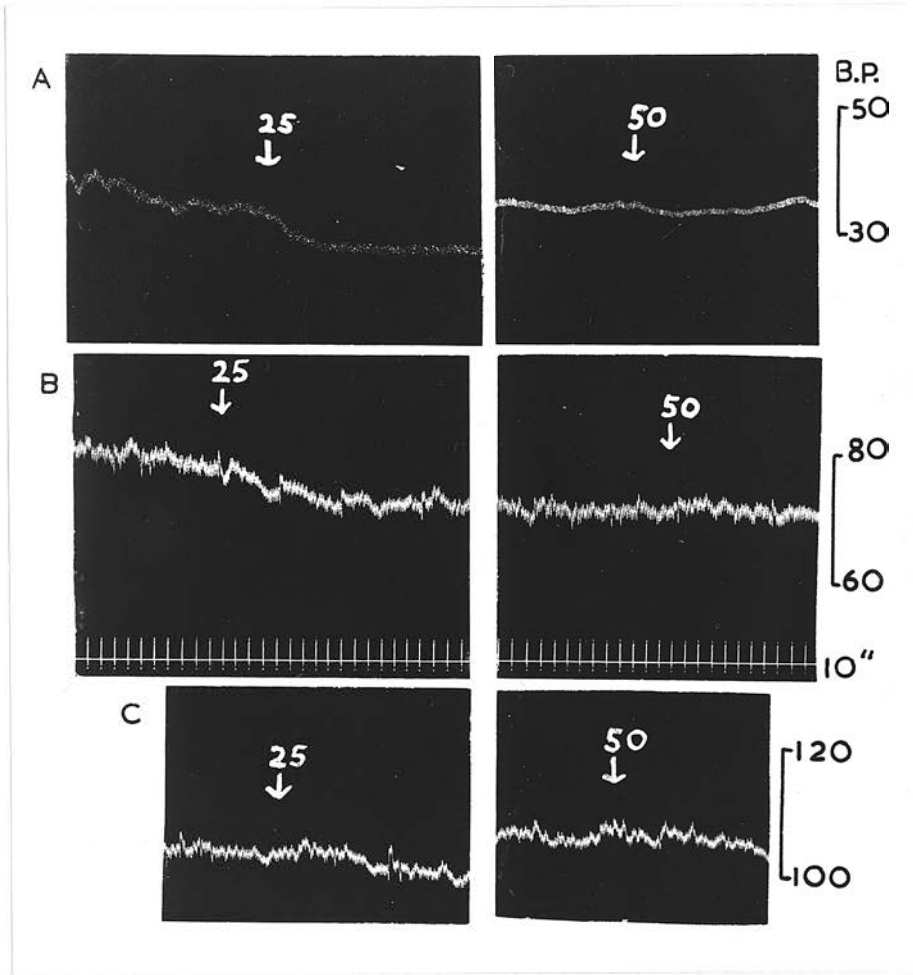

Figure 7. The effect of oxytocin on the blood pressure of the rat, during parturition and in the puerperium.

A. Parturition.

B. Day 1 post partum.

C. Day 2 post partum.

Arrows indicate time of intravenous injection, the figures the mU of oxytocin injected.

RESPONSES TO OTHER TEST SUBSTANCES.

Similar tests were made in nearly all the different series, with acetyl choline and nor-adrenaline. With acetyl choline, no cyclic difference in depressor effect was seen (9 rats tested), neither was there any change in this with ovariectomy, (15 rats), or administration of ovarian hormones (10 rats). The threshold dose for a depressor response was approximately 0.01µg in all cases. With nor-adrenaline, which was used in all experiments so far described, differences in pressor action were observed. These were similar to those found with vasopressin. i.e. there was an enhanced pressor response to a given dose during oestrus, and after ovarian hormone administration, and a reduced response after ovariectomy. Typical instances of this are shown in figure 8.

INVESTIGATION INTO THE MECHANISMS INVOLVED.

A few experiments were carried out to try to elucidate the mechanisms involved, both in the vascular responses to the posterior pituitary hormones, and in the effects of ovarian hormones on these responses. The fact that the dilator and constrictor effects in the meso-appendix preparation could be obtained by topical application of oxytocin and vasopressin suggested that their effect was, at least in part, peripheral.

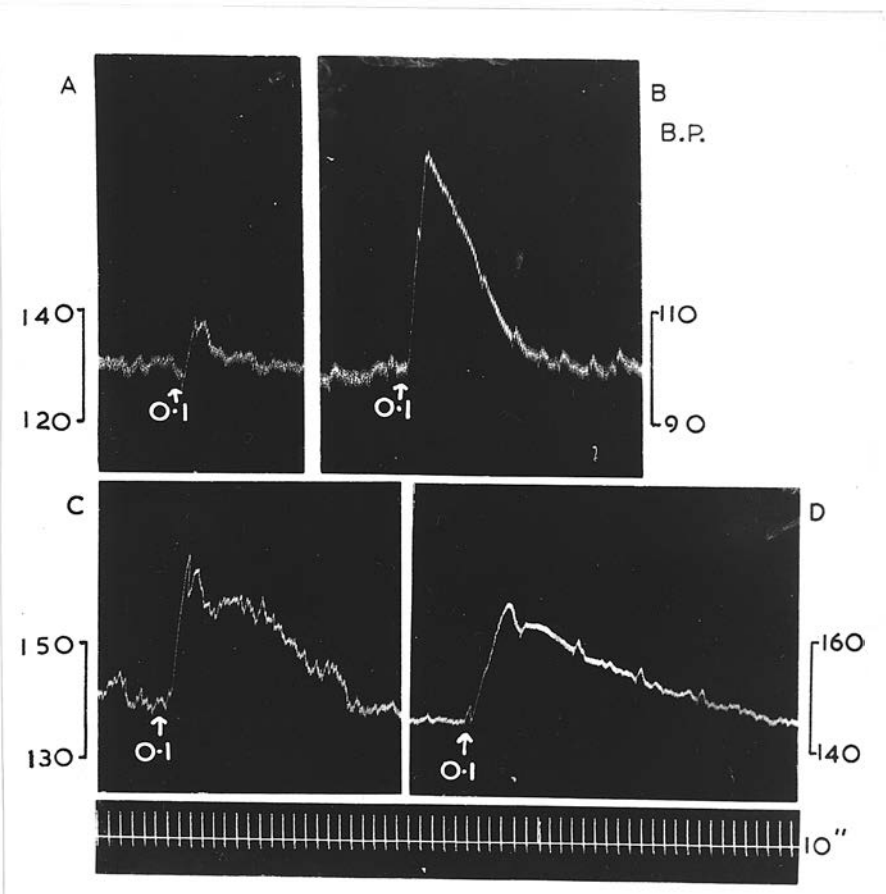

Figure 8. The effect of intravenous injection of 0.1ug nor-adrenaline on the blood pressure of the female rat.

A. Dioestrus.

B. Oestrus.

C. After stilboestrol, 3.5µg per 100g.

D. After progesterone, 90µg per 100g.

Effect of general blood pressure.

No correlation existed in any of the series between differences in general blood pressure levels and the responses obtained to posterior lobe hormones. The range of blood pressures was wide, (30-160mm Hg), and did not appear to be affected by ovariectomy, or ovarian hormone administration. In two rats the blood pressure was lowered by amyl nitrite inhalation, and in two by haemorrhage. Whilst the blood pressure was reduced by these procedures, vasopressin and oxytocin were injected. Though the results of such injections were obscured to some extent by the recovery in blood pressure levels, there did not seem to be any change in response to the posterior lobe hormones compared with those obtained before the blood pressure was lowered.

The effect of vagotomy.

Observations were made on the effect of bilateral vagotomy on the blood pressure responses of six rats. In the four dioestrous animals before vagotomy, oxytocin had as usual no pressor effect, but after vagotomy a pressor response was obtained with doses of oxytocin above 10mU. (Figure 9). In the two oestrous animals, in which there was a pressor response to oxytocin, vagotomy produced no enhancement of the effect, 10-20mU being the threshold pressor dose both before and after

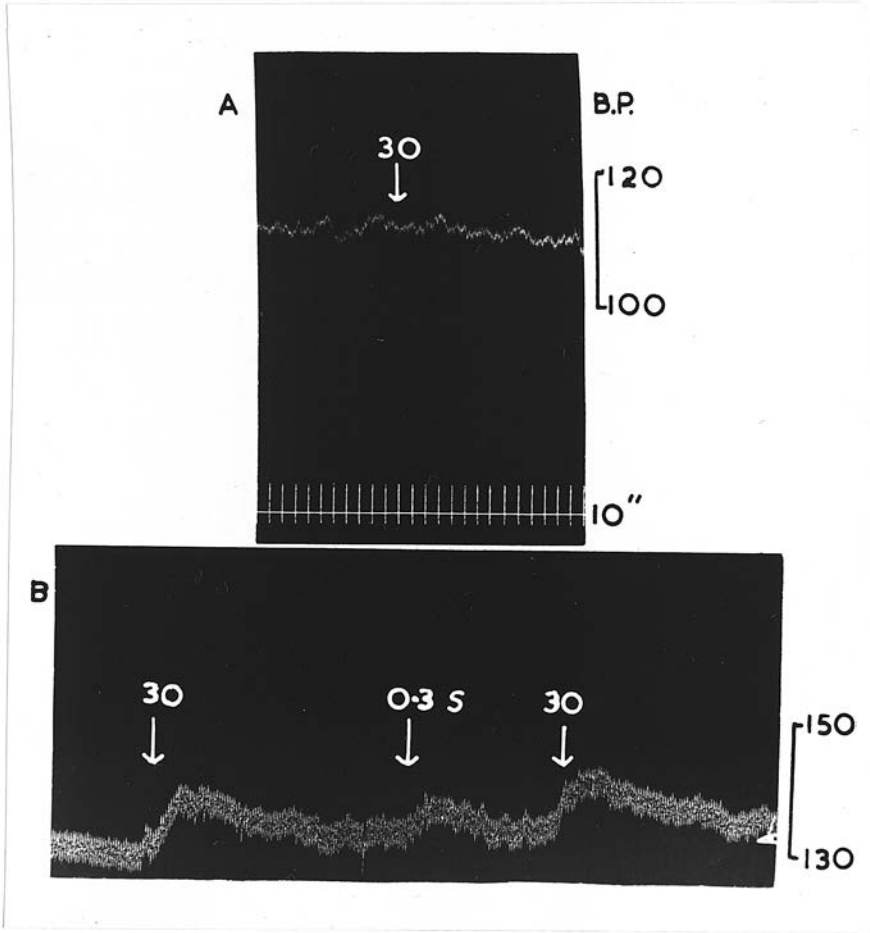

Figure 9. The effect of 30mU oxytocin on the blood pressure of the dioestrous rat, before and after bilateral vagotomy.

A. Before vagotomy.

B. After vagotomy.

Arrows indicate time of injection.

S indicates control injection of 0.3ml 0.9% saline.

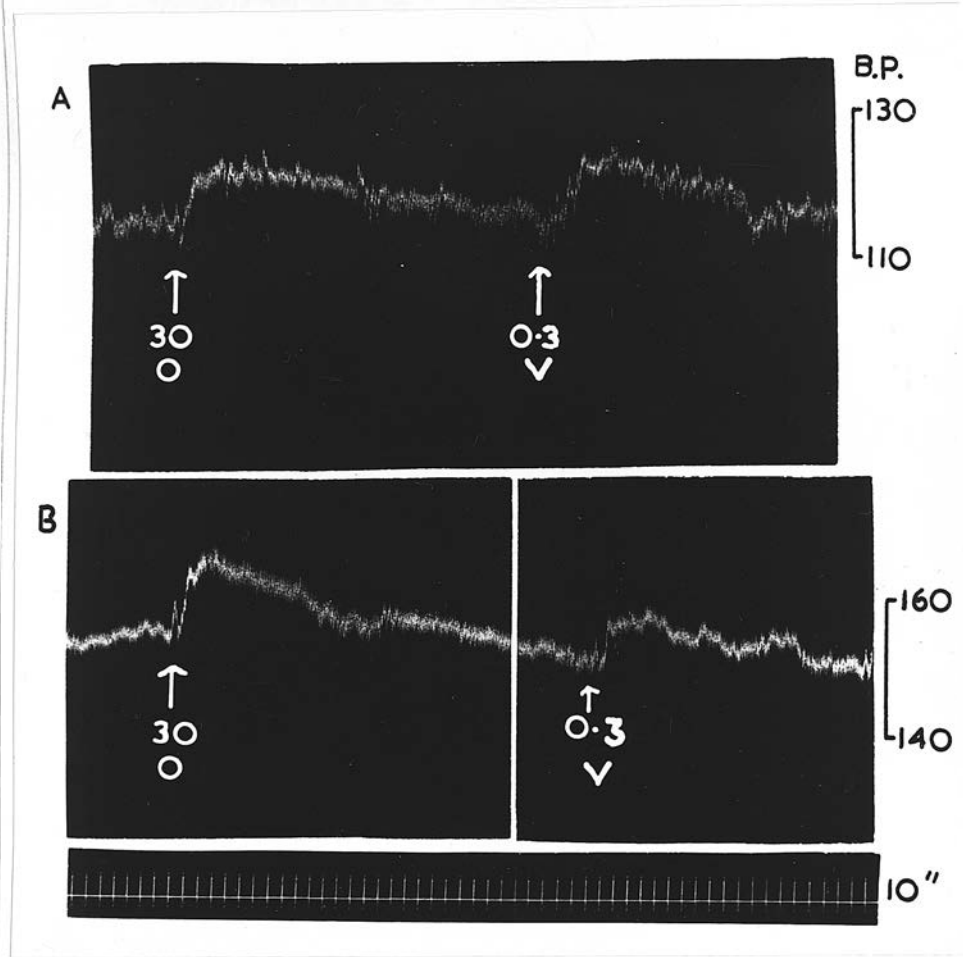

Figure 10. The effect of bilateral vagotomy on the blood pressure responses to 30mU oxytocin (O) and 0.3mU vasopressin (V) in the oestrous rat.

A. Before vagotomy.

B. After vagotomy.

nerve section. Vagotomy had no effect on the magnitude of the pressor response to vasopressin in any of the animals. (Figure 10).

The effect of decerebration.

8 rats were tested for the effects of decerebration. After this procedure, all animals gave a pressor response to oxytocin, and in one of the eight, which had been ovariectomised, this occurred with as little as 5mU. (Figure 11). Thus in both the three dioestrous animals, and the ovariectomised one, the normal dilator response was converted to a pressor one. The pressor responses to oxytocin and vasopressin of four rats in oestrus were still pressor after decerebration (Figure 12), and in two of the four, it appeared that the normal pressor response to oxytocin was enhanced by decerebration. The magnitude of the pressor response was not increased to a given dose of vasopressin or nor-adrenaline in any of the 8 animals in this series. (Figure 12).

The effect of atropine administration.

This was tested in six animals, three dioestrous, and three in oestrus. 0.2-0.4mg, injected intravenously, was found to be sufficient to block the depressor response to acetyl choline for at least one hour, and during this time vasopressin and oxytocin were also injected. The responses in all animals were the same as those found before atropinisation. (Figures 13 and 14)

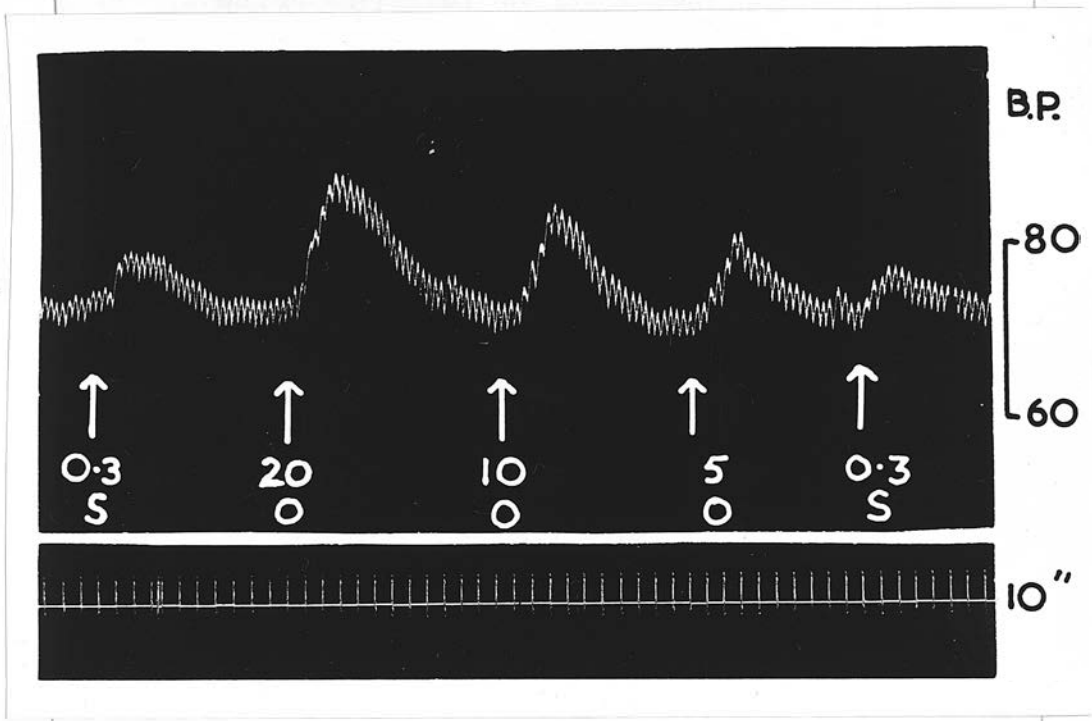

Figure 11. The effect of decerebration on the blood pressure responses to oxytocin.
Rat ovariectomised 9 days previously.
Arrows show times of injections, and figures the mU of oxytocin injected.
(0.3S is control injection of 0.3ml 0.9% saline.)

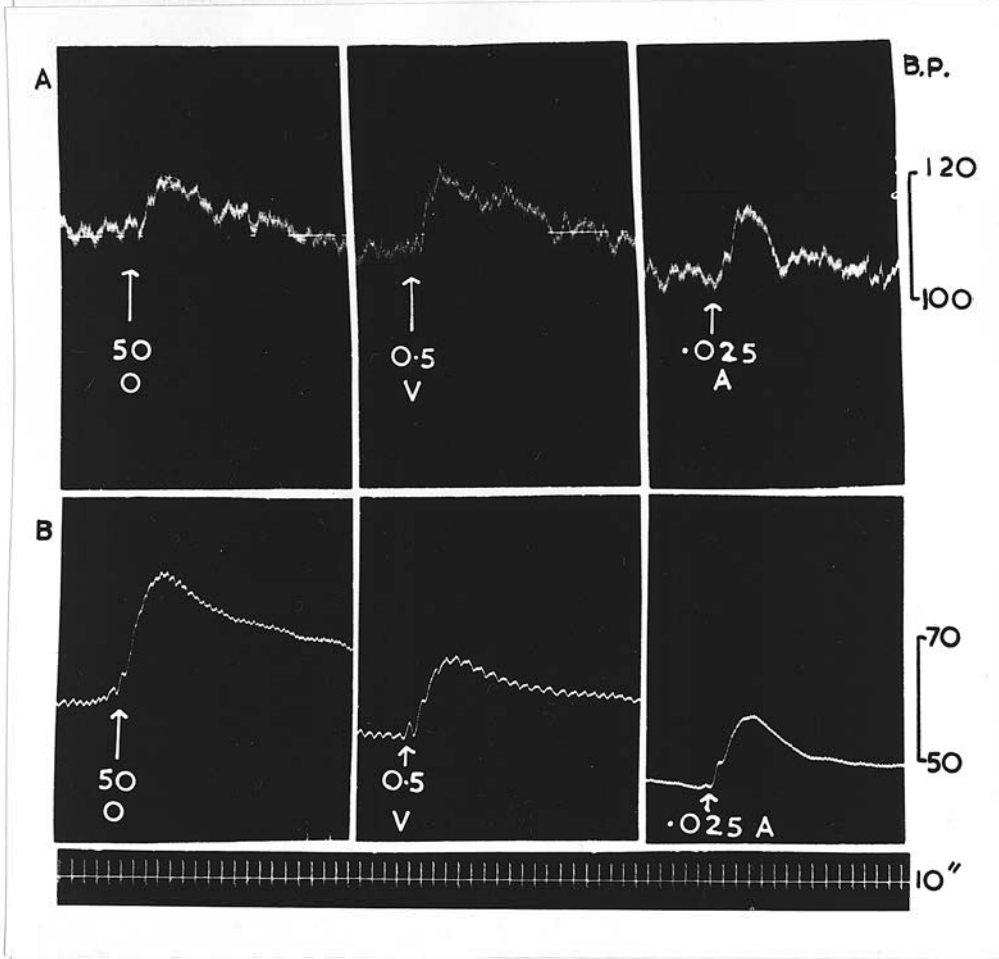

Figure 12. The effect of decerebration on the blood pressure responses of the oestrous rat. All records from the same animal.

A. Before decerebration.

B. After decerebration.

Arrows indicate times of injections.

50 O = 50mU oxytocin.

0.5 V = 0.5mU vasopressin.

.025 A = 0.025ug nor-adrenaline.

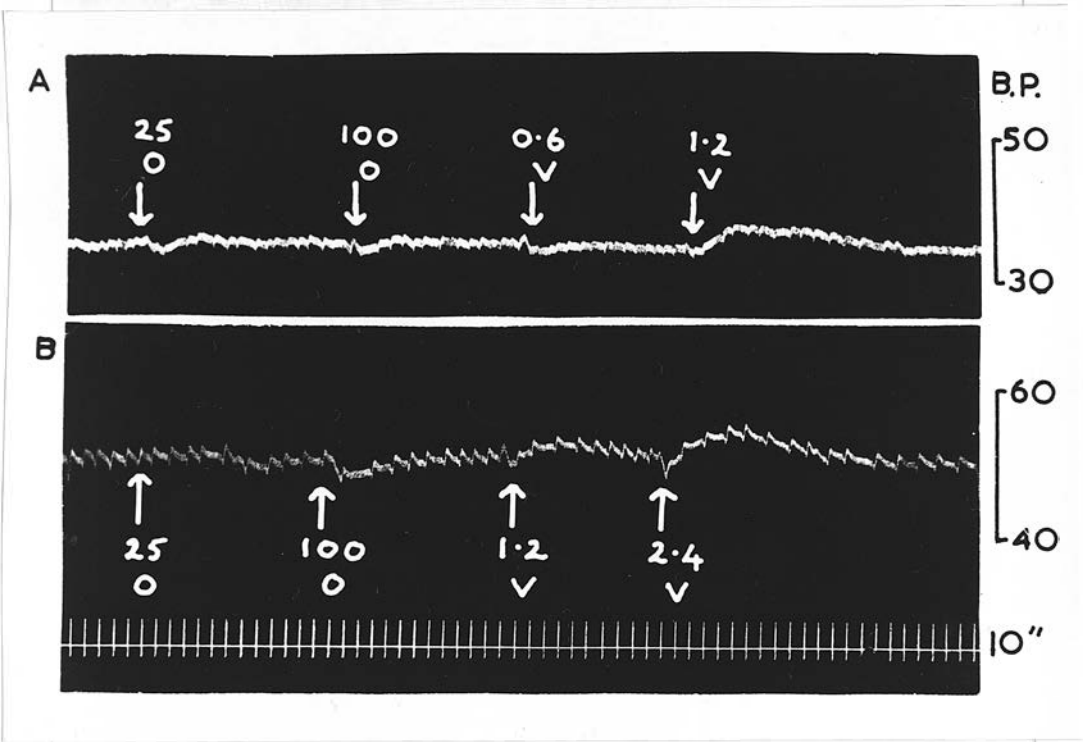

Figure 13. The effect of atropine on the blood pressure responses of the ovariectomised rat.

A. Before atropine.

B. After atropine. (0.2mg i.v.)

Arrows indicate times of injections, and figures the mU of oxytocin (O) and vasopressin (V) injected.

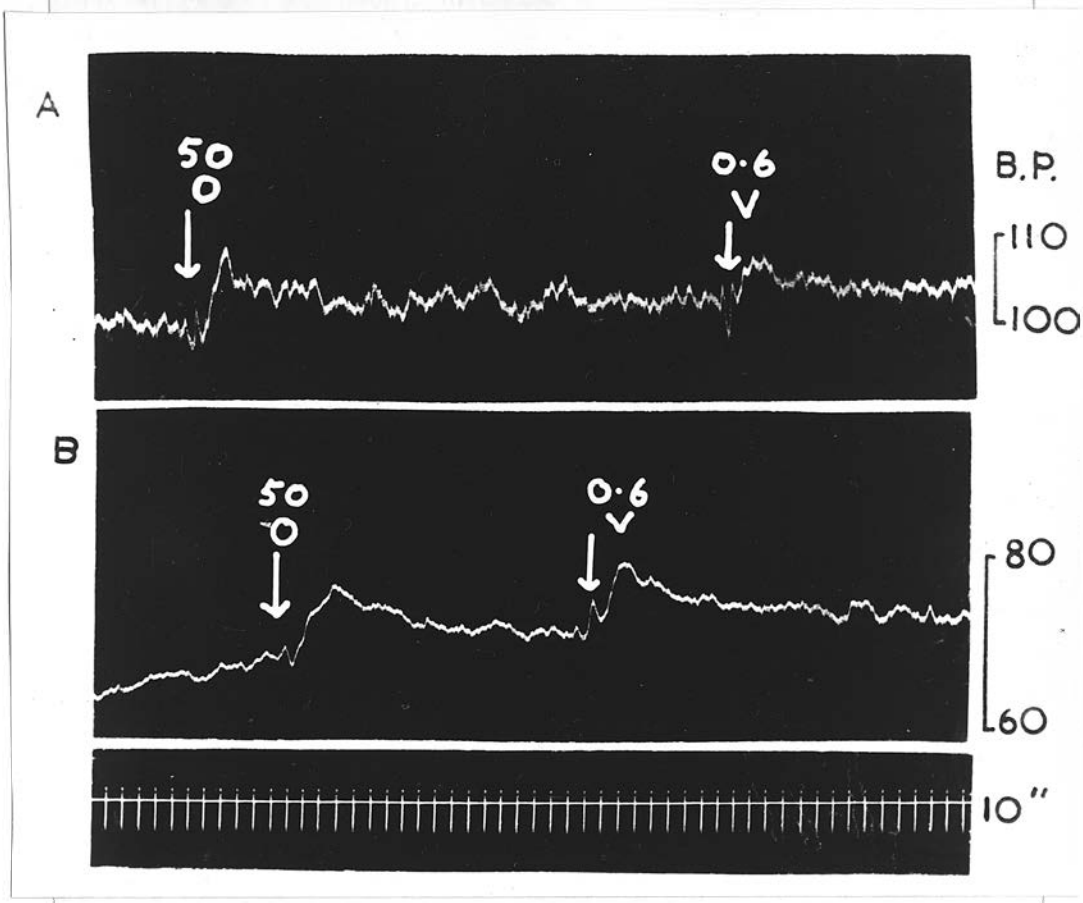

Figure 14. The effect of atropine on the blood pressure responses of the oestrous rat.

A. Before atropine.

B. After atropine. (0.2mg i.v.)

Arrows indicate times of injections, and figures the mU of oxytocin (O) and vasopressin (V) injected.

The effect of Priscol administration.

Observations have been made on only two dioestrous rats. This compound is known to have a peripheral vasodilator action, though the mechanism by which this is produced is complex, and involves more than one tissue. (Alquist, Huggins, and Woodbury, 1947, Lum, Nickerson, 1954). A fall in blood pressure was noted immediately after its administration. Oxytocin and vasopressin were administered in various doses both during and after the Priscol-induced fall in blood pressure, and both produced their normal effects.

The effect of tetra-ethyl ammonium bromide (T. E. A.)

This compound produces a fall in blood pressure by peripheral sympathetic blockade, (Acheson and Moe, 1946), and its effects were tested in three rats. The dilator response of two dioestrous animals to oxytocin was converted to a pressor response, while in the one oestrous rat the usual pressor response was unaffected. (Figures 15 and 16)

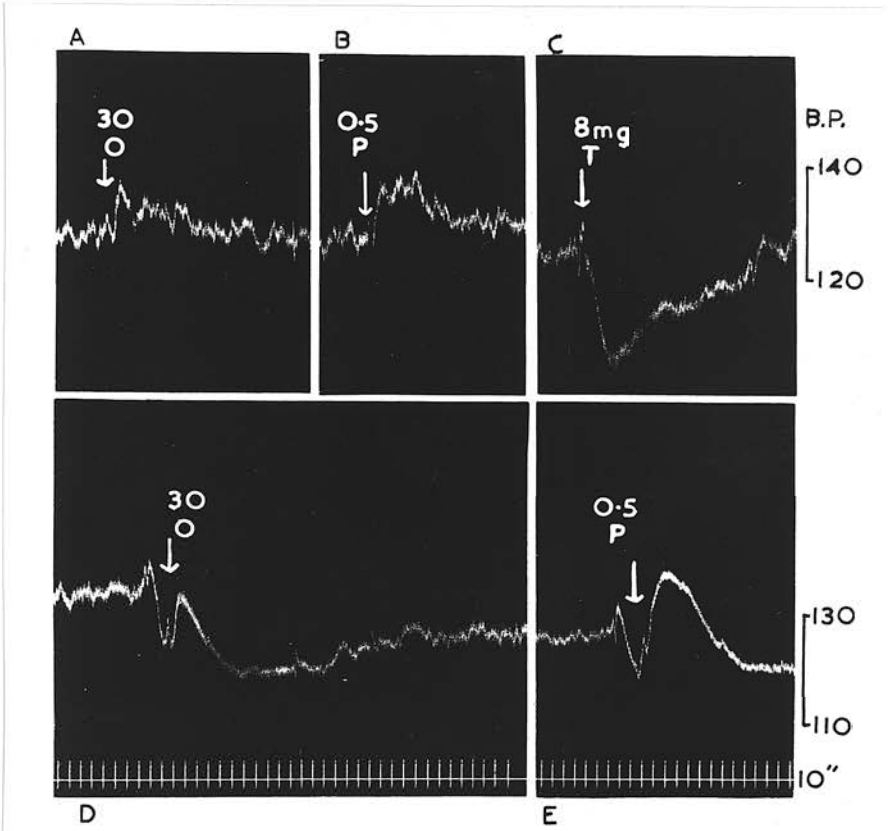

Figure 15. The effect of T.E.A. on the blood pressure responses of the oestrous rat.

A,B,C, Normal response to 30mU oxytocin, 0.5mU Pitressin, and 8mg tetra-ethyl ammonium bromide.

D,E, Effect of oxytocin and Pitressin during the depressor response to T.E.A.

Arrows indicate times of injections.

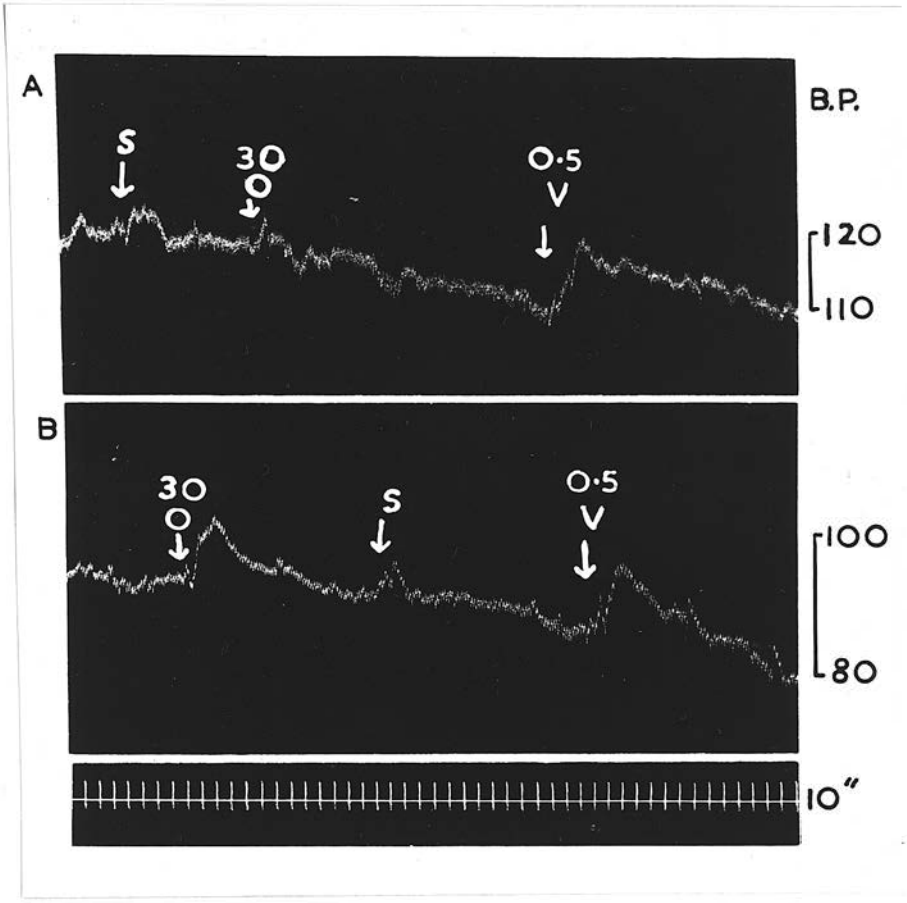

Figure 16. The effect of T.E.A. on blood pressure responses of the dioestrous rat.

A. Before T.E.A. administration.

B. After 8mg T.E.A. given i.v.

Arrows indicate time of intravenous injections.

S = control injection of 0.3ml 0.9% saline.

Figures indicate mU of oxytocin (O) and vasopressin (V).

HUMAN WORK.

The results so far described have been obtained with rats. It was of interest to know how far they could be applied to humans, and a few preliminary observations have been made on human subjects. This work was carried out in conjunction with Dr. Kitchin at the Western General Hospital, the subjects being members of the hospital staff, and patients.

Blood flow in hand and forearm was measured by venous occlusion plethysmographs, readings being taken every half minute during the course of each experiment, and the response to oxytocin administration investigated. Oxytocin was given either as a single injection, or as an infusion lasting for 8 to 20 minutes. The needle through which the drugs were administered was left in the vein throughout the experiment, to prevent the subject knowing when the injection or infusion was made. In all cases, in 8 normal healthy male and female subjects, there was a transient, but well marked dilatation, as shown by the fact that blood flow was increased in both hand and forearm (Table 7). The minimal doses required to produce the response was of the order of 6 or 7mU per kilo. for a single injection, and approximately 3mU per kilo. per minute for infusions, but these values are approximations only, as the weight of the subjects was not noted in these preliminary

Subject	Method of admin.	Dose	Blood flow ml/min.			
			Hand		Forearm	
			Before	After	Before	After
L.	Injection	0.4U	8	13	4	7
		0.4U	9	20		
2.	Infusion	0.5U/min	6	22	1	3
3.	Infusion	0.5U/min	5	14		
4.	Infusion	0.15U/min	2	2	3	3.5
5.	Infusion	0.15U/min	2.5	8	2	4
6.	Infusion	0.5U/min	6	14	4	10
7.	Injection	0.4U	3	6	4	13
		0.4U	2	5	1	8
		0.4U	2	4	1	5
8.	Infusion	0.5U/min	2	7.5	1	3.5

Table 7. The effect of oxytocin on blood flow in hand and forearm of eight normal human subjects.

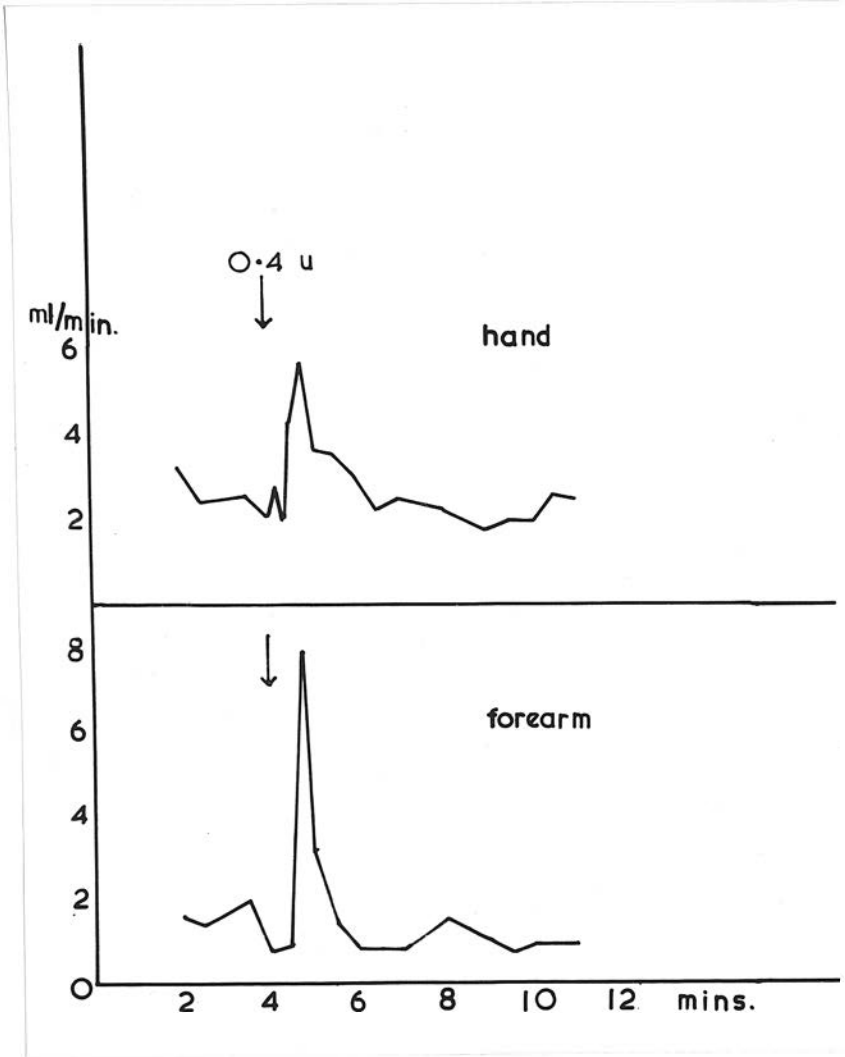

Figure 17. The blood flow response in hand and forearm of a normal human subject to a single intravenous injection of 0.4U oxytocin.

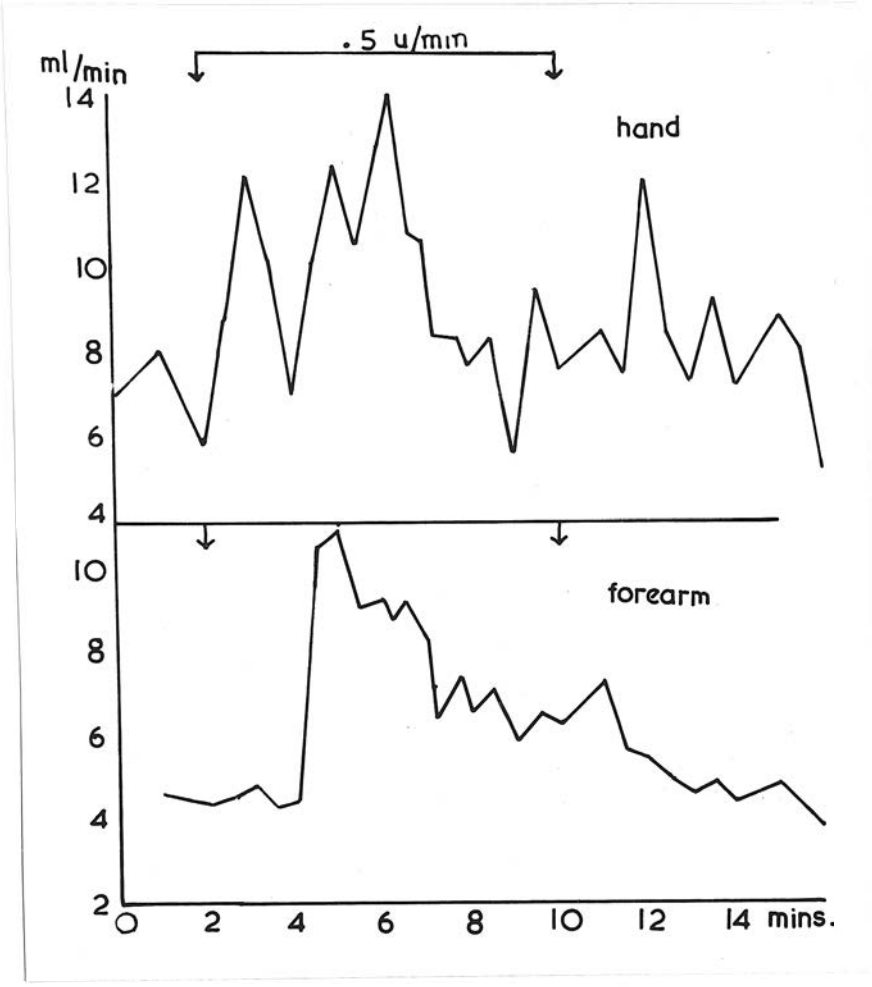

Figure 18. The blood flow response in hand and forearm of a normal human subject to the intravenous infusion of 0.5U/min of oxytocin.

observations. The duration of the dilatation was greater in the infusion experiments than when a single injection was given, though the height of the increase in flow was not maintained throughout the infusion. The results shown in figures 17 and 18 are typical of the 8 experiments. The subjective sensation was one of sudden and transient flushing, particularly of the face and ears.

Since ovarian hormones had been shown in rats to abolish the dilator response to oxytocin, it was of interest to determine what effects such hormones would have in human subjects. Five post-menopausal women, who were to be given stilboestrol for medical reasons, were tested for dilator response to oxytocin before and after the start of treatment, which consisted of the administration of 10mg stilboestrol diproprionate per day for six weeks. All showed dilator responses to oxytocin before treatment, and the magnitude of such responses did not appear to differ from those of normal subjects, though again, threshold doses could not be calculated accurately, as the subjects were not weighed. On the day following the start of treatment, the dilator response to similar doses was much reduced in all cases. (Table 8). Figure 19 shows the response in one of these subjects. Before stilboestrol, in this patient, the hand and forearm flows were increased by 0.4U oxytocin by 230% and 180% respectively; on the

Subject	Dose	Blood flow			
		Hand		Forearm	
		Before	After	Before	After
L	0.4U	4.0	10.0	3.0	3.0
	0.6U	4.0	10.0	3.0	5.0
+S	0.4U	1.5	2.0	3.0	2.5
	0.6U	2.0	3.5	2.0	3.0
2	0.4U	2.5	10.0	2.5	7.0
+S	0.4U	2.5	7.5	2.0	2.0
		Left hand		Right hand	
3.	0.5U	2.0	7.5	1.0	4.0
+S	0.5U	5.0	8.0	4.2	7.5
4.	0.5U	6.0	9.0	5.0	10.0
+S	0.5U	8.0	8.0	8.0	8.0

Table 8. The effect of stilboestrol treatment on the blood flow responses to a single intravenous injection of oxytocin in four post-menopausal women. Blood flow in ml/min; +S indicates results obtained on the first day after start of treatment.

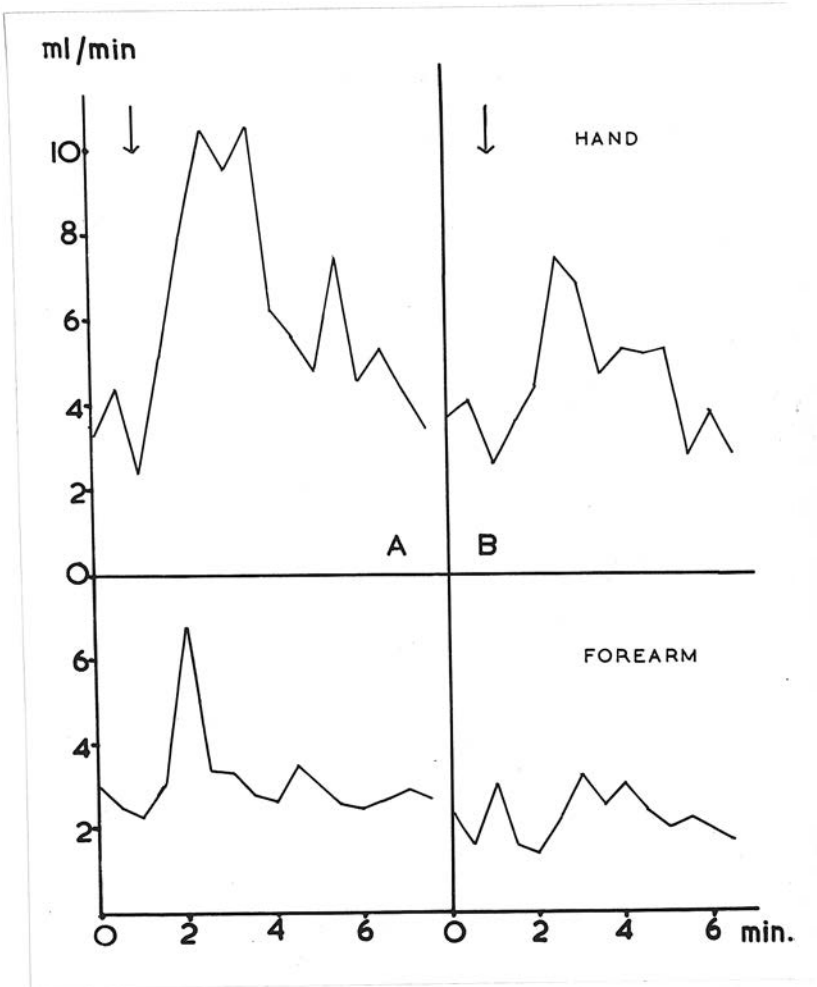

Figure 19. The blood flow response of a post-menopausal subject to a single intravenous injection of 0.4U oxytocin, before and after stilboestrol therapy.

A. Before stilboestrol.

B. On the first day after stilboestrol.

day following stilboestrol, the increases were 100% and 0%. This drop in response was seen regularly, and was always of this order. In one patient in whom responses were tested daily after the start of treatment, return of the dilator response was almost complete by the fifth day.

DISCUSSION.

Before considering the implications of the effects of the ovarian hormones on the vascular system, the actions of the main test substances used in the present work, namely oxytocin and vasopressin, must be clarified. Previous work has indicated that a peripheral effect is involved, and the experiments described here support this view. Oliver and Schafer (1895) obtained a pressor response in mammals to pituitary extracts whether the animal was intact, or had the spinal cord cut or the bulb destroyed. As early as this, therefore, the inference was that the pressor action was peripheral, due to arteriolar constriction. Dilatation of the arterioles in birds was held by Paton and Watson (1912) to be the reason for the depressor action obtained. The experiments of Campbell (1911) on isolated vessels also point to a peripheral action, and the fact that dilatation or constriction of the mesenteric vessels was obtained by topical application in the experiments described here confirm this hypothesis. There are also reasons for believing that oxytocin may have some central action. Benson and Folley (1957) and Cowie and Folley (1957) have suggested a central action affecting the production of prolactin, and Brooks and Pickford (1958) have described a central action affecting the excretion of electrolytes by the kidney.

That the vascular responses to posterior pituitary hormones may be affected by ovarian hormones has some previous experimental support. Reynolds (1952) making observations in man, found that the rise in blood pressure caused by Pituitrin was greater if oestrogens had first been administered. Since this preparation is a purified extract of mammalian posterior lobe, the observations gave no indication as to whether one, or both, hormones were involved. The experiments described described here show that similar changes in response may be produced in the rat also, and further, that both the hormones are involved, vasopressin quantitatively, and oxytocin qualitatively. The change in response to the hormones occurs naturally during oestrus, and the second half of pregnancy, and can also be induced in the dioestrous rat by the administration of exogenous ovarian hormones. A similar increased pressor response after treatment with oestrogens has been found for adrenaline in dogs. (Boxill and Brown, 1955). This only occurred in dogs with intact ovaries, and progesterone therefore seemed to be implicated in the effect. In the observations reported here, this also appears to hold, since during natural oestrus it is known that the oestrogen level is high, and in addition progesterone is present. Further, in ovariectomised rats it was not possible to produce the altered vascular

responses by the injection of stilboestrol alone - it was necessary to give progesterone also; and finally, only in rats with intact ovaries would either oestrogens or progesterone alone produce the oestrus type of response to the posterior lobe hormones. The results obtained during pregnancy and lactation also suggest the necessity for the presence of both ovarian hormones. It is known that progesterone levels rise through pregnancy, and fall just prior to parturition, although oestrogen levels remain high until after delivery. The change in vascular response was not apparent until the second half of pregnancy, so that it must be dependent on the ratio of oestrogen to progesterone, rather than the absolute amount of either alone. Once the critical ratio was reached, it was maintained until just before parturition. The observation made during parturition shows that reversion to the dioestrus type of response had already occurred. Since the progesterone level would have fallen by that time, giving a relatively high oestrogen level, it is difficult to see why this state of affairs did not result in the oestrus type response. One possibility suggests itself to account for these observations; that is, that provided both ovarian hormones are present, an excess of either oestrogen or progesterone converts the response from the dioestrus to the oestrus type.

Oestrogens are known to give rise to vasodilatation, but few theories have been postulated on the mechanism of this action. Reynolds (1939) produced evidence to support the theory that the peripheral hyperaemia is secondary to the acetylcholine releasing properties of the oestrogens. He found that in the rabbit uterus, hyperaemia caused by oestrogen administration was accompanied by an increase in the acetyl choline content of the organ. However, the hyperaemia persisted for longer than the relatively transient increase in acetyl choline content. The preparation he originally used was Amniotin, but similar results were later obtained with other oestrogen compounds, with the exception of stilboestrol, which was found to have no appreciable cholinergic action. (Reynolds and Foster, 1939c). In his original observations, which were made on ovariectomised animals, the accumulation of acetyl choline preceded the onset of uterine motility, and on such grounds he suggested that the effects of oestrogens could be classified into two groups; those depending on hyperaemia, and therefore on their acetyl choline releasing properties, and those which do not release acetyl choline. The results in the rabbit were obtained equally well independently of connection with the central nervous system. Later, however, it was found that species differences existed, oestrogens having no

cholinergic action on the uterus of the cat or rat. (Reynolds and Foster, 1940a). In both cats and rabbits there was an increase in the content of an acetyl choline-like substance in the nasal mucosa accompanying vasodilatation caused by oestrogen administration. (Reynolds and Foster, 1940b). Such a theory, that the vasodilatation depends on local acetyl choline release independently of central nervous mechanisms, although a possibility, has never been proved satisfactorily, and it is difficult to see why the hyperaemia should persist after the acetyl choline accumulation has ceased. Further, it is difficult to see why an oestrogenic substance such as stilboestrol, which has vascular actions, should be ineffective in releasing acetyl choline in the rabbit uterus, if this is indeed the mechanism by which hyperaemia is produced. Another factor complicating this hypothesis is the existence of species differences. Presumably some other dilator substance would be released in the animals in which there is no acetyl choline accumulation, though no candidate for this role has been suggested. If, as Reynolds says, stilboestrol has no such cholinergic action, and other oestrogens are also ineffective in this respect in rats, the results obtained in this study cannot, presumably, be due to choline/rgic dilatation, since the experimental animals used were rats, and stilboestrol was

employed as the oestrogenic substance. Whatever the cause of the action of stilboestrol, the results of its administration were very similar to those of natural oestrus; and that the stilboestrol caused vascular dilatation was very evident during dissection, and complicated the preparation for experiments.

The results obtained in the studies on the effects of vagotomy and decerebration suggest that the action of the oestrogens may not be entirely peripheral. A central action has been reported by Harris and Michael (1958), who found that implants of minute doses of oestrogens into the posterior hypothalamus produced mating behaviour in castrate female cats, without any effect on vaginal smears. Other indications of a central action of oestrogens have been shown by Herren and Haterius (1931). They found that there was a long Achilles reflex time in oestrous rats, but that this was short during dioestrus. During pregnancy, there was a gradual decrease in this reflex time until the 10th or 11th day, after which a gradual lengthening occurred, with an extremely long reflex time at parturition. They concluded that this variation was caused by a central action of the ovarian hormones, but were unable to state whether this was a direct action, or mediated indirectly through other nervous connections with higher levels. In later studies, (Herren and Haterius,

1932) they tested the effect of bilateral sympathectomy on the variation of reflex time, and concluded that the influence on the higher nervous levels were effected through the sympathetic connections. Chauchard (1943) suggested that the ovarian hormones had a depressive action on the brain. If the responses to oxytocin in the experiments reported here are considered, it is seen that a similar response to that obtained in the oestrous rat with intact central nervous system, was obtained in the dioestrous animal after either bilateral vagotomy or decerebration. Since the former increases the general blood pressure, and the latter decreases it, and such a range of pressures was found to have no effect on the responses to posterior lobe hormones in the normal rat, this can be discounted as a cause of the change in response. In the oestrous state, two out of four times, there was an increased pressor response to oxytocin following decerebration. The mechanism involved in the alteration of these responses by vagotomy and decerebration may not be the same as those producing similar results during oestrus, or after ovarian hormone administration; nevertheless the possibility exists that it might be the same. It must, then, be postulated that the ovarian hormones have a central action, and inhibit some (depressor) part of the control of the vascular system, which is

normally transmitted by the vagi. Vagotomy would also remove this control, and hence vascular responses to the posterior lobe hormones would be similar to those seen in oestrus. That decerebration, as well as vagotomy, alters the response to oxytocin suggests that this substance has an action at a site above the origin of the vagus nucleus, and a possible explanation of the results seen is an inhibition of depressor and/or a stimulation of the pressor centres. The issue is confused by the fact that the vagus nerve is composed of many kinds of fibres, both efferent and afferent, and also that in three experiments on the atropinised rat the vascular responses to oxytocin and vasopressin were no different from those seen in normal animals. It appears, therefore, that the nervous impulses which may be inhibited by oestrogens cannot be carried by cholinergic fibres. The conversion of response in the dioestrous animal following sympathetic blockade with T.E.A., though tested in only two animals, was clear and definite, and suggests that sympathetic fibres are concerned.

It is unknown how far oestrogen induced changes in the anterior pituitary may have modified the response of central and peripheral tissues. Clearly a firm conclusion on the mode and site of action of posterior pituitary hormones cannot be reached without a great deal

more work. Helpful information might be obtained by inducing peripheral sympathetic blockade with C_6 and in particular by using pithed rat preparations, with and without preliminary treatment with oestrogens.

The results of other workers on the effects of nervous blockade on the vascular responses to posterior lobe hormones must also be considered. In the duck, Paton and Watson (1912) found that following atropine administration, the fall in blood pressure caused by the injection of whole posterior pituitary extracts was more prolonged than in the normal animal, and they considered this to be due to abolition of a cardiac stimulating effect. With oxytocin, Coon (1939) found that in the chicken atropine prolonged and augmented the depressor action. This last result also followed vagotomy. Vagotomy, or destruction of the carotid sinus nerves was observed by Larson (1938) to increase the pressor response of dogs to pituitary extract, showing that these mechanisms normally inhibited the response to some extent. The results of Larson are in keeping with those obtained in rats in this study. In the results reported here, the cardiac effects have not been considered, though obviously they may modify the results obtained after the various procedures employed.

Other experiments which may have some bearing

on the effects of oestrogens are those which have been made on the smooth muscle of the uterus. In this organ, it has been shown in vivo that the effects of electrical stimulation vary with the type of ovarian hormone domination, (Csapo and Goodall, 1954), and this has been confirmed in vitro by Schofield. (1954). The threshold changes which occurred were investigated, and it was concluded that ovarian hormones alter the potassium gradient of the myometrium. (Csapo,1956). Whether such gradients in the vascular musculature are similarly affected is not yet known.

Whatever the mechanisms involved in the modifying activity of ovarian hormones, and apart from the complex question of the importance of the oestrogen/progesterone ratio, some aspects of the activities of the posterior pituitary hormones themselves may have a bearing on vascular physiology under certain conditions. The reasons for the circulatory disturbances which accompany the menopause in women remain as yet unsolved. Overproduction of anterior pituitary gonadotrophins has been shown by Albright (1936) to accompany the condition, and Reynolds, Kaminester, Foster and Schloss (1941) have pointed out that psychogenic factors may be contributory. Oestrogen therapy is often effective in the clinical treatment of the flushes, presumably by restoring a more normal level of the hormone, and depressing

gonadotrophin liberation. But the gonadotrophins are not themselves dilator, and cannot be regarded as the direct cause of the flushes. Neither can the vascular actions of oestrogens, which are dilator, be the direct cause of alleviation of the symptoms. A possible explanation of the actual flushes is the following; that when the normal oestrogen level is declining there is an increased sensitivity to some dilator substance. Since the vascular disturbances eventually disappear, restoration to normal sensitivity must occur naturally, or some other compensatory factor appear. That such changes in sensitivity may occur has been shown in the experiments described, and though the evidence is far from conclusive, it is possible that the hormones of the posterior pituitary may in fact be involved. Rats in which endogenous oestrogens were removed by ovariectomy showed a marked increase in their sensitivity to the dilator action of oxytocin for a few days, and a decreased pressor response to vasopressin, after which normal sensitivity was restored. It has been shown by Abrahams and Pickford (1954) and by Harris (1955) that under conditions in which one posterior lobe hormone is released, it is always accompanied by the other. In the experiments described here only the separated individual hormones have been used, as the ratio in which the two are released seems to vary in different

species, and was not known in the case of the rat, though Dicker (private communication) has since intimated that, for osmotic stimuli, the ratio of vasopressin to oxytocin liberated may be 1/20 to 1/30 in this animal. However, since the effect of ovariectomy is to increase the dilator effect of oxytocin, and at the same time to reduce the pressor effect of vasopressin, the mixture of the two in the natural ratio would presumably have both these actions, being less constrictor, and more dilator under these conditions than in the normal animal. Ideally, the sensitivity to a mixture of the two, in the correct proportions, should be tested.

That the above hypothesis is not outside the bounds of possibility is shown by the results of the experiments on humans, where the dilator response to oxytocin of the vessels of the hand and forearm has been established, showing that the vessels of the skin, and possibly also of the muscles, are involved in the response. As yet, the experiments on humans have been limited in number, but the results are definite and suggest that further work may be profitable. The reduction of the dilator response to oxytocin after oestrogen therapy is in keeping with the results in rats, as are also recent experiments, not yet fully analysed, on four menopausal patients in whom an increased sensitivity to oxytocin was found.

Here again, it would be desirable to test the correctly proportioned mixture of the two posterior lobe hormones. It has been noted by Reynolds, Kaminester, Foster and Schloss (1941) that the menopausal flushes are particularly common during exitement, emotion, exercise, and sometimes eating and also at night, and these are conditions often accompanied by antidiuresis, suggesting posterior lobe hormone release. While blood flow measurements have not been made in humans of areas other than the hand and forearm, it is certain from the appearance that the face is particularly affected, and the duration and sometimes the intensity of the flush, after a single injection of oxytocin, are similar to those of the menopause.

However, the experimental evidence does not wholly support the idea that oxytocin is the agent responsible for the menopausal flushes; for instance, within a few days of ovariectomy rats show normal dioestrous vascular responses to oxytocin, without any form of oestrogen treatment. As against this, experience shows that a surgical menopause in humans has a prolonged effect on the vascular system. Then in human females, oxytocin was found to have a reduced dilator action only for a brief period at the beginning of treatment with stilboestrol. The dilator agent immediately causing the flushes does not appear to be acetyl choline, since the vascular response to this

substance is unaffected by oestrogens or castration. It can only be said that the dilator substance may be oxytocin, or something else so far unsuspected. The only certain answer would be the discovery of high concentrations of oxytocin in the circulation of menopausal women, and this cannot be given until the technique of oxytocin extraction from tissue fluids has improved. Nevertheless, the results have demonstrated that oestrogens can convert a dilator response to a constrictor one, and thus indicate a reason for the success of stilboestrol in the treatment of menopausal flushes. It is unknown how far this action of stilboestrol is mediated by depression of gonadotrophin production by the anterior pituitary.

PART II.

THE EFFECTS OF OVARIAN HORMONES ON WATER AND ELECTROLYTE EXCRETION.

INTRODUCTION.

Water and electrolyte retention is another aspect of ovarian hormone action which has been extensively studied experimentally, and which is also clinically recognised in conditions such as pregnancy, where ovarian hormone levels are high. Though now a well established action, here again the mechanism by which such retention occurs has not been elucidated.

Thorn and Harrop (1937) were the first to investigate the effect of ovarian hormone administration on the 24 hour water and electrolyte excretion of dogs maintained on a constant diet. They found that both oestrogens and progesterone caused a retention of water and sodium, and also reported that recovery to normal excretion levels occurred even if hormone administration was continued. It was in this year also that Krohn and Zuckerman, studying the water metabolism of the female pig-tailed macaque during the menstrual cycle, found that the body weight fluctuated with the swelling and subsidence of the sex skin. The calculated water retention was found to correlate well with the weight increase during swelling. In 1938 Thorn and Engel confirmed the fall in sodium

and chloride excretion following oestrogen administration to male dogs, and also reported that phosphate and nitrogen excretion were similarly reduced, though the potassium excretion appeared to be increased slightly. The decrease in urinary output of sodium, nitrogen and phosphate was also found by Knowlton, Kenyon, and Sandiford (1942) when oestrogens were given to two eunuchoid men, one hypogonadal subject, and one normal woman. The gain in weight found pre-menstrually in some women has also been attributed to the high oestrogen levels prevailing at this time, as has also the oedema found in many pregnancies.

Further work has shed some light on the location of the water and electrolytes retained, and the evidence suggests that they are to be found in an expanded extracellular fluid space. Witten and Bradbury (1951) studied blood samples taken from 16 women treated with oestrogens in the intermenstrual period (to eliminate errors due to blood loss). They found a consistent lowering of the red blood cell count, the haemaglobin level, and the venous haematocrit, which could be accounted for by the increased blood volume also observed. They suggested that the raised oestrogen levels during pregnancy might account for the 'physiologic anaemia' by haemodilution. Cohen and Thomson (1939) had already demonstrated this increased blood volume, together with a reduction

in blood count, viscocity, haemoglobin content, and haematocrit during human pregnancy. Newman, (1955) made similar observations during the menstrual cycles of 34 women, but could find no consistent change in the haematocrit, haemoglobin or electrolyte content of the blood during the cycles. Preedy and Aitken (1956) similarly failed to find any significant change in plasma sodium and chloride levels in 11 women treated daily with oestrogens, though urinary excretion of these electrolytes was decreased. They confirmed the fall in serum protein and venous haematocrit, which suggested an increase in plasma volume, though this was not measured. These workers concluded that the reduction in water and electrolyte output was associated with an increase in the extracellular fluid volume. Horger and Zarrow, (1951) reported an increase in blood and plasma volumes in the anaemia of late pregnancy in the rabbit, without an increase in the thiocyanate space, though the latter was found in normal rabbits after oestrogen treatment. Progesterone was found by these workers to have no effect alone, but to modify the action of oestrogens on the blood and plasma volumes. The effect was synergistic at a progesterone/oestrogen ratio of 40/1, and antagonistic at a ratio of 4/1. As has already been mentioned, Thorn and Harrop had found a water and electrolyte retaining action of pro-

gesterone in dogs, and this was confirmed by Thorn, Nelson, and Thorn (1938), and by Thorn and Engel (1938). Selye and Basset (1940) however, found that progesterone administration to rats caused an increase in urine output in these animals.

While it therefore seems amply demonstrated that oestrogens can cause a retention of water and electrolytes, and that an expanded blood volume and haemodilution may accompany such effects, the action of progesterone is less sure. Confusion also exists in reports of the studies which have been made on renal function in attempts to discover how such retentions occur.

Chesley and Chesley (1939) compared the diodrast and endogenous creatinine clearances of a group of 9 normal non-pregnant women with those of 8 normal pregnant subjects, within 4 weeks of term. They found an increase in endogenous creatinine clearance, and though they did not consider this to be a true measure of the glomerular filtration, they suggested that it might indicate a rise in this factor. The renal plasma flow (measured by diodrast clearance) was also higher in the pregnant group, but as the haematocrit was lowered, the calculated renal blood flow was unaltered. Welsh, Wellor and Taylor (1941) failed to find any change in filtration rate, renal blood flow. or tubular excretory mass during pregnancy

or in the puerperium of normal women, while Dill, Isenhour, Cadden, and Robinson (1942) found that pregnancy in women was usually accompanied by higher glomerular filtration rate and renal blood flow than were found post partum. The results obtained after direct oestrogen administration are equally confusing. In 18 post-menopausal women, repeated intramuscular oestrogen injections caused no change in glomerular filtration rate (hereafter referred to as G.F.R.) or renal plasma flow(R.P.F.) (Dignam, Voskian, and Assali, 1942). These authors also noted a decrease in electrolyte excretion after oestrogen treatment in these patients, though urine flow was not altered by this procedure. A similar lack of effect on G.F.R. (measured by mannitol clearance) or R.P.F. (P.A.H. clearance) was observed by Dean, Abelks and Taylor (1945) after oestrogen administration over several days in women. In contrast to these results, however, are those of Selkurt, Talbot, and Houck (1943). They found that there was a 13-23% increase in G.F.R. (measured by creatinine clearance) after oestrogen administration to 3 normal bitches. They considered this increase in filtration to result from increased plasma volume.

Recent work by Dance, Lloyd and Pickford (unpublished) has given a more complete picture of the changes in renal function after oestrogen treatment in dogs, and Dance and Pickford (unpub

lished) studied the excretory events occurring during natural oestrus in dogs. These workers have shown that a depression of water excretion normally occurred within 48 hours of a subcutaneous dose of stilboestrol diproprionate, and that recovery usually occurred within 72 hours. A fall in the excretion of sodium, potassium, and chloride was also observed, usually persisting for several days. No change in plasma electrolyte levels, or in venous haematocrit were observed in animals in which these factors were investigated. In normal dogs the renal clearances of creatinine and diodone were markedly increased after stilboestrol treatment, and this elevation lasted for several days. Similar observations during saline diuresis showed that water and sodium were retained after oestrogen treatment, but that potassium was not much affected. In natural oestrus marked water retention was seen only on the day before vulval oedema was apparent; there was a retention of sodium on the first day of bleeding, and an increased loss of sodium on the second day. Thereafter any difference from the normal was small.

METHOD OF APPROACH TO PROBLEM.

While disagreement exists between previous workers as to whether the glomerular filtration rate is, or is not, raised in cases of high oestrogen levels in the body, either by exogenous administration or in conditions such as pregnancy, none has described a fall in this factor. It would thus appear that a reduced glomerular filtration cannot be the cause of the reduction in fluid output in such conditions. The possibility therefore arises that the tubular reabsorption may be affected. Among the factors known to affect this are the hormones of the adrenal cortex, and those of the posterior pituitary. Hypertrophy of the adrenal cortex has been shown to follow oestrogen administration in rats (Vogt,1945), and it has also been shown that the weight of the glands fluctuates with the oestrous cycle in these animals.(Bourne and Zuckerman, 1940). However, in experiments on adrenalectomised dogs, Thorn and Engel (1938) showed that the effect of the sex hormones on renal excretion was not necessarily mediated via the adrenals. The object of the experiments to be reported here was to investigate the possibility that the hormones of the posterior pituitary might be involved, and two lines of approach were made.

In one series of experiments, an attempt was made to investigate any changes which might occur

in the renal response to injected posterior lobe hormones after oestrogen treatment. In the other series a study was made of the water and electrolyte excretion, and of glomerular filtration and renal plasma flow, in dogs with experimental diabetes insipidus, before and after oestrogen treatment.

EXPERIMENTAL METHODS.

The procedure used in all experiments on dogs was essentially the same. Conscious animals were used, as the use of anaesthetics may affect urine flow, and observations were made in an isolated partially sound proof room, to minimise emotional disturbance in the animals. The dogs were trained to lie quietly on a table throughout the period of observation. A further precaution which was taken to avoid spontaneous changes in the results was the maintainance of a constant dietary intake of electrolytes, by keeping the dogs on a diet of tinned dog food with the addition of a constant volume of milk. Water was supplied ad lib in the evening and at night. 2-2½ hours before the start of each observation, a hydrating dose of 250ml water was given by stomach tube, and food and water then witheld until the experiment was begun. A water diuresis was then induced by giving 300-350ml water (depending on the size of the dog) by stomach tube. A catheter was then inserted into the bladder, which was drained, and thereafter 10 minute samples of urine were collected in graduated measuring cylinders. (The perineum had been slit dorsally to facilitate catheterisation.) Glomerular filtration rate, and renal plasma flow were measured by the renal clearances of creatinine and diodone respectively. In the experiments where these were to be estimated,

4-5g creatinine, and 9-12ml 30% diodone were injected subcutaneously in aqueous solution 30 minutes before the start of the experiment, as this had been found by previous experiments to give a fairly steady level of the substances in the blood by the time blood samples were taken. Two or three blood samples were taken from the saphenous vein at intervals at the height of the diuresis, and the urine samples collected between blood samples used to calculate the clearances.

Analysis of samples for sodium and potassium was done on a flame photometer, and chlorides were estimated by the method of Prout-Winter. (See Cole, 1919). Creatinine estimations were performed according to the method of Rehberg (1926) for blood, and Folin (1914) for urine. and diodone by the method of Alpert (1941). The oestrogen preparation used was Stilboestrol diproprionate, and was given in two consecutive doses of 30µg per kilo. body weight, subcutaneously in oil.

A non-diabetic ovariectomised bitch was used for testing the renal responses to oxytocin and vasopressin before and after treatment with stilboestrol. In this animal no clearance measurements were made, since observations on the G.F.R. and R.P.F. of other normal ovariectomised oestrogen treated dogs gave constant results which have been described on page48.. The observations on the effect of oxytocin were made with the dog in the non-diuretic state, since Brooks and

Pickford (1957, 1958) found that to be the optimal condition.

For purposes of comparison between the different animals, it was found convinient to calculate electrolyte excretion as the total output during a standard hour of diuresis. As the excretion rate of electrolytes tends to fall over the first few samples, these were omitted from the calculations, the standard hour being taken as 30 to 90 minutes after the induction of diuresis.

The pperations for the production of diabetes insipidus were performed by Dr. Mary Pickford. Two of the animals had the supraoptico-hypophysial tracts sectioned immediately posterior to the optic chiasma, ('Thisbe' and 'Chris') and the other ('Jess') had the posterior pituitary only removed.

RESULTS.

THE EFFECT OF STILBOESTROL ON THE RENAL RESPONSE TO VASOPRESSIN AND OXYTOCIN IN THE NORMAL DOG.

Vasopressin.

The dose of vasopressin used was 1.2mU given intravenously, since this dose was found to give a suitable sub-maximal antidiuresis during water diuresis. Table 9 summarises the results, before and after stilboestrol administration, and figures 20 and 21 show the effect on urine flow and sodium excretion. The normal curves shown are typical of several observations made before oestrogen therapy. It was found that such treatment had little or no effect on the pattern of urine flow; but a distinct change was found in the electrolyte excretion response to vasopressin. In the pre-treatment observations there was always an increase in the sodium excretion in the samples following the injection of vasopressin, but on the first and second days after oestrogen therapy vasopressin was inactive in this respect. By the third day the response was again normal, and remained so in subsequent observations. The potassium excretion was affected similarly to that of sodium, but was always smaller in degree both before and after stilboestrol. Chloride excretion was increased even on the first and second days after stilboestrol, though to a lesser extent than in the pre-treatment observations.

Day of expt.	Sample.	1	2	3	4	5	6	7	8	9	10
Normal V at 0.53	Time(mins)	20	30	40	54	60	70	80	90	100	110
	Flow rate	2.15	2.3	2.7	2.97	1.67	0.87	1.5	1.6	1.87	1.9
	Na	12.2	6.0	8.9	10.6	8.5	18.0	10.7	7.1	7.4	7.4
	K	6.2	5.2	4.9	5.1	4.3	8.5	5.8	3.5	3.5	3.5
	Cl	16.8	11.1	13.7	14.8	11.4	33.1	19.1	11.2	9.6	8.7
Normal V at 0.56	Time(mins)	20	30	40	50	57½	60	70	80	90	100
	Flow rate	1.45	1.77	2.05	2.2	2.6	1.9	0.4	0.8	1.2	1.5
	Na	8.9	4.2	5.2	6.5	8.1	6.8	4.0	11.2	9.0	
	K	7.9	6.6	6.5	5.5	5.3	4.3	3.0	6.3	3.5	
	Cl	7.0	4.4	5.1	6.0	7.3	6.7	4.2	11.2	9.6	6.8
Normal V at 0.55	Time(mins)	20	30	40	50	56	60	70	80	90	100
	Flow rate	1.71	2.2	2.7	3.1	3.5	2.18	0.35	1.4	1.55	2.0
	Na	6.3	5.9	7.5	10.2	15.8	10.9	6.7	15.7	5.3	5.8
	K	10.1	9.4	9.6	10.0	10.3	8.9	5.2	14.6	8.5	9.0
	Cl	4.3	4.2	5.1	5.8	8.4	7.4	6.5	17.4	6.4	6.0
Day 1 V at 0.54½	Time(mins)	20	30	40	50	56	60	70	80	90	100
	Flow rate	1.77	1.9	2.0	2.2	2.25	0.7	0.2	0.57	1.1	1.57
	Na	3.9	2.9	2.8	2.9	3.4	1.9	1.5	4.2	3.6	4.1
	K	5.9	5.0	4.3	4.5	4.5	2.0	2.1	5.3	2.7	3.5
	Cl	5.8	4.2	4.4	4.2	4.5		2.4	11.4	8.4	6.6
Day 2 V at 0.56½	Time(mins)	20	30	40	50	57	60	70	80	90	100
	Flow rate	2.06	2.05	2.4	2.2	2.43	1.67	0.25	0.77	1.35	1.6
	Na	5.3	4.2	4.9	5.1	6.1	4.4	2.9	5.4	6.1	4.0
	K	5.1	5.6	7.1	5.4	5.5	3.9	2.7	5.6	4.7	3.3
	Cl	6.4	4.9	5.8	5.0	6.3	5.6	4.4	13.0	9.5	6.6
Day 3 V at 0.54½	Time(mins)	20	31	40	50	55	60	70	80	90	100
	Flow rate	1.87	2.27	2.28	2.4	2.6	1.46		0.9	1.25	1.55
	Na	10.3	8.5	5.6	5.4	7.5	5.7		12.0	6.0	4.8
	K	6.3	8.5	5.9	5.1	4.9	3.3		6.9	3.6	2.9
	Cl	9.0	9.1	7.1	6.5	8.3	6.8		17.6	11.8	8.7
Day 5 V at 0.56½	Time(mins)	20	30	40	50	57½	60	70	80	90	100
	Flow rate	1.36	1.75	2.3	2.3	2.54	2.2	0.7	1.05	1.6	1.8
	Na	5.2	5.2	6.1	6.0	9.5	9.2	14.8	16.8	7.6	6.7
	K	4.3	4.7	4.9	4.5	4.8	4.7	5.5	4.9	5.5	3.0
	Cl	8.3	8.8	10.6	10.6	13.7	13.2	25.2	28.9	13.1	9.8
Day 10 V at 60½	Time(mins)	20	30	40	50	61	70	80	90	100	110
	Flow rate	1.7	1.85	2.4	2.4	2.71	1.05	0.8	1.4	1.78	1.9
	Na	11.5	7.2	12.8	10.7	15.0	7.4	13.9	6.5	7.4	5.2
	K	8.9	8.5	8.5	8.1	7.6	4.5	7.8	6.1	5.5	4.8
	Cl	19.0	14.8	18.9	19.2	22.2	11.8	36.0	17.5	12.4	9.2

Table 9. The effect of stilboestrol on the renal response to 1.2mU vasopressin.
Injection given at time indicated in first column, which also gives day of experiment. Stilboestrol given on days 0 and 1.
Rate of flow is in ml/min, and all electrolytes in µE/min.

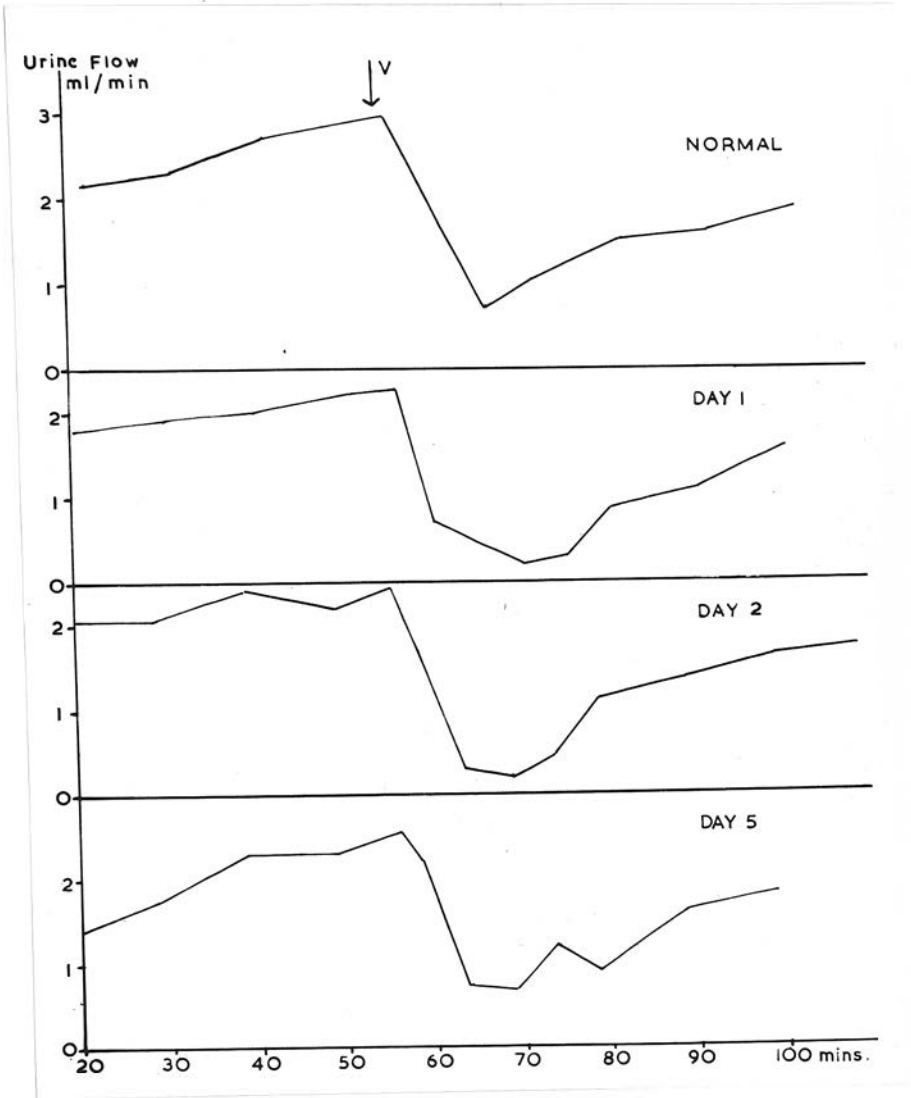

Figure 20. The effect of intravenous injection of 1.2mU vasopressin on the urine flow during water diuresis, before and after stilboestrol.

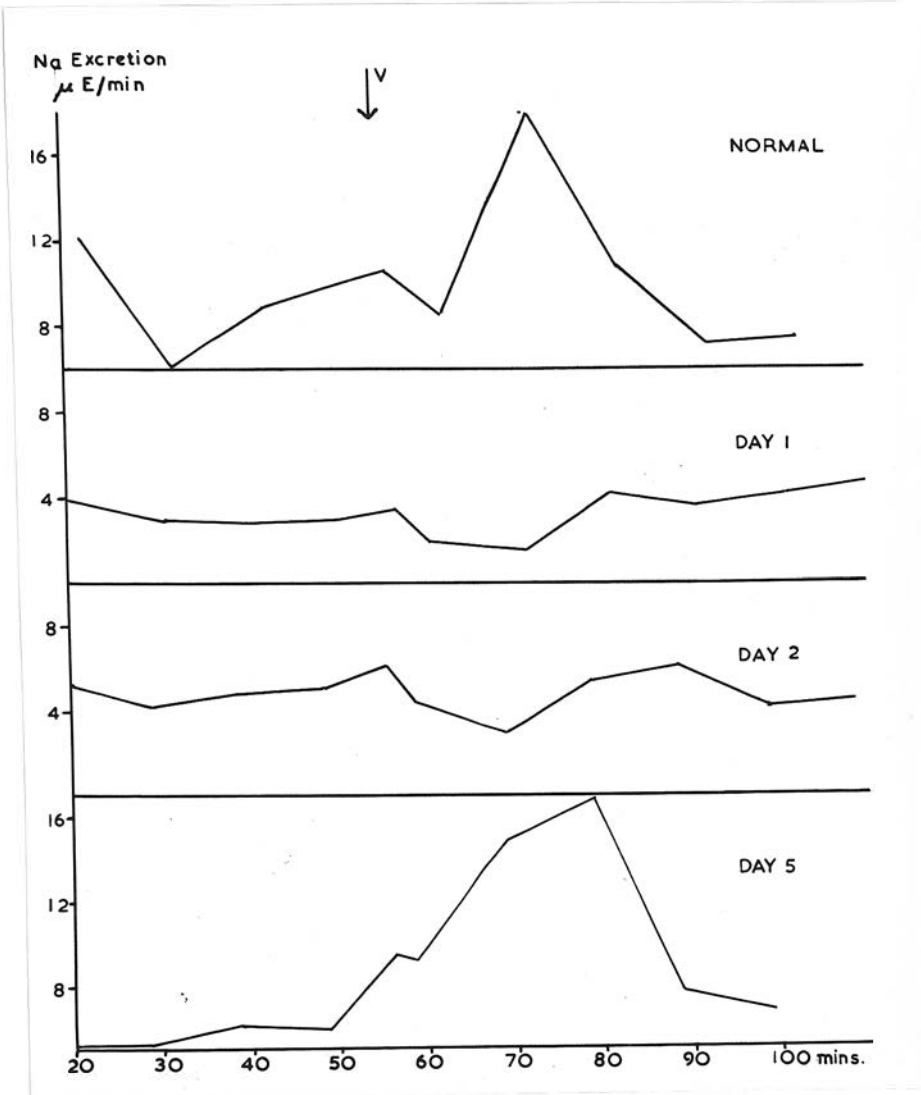

Figure 21. The effect of intravenous injection of 1.2mU vasopressin on the sodium excretion during water diuresis, before and after stilboestrol.

Oxytocin.

The procedure was similar to that used in testing the response to vasopressin, but in this case observations were made at a resting rate of urine flow, and not during diuresis. The oxytocin used was the preparation 'Pitocin' (from Parke Davies). 150mU were injected into the saphenous vein after several control samples had been collected. The results are given in table 10, and the figures for sodium excretion shown in figure 22. It can be seen from the table that a slight antidiuresis followed oxytocin injection, but this did not appear to be altered by stilboestrol administration. On the first to third days inclusive after stilboestrol was given there was a change in the effect of oxytocin on electrolyte excretion. Figure 22 shows that on these days oxytocin caused a far greater than normal increase in the excretion of sodium; by the fifth day the response had returned to the normal range, and remained within it. The effect on potassium and chloride excretion was similar in type and degree to the effect on sodium.

Day of expt.	Sample	1	2	3	4	5	6	7	8	9	10
Normal 0 at 0.36½	Time(mins)	10	20	36	45	55	65	75	85	95	
	Flow rate	0.9	0.95	1.0	0.39	0.18	0.6	0.53	0.56	0.5	
	Na	6.4	2.57	4.27	1.89	8.75	5.12	6.75	8.95	6.28	
	K	6.57	3.59	3.97	1.48	8.42	5.75	5.55	5.4	5.25	
	Cl	11.5	4.95	7.2	2.74		11,1	14.3	17.7	12.7	
Normal 0 at 0.33	Time(mins)	10	20	34	45	55	65	75	85	95	
	Flow rate	0.8	1.0	1.11	0.14	0.22	0.57	0.46	0.37	0.28	
	Na	3.85	4.38	5.9	1.26	8.45	6.05	5.2	5.83	7.32	
	K	4.19	3.63	3.76	0.74	7.32	6.6	5.62	4.13	5.45	
	Cl	7.0	6.79	8.88		11.0	14.3	14.6	13.1	16.0	
May 1. 0 at 0.34	Time(mins)	10	20	35	45	55	65	75	85	95	105
	Flow rate	0.55	0.42	0.54	0.12	0.33	0.45	0.33	0.38	0.39	0.39
	Na	6.3	6.28	9.95	4.56	30.6	21.6	27.0	27.6	21.7	20.0
	K	3.56	2.76	2.55	0.76	6.2	3.3	2.98	3.58	3.31	3.47
	Cl	10.5	11.1	15.7		30.0	26.0	30.0	32.4	23.8	22.2
Day 2 0 at 0.32	Time(mins)	10	20	33	45	55	65	75	85	95	105
	Flow rate	0.94	0.85	0.81	0.17	0.32	0.54	0.39	0.36	0.4	0.24
	Na	4.1	4.62	5.5	3.82	19.4	14.3	10.1	11.2	10.4	4.3
	K	4.73	4.56	4.57	1.63	11.6	7.2	5.5	6.1	5.65	3.7
	Cl	13.2	12.8	14.6		28.6	28.5	24.2	25.5	22.4	12.3
Day 3 0 at 0.33	Time(mins)	10	20	34	45	55	65	75	85	95	105
	Flow rate	1.35	1.2	1.05	0.18	0.2	0.46	0.64	0.52	0.48	0.37
	Na	5.17	4.9	5.34	4.85	17.9	14.9	15.1	17.3	14.9	10.2
	K	5.42	4.95	4.95	2.21	12.3	9.9	8.1	7.8	7.0	6.12
	Cl	13.8	12.5	15.5		29.4	35.4	30.7	32.8	26.8	20.7
Day 5 0 at 0.39½	Time(mins)	10	20	40	50	60	70	80	90	100	
	Flow rate	1.35	0.95	0.77	0.12	0.12	0.4	0.59	0.38	0.4	
	Na	37.6	13.2	5.75	0.94	5.3	7.7	7.1	5.9	5.42	
	K	17.2	6.2	2.43	0.42	2.02	3.61	2.82	2.26	2.38	
	Cl	52.9	17.6	8.85			13.2	12.4	12.2	12.0	
Day 8 0 at 0.31½	Time(mins)	10	20	32	40	50	60	70	80	90	100
	Flow rate	0.6	0.55	0.66	0.15	0.07	0.37	0.5	0.42	0.3	0.4
	Na	5.23	1.19	1.43	0.58	0.75	2.72	2.5	1.96	2.22	1.97
	K	4.28	1.69	1.66	0.53	1.09	3.54	2.43	1.83	1.84	1.66
	Cl	6.9	2.75	3.56			5.92	5.28	5.9	7.65	6.6

Table 10. The effect of 150mU oxytocin, before and after stilboestrol treatment, in the normal dog.
Times of intravenous injection of oxytocin given in first column, with day of experiment. Stilboestrol given on days 0 and 1
Rate of urine flow is in ml/min, and all electrolytes in µE/min.

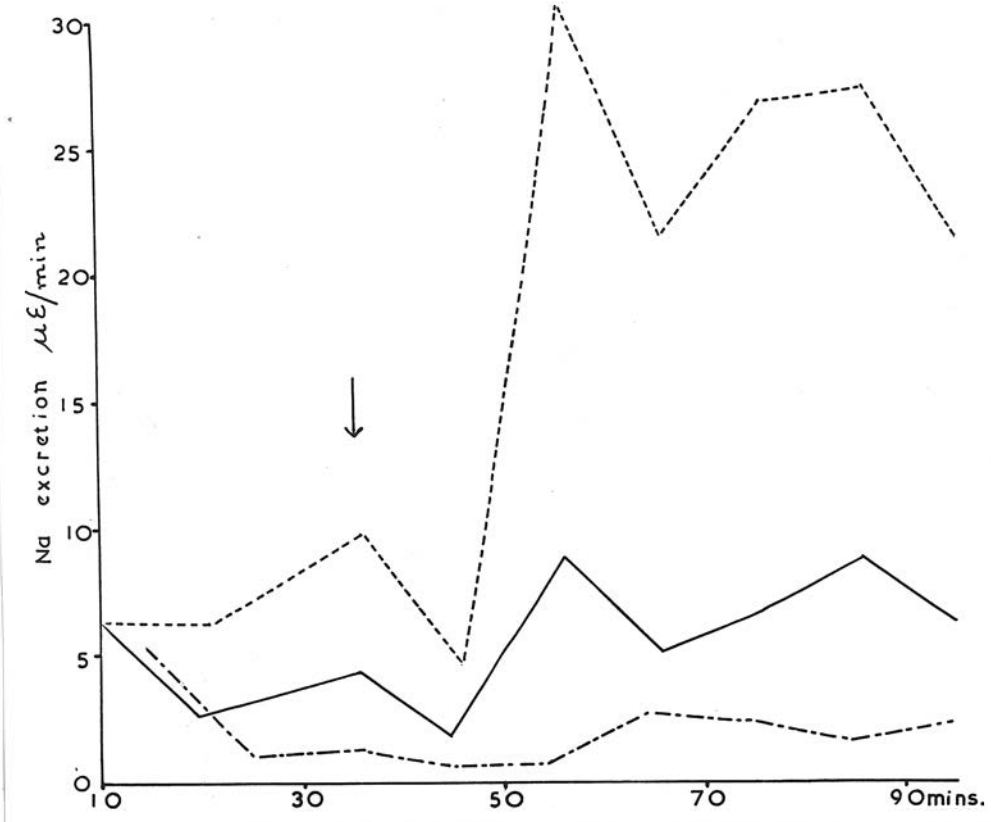

Figure 22. The effect of a single intravenous injection of 150mU oxytocin on the sodium excretion at a low rate of urine flow, before and after stilboestrol.

——— = Normal pretreatment response.

------ = First day after treatment.

-·-·- = Eighth day after treatment.

Arrow indicates time of injection.

THE EFFECT OF STILBOESTROL ON RENAL FUNCTION IN DOGS WITH DIABETES INSIPIDUS, AND AFTER POSTERIOR LOBE REMOVAL.

In each experiment water excretion, electrolyte output, and renal clearances were studied simultaneously, but it will be more convenient to describe the results separately. The findings in normal dogs, with which the following results must be compared, have been given on page 48.

Water excretion.

The effect of stilboestrol on the urine excretion during water diuresis differed in the three animals tested. In 'Chris', in whom diabetes insipidus had been induced two months previously, there was an increase in the height of diuresis on the second day after stilboestrol administration. Figure 23 shows the normal pre-treatment excretion curve, and those obtained on the second and fourth days after medication. The higher peak rate of excretion was present only on the second day. Thereafter the curves were similar to the normal. In the two observations on 'Thisbe', 6 and 8 months after operation, there was a depression in the maximal rate of urine output during water diuresis, similar to that of normal dogs. On the first occasion the depression lasted throughout the 6 days of observation, but recovery had occurred before the start of the second observations 7 weeks later. In the dog from which the

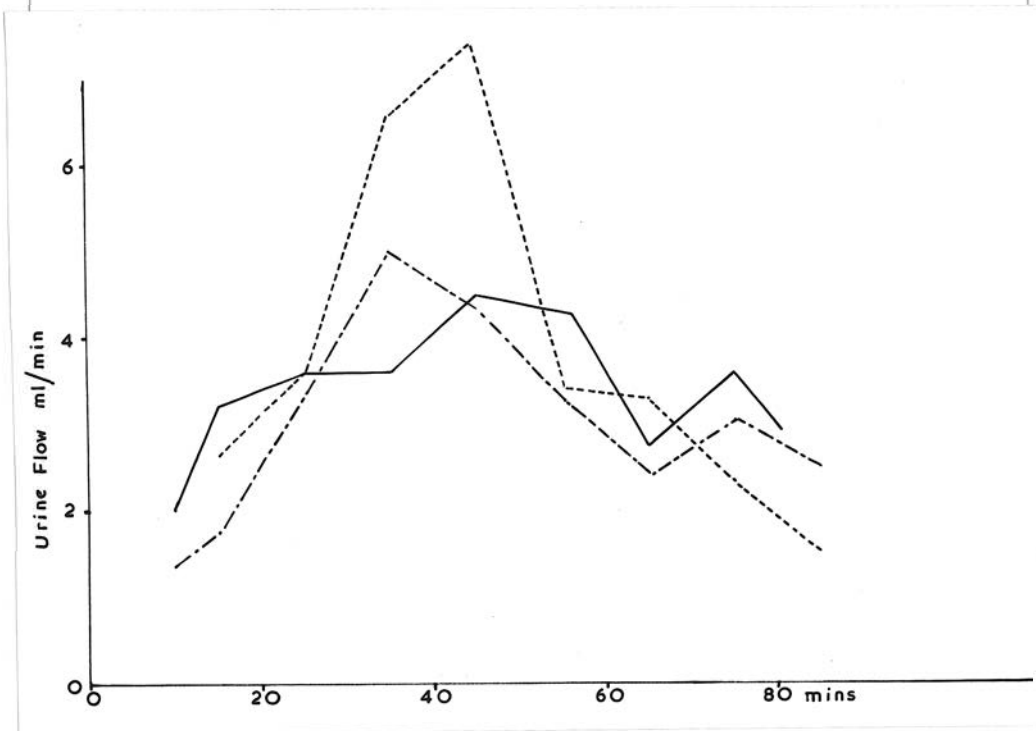

Figure 23. Urine flow during water diuresis in 'Chris', before and after stilboestrol treatment.

——— = Normal pattern of urine flow.

------ = First day after stilboestrol.

—·—· = Fourth day after stilboestrol.

350ml water given at zero time.

posterior lobe only had been removed, the effect of stilboestrol on water excretion was variable. There was a depression of excretion on the first day after stilboestrol, and enhancement on the fifth day. On other days, the pattern of flow was not different from the normal.

Renal clearances.

Though both G.F.R. and R.P.F. were increased in all cases after stilboestrol treatment, the extent and duration of this increase varied among the different animals. (Figure 24). In the dog with diabetes insipidus of two months duration, the rise in both values was considerable on the second day after stilboestrol, with a return to normal pre-treatment values by the 8th day. In the animal which had been polyuric for six months, there was a marked rise in G.F.R. on the second day, and a smaller rise in R.P.F. The values were normal by the third day. In 'Jess', with the posterior lobe alone removed, there was some rise in both G.F.R. and R.P.F. on the second day after treatment, and a fall by the fourth day. Figure 24 shows the clearance values in all animals.

Electrolyte excretion.

This again varied in the three dogs tested. (Figure 25). In 'Chris' (diabetic for two months) there was an increase in the excretion of sodium, potassium, and chloride, over a standard hour of water diuresis, which was first apparent on the

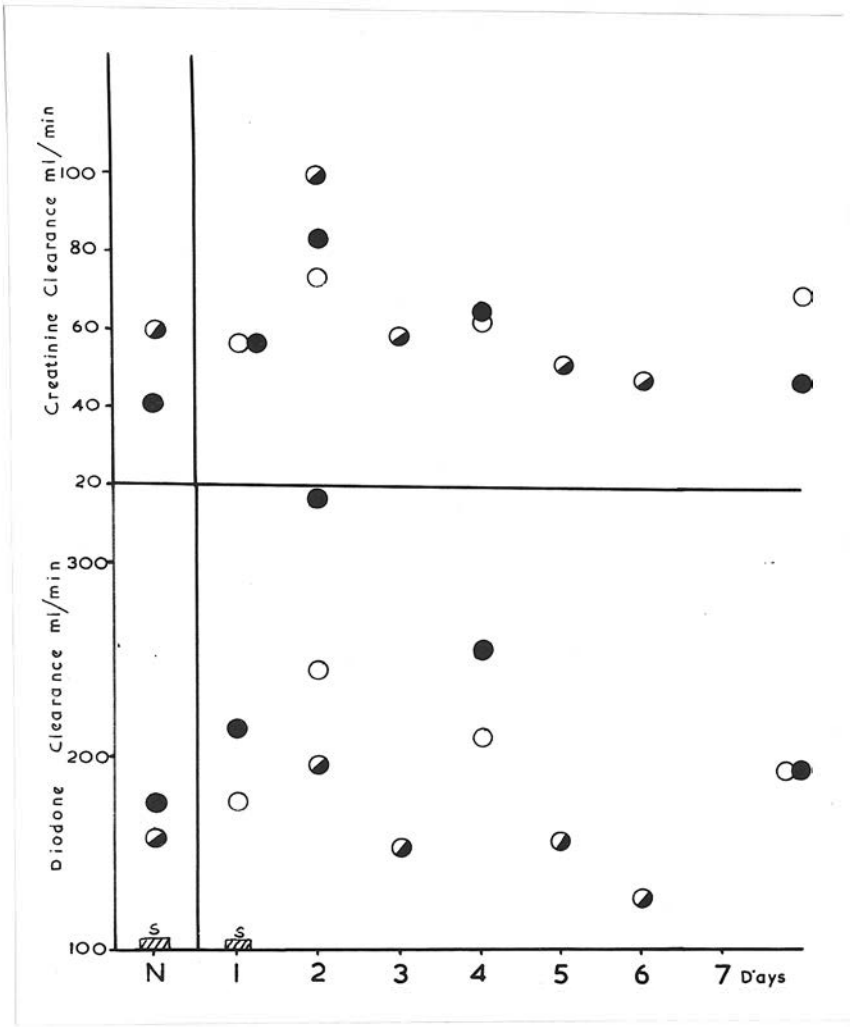

Figure 24. The effect of stilboestrol on renal clearances.

● = Diabetes insipidus of 2 months duration.

◐ = Diabetes insipidus of 6 months duration.

○ = Posterior lobe removed 6 months previously.

S Indicates days on which stilboestrol was given.

N = Normal pretreatment controls.

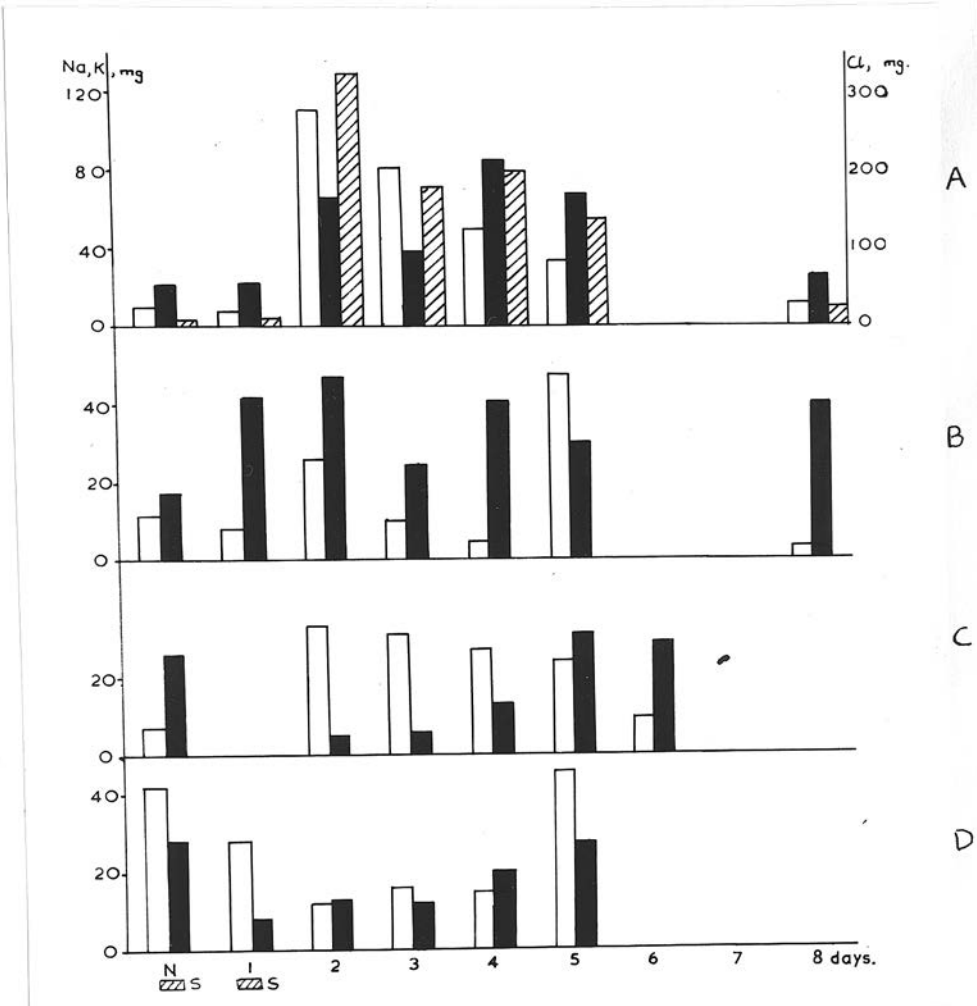

Figure 25. The effect of stilboestrol on electrolyte excretion in a standard hour.

White columns = Na, Black = K, Hatched = Cl.

A. Diabetes insipidus of 2 months duration.

B. Posterior lobe removed 6 months previously.

C. Diabetes insipidus of 6 months duration.

D. Same dog as C, diabetes insipidus of 8 months duration.

▨ S indicates days on which stilboestrol given.

N = Typical normal pretreatment controls.

second day after stilboestrol administration, and which lasted at least until the fifth day. (figure 25A). The results in 'Thisbe', six months after induction of diabetes insipidus, show that here also stilboestrol caused a rise in sodium excretion, but there was a depression in potassium excretion; (Figure 25C), but eight months after the onset of diabetes insipidus the response to stilboestrol was normal. (Figure 25D). In 'Jess' (posterior lobe removed) there was a fairly constant increase in potassium excretion after stilboestrol, but sodium excretion was very variable, increases being apparent on the second and fifth days only. (Figure 25B).

DISCUSSION.

Before considering the significance of the results obtained, some comment must be made on the methods used. The use of clearance methods for the estimation of G.F.R. and R.P.F. is open to criticism. Changing blood level of the substances used, and abrupt changes in the rate of urine flow are only two of the factors which may lead to errors in the calculations. With regard to the former, it has been found that blood levels were in most cases reasonably constant when the clearance substances were given subcutaneously some time before the start of the experiment. (Brooks and Pickford, 1958), and the latter factor was minimised by making measurements at the height of water diuresis. Since exact measurement of G.F.R. and R.P.F. was not the object of the present studies, but the search for a qualitative change, it was enough to use a method giving reproducible results in a normal animal in constant conditions, and causing the animal the minimum of inconvenience. In this way no resentment or other emotional disturbance was engendered, and the clearance changes observed could be accepted with some confidence as being genuine.

Although, in the first series of experiments, only one observation has so far been made with oxytocin, and one with vasopressin, the results were clear and definite, and, while needing

confirmation, show that, in these experiments at least, the renal response to oxytocin and vasopressin was altered by stilboestrol treatment. The conditions under which observations were made were such that in untreated animals, in an appropriate state of hydration, the injection of either posterior pituitary hormone caused an increase in sodium excretion in the urine samples immediately following the injection. After stilboestrol administration, however, this increase in sodium output was altered, apparently in opposite ways, being abolished in the case of vasopressin, and augmented in the case of oxytocin. The latter result is, however, subject to some suspicion, as the basic rate of sodium excretion was higher after stilboestrol than before, and there was thus no sign of the sodium retention which usually occurs in normal dogs. It cannot, therefore, be assumed that this type of alteration in the response to oxytocin is typical. Where vasopressin was used, urine output and sodium excretion showed the usual depression after stilboestrol treatment, and the dog therefore seemed to be responding normally to such treatment. Why this was not so in the oxytocin experiment cannot be explained - the diet on which the dog was maintained was identical in both cases, and the dose of stilboestrol given was also the same. The interval of eight weeks between the two experiments should have been sufficient for

complete recovery from the effects of the first administration of stilboestrol. However, the results of these experiments in the normal animal do point to the fact that the renal action of the posterior lobe hormones may be altered by oestrogens, and the results of the second series of experiments on animals with diabetes insipidus or after posterior lobe removal further suggests that these hormones may be implicated in the retention of water and electrolytes caused by oestrogens. Thus, following stilboestrol treatment, the dog with diabetes insipidus of two months duration showed an increase, and not a decrease, in excretion of water and electrolytes. In the animal whose posterior lobe alone had been removed, the water retention was small, and electrolyte excretion was irregular; this animal was found to respond to osmotic stimuli by antidiuresis, (Brooks and Pickford,1958), suggesting that it was not completely lacking in vasopressin. In the other animal with diabetes insipidus, water retention was observed after stilboestrol in both experiments, but during the first, sodium excretion was increased. This suggests that sodium and water are not necessarily identically affected. During the second experiment on this animal, the response to stilboestrol was similar to that of the normal animal, i.e. water and electrolytes were similarly retained. Either recovery or

compensation must have taken place. How far the duration of diabetes insipidus is responsible for the varying results in the different experiments is not known. Another factor which might conceivably have affected the response is the initial pre-treatment excretion rate of electrolytes. It can be seen from figure 25 that in the two cases where sodium excretion was increased after stilboestrol, the dogs initially excreted only moderate amounts of sodium, but that where retention of sodium occurred, the pre-treatment excretion level was considerably higher. Why this was so is unknown, as the dogs were maintained on the same diet. However, the dog without a posterior lobe was also excreting only moderate amounts of sodium, but the response to stilboestrol was erratic; thus such differences cannot be the sole reason for the difference of response.

As in normal dogs, all the operated animals showed a rise in G.F.R. and R.P.F. following stilboestrol treatment, but compared with normal animals, these changes erratic, and relatively transient. From experiments in which oestrogens were administered to normal dogs, Selkurt, Talbot and Houck (1943) concluded that the observed alteration in renal clearances was not an integral part of the changes in excretory activity. This is bourne out by the results described here, as electrolyte excretion was in some cases increased,

and in some reduced during such changes in clearances.

That interference with the supraoptico-hypophysial tracts alters the renal response to stilboestrol, suggests that the response to stilboestrol in the normal dog may well be related to some function of the pars nervosa. Moreover, if changes in G.F.R. and R.P.F. are not causative, the effect must be on tubular function. That the renal response to oxytocin and vasopressin may be altered by stilboestrol has been shown in the two experiments on normal dogs, and as there is no evidence that such small doses of vasopressin affect renal clearances, the effect of vasopressin at least must be tubular.

The experiments described do not attempt to prove that the hormones of the posterior pituitary, and more particularly vasopressin, are responsible for the water and electrolyte retention observed after oestrogen treatment, but they do suggest that changes in renal activity may be a part of the mechanism of water and electrolyte retention. Among other factors which have been cited as having a bearing on the water retention is the theory that hyaluronic acid may first cause the retention of serum proteins in the extracellular fluid space, which in turn causes the retention of water to promote osmotic balance. How far changes in reabsorption by the kidney are of

importance in this change in distribution is not known. They may serve merely to increase the return of filtrate to the circulation, thus enabling water and solutes to be passed on to other tissues with greater ease.

As yet no single hypothesis can explain all the isolated pieces of experimental observation in this field. The collection of evidence may help to produce a working hypothesis, but at present the facts make no clear pattern.

GENERAL CONSIDERATIONS.

While the experiments described in the two parts of this thesis were undertaken to provide information about two different aspects of ovarian hormone action, certain factors common to both emerge. These are the changes in the actions of posterior pituitary hormones, on both the vascular system and the kidney, which occur at different ovarian hormone levels. Moreover, most of the changes are maximal at approximately the same time after stilboestrol administration. This raises the question as to whether other functions of oxytocin and vasopressin are similarly affected, and it has long been known that this is so in at least one other instance; the action of both oxytocin and vasopressin on the uterus is affected by the ovarian hormones. Studies on this organ under predominantly oestrogen or progesterone control have shown that uterine reactivity to oxytocin is in general increased under the influence of oestrogens, and that to vasopressin diminished, and that this may be counteracted by progesterone in some species. Whether changes in vascular reactions are the basic cause of changes in all organs is not known. This may be so, or the vascular tissues, like the others, may be subject to some influence which affects all. It seems, then, that many of the functions of the posterior pituitary hormones are affected by those of the

ovary, and further experiments to discover whether this action is indeed a general one would be of great interest. In the light of this possibility, reports of different levels of posterior pituitary activity in relation to ovarian hormone levels are of interest.

Heller (1957) showed that in the rat there was a variation in the amounts of oxytocin and vasopressin found in the gland, and that this variation was correlated with the oestrous cycle. The vasopressin/oxytocin ratio remained about the same, but the actual content of the hormones was higher during oestrus than during dioestrus. From these determinations it is not possible to say whether the difference is caused by a change in rate of production, or in rate of release from the gland into the circulation. Hawker (1953a)measured the quantity of antidiuretic substance (A.D.S.) in the sera of sheep and cattle, and found that it was higher during pregnancy than in the non-pregnant state, and in cows the level was lowest prepubertally, and increased step-wise with sexual maturity, pregnancy, and lactation. In humans, (Hawker, 1953b) the level was higher during pregnancy than during the normal menstrual cycle, and fell again after parturition, just prior to the onset of lactation. It seems therefore, that under the influence of ovarian hormones, not only are the actions of posterior pituitary hormones

altered, but there is also a change in the production and/or liberation of these hormones.

While the evidence for pars nervosa implication in vascular disturbances such as the menopause, and in water and electrolyte retention is not conclusive, it is at least suggestive, and indicates that further work along similar lines may be profitable, and assist in the understanding of ovarian hormone effects on tissues other than those directly concerned with reproductive function.

SUMMARY OF RESULTS.

Variations occurred in the vascular responses of normal female rats to posterior pituitary hormones, and these were found to be correlated with the oestrous cycle. During oestrus, both oxytocin and vasopressin were pressor and vaso-constrictor; at all other times vasopressin had a less marked pressor action, while oxytocin was dilator, with no effect on the blood pressure.

For a few days after ovariectomy there was an increased sensitivity to the dilator action of oxytocin, and a decreased sensitivity to the pressor action of vasopressin.

In normal rats, the administration of either stilboestrol or progesterone induced a pressor response to oxytocin; in ovariectomised rats it was necessary to give both ovarian hormones together. In all cases where a pressor response to oxytocin was present, there was an enhanced pressor response to vasopressin.

During the first half of pregnancy, the responses were of the dioestrous type. From then until parturition the oestrous type of response occurred, reverting to the dioestrous type at parturition.

The depressor action of acetyl choline was not affected by ovarian hormone levels, but the pressor action of nor-adrenaline varied in a similar manner to that of vasopressin.

Neither atropine nor Priscol administration affected the vascular responses to posterior lobe hormones. After T.E.A. administration, however, or after decerebration or bilateral vagotomy, the dilator action of oxytocin was coverted to a pressor action.

In human subjects oxytocin was found to be vasodilator in the hand and forearm; this dilatation was reduced by stilboestrol therapy, and may be increased during the menopause.

In normal dogs, the electrolyte excretion response to oxytocin and vasopressin was altered by stilboestrol treatment. Antidiuretic action was not altered by such treatment.

For a few months after the induction of diabetes insipidus in dogs, stilboestrol increased sodium loss, and might also increase the rate of water loss for a few days. Renal clearances in these animals were irregularly increased by stilboestrol.

In a dog whose posterior pituitary was removed, stilboestrol caused minimal changes in electrolyte excretion and renal clearances.

REFERENCES.

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

ABRAMSON,D.I. ZALEELA,H, and SCHKLOVEN,N. (1941) Amer. Heart J. 21 756.

ACHESON,G.H. and MOE,G.K. (1946) J.Pharmacol. 87 220.

ALBRIGHT,F. (1936) J.Endocr. 20 24.

ALPERT,L.K. (1941) Johns Hopk. Hosp. Bull. 68 522.

ALQUIST,R.P. HUGGINS,R.A. and WOODBURY,R.A. (1947)J.Pharmacol 89 271

BENSON,G.Kand FOLLEY,S.J.(1956) Nature, Lond. 177 700.

BROOKS,H. and PICKFORD,M. (1957) The Neurohypophysis, London: Butterworths Scientific Publications.

BROOKS,H. and PICKFORD,M. (1958) In press. J.Physiol.

BOURNE,G. and ZUCKERMAN,S. (1940) J.Endocr. 2 283.

BOXILL,G.C. and BROWN,R.V. (1955) J.Pharmacol. 115 1.

BYROM,F.B. (1938) Lancet. Jan. 15. 129.

CAMPBELL,J.A. (1911) Quart. J. exp. Physiol. 4 1.

CHAUCHARD,P. (1943) Ann. Endocr., Paris. 4 133.

CHESLEY,L.C. and CHESLEY,E.R. (1939) Amer. J. Physiol. 127 731.

COHEN,M.E. and THOMSON,K.J. (1939) J.Amer. med. Ass. 112 1556.

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

COON,J.M. (1939) Arch. int. Pharmacodyn. 62 79.

COWIE,A.T. and FOLEEY,S.J. (1957) The Neurohypophysis, London: Butterworths Scientific Publications.

CSAPO,A. (1956) J.Physiol. 133 145.

CSAPO,A. and GOODALL,M. (1954) J.Physiol. 126 384.

DEAN,A.L. ABELS,J.C. and TAYLOR,H.C. (1945) J.Urol. 53 647.

DIGNAM,W.S. VOSKIAN,J. and ASSALI,N.S. (1956) J.clin.Endocr.16 1032

DILL,L.V. ISENHOUR,C.E. CADDEN,J.F. and ROBINSON,C.E Amer.J.Obstet.Gynec. 44 66.

FOLIN,O. (1914) J.biol. Chem. 17 469.

GADDUM, J. H. (1928) J. Physiol. 65 434.

GROLLMAN, A. HARRISON, T. R. and WILLIAMS, J. R. (1940) J. Pharmacol. 69 149.

HARRIS, G. W. (1955) Neural Control of the Pituitary Gland. London: Edward Arnold Ltd.

HARRIS, G. W. and MICHAEL, R. P. (1958) Proc. Physiol. Soc. J. Physiol. 142 no. 1. 26P.

HAWKER, R. W. (1939a) Endocrinology. 52 115.

HAWKER, R. W. (1939b) Endocrinology. 52 115.

HELLER, H. (1957) Ciba Foundation Colloquia on Endocrinology No. 11 London: Churchill Ltd.

HERREN, R. Y. and HATERIUS, H. O. (1931) Amer. J. Physiol. 96 214.

HERREN, R. Y. and HATERIUS, H. O. (1932) Amer. J. Physiol. 100 533.

HORGER, L. M. and ZARROW, M. X. (1957) Amer. J. Physiol. 189 407.

KNOWLTON, K. KENYON, A. T. SANDIFORD, I. LOTWIN, G. and FRICHER, R. (1942) J. clin. Endocr. 2 671.

KROHN, P. L. and ZUCKERMAN, S. (1937) J. Physiol. 88 369.

LARSON, A. (1938) J. Pharmacol. 62 346.

LEATHEM, J. H. and DRILL, V. A. (1943) Amer. J. Physiol. 139 17.

LUM, B. K. and NICKERSON, M. (1954) Fed. Proc. 13 381.

McGRATH, E. J. (1935) J. Amer. med. Ass. 105 854.

McGRATH, E. J. and HERRMANN, L. G. (1944) Ann. Surg. 120 607.

NEWMAN, R. L. (1955) J. Obstet. Gynaec. 6 615.

OLIVER, G. and SCHAFER, E. A. (1895) J. Physiol. 18 277.

PATON, D. N. and WATSON, A. (1912) J. Physiol. 44 413.

PREEDY, J. R. K. and AITKEN, E. H. (19) J. clin. Invest. 35 440.

REHBERG, P. B. (1926) Biochem. J. 20 447.

REYNOLDS, S. R. M. (1939) J. Physiol. 95 258.

REYNOLDS, S. R. M. and FOSTER, F. I. (1939a) Amer. J. Physiol. 126 606.

REYNOLDS, S. R. M. and FOSTER, F. I. (1939b) J. clin. Invest. 18 649.

REYNOLDS, S. R. M. and FOSTER, F. I. (1939c) Amer. J. Physiol. 128 147.

REYNOLDS, S. R. M. and FOSTER, F. I. (1940a) Amer. J. Physiol. 131 200.

REYNOLDS, S.R.M. and FOSTER, F.I. (1940b) Amer.J.Physiol. 131 422.

REYNOLDS, S.R.M. HAMILTON, J.B. diPALMA, J.R. HUBERT, G.R. and FOSTER, F.I. (1942) J. clin. Endocr. 2 228.

REYNOLDS, S.R.M. KAMINESTER, S. FOSTER, F.I. and SCHLOSS, S (1941) Amer.J. Obstet. Gynec. 41 1022.

REYNOLDS, S.R.M. (1952) in Physiological Bases of Gynecology and Obstetrics. Springfield: Thomas.

SAWYER, W.H. and SAWYER, M.K. (1952) Physiol. Zool. 25 84.

SCHOFIELD, B.M. (1954) Endocrinology. 55 142.

SELKURT, E.E. TALBOT, L.J. and HOUCK, C.R. (1943) Amer.J.Physiol 140 260.

SELYE, H. and BASSETT, L. (1940) Proc.Soc.exp.Biol, N.Y. 44 502.

SUZMAN, M.M. FREED, C.C. and PRAG, J.J. (1938) S.Afr.J. med Sci. 3 29.

THORN, G.W. and ENGEL, L.L. (1938) J. exp.Med. 68 299.

THORN, G.W. NELSON, K.R. and THORN, D.W. (1938) Endocrinology 22 155

THORN, G.W. and HARROP, G.A. (1937) Science. 86 40.

VICK, J. EDERSTROM, H.E. and VERGER, T. (1956) Proc.Soc.exp.Biol 93 536.

VOGT, M. (1945) J.Physiol. 104 60.

WELSH, C.A. WELLOR, I. and TAYLOR, H.C. (1941) J. clin. Invest. 21 57.

WITTEN, C.L. and BRADBURY, J.T. (1951) Proc.Soc. exp.Biol. 78 626.

WOODBURY, R.A. and ABREU, B.E. (1944) Amer.J.Physiol. 142 114.

ZWEIFACH, B.W. and CHAMBERS, R. (1944) Amer.J.Anat. 75 173.