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THE HYPOTHALAMIC NEUROSECRETORY PATHWAYS FOR THE RELEASE OF OXYTOCIN AND VASOPRESSIN IN THE CAT

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SUMMARY

1. The neurones of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) were stimulated electrically in lactating cats under chloralose anaesthesia. Milk-ejection responses were used to monitor the release of oxytocin and vasopressin and both hormones were assayed in samples of blood collected during stimulation. The position of the tip of the stimulating electrode was confirmed from brain sections stained selectively for cystine-rich neurosecretory material.

2. A previous finding that stimulation of the SON in the cat releases vasopressin without oxytocin was confirmed.

3. Stimulation of the PVN caused both hormones to be released. The ratio of their concentrations in blood was variable; this suggests release from separate neurones.

4. Both hormones were also released on stimulation of the median eminence but not of the zone lying vertically between this structure and the PVN. No neurosecretory material was detected in this zone. These findings argue against the existence of a direct or medial paraventriculo-hypophysial pathway running downwards along the wall of the third ventricle.

5. Study of sections from unstimulated brains confirmed that the tractus paraventricularis cinereus of Greving which runs ventro-laterally from the PVN towards the SON, represents the principal efferent pathway for neurosecretory fibres from the PVN.

6. The results are discussed in relation to the problem of the independent release of oxytocin and vasopressin in response to physiological stimulation of the neurohypophysis.

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INTRODUCTION

Oxytocin and vasopressin are produced, and their release is controlled by neurosecretory cells in the anterior hypothalamus. These cells are grouped in two nuclei, the supraoptic nucleus (SON) and the paraventricular nucleus (PVN). It is still a controversial question whether there is any differentiation of function between the two nuclei. Does one produce oxytocin and the other vasopressin? A clearly related problem is whether the two hormones can be released independently in response to specific stimuli. There is now considerable evidence for independent release. Vasopressin can be released without oxytocin in response to haemorrhage in the rat (Ginsburg & Smith, 1959), the cat (Beleslin, Bisset, Haldar & Polak, 1967) and the dog (Schrier, Verroust, Jones, Fabian, Lee & de Wardener, 1968) and to carotid occlusion in the cat (Clark & Rocha e Silva, 1967); oxytocin can be released without vasopressin in response to suckling (Bisset, Clark & Haldar, 1970) and parturition (Haldar, 1970) in the rabbit. It is possible that a given stimulus might release one or other hormone from the same neurone but independent release could be more easily envisaged if the two hormones were contained in different neurones and these neurones were in turn distributed in separate neurosecretory pathways.

Bisset, Hilton & Poisner (1967) attempted to define neurosecretory pathways for oxytocin and vasopressin in the cat, by estimating both hormones in samples of blood collected during electrical stimulation of discrete points within the hypothalamus. The hormones were estimated by assaying blood extracts for antidiuretic activity (ADA) and milk-ejecting activity (MEA). Oxytocin has negligible ADA, but vasopressin has MEA which amounts in some species to about one fifth of its ADA. Stimulation of the SON in the cat caused a large increase of ADA in the blood, indicating release of vasopressin and the concentration of this hormone was sufficiently high to account for any MEA. It was concluded that in the cat the neurones of the SON constitute a specific neurosecretory pathway for vasopressin. This implies that oxytocin is produced by the PVN, but no MEA could be detected in the blood on stimulation of this nucleus although moderate increases of ADA were sometimes observed. However, the assay for MEA, using the lactating guinea-pig, was relatively insensitive and the lowest concentration of oxytocin which could be detected was about 20 μ-u./ml blood.

In the present experiments, which are an extension of the previous work by Bisset, Hilton & Poisner (1967), two methods have been used to detect release of neurohypophysial hormones in response to a stimulus. One is to observe the response of the animal's own target organs and the other is to assay the hormones in blood. The hypothalamus was stimulated electrically

in lactating cats and the release of hormone monitored by recording milk-ejection pressure from a cannulated teat duct. Blood samples were collected during stimulation. The lactating rat was used to assay MEA and this reduced the lowest concentration of oxytocin which could be detected to 3 μ-u./ml blood or less. In addition, sections of the brain were stained selectively for cystine-rich neurosecretory material (Sloper, 1966) in order to reveal more clearly the position of the stimulating electrode in relation to the cell bodies and axons of neurosecretory cells. The same staining technique was used to study the normal anatomy of the paraventricular neurones in the unstimulated brain.

METHODS

Experiments were performed on eleven lactating cats weighing between 3·0 and 4·2 kg. Anaesthesia was induced with ethyl chloride and ether and maintained with chloralose (60–70 mg/kg) given intravenously. Additional injections of one fifth of the initial dose were given when necessary. In all experiments the trachea was cannulated to maintain a free airway, but artificial respiration was not applied. Arterial blood pressure was recorded from a cannulated femoral artery and milk-ejection pressure from a cannulated teat duct by means of strain gauge transducers (Statham, P. 23 A) and potentiometric recorders (Goertz, type RE 511). Blood samples were collected from a cannulated external jugular vein. Each sample (5 ml.) was withdrawn at a steady rate over a period of 2 min with simultaneous replacement into a cannulated femoral vein of an equal volume of dextran solution heated to body temperature. Cannulae, needles and syringes were siliconed before use to avoid the formation of plasma kinins.

The intravenous dose of oxytocin required to elicit a threshold milk-ejection response was established at the beginning of each experiment. Discrete points within the hypothalamus were stimulated by means of a sterotactically placed monopolar steel electrode. Electrodes were prepared from steel sewing needles by electrolytic erosion (Bishop & Collin, 1951) to a tip size of 5–10 μ, and were varnished, except for 0·4 mm at the tip, with a single coat of vinyl lacquer. The indifferent electrode was a similar steel needle pushed under the skin of the neck. The stimuli were square-wave pulses of 2 msec duration, at a frequency of 80 Hz and of sufficient voltage to give a current of 200 μA.

Stimulation was applied at points 0·2–1 mm apart in the same vertical plane for periods of 10 sec. It was necessary in some experiments to explore two or three planes, separated by at least 1 mm in the sagittal plane, before an active zone was located. When a milk-ejection response was obtained, a period of 30 min was allowed to elapse, at the end of which a sample of blood was collected. Stimulation was then applied for 1 min and a blood sample withdrawn, commencing at the beginning of the stimulation period. After a further 30 min the exploration continued; blood samples were collected at each point at which a milk-ejection response was obtained.

The β-receptor blocking drug Trasicor 1-2 mg was given by intravenous injection routinely at the beginning of each experiment and repeated when necessary as it had been observed that stimulation of the paraventricular nucleus frequently elicited signs of sympathetic activity and that adrenaline inhibited the response to oxytocin (Cross, 1958; Bisset, Clark & Lewis, 1967).

At the end of each experiment the animal's head was perfused, with the electrode

in situ, first with 0·9% NaCl solution and then with 10% formol saline. After withdrawal of the electrode, the brain was dissected from the cranial cavity and immersed in 10% formol saline for several days to ensure complete fixation of the tissue. Serial frozen sections 20 or 25 μ in thickness were cut in the coronal plane and alternate sections stained with luxol fast blue-cresyl fast violet (Klüver & Barrera, 1953) and performic acid – Victoria blue – phloxine (J. C. Sloper, personal communication). The latter technique is a modification of that described by Adams & Sloper (1956) for the demonstration of cystine-rich neurosecretory material. With this method, sulphonates, derived from oxidation of cystine with performic acid, react with a basic dye, Victoria blue, at pH 0·2 to give a deep blue colouration of neurosecretory material contained in cells and fibres in the hypothalamus. Control sections prepared without oxidation did not stain. For the graphic demonstration of the stimulation points in relation to the neurones of the paraventricular and supraoptic nuclei, sections showing the electrode track were projected on to a screen at a constant magnification (× 10). By marking in the position of the third ventricle, optic chiasma or tracts, the fornix and the electrode track, a composite picture of the region stimulated was constructed on which cells and fibres containing neurosecretory material, observed on microscopic examination of the sections, were superimposed. Stimulation sites were located by measurement from the position of the electrode tip as indicated by the lowest point of tissue damage.

Extraction and assay of blood samples. Blood samples were extracted with alcohol according to the method of Bisset, Hilton & Poisner (1967), and the extracts assayed for vasopressin and oxytocin against pituitary (posterior lobe) extract (PPLE).

Extracts were assayed for vasopressin by their antidiuretic activity in water-loaded rats under ethyl alcohol anaesthesia, using intravenous injections. The method is basically that of Dicker (1953) with the modifications described by Bisset (1962) and Clark & Rocha e Silva (1967). This preparation is practically free from interference by other naturally occurring substances in blood (Bisset, Hilton & Poisner, 1967) and oxytocin itself has negligible ADA (Berde & Boissonnas, 1968). All the ADA in the extracts was therefore assumed to be due to vasopressin.

Extracts were assayed for oxytocin by their milk-ejecting activity in the lactating rat using retrograde arterial injections (Bisset, Clark, Haldar, Harris, Lewis & Rocha e Silva, 1967). The MEA detected in an extract could not always be attributed entirely to oxytocin since vasopressin possesses MEA equivalent, in the rat, to 13·6% of its ADA (Bisset, Clark, Haldar, Harris, Lewis & Rocha e Silva, 1967). The amount of oxytocin present in samples containing vasopressin was calculated by subtracting from the total MEA, the MEA of the vasopressin present. Possible interference by 5-hydroxytryptamine or adrenaline in the blood extracts was eliminated by injecting 2-bromo-lysergic acid diethylamide (BOL) 0·2 mg and the β-receptor blocking agent Trasicor 0·2 mg intravenously into the rat at the beginning of each assay (Bisset, Clark & Haldar, 1970).

The MEA in the blood samples was identified with the neurohypophysial hormones by means of the antagonist 1-N-carbamoyl-hemicystine-2-*O*-methyltyrosine-oxytocin (N-carbamoyl-*O*-methyloxytocin) synthesized by Chimiak, Eisler, Jošt & Rudinger (1968). The analogue has been shown to antagonise the MEA of oxytocin and vasopressin, but not that of bradykinin, 5HT or acetylcholine (Smyth, 1967; Bisset & Clark, 1968; Bisset, Clark, Krejčí, Poláček & Rudinger, 1970). When the analogue was injected intravenously 1 min before an extract containing MEA, the activity of the extract was abolished or reduced by the same amount as that of an equipotent dose of PPLE. Responses to the extract and PPLE recovered at the same rate.

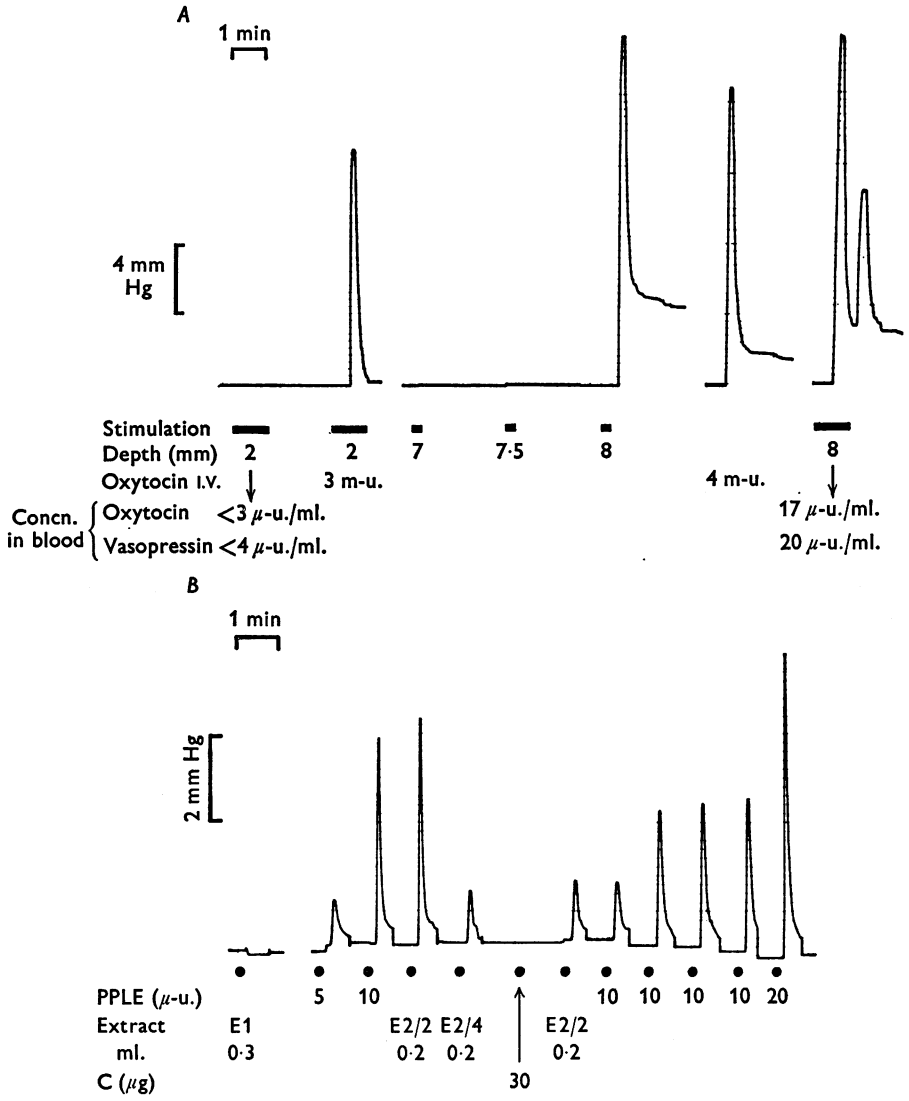
Materials. The pituitary (posterior lobe) extract (PPLE) which was used to assay

blood extracts was a laboratory standard containing 2 u./ml., prepared from a sample of the 3rd International Standard for Oxytocic, Vasopressor, and Antidiuretic Substances (Bangham & Mussett, 1958), according to the method prescribed in the British Pharmacopoeia (1953). Other substances used were oxytocin (Syntocinon: Sandoz), 1-iso-propyl-amino-2-hydroxy-3-(*O*-alloxy-phenoxy)-propane hydrochloride (Trasicor, CIBA), 2-bromo-lysergic acid diethylamide (BOL Sandoz), dextran solution (Intradex, Glaxo) and heparin (Pularin, Evans Medical). The synthetic oxytocin analogue, N-carbamoyl-*O*-methyloxytocin was a gift from Professors Rudinger and Jošt of the Czechoslovak Academy of Sciences.

RESULTS

The threshold dose of oxytocin by intravenous injection for eliciting a milk-ejection response (MER) was 2 m-u. in six of the eleven cats, 3 m-u. in three and 4 m-u. in two. For doses of 2–8 m-u. injected rapidly, the latent period from the start of the injection to the rise of milk-ejection pressure was 12–24 sec. The pressure rose in a single peak and fell to within 20 % of its maximum value in 0·5–2 min (Text-fig. 1*A*). Injections could be repeated at intervals of 5 min without tachyphylaxis. In two cats in which the response of the mammary gland to vasopressin was tested, the threshold dose was 40 m-u. It was not possible to assay the milk-ejecting activity of vasopressin because doses above the threshold inhibited the response to subsequent doses of oxytocin. Doses of 40–80 m-u. vasopressin caused a rise of blood pressure of 10–20 mm Hg, but since this rise lasted only for 1–2 min, the inhibitory effect of vasopressin on the milk-ejection response to oxytocin was unlikely to have been caused by vasoconstriction of the mammary blood vessels. It was probably due to saturation by vasopressin of a common receptor in the myoepithelial cells of the gland and analogous to the tachyphylaxis which is observed when large doses of vasopressin are tested at short intervals on the blood pressure of the cat.

Two experiments were carried out to measure the concentration of oxytocin and vasopressin in the blood following an intravenous injection of these hormones in the cat. Samples of 5 ml. blood were collected over a period of 2 min as in the experiments on electrical stimulation. When 4 m-u. oxytocin was injected 20 sec after the commencement of blood sampling, the concentration in the sample was 4 μ-u./ml.; control samples taken 10 min before and after the injection contained $< 1{\cdot}3$ μ-u./ml. When 20 m-u. vasopressin, a dose too small to elicit milk ejection, was injected at the start of sampling, the concentration was 22 μ-u./ml. compared with 12 μ-u./ml. in a control sample taken 15 min before and 6 μ-u./ml. in one taken 15 min after sampling. The results of these experiments suggest that a concentration of oxytocin in the blood as low as 4 μ-u./ml. is compatible with a milk-ejection response whereas vasopressin is unlikely to elicit a response at blood concentrations below 20 μ-u./ml.



Text-fig. 1. *A*, Milk-ejection responses to electrical stimulation of the hypothalamus (square-wave pulse, 2 msec, 80 Hz, 200 μ-A) in a lactating cat. Milk-ejection pressure was recorded from a cannulated teat duct. The horizontal bars indicate periods of stimulation. The depth refers to the position of the tip of the stimulating electrode below the Horsley–Clarke zero. The Fig. gives the concentration of oxytocin and vasopressin in samples of blood (5 ml.) collected over 2 min from the start of stimulation for 1 min at 2 mm and 8 mm.

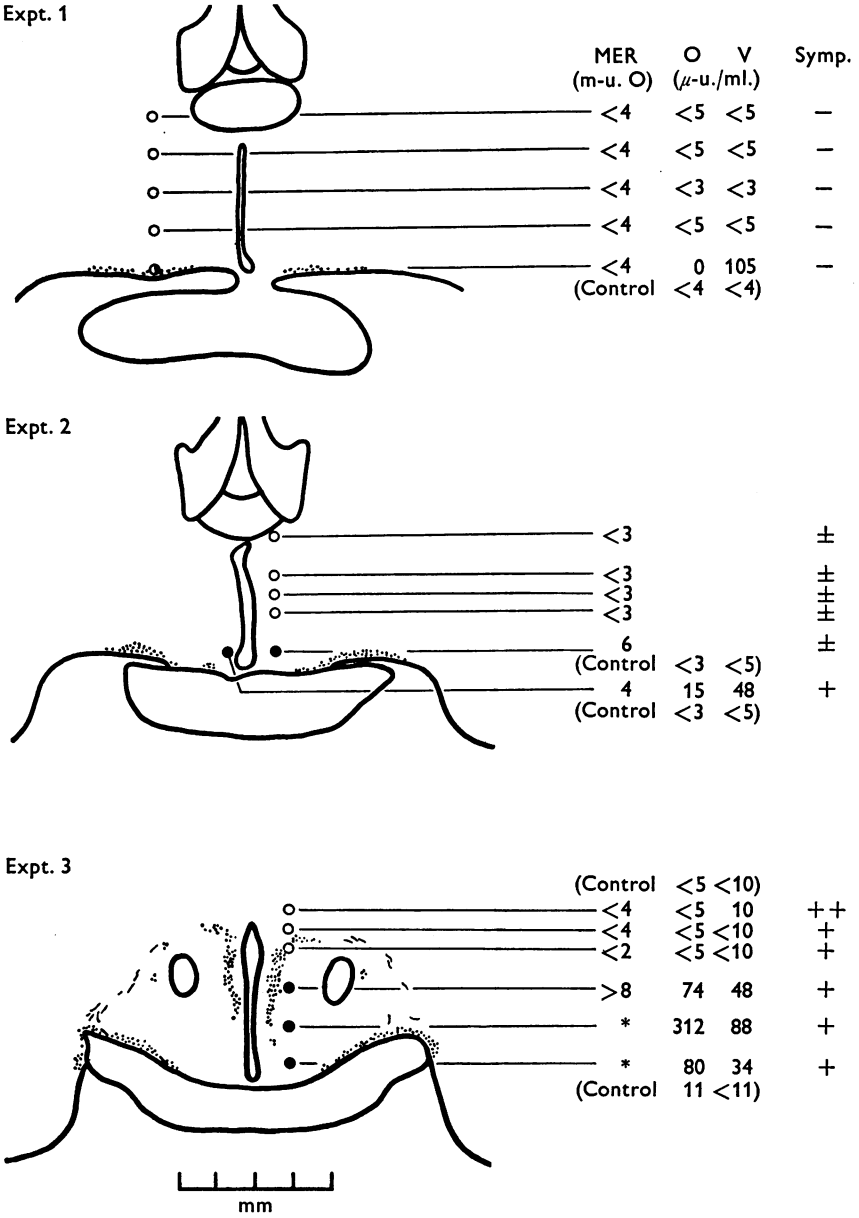
B, Milk-ejection pressure in a lactating rat recorded from a cannulated teat duct. Assay of the milk-ejecting activity of extracts (1 ml.) from the blood samples collected during electrical stimulation at 2 mm (E 1) and 8 mm (E2) in the experiment illustrated in *A*. PPLE = pituitary (posterior lobe) extract. C = N-carbamoyl-*O*-methyloxytocin.

Electrical stimulation produced milk ejection, or an increased concentration of hormone in the blood, or both effects, in nine of the eleven cats. In these nine cats, the point of the stimulating electrode was found to have been placed within 0·5 mm of the cell bodies of the supraoptic or paraventricular neurones in the SON and PVN or in contact with their axons in the median eminence. In the other two cats in which there was no evidence for release of hormone, the electrode lay more than 0·5 mm from the neurones. The latent period for a milk-ejection response to electrical stimulation was 22–66 sec, and the response closely resembled that to an injection of oxytocin.

A typical experiment (Expt. 8) is illustrated in Text-figs. 1 and 4; Text-fig. 1*A* shows milk-ejection responses (MERs) in the cat and Text-fig. 1*B* part of the assay of blood extracts for milk-ejecting activity (MEA) in the rat. Electrical stimulation of the hypothalamus in the anteroposterior plane indicated in Text-fig. 4 commenced at a point 2 mm below the Horsley-Clarke zero line and 0·5 mm from the mid line. No MER occurred. (Text-fig. 1*A*). Signs of sympathetic stimulation were marked but an intravenous injection of 3 m-u. oxytocin given during a second stimulation at this point induced a normal response. This confirmed that the dose of Trasicor given at the beginning of the experiment had effectively prevented a possible inhibitory action of adrenaline on the mammary gland. The electrode was lowered to 6 mm in 1 mm steps (not shown). Each stimulation failed to induce a MER. No response was obtained at 7 or 7·5 mm. Stimulation for 10 sec at 8 mm produced a large monophasic MER, greater than that after an intravenous injection of 4 m-u. oxytocin. When stimulation was repeated at 8 mm for 1 min a biphasic MER occurred. Blood samples (5 ml.) were withdrawn during stimulation for 1 min at 2 mm and during the second period of stimulation at 8 mm. The extracts (1 ml) were asayed for MEA and ADA. An injection of 0·3 ml. of the first extract (E 1) did not produce a MER (Text-fig. 1*B*) although the rat responded to 5 μ-u. PPLE. The blood sample therefore contained < 3 μ-u. MEA/ml. One group of a four-point assay of the second blood extract (E 2) is shown in which two doses of the extract are matched against 5 and 10 μ-u. PPLE. The MEA in the blood sample was calculated to be equivalent to 20 μ-u. PPLE/ml., but 3 μ-u. ml. could be attributed to the vasopressin (20 μ-u./ml.) present .The sample therefore contained 17 μ-u./ml. oxytocin. The tracing continues to show a 50 % reduction in the response to both E 2 and a matching dose of PPLE (10 μ-u.) following an intravenous dose of 30 μg N-carbomoyl-*O*-methyloxytocin, with partial recovery of the response to PPLE. The residual inhibition was surmounted with 20 μ-u. PPLE.

The results of the nine positive experiments are illustrated in Text-figs. 2–4 which represent coronal sections of the hypothalamus arranged in

order antero-posteriorly. The symbols indicate the position of the electrode tip in relation to the cell bodies and, where they could be discerned, the axons of the supraoptic and paraventricular neurones. Those points from which a MER was elicited, are distinguished by a filled symbol. The



Text-fig. 2. For legend see opposite page.

tables show the equivalence of the MER in terms of intravenous injections of oxytocin, the concentrations of oxytocin and vasopressin in the blood and the incidence of sympathetic activation, as assessed by pupillary dilatation, retraction of the nictitating membrane and cardiovascular effects. Seventeen control samples of blood were tested: in ten, neither oxytocin nor vasopressin was detected (< 3–< 10 μ-u./ml.); in seven, one or other was present at a concentration of 4–16 μ-u./ml.

In Expt. 1 (Text-fig. 2), five points were stimulated in a single track 2·5 mm from the mid line in the plane of the optic chiasma, anterior to the paraventricular nucleus. The lowest point was in the anterior part of the SON. Stimulation at this point resulted in a just detectable increase in milk-ejection pressure which was of doubtful significance and is indicated by the symbol ◑. The concentration of vasopressin in the blood increased from < 5 to 105 μ-u./ml. and, since the MEA in the blood sample did not exceed the intrinsic MEA of the vasopressin, the release of oxytocin could be excluded. A similar series of points was stimulated on one side of the brain, 0·5 mm from the mid line, in Expt. 2 (Text-fig. 2). A MER was obtained from the lowest point which lay 0·5 mm from the extreme medial edge of the SON. From a symmetrically opposite point on the other side of the brain, a MER was also elicited and this was accompanied by an increase in the concentration of oxytocin in the blood from < 3 to 15 μ-u./ml. and of vasopressin from < 5 to 48 μ-u./ml. This point lay close to a small cluster of neurosecretory cells which appeared to be distinct from the SON and

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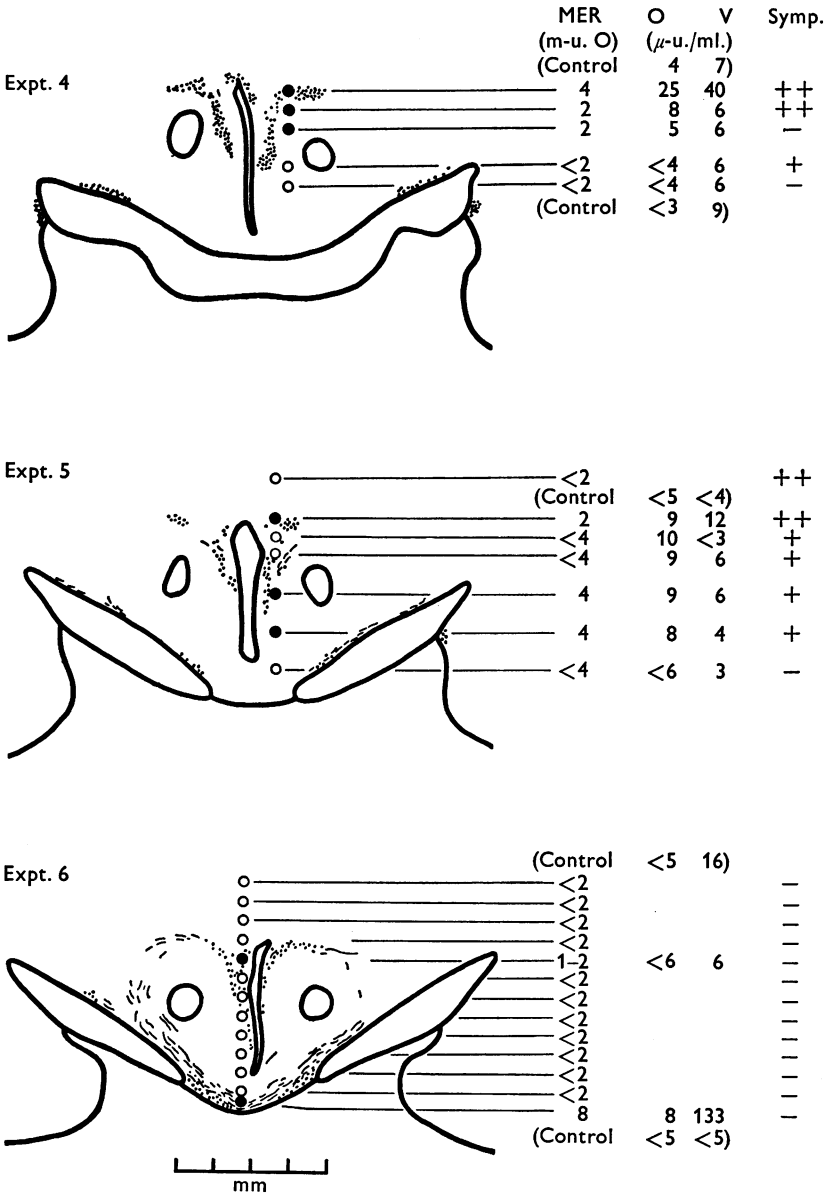
Legend to Text-fig. 2.

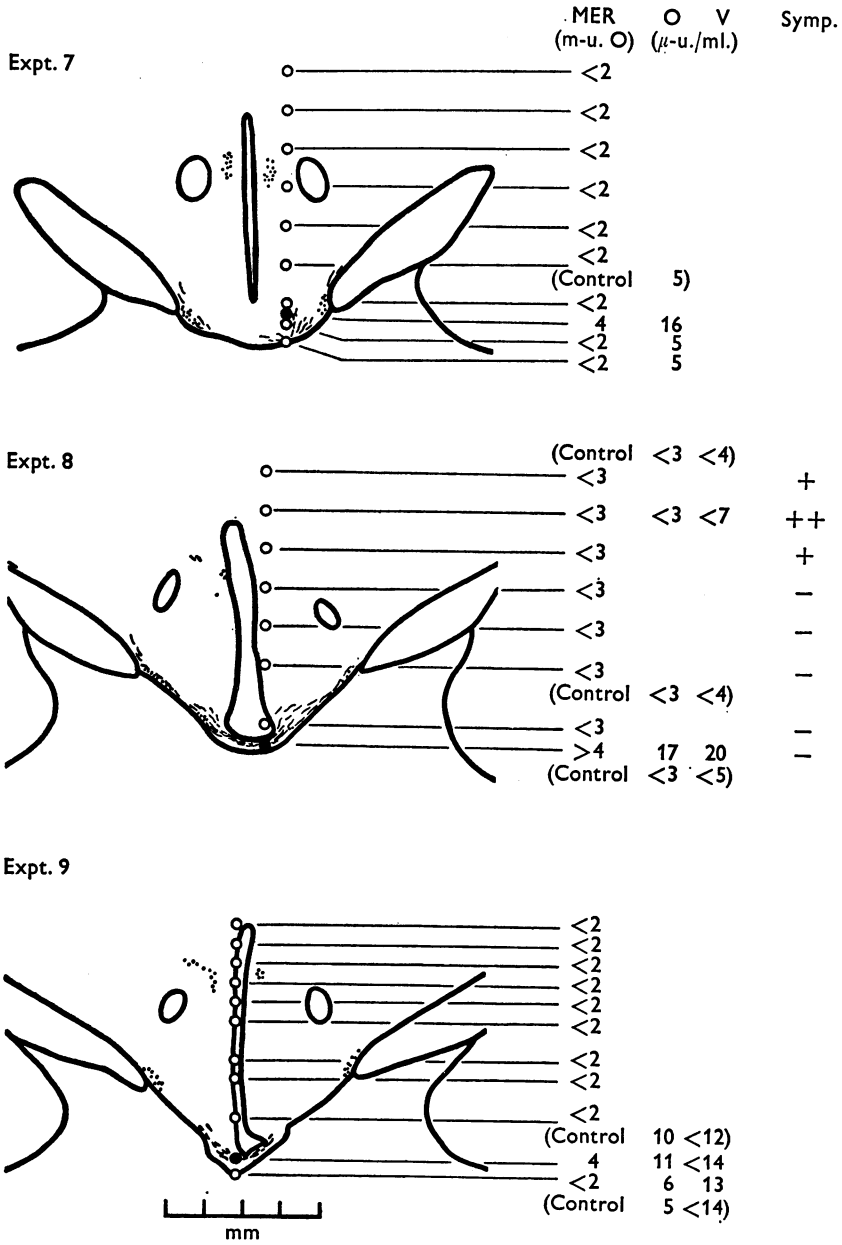
Diagrams of coronal sections of the hypothalamus in three cats (Expts. 1–3) to show the effects of electrical stimulation. The diagrams were constructed from histological sections stained alternately with luxol fast blue – cresyl fast violet and performic acid – Victoria blue – phloxine. The small dots represent the cell bodies of the supraoptic and paraventricular neurones; where they could be discerned the axons of these neurones are also depicted (for a guide to the anatomy of the hypothalamus see Text-fig. 5). The symbols indicate the positions of the tip of the stimulating electrode; ● denotes a milk-ejection response (MER) and ○, no MER (stimulation at the lowest point in Expt. 1 produced an equivocal MER which is indicated by ◑). The Table gives the magnitude of the MER in terms of an intravenous injection of oxytocin (O); where the symbol $< x$ m-u. is used, x indicates the threshold dose of oxytocin for eliciting a MER. Also shown in the Table are the concentrations of oxytocin (O) and vasopressin (V) in blood samples collected during stimulation and the incidence of signs of sympathetic activation (Symp). In Expt. 3, stimulation at the two lowest points (*) caused movements in the cat which obscured the pattern of the MERs; they appeared, however, to be equivalent to the intravenous injection of at least 8 m-u. oxytocin.

was thought to constitute an anterior extension of the tip of the PVN (see the section on Anatomy and Discussion).

The brain sections from Expts. 3 and 4 (Text-figs. 2 and 3) show the optic tracts beginning to emerge from the optic chiasma. It is in this zone



Text-fig. 3. Diagrams of coronal sections of the hypothalamus in three cats (Expts. 4–6). For key to symbols and Tables see legend to Text-fig. 2.



Text-fig. 4. Diagrams of coronal sections of the hypothalamus in three cats (Expts. 7–9). For key to symbols and Table, see legend to Text-fig. 2.

that the PVN is most prominent. The anterior part of the SON is also present lying along the superolateral border of the optic tract. In Expt. 3, large MERs equivalent to intravenous injections of at least 8 m-u. oxytocin, were obtained at three points lying within 0·5 mm of the lateral edge of the PVN and high concentrations of both hormones were present in the blood. The ratios of the concentration of oxytocin to vasopressin in the same blood samples were 1·5, 3·6 and 2·4. The three positive points were more than 0·5 mm from the medial edge of the SON. Since the spread of stimulus is less than 0·5 mm, as will be shown later, the release of hormones can be attributed to stimulation of the PVN. The three points in Expt. 4 from which MERs were elicited, lay above and far medial to the supraoptic neurones. The largest MER, and also the highest concentration of oxytocin and vasopressin in blood, were produced by stimulation of the uppermost point. The concentration of both hormones was increased about sixfold compared with that in the initial control sample taken before stimulation. The fact that a MER occurred despite pronounced sympathetic activity confirms again the effectiveness of Trasicor.

In the brain section from Expt. 5 (Text-fig. 3) the optic tracts have separated; the PVN is still well defined. Five stimulation points were within 0·5 mm of the PVN. At three, MERs were elicited equivalent to intravenous injections of 2-4 m-u. oxytocin. Oxytocin was detected in all the blood samples collected during stimulation of these five points but not in a control sample or in one collected during stimulation of a point 1·5 mm below the PVN. At one of the five points, the concentration of vasopressin was increased at least threefold compared with the control. However, from the results reported in a previous section, it seems probable that the MERs in this experiment were produced by release of oxytocin and not of vasopressin.

The brain sections from Expts. 6–9 (Text-figs. 3 and 4) pass through the median eminence. Scattered cell bodies of paraventricular neurones can be seen at the level of the fornix in Expts. 7, 8 and 9 and extending above and below this level in Expt. 6. In the section of Expt. 6, the axons of the paraventricular neurones are seen particularly clearly running in an arcuate course above and then lateral to the fornix, turning ventromedially along the upper border of the optic tract and finally lying in close apposition to the cell bodies and axons of the SON. Although this tract could not be traced in its entirety in the remaining sections (Text-fig. 4), fibres were seen in the median eminence which appeared to represent its distal end. In all four experiments MERs were obtained on stimulation of the median eminence. In Expt. 6 a MER was also elicited from a point near the PVN above the level of the fornix, but in none of the experiments was a MER obtained from the intermediate zone lying vertically between the PVN and

the median eminence, nor was there any histochemical evidence for the presence of neurosecretory material in this zone. The lower point from which a MER was elicited in Expt. 6 lay in contact with cell bodies of the SON and the concentration of vasopressin was about 16 times that of oxytocin; it is probable that the MER was due at least in part to vasopressin. Vasopressin was detected also in Expt. 8 but the level was too low for it to have contributed to the MER. In Expt. 7, however, vasopressin was not estimated, and although a threefold increase in MEA was found, the MER could not be attributed entirely to the release of oxytocin. In this experiment, the positive point shown was only 0·2 or 0·3 mm from the two points above and below it in the same electrode track, showing that the spread of stimulus is limited to this distance. Nine planes additional to those shown in Text-figs. 2–4 were explored in Expts. 8 and 9, but no MERs were obtained. In each case the electrode was found to have passed 0·5 mm or more lateral to the PVN.

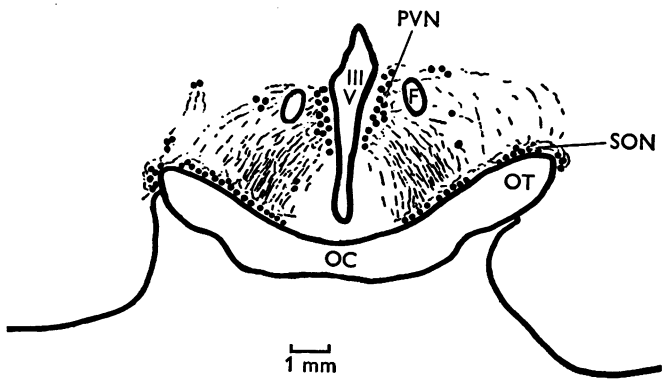
Anatomy of the paraventricular neurones in the cat

In the series of sections shown in Text-figs. 2, 3 and 4, cell bodies constituting the two nuclei were clearly visible, although not deeply stained with the performic acid–Victoria blue–phloxine technique, whereas, with the exception of the section from Expt. 6, only occasional fibres could be distinguished. It is probable that neurosecretory material contained in the fibres had been discharged during stimulation.

In order to obtain a more precise determination of the normal anatomy, serial brain sections were examined from lactating cats which had not been used for stimulation experiments or in which the electrode tracks had been placed at some distance from the PVN. There is some uncertainty in the literature about the number and course of the fibre tracts from the PVN. The present study revealed a broad tract which corresponded closely with that originally described for the human by Greving (1923), who used silver staining, and confirmed by Bargmann (1949) in the cat using a modification of Gomori's chrome alum-haematoxylin-phloxine technique. In addition, scattered fibres were seen which were thought to constitute the lateral pathway described by Laqueur (1954). These fibres were directed laterally, dorsal to the fornix and pierced the internal capsule before curving ventromedially towards the dorsolateral margin of the SON. Text-fig. 5 illustrates diagrammatically the path of the paraventricular neurones in a section cut in a plane similar to that of Expts. 3 and 4. Greving's tract from the PVN is clearly defined. This runs as a broad sheet of fibres sweeping out fan-wise from the lateral border of the nucleus in a ventro-lateral direction towards the SON. Fibres from the dorsal tip of the nucleus pass dorsal and lateral to the fornix, those from the middle third pierce the fornix, whilst those arising from the ventral tip pass ventral to the fornix. Scattered neurosecretory cells were observed along the dorsal portion of the tract, usually associated with blood vessels. A group of cells occurred regularly dorsolateral to the fornix. Photomicrographs of the section from which Text-fig. 5 was constructed are shown in Pls. 1 and 2. Pl. 1*a* shows the PVN in its entirety. The lower pole of the nucleus, included in the inset, is shown at higher magnification in Pl. 1*b*. The cell bodies of the paraventricular neurons are clearly seen; the beaded appearance of the axons is due to aggregations of neurosecretory material. Pl. 2*a* shows the most medial fibres of the tract running ventro-laterally towards the cell bodies at the medial border of the anterior part of

the SON. It is to be noted particularly that there is an absence of neurosecretory material in the triangular zone occupying the lower left half of the plate. That is, there is no evidence of any direct paraventriculohypophysial pathway such as that described by Laruelle (1934), running vertically downwards from the PVN along the wall of the third ventricle. Pl. 2*b* shows the parallel and diagonal distribution of the neurosecretory fibres in the middle part of the tract.

In another section corresponding with that illustrated in Expt. 2, neurosecretory cells forming the rostral tip of the anterior part of the PVN, were observed to lie in close proximity to the medial border of the SON. These cells were thought at first to constitute part of the supraoptic nucleus itself, but in successive sections cut caudally, the cells became more numerous and formed a discrete cluster separate from the SON and eventually attaining the typical appearance of the PVN as seen in Expts. 3 and 4 (Text-figs. 2 and 3).



Text-fig. 5. Diagram of coronal section of hypothalamus in an unstimulated cat's brain. Constructed from sections stained with performic acid-Victoria blue – phloxine, to show the distribution of the axons of the paraventricular neurones. PVN, paraventricular nucleus; SON, supraoptic nucleus; OT, optic tract; F, fornix; III V, third ventricle; OC, optic chiasma.

DISCUSSION

The aim of the present study was to define the respective neurosecretory pathways for the release of oxytocin and vasopressin in the cat. Despite numerous studies in several different species, the distribution of the two hormones between the neurones of the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) is still uncertain. There are three main approaches to this problem – estimating the hormone content of the two nuclei, studying the effects of lesions placed in one or other nucleus and detecting the release of the hormones in response to electrical stimulation of discrete points within the hypothalamus (Bisset, 1968). In much of the previous work in which the last approach was used, reliance has been placed on the response of the target organs of the stimulated animal. This method has several limitations. Stimulation can release pharmacologically active substances which mimic or interfere with the responses of the target

organs to oxytocin or vasopressin. The organs or responses which are used to detect the hormones might not be of equal sensitivity; for example, the threshold dose of oxytocin for a milk-ejection response is far less than that of vasopressin for a pressor response. Finally, a particular response might not be specific for one hormone; for example, a milk-ejection response does not necessarily indicate release of oxytocin, because vasopressin has a considerable degree of intrinsic milk-ejecting activity (Bisset, 1968).

In the present investigation, we have studied the release of oxytocin and vasopressin in response to electrical stimulation of the hypothalamus, using a combination of two methods. The response of a target organ, in this case the mammary gland, was used to detect the release of hormone and monitor the position of the stimulating electrode and, in addition, the concentrations of both oxytocin and vasopressin were estimated in samples of blood collected during stimulation. This we consider to be a more reliable means of measuring the ratio in which the hormones are released than recording the responses of the target organs alone.

The validity of the methods used in the present work for estimating oxytocin and vasopressin in blood by assaying extracts for MEA and ADA, has been established in previous work (Bisset, Hilton & Poisner, 1967). The use of the lactating rat has increased the sensitivity of the assay for MEA and has made it possible to assay oxytocin in blood at as low a concentration as vasopressin: the threshold for both hormones is about 3 μ-u./ml. The use of the analogue carbamoylmethyloxytocin has further assisted in identifying the active principles in blood extracts with the neurohypophysial hormones. The previous experiments by Bisset, Hilton & Poisner (1967) were carried out under barbiturate anaesthesia. Barbiturates are known to stimulate the neurohypophysis to release vasopressin (de Bodo & Prescott, 1945) and it was found that vasopressin was usually present in control samples of blood at concentrations up to 80 μ-u./ml. At such high levels of vasopressin, its intrinsic MEA would certainly have been detected using the lactating rat preparation and this would have made the precise determination of small quantities of oxytocin difficult. To overcome this complication our experiments were performed under chloralose anaesthesia. In only a few control samples was any vasopressin or oxytocin detected.

The present experiments show that in the cat, oxytocin is derived from the PVN alone and vasopressin is present in both the SON and the PVN.

The first experiment in the present series confirmed the finding of Bisset, Hilton & Poisner (1967) that stimulation of the SON in the cat releases vasopressin only, but the second experiment seemed at first to contradict this finding. Both hormones were detected in the blood on stimulation of a point which lay close to a small cluster of cells about 0·5 mm

from the SON and it was thought that these cells formed part of the SON. However, a study of the anatomy in brain sections from a normal cat suggested that this cluster of cells probably represented the extreme rostral tip of the PVN which is known to extend almost to the level of the SON (see Bleier, 1961 and Diepen, 1962). Stimulation in the region of the PVN in Exp. 3, 4, 5 and 6 resulted in a milk-ejection response, or an increase in the concentration of oxytocin in the blood, or both effects. In many cases, the concentration of vasopressin was also increased. The exceptionally high levels of both oxytocin and vasopressin in Expt. 3 might have been due to the fact that the cat in this experiment was lightly anaesthetised and this might possibly have rendered the neurosecretory cells particularly excitable. In Expt. 6 the mammary gland of the cat was relatively sensitive. Stimulation at the uppermost point resulted in a milk-ejection response equivalent to between 1 and 2 m-u. oxytocin, but there was no detectable increase in the concentration of oxytocin in the blood. This was not altogether surprising since it was shown that the intravenous injection of 4 m-u. oxytocin, which produced a MER in the cat, raised the concentration of oxytocin in the blood to only 4 μ-u./ml. The relatively high ratio of vasopressin to oxytocin (approximately 17:1) in the blood sample collected during stimulation of the lowest point in this experiment can be explained by the fact that the tip of the simulating electrode was in contact with the posterior or tuberal part of the SON.

The finding that the release of oxytocin in the cat is confined to the PVN is in harmony with the report that bilateral destruction of this nucleus in the cat leads to a depletion of at least 90% in the content of oxytocin in the posterior pituitary, and to the occurrence in pregnant cats of dystocia without disturbance in water balance (Nibbelink, 1961). A selective depletion of oxytocin after lesions in the PVN was also reported in the rat by Olivecrona (1957). Tindal, Knaggs & Turvey (1968) reported release of oxytocin without vasopressin in the guinea-pig, and Aulsebrook & Holland (1969), release of both hormones in the rabbit, on electrical stimulation in the region of the SON, but both groups of workers admit the possibility that the release of oxytocin could have been caused by stimulation of fibres from the PVN approaching the SON in their passage to the neurohypophysis. Their experiments do not therefore necessarily contradict the hypothesis that oxytocin is derived from the PVN and that the SON releases vasopressin only. On the other hand, milk-ejection responses, presumed to be due to the release of oxytocin, were observed in the goat (Andersson, 1951; Andersson & McCann, 1955) and the rabbit (Cross, 1958) on electrical stimulation of both the SON and PVN. However, the possibility that these responses were due to vasopressin cannot be excluded (Bisset, 1968). The point is illustrated by an experiment in the present

investigation, in which the responsiveness of the neurohypophysis was tested by applying the stimulus of haemorrhage. Haemorrhage has been shown to act as a specific stimulus for the release of vasopressin without oxytocin (Ginsburg & Smith, 1959; Beleslin *et al.* 1967). The withdrawal of 100 ml. blood from the cat resulted in an abrupt fall in blood pressure of 100 mm Hg, accompanied by a milk-ejection response which was matched by the intravenous injection of 3 m-u. oxytocin. A sample of blood collected from the cat during and immediately after haemorrhage was found to contain 780 μ-u. ADA/ml. and 91 μ-u. MEA/ml. The ADA represents vasopressin which, at a concentration of 780 μ-u./ml., would contribute about 110 μ-u. MEA/ml. (14%). The blood sample therefore contained vasopressin only and the milk-ejection response observed in the cat could be attributed entirely to the release of vasopressin.

In our experiments, vasopressin was released as well as oxytocin on stimulation of the PVN. This is consistent with the presence of both hormones in extracts of the PVN in this species (Bisset, Hilton & Poisner, 1967) and the observation of antidiuretic responses to electrical stimulation of the PVN in the cat (Koella, 1949), the dog (Fang, Liu & Wang, 1962) and the goat (Andersson & McCann, 1955). However, Tindal *et al.* (1968), in the guinea-pig and Aulsebrook & Holland (1969) in the rabbit observed milk-ejection responses without pressor responses on electrical stimulation of the PVN and argued that this nucleus releases oxytocin alone. The failure to detect vasopressin might have been due to the relative insensitivity of the method used for detecting the hormone or to a species difference.

In the literature, there is some uncertainty about the course of the nerve fibres from the PVN. A number of tracts has been described (see Diepen, 1962; Christ, 1966; Sloper, 1966). This is of importance in the present context since any one of these tracts might constitute a specific pathway for oxytocin. As part of the present investigation, the anatomy of the neurones was studied histochemically using a stain which is specific for cystine-rich neurosecretory material. Laqueur (1954) (see also Fig. 6, Diepen, 1962) described three pathways from the PVN in the dog. One corresponds with Greving's tractus paraventricularis cinereus (Greving, 1923), a second lateral path runs towards the internal capsule and then emerges at the lateral border of the SON and a third, or medial path, which represents the shortest route to the hypophysis, arises from the dorso-caudal part of the PVN and passes dorsal, then lateral to the fornix before entering the infundibulum. Greving's tract was clearly defined in most sections but only fragments of Laqueur's lateral tract were seen. The illustration of the medial tract in Fig. 2 of Laqueur's paper bears a resemblance of the section of Expt. 6 in the present paper. However, the fibres shown in this section were

regarded simply as the caudal limit of Greving's tract. Laruelle (1934) described a direct paraventriculo-hypophysial pathway running vertically downwards along the wall of the third ventricle and this was confirmed also in the rabbit by Ford & Kantounis (1957). In the present work there was no histological evidence of such a pathway. Moreover, it was shown that milk-ejection responses and increased concentrations of neurohypophysial hormones in the blood could be obtained on electrical stimulation of either the PVN or paraventricular and supraoptic fibres in the median eminence, but not on stimulation of the intermediate zone lying vertically between the PVN and the median eminence. It is concluded from both histological evidence and the results of electrical stimulation that the principal pathway from the PVN is that represented by Greving's tract. Since the release of oxytocin in the cat is confined to the PVN, it follows that this tract in the cat must contain the efferent limb of the milk-ejection reflex.

Brooks, Ishikawa, Koizumi & Lu (1966) have shown that suction applied to the nipple and distension of the uterus in the cat under chloralose anaesthesia result in milk ejection accompanied by an increase in unit activity recorded from the cell bodies of the PVN. This work establishes that there are afferent pathways from the mammary gland and the uterus to the PVN. In the guinea-pig and rabbit the afferent pathway of the milk-ejection reflex has also been traced to the paraventricular nucleus (Tindal *et al.* 1967, 1969).

It has been shown recently in the rabbit that suckling (Bisset, Clark & Haldar, 1970) and parturition (Haldar, 1970) cause release of oxytocin without vasopressin. Independent release of oxytocin could be readily envisaged if the afferent fibres from the mammary gland and the uterus to the hypothalamus innervated the PVN and this nucleus released only oxytocin. The present experiments show that, at least in the cat, the PVN releases both hormones, but the variability in the ratio of their concentrations in blood suggests that they are released from separate neurones. It must be supposed that the afferent fibres from the mammary gland and uterus selectively innervate those neurones which release oxytocin. It is possible that there is an anatomical segregation of neurones in the PVN which is not revealed by the effects of electrical stimulation.

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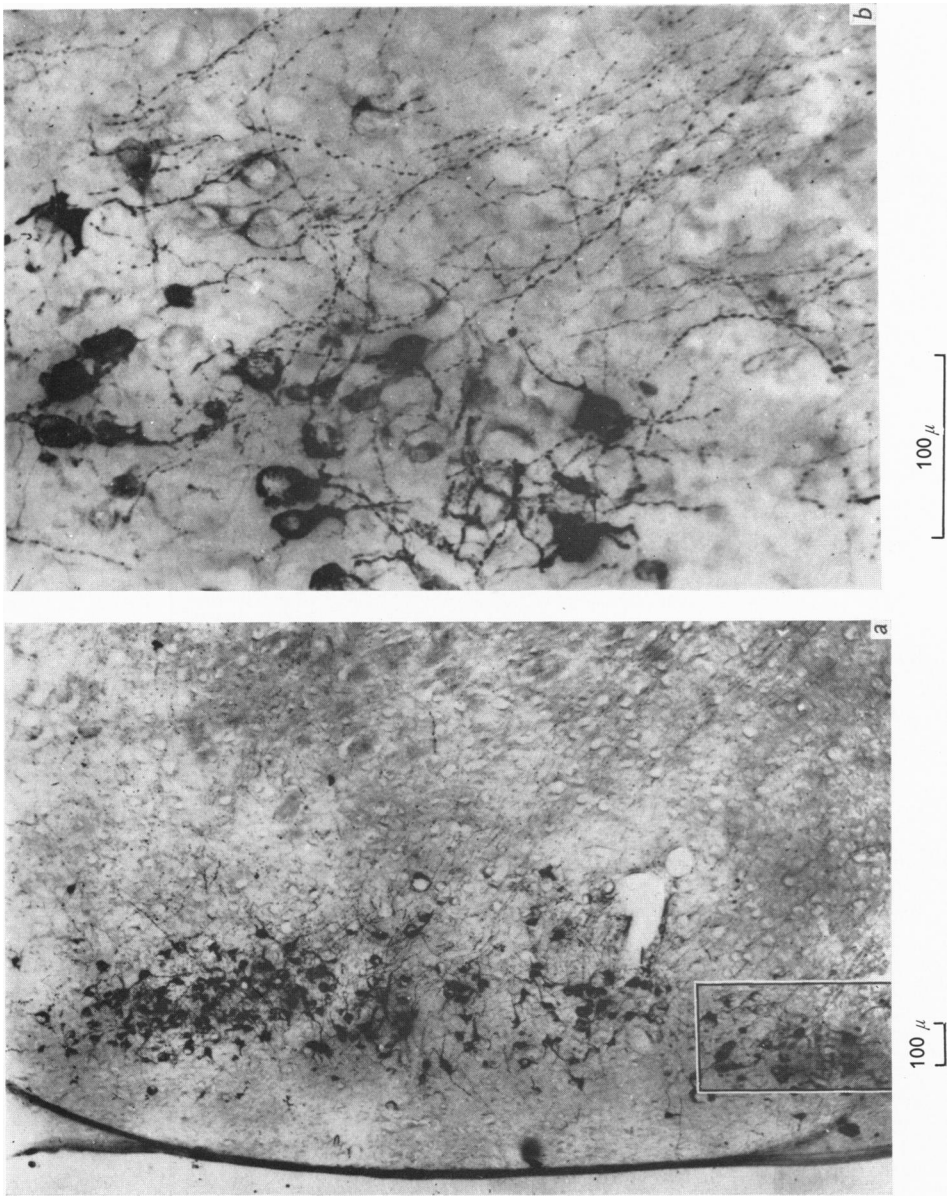
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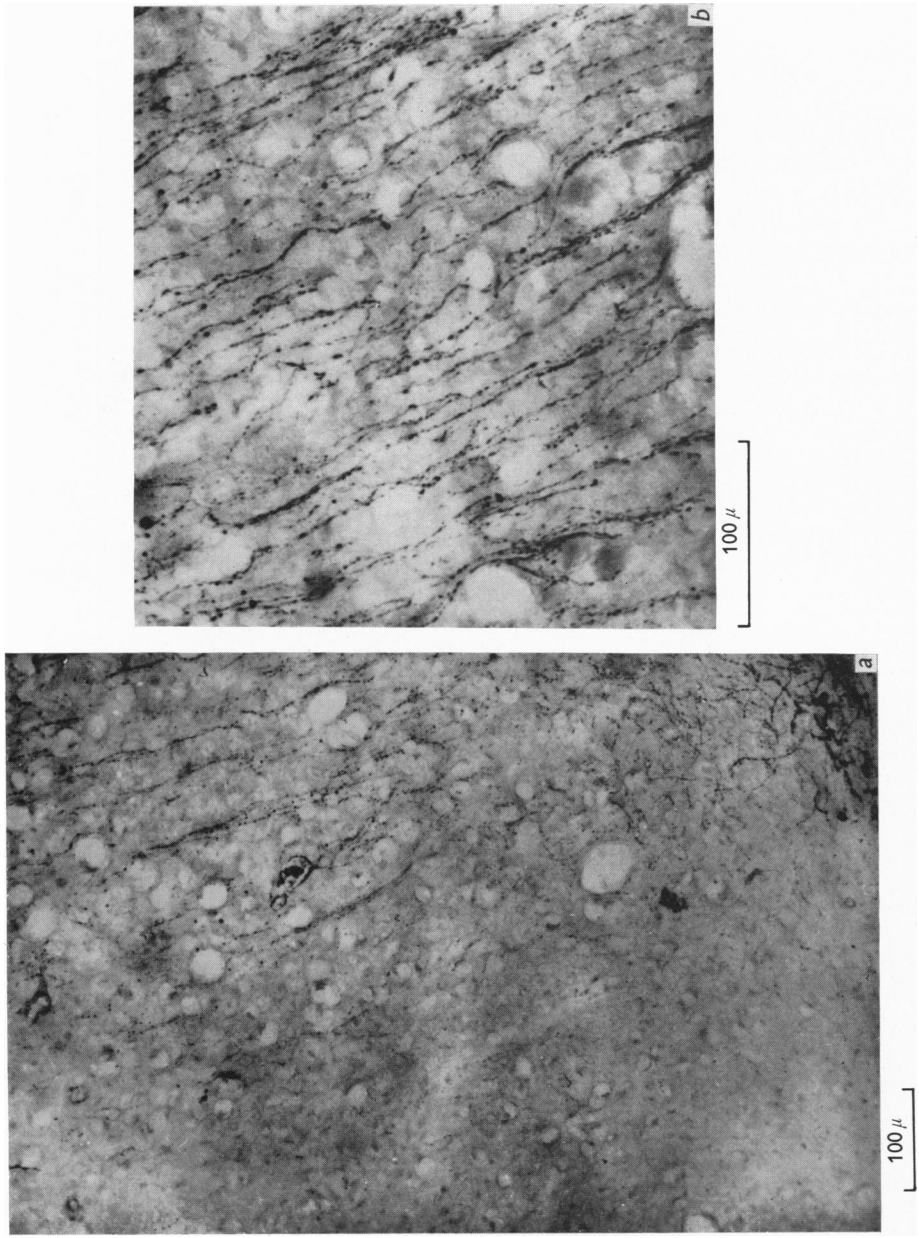
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EXPLANATION OF PLATES

PLATES 1 AND 2

Photomicrographs of histological sections from which Text-fig. 5 was prepared. Pl. 1*a* shows the whole of the PVN on the left side of the brain and 1*b* the area included in the inset of 1*a*. Pl. 2*a* shows the medial border of the tractus paraventricularis cinereus (Greving) with the axons of the paraventricular neurones coursing towards the cell bodies of the SON (lower right); 2*b* illustrates in greater detail the parallel and diagonal distribution of the axons in this tract.