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The distribution of vasopressin and oxytocin in the hypothalamoneurohypophysial system of the guinea-pig

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Summary

1. The ratio of the content of vasopressin to that of oxytocin (V/O ratio) was estimated in the supraoptic nucleus (SON), paraventricular nucleus (PVN) and posterior pituitary gland (PIT) of guinea-pigs.

2. Extracts were assayed for antidiuretic activity to estimate vasopressin and for milk-ejecting activity to estimate oxytocin. In assays for milk-ejecting activity, trypsin was used to inactivate vasopressin in the extracts.

3. The mean V/O ratios in the SON, PVN and PIT were 28, 8·5 and 7·0 respectively in male guinea-pigs, 6·8, 7·4 and 6·9 in non-lactating females, and 5·1, 3·3 and 6·6 in lactating females.

4. The distribution of the hormones within the hypothalamus is discussed in relation to their independent release in response to electrical stimulation of the SON and PVN.

Introduction

Although both oxytocin and vasopressin are released from the neurohypophysis and thus share a common terminal pathway, they are released independently by appropriate stimuli. For example, suckling causes the release of oxytocin without vasopressin (Bisset, Clark & Haldar, 1970), and haemorrhage and carotid occlusion the release of vasopressin without oxytocin (Ginsburg & Smith, 1959 ; Bisset, Hilton & Poisner, 1967 ; Clark & Rocha e Silva, 1967). The release of these two hormones is governed by the activity of neurosecretory cells whose cell bodies lie in the supraoptic and paraventricular nuclei (SON and PVN, respectively). However, it is not yet clear whether each nucleus is concerned with the synthesis and release of only one or both of the two hormones. One approach to this problem is to stimulate the nuclei electrically and estimate the ratio in which the hormones are released into the circulation. Another is to remove the nuclei and measure the ratio in which the hormones are contained within them. In the cat, it has been shown by direct estimation of the hormones in blood that stimulation of the SON releases vasopressin alone ; stimulation of the PVN releases both hormones (Bisset *et al.*, 1967 ; Bisset, Clark & Errington, 1971). Yet in the same species, oxytocin and vasopressin have been detected in the region of the SON as well as in the PVN (Bisset *et al.*, 1967). However, in dissecting coronal sections through the anterior hypothalamus it is difficult to separate the SON from the axons of the

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PVN which run ventrolaterally between the two nuclei in the tractus paraventricularis cinereus of Greving (1923). The oxytocin present in extracts of the SON and its surrounding tissue might, therefore, have been derived from the PVN.

Electrical stimulation in the region of the PVN and SON has also been used in experiments on lactating guinea-pigs (Tindal, Knaggs & Turvey, 1968) and rabbits (Aulsebrook & Holland, 1969). In these experiments milk ejection and pressor responses were used to detect the release of oxytocin and vasopressin. Electrical stimulation in the region of the PVN released oxytocin alone whereas electrical stimulation in the region of the SON released both hormones in the rabbit but oxytocin alone in the guinea-pig. However, both groups of authors recognized the possibility that oxytocin had been released by stimulation of the axons of the paraventricular neurones which form Greving's tract. Their results imply that in these two species vasopressin is confined to the SON and oxytocin to the PVN.

The present experiments on guinea-pigs were carried out to determine if such a distribution is reflected in the hormonal contents of the two nuclei. An attempt was made to dissect the SON free from the PVN and oxytocin and vasopressin were estimated in extracts of the two nuclei and of the pituitary gland.

To detect the small amounts of oxytocin present in the hypothalamus, extracts were assayed for milk-ejecting activity in the lactating rat. Unfortunately, vasopressin also has some significant milk-ejecting activity. To avoid this interference, vasopressin was inactivated by treatment with trypsin.

Methods

The animals used were male guinea-pigs (350–475 g), non-lactating females (490–575 g) and lactating females (800–1,080 g) from the Heston strain 2.

Dissection of the supraoptic nucleus and paraventricular nucleus

The animals were killed by a blow on the back of the neck. The entire brain was rapidly removed from the skull and the pituitary gland detached. A block of tissue containing the anterior hypothalamus was cut from the brain by making two cuts in the coronal plane about 0·5 mm anterior and posterior to the pituitary stalk. A glass guide was rested against the block of tissue and successive coronal sections of about 0·5 mm thickness were sliced off with a razor strip, according to the method described by McIlwain & Rodnight (1962) for preparing isolated slices of cortex from the guinea-pig.

Supraoptic nucleus The supraoptic nuclei were dissected from the coronal sections corresponding with planes A14 to A15 in Tindal's paper (1965) on the forebrain of the guinea-pig in stereotaxic coordinates. In plane A14 two small areas enclosing the supraoptic nuclei were dissected from the base of the brain on the lateral edges of the optic chiasma as illustrated in Figure 1a. The procedure used with more anterior sections in planes 14·5 and 15 was to detach the optic chiasma with the adherent supraoptic nuclei from the base of the brain by gentle traction on the optic nerves. The optic chiasma was then divided in two and the anterior half extracted.

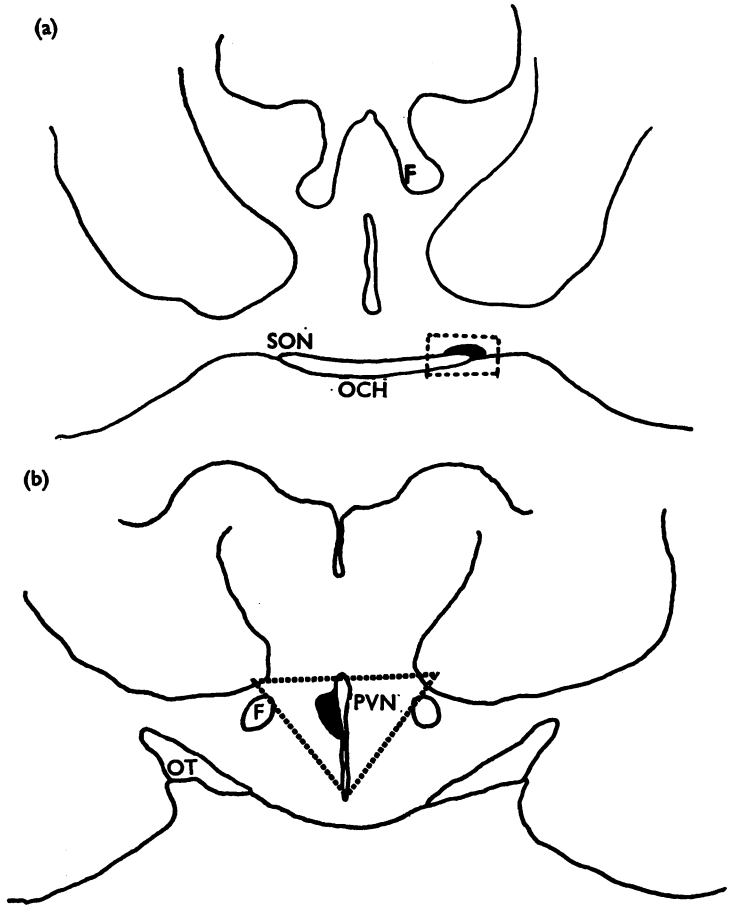

FIG. 1. Coronal sections through the forebrain of the guinea-pig. The rectangle in (a) and the triangle in (b) indicate the areas dissected from the hypothalamus to include the supraoptic nuclei (SON) and paraventricular nuclei (PVN) respectively. The other anatomical features labelled in the figure are the fornix (F), the optic chiasma (OCH) and the optic tract (OT).

Paraventricular nucleus A triangular area enclosing both paraventricular nuclei was dissected from sections corresponding with plane A12·5, as illustrated in Figure 1b.

Extraction of vasopressin and oxytocin from brain tissue and the treatment of extracts with trypsin

Samples of neurosecretory tissue from three animals were pooled. The brain tissues dissected as described in the previous sections and the whole pituitary glands were homogenized with a solution containing 0·25% acetic acid (v/v) and 0·9% NaCl (w/v) in distilled water. The suspension was placed in a boiling water bath for 5 min then cooled and centrifuged and the supernatant recovered. The precipitate was resuspended in 0·5 ml of 0·9% NaCl solution and centrifuged. The second supernatant was added to the first and the final volume of the extract adjusted to 2 ml with 0·9% NaCl solution.

The interference with the assay of oxytocin by its milk-ejecting activity that is caused by the intrinsic milk-ejecting activity of vasopressin (Bisset, 1968) was

avoided by treating a portion of each extract with trypsin. This enzyme inactivates vasopressin but not oxytocin because it acts only on peptides containing arginine residues; of the two hormones only vasopressin contains arginine (Lawler & du Vigneaud, 1953). Trypsin has also been used for the estimation of oxytocin in blood (Roberts & Share, 1968) and in urine (Frandsen & Jensen, 1971). The procedure adopted was as follows. Each extract was divided into two equal portions to one of which 100 μg trypsin was added in 0·1 ml 0·9% NaCl solution; this portion was incubated at pH 7 for 60 min at 37° C. All extracts were acidified to pH 4 with N H_2SO_4, and stored at 4° C until required for assay when they were neutralized with N Na_2CO_3.

Assay of extracts

The extracts were assayed for antidiuretic activity by intravenous injection in the water-loaded rat under ethanol anaesthesia, and for milk-ejecting activity by intravenous or retrograde arterial injection in the lactating rat. Interference from adrenaline and 5-hydroxytryptamine in the assay for milk-ejecting activity was prevented by the use of specific antagonists. Details have been given in previous work (Bisset *et al.*, 1971). In the lactating rat, atropine sulphate 0·2 mg i.v. was also given to block the action of any acetylcholine in the extracts.

Materials

The standards used for assay of antidiuretic activity and milk-ejecting activity were synthetic arginine vasopressin (Sandoz; batch no. 63104), the potency of which was confirmed by pressor assays against the Third International Standard for Posterior Pituitary (Bangham & Mussett, 1958) and synthetic oxytocin (Syntocinon, Sandoz; batch no. 62099). Trypsin was a specimen of the crystallized enzyme (Sigma, Type XI, DCC treated lot 108B–8130) known to be free from chymotrypsin which would inactivate oxytocin.

Results

Incubation of vasopressin and oxytocin with trypsin

The results of incubating vasopressin and oxytocin with trypsin in 0·9% NaCl solution are shown in Table 1. When studying the effect of an enzyme on these hormones it is usual to acidify and boil the incubation mixture after a given time

TABLE 1. *Recovery of antidiuretic activity (ADA) and milk-ejecting activity (MEA) from samples of vasopressin and oxytocin incubated in 0·9% NaCl solution with 100 μg trypsin at pH 7, 37° C for 60 minutes*

Sample	Amount of hormone incubated (mu)		Assay	Activity recovered (mu)	
	Vasopressin	Oxytocin		ADA	MEA
1	200	—	1	10	1
			2	8	1
2	—	20	1	0·2	21·2 (19·0–23·8)
			2	0·2	22·0 (20·4–23·6)
3	200	20	1	10	21·6 (18·8–24·8)
			2	—	21·6 (17·4–26·8)

Two assays of each sample were carried out, the second three days after the first. The figures in parentheses indicate the 95% fiducial limits of the assays.

in order to inactivate the enzyme and stop the reaction. This has been done for example with tyrosinase (Bisset, 1962); trypsin, however, is stable to boiling in acid solution (Northrop, 1932). In the present experiments, therefore, no attempt was made to inactivate the trypsin after the initial period of incubation for one hour at 37° C, but in order to detect any continuing or delayed action of the enzyme in the incubation mixtures, two assays were carried out, one as soon as possible after the initial incubation and the other three days later. In the interval the mixtures were kept at pH 4 and 4° C. After incubation of 200 mu vasopressin the antidiuretic activity was reduced to 10 mu in the first and 8 mu in the second assay: this represents a loss of activity of at least 95%. Vasopressin has an intrinsic milk-ejecting activity (MEA) equivalent to about 20% of its antidiuretic activity (ADA). This figure of 20% represents the approximate value found for three estimations of the intrinsic MEA of vasopressin assayed against oxytocin on the lactating rat. The actual values found were 18·7%, 21·4% and 18·9%: 200 mu vasopressin should therefore contribute 40 mu MEA. After incubation the MEA was equivalent to 1 mu or 2·5%. When 20 mu oxytocin was incubated there was no loss of MEA in either of the two assays. Oxytocin has intrinsic ADA equivalent to about 1% of its oxytocic or milk-ejecting activity: 20 mu oxytocin should, therefore, contribute 0·2 mu ADA and this was the amount present after incubation. When a mixture of 200 mu vasopressin and 20 mu oxytocin was incubated, there was the same loss of ADA as with vasopressin alone and the recovery of MEA was similar to that obtained with oxytocin alone. These experiments showed that in 0·9% NaCl solution, trypsin causes at least 95% inactivation of vasopressin with no loss of activity of oxytocin. Trypsin itself was found to be devoid of ADA and MEA.

Table 2 shows the results of experiments to test the recovery of oxytocin and vasopressin extracted from brain tissue and the effectiveness of trypsin in eliminating vasopressin from the extracts. Endogenous MEA equivalent to 0·2 mu oxytocin was detected in homogenate 1 (sample 1) from the posterior hypothalamus; it was not reduced by incubation with trypsin and it was probably due to oxytocin derived from paraventricular neurones. No endogenous ADA or MEA was detected in homogenate 2 (sample 1) from the cortex. When the hormones were added separately to the homogenates (sample 2 and 3) at least 90% of the ADA of vasopressin and the MEA of oxytocin was recovered before incubation with trypsin. After

TABLE 2. *Recovery of antidiuretic activity (ADA) and milk-ejecting activity (MEA) from extracts of brain homogenates before and after incubation with* 100 *μg trypsin at pH* 7, 37° *C, for* 60 *minutes*

Homogenate	Sample	Amount of hormone added (mu)		Activity recovered (mu) Before incubation		After incubation	
		Vasopressin	Oxytocin	ADA	MEA	ADA	MEA
1	1	—	–	<0·2	0·2	<0·2	0·2
	2	10	–	10·9	2	0·5	0·2
	3	—	2	—	1·8 (1·40–2·26)	—	1·8 (1·46–2·18)
	4	10	2	—	—	—	2·3 (1·80–3·04)
2	1	—	–	<0·2	<0·05	—	—
	2	10	–	9·4 (7·6–11·4)	0·6	0·4	<0·05
	3	—	2	—	—	—	1·9 (1·64–2·20)
	4	10	2	10·7 (9·1–12·8)	3·6 (3·12–4·00)	0·4	2·5 (2·08–3·20)

The homogenates were divided into samples of 1 ml, each containing 50 mg tissue. One sample was used for the assay of endogenous activity and known amounts of vasopressin and oxytocin were added to the remaining samples. Homogenate 1 was prepared from the posterior hypothalamus and 2 from the cortex. The figures in parentheses indicate the 95% fiducial limits of the assays.

incubation at least 95% of the ADA in the extracts was removed. In sample 2 of homogenate 1, the MEA after trypsin probably represents oxytocin originally present in the homogenate (cf. sample 1). In sample 2 of homogenate 2 to which 10 mu vasopressin had been added, no MEA was detected after trypsin (<0·05 mu). Incubation with trypsin did not cause any significant loss of the MEA of oxytocin added to the homogenates.

Vasopressin and oxytocin in the supraoptic and paraventricular nuclei and in the posterior pituitary gland

Table 3 gives the calculated ratio of the amount of vasopressin to that of oxytocin (V/O) in the supraoptic nuclei (SON) paraventricular nuclei (PVN) and the pituitary gland (PIT) in guinea-pigs. Antidiuretic activity (ADA) was assayed both before (a) and after (b) incubation with trypsin, and the percentage loss of activity after incubation was calculated (c); ADA before incubation (a) represents vasopressin. Milk-ejecting activity (MEA) was asayed in the incubated extracts only (d). Oxytocin was estimated as the net MEA (e) after deducting from the total (d) 20% of the ADA remaining in the extracts after incubation with trypsin (b). Since trypsin caused at least 95% loss of ADA in every assay, the correction

TABLE 3. *Antidiuretic activity (ADA), milk-ejecting activity (MEA) and estimated ratio of vasopressin/oxytocin (V/O) in supraoptic nuclei (SON), paraventricular nuclei (PVN) and posterior pituitary gland (PIT) of guinea-pigs (expressed as content per animal)*

			ADA (mu)			MEA (mu) after trypsin		V/O
Group	Expt.	Region	Before trypsin	After trypsin	% Loss	Total	d less 20% b	(a/e)
			a	b	c	d	e	f
Male	1	SON	11·0	0·75	93	0·42	0·27	41
	2	PVN	1·8	0·10	94	0·32	0·30	6·0
	3	SON	6·5	0·33	95	0·45	0·38	17·1
		PVN	3·1	0·26	92	0·47	0·42	7·4
		PIT	385	20	95	36	32	12·0
	4	SON	2·2	0·12	95	0·10	0·08	28
		PVN	0·45	0·033	93	0·038	0·031	14·5
		PIT	855	20	98	215	211	4·1
	5	SON	1·1	0·068	94	0·067	0·053	21
		PVN	0·066	<0·017	>75	0·014	0·011	6·0
		PIT	875	33·0	97	192	185	4·7
Non-lactating female	1	SON	0·74	0·083	90	0·12	0·10	7·4
		PVN	0·74	0·066	91	0·093	0·080	9·2
		PIT	1070	37	97	190	181	5·9
	2	SON	0·72	0·066	91	0·13	0·12	6·0
		PVN	0·37	0·017	95	0·05	0·05	7·4
		PIT	1140	51	96	150	140	8·0
	3	SON	0·86	0·057	93	0·13	0·12	7·0
		PVN	0·56	0·033	94	0·11	0·10	5·6
		PIT	664	25	96	101	96	6·9
Lactating female	1	SON	1·1	0·067	94	0·19	0·18	6·1
		PVN	0·088	0·008	91	0·032	0·030	2·9
		PIT	1040	39	92	120	110	9·5
	2	SON	1·14	0·071	94	0·18	0·16	6·9
		PVN	0·23	0·012	93	0·053	0·05	4·6
		PIT	100	5·5	95	16	15	6·7
	3	SON	0·56	0·033	94	0·23	0·22	2·5
		PVN	0·11	0·013	90	0·049	0·046	2·4
		PIT	280	15	94	83	80·0	3·5

applied for the intrinsic MEA of residual vasopressin is in most cases negligible; however, it was of significance in cases where the ratio of ADA/MEA was very high and for this reason it was applied to all estimations.

The mean V/O ratios and the ranges are given in Table 4. In all three groups investigated, males, non-lactating females and lactating females, vasopressin was detected in both supraoptic and paraventricular nuclei in greater amount than oxytocin. The V/O ratio in the PVN was 8·5 in males and 7·4 in non-lactating females, while in lactating females it was only 3·3. Although the number of results is too small to make a statistical analysis, it may be noted that the highest ratio in this group (4·6) is less than the lowest ratio in either of the other two groups. The V/O ratio in the SON was 28 in male animals compared with 6·8 in non-lactating females and 5·1 in lactating females; the lowest ratio in the male group is considerably greater than the highest ratio in the other groups. The V/O ratio in the posterior-pituitary varied only slightly from 6·6–7·0 in all three groups.

TABLE 4. *The ratio of vasopressin/oxytocin in supraoptic nuclei (SON), paraventricular nuclei (PVN) and posterior pituitary gland (PIT) of guinea-pigs*

	SON	PVN	PIT
Male	28 (17–41)	8·5 (6·0–14·5)	7·0 (4·1–12·0)
Non-lactating female	6·8 (6·0–7·4)	7·4 (5·6–9·2)	6·9 (5·9–8·0)
Lactating female	5·1 (2·5–6·9)	3·3 (2·4–4·6)	6·6 (3·5–9·5)

Calculated from results in Table 3. Values represent means and ranges.

Discussion

If pressor and oxytocic assays are used to estimate vasopressin and oxytocin in extracts of the hypothalamus, it is necessary to separate them from interfering substances such as substance P, 5-hydroxytryptamine and catecholamines (Lederis, 1961). They can be separated by paper chromatography, but with small amounts of hormone this procedure is liable to cause large and variable losses of activity (Fitzpatrick, 1961; Lederis, 1961). The high degree of specificity of the anti-diuretic and milk-ejecting assays used in the present investigation (Bisset, 1968; Fitzpatrick & Bentley, 1968; Smith, 1970), coupled with the use of appropriate antagonists, permitted the use of crude extracts giving consistently high recoveries. Pretreatment of the extracts with trypsin reduced the intrinsic MEA of vasopressin to negligible proportions and this simplified the assay of oxytocin.

The total content of vasopressin and oxytocin in the hypothalamus varies in different species from about 0·5% to 9% of that in the posterior pituitary gland (Lederis, 1961). The object of the present work was to estimate the ratio rather than the total amounts of the two hormones in the SON and PVN. To make the dissections as precise as possible, only minimal amounts of tissue surrounding the two nuclei were taken and intermediate zones containing the nerve tracts were excluded. For this reason, in some of the experiments shown in Table 3, the total content of hormone in the nuclei amounts to only about 0·1% of that in the posterior lobe.

Vasopressin : oxytocin ratios

The V/O ratios in the SON, PVN and posterior pituitary of a number of species are given in Table 5 for comparison with the data for the guinea-pig in Table 4.

In the male guinea-pig, the V/O ratios in the SON and PVN are relatively high and of the same order as those in the dog. The fact that vasopressin and oxytocin occur in practically equal amounts in the posterior pituitary of the dog, despite the high V/O ratio in the hypothalamus, led Vogt (1953) to suggest that in this species, oxytocin is synthesized in the posterior pituitary itself or during the passage of a precursor in the hypothalamo-hypophysial tract. In this connexion, it is interesting that, in the male and non-lactating female guinea-pig the V/O ratio in the posterior pituitary is only slightly lower than that in the PVN and, in the lactating female, it is twice as high. Moreover, the V/O ratio in the PVN is lower in lactating females than in the other two groups, while that in the SON is considerably lower in the two groups of females than in the males. These differences suggest that the rates of synthesis or transport of the hormones might vary in different species, or even between the two sexes or under different physiological conditions in the same species. This would be consistent with independent mechanisms for their release (Bisset *et al.*, 1971). The V/O ratio in the posterior pituitary of the guinea-pig is higher than that reported for the other species in Table 5.

TABLE 5. *The ratio of vasopressin/oxytocin in the supraoptic nuclei (SON), paraventricular nuclei (PVN) and posterior pituitary gland (PIT) of species other than the guinea-pig*

Species	SON	PVN	PIT	Reference
Dog	24	15	1·5	1
Dog	18	9	1·2	4 & 2
Camel	2·6	0·26	4·3	2 & 3
Sheep	3·3	0·7	1·8	4
Elephant*	15·4	2·7	1·0	2
Cat†	6·8	1·9	3·0	5

* 1 experiment. † Mean of 2 experiments. References: 1. Van Dyke, Adamsons & Engel (1955); 2. Lederis (1962); 3. Adamsons, Engel, Van Dyke, Schmidt-Nielsen & Schmidt-Nielsen (1956); 4. Lederis (1961); 5. Bisset, Hilton & Poisner (1967).

Vasopressin has been found in the PVN of all species investigated and there is no anatomical reason for suspecting that this hormone could have been derived from supraoptic neurones. This finding is consistent with the fact that electrical stimulation of the PVN in the cat (Koella, 1949 ; Bisset *et al.*, 1967 ; 1971), the dog (Fang, Liu & Wang, 1962) and the goat (Andersson & McCann, 1955) has been shown to produce antidiuresis or to increase the concentration of vasopressin in the blood. These results suggest that the PVN is concerned with the synthesis and release of both hormones.

However, the presence of vasopressin in the PVN is difficult to reconcile with failure to release the hormone in the guinea-pig (Tindal *et al.*, 1968) and also in the rabbit (Aulsebrook & Holland, 1969) on electrical stimulation. The simplest explanation is that vasopressin is released but the amount is too small to detect by the relatively insensitive pressor response which was used as an indicator of release ; in the cat, the release of oxytocin from the PVN could not be demonstrated until a sufficiently sensitive assay had been developed to detect low concentrations of the hormone in blood (Bisset *et al.*, 1967, 1971). Another possibility is that electrical stimulation in the immediate vicinity of the PVN might involve the terminal part of the afferent path of the milk-ejection reflex which has been shown in the rabbit (Aulsebrook & Holland, 1969 ; Tindal, Knaggs & Turvey, 1969) and guinea-pig (Tindal, Knaggs & Turvey, 1967) to subserve a preferential release of oxytocin.

The evidence concerning the function of the SON is contradictory. Release of vasopressin without oxytocin on electrical stimulation of the SON in the cat (Bisset *et al.*, 1967, 1971) and the selective depletion of oxytocin from the posterior pituitary after lesions in the PVN in the cat (Nibbelink, 1961) and rat (Olivecrona, 1957), suggest that the SON is involved principally or entirely with the synthesis and release of vasopressin. The very high V/O ratios in the SON of the dog and the male guinea-pig support this idea but in every species studied so far, oxytocin has been reported to occur in extracts prepared from the region of the SON. It is probable that an incomplete separation of the SON from the fibres of Greving's tract was obtained in the present and in previous work. To test this possibility further work is in progress to estimate the content of vasopressin and oxytocin in the supraoptic region of the hypothalamus in the guinea-pig after bilateral lesions in the paraventricular nuclei.

In conclusion, we have found that the PVN contains both oxytocin and vasopressin and is therefore presumably concerned with the synthesis and release of both hormones. The SON, however, may be solely concerned with the synthesis and release of vasopressin.

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