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THE OCCURRENCE OF VESICULATED NEURONES IN THE HYPOTHALAMUS OF THE DOG

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Verney (1947) showed that the release of antidiuretic hormone from the posterior lobe of the pituitary gland is directly related to the osmotic pressure of the blood reaching the brain. It was postulated that sensory elements, which were termed osmoreceptors, and which are functionally linked with the neurohypophysis, must exist somewhere in the field of distribution of the internal carotid artery. In searching for such structures attention was first directed towards the main group of cells from which the supraoptico-hypophysial tract originates—the supraoptic nucleus of the hypothalamus. In an histological examination of this nucleus in the dog Verney (1947) observed small vesicles with features which might fit them for an osmoreceptive function. A few observations on their nature were recorded. They were present in the supraoptic nuclei of four animals in which the brain had been fixed by immediate perfusion. In a dog in which fixation of the brain was delayed none were to be seen. In one of the former animals eighty-nine vesicles were counted, and when the distribution of their sizes was plotted it approximated in form to a normal distribution curve; whilst in another animal, whose posterior lobe had been removed 51 days before fixation of the brain, only thirty-two vesicles were counted. The vesicles were roughly spherical and of a mean diameter of 68 μ: they contained no fat.

The purpose of the present investigation was to examine these vesicles in greater detail, in order to learn more of their histological structure and relation to neighbouring cellular elements, and to determine the extent of their distribution in the hypothalamus. In addition, their fate following lesions to the neurohypophysis was investigated, since such lesions interfere with the animal's ability to release antidiuretic hormone.

MATERIAL AND METHODS

Animals

Normal animals. Serial sections of the hypothalamus in thirteen normal animals have been examined. Dogs and bitches were included in the group; their weights varied between 5 and 28 kg. One of the animals ('Nicky') had been subjected to certain operations and had had its osmotic responses determined (see Verney, 1947).

Animals subjected to operative interference with the neurohypophysis. This group of animals was composed of five bitches which had been used in survival experiments to determine the role of the neurohypophysis in the excretion of water or salts. Two were post-hypophysectomized and three had suffered section of the supraoptico-hypophysial tracts. The relevant data for these animals is as follows:

'Sally' was prepared by Prof. E. B. Verney and has already been reported upon (Verney, 1947): the sections of the hypothalamus of this animal were re-examined to provide some additional data for the present paper. The other four animals were prepared by Dr W. J. O'Connor who kindly allowed the brains to be used for the present investigation.

'Bessie' (17 kg) was killed 14½ months after removal of the posterior lobe. Her post-operative daily urine volume was rather above the pre-operative level but with no definite polyuria (see O'Connor, 1952, fig. 1).

'Diana' (17 kg) was killed 8 months after tract section and was at that time exhibiting a permanent polyuria of 2·5 l. a day.

'Ruby' (13 kg) was killed 3 months after attempted tract section. No polyuria appeared.

'Sheila' (14 kg) was killed 3 months after tract section and exhibited a polydipsia of 5 l. a day during this period (see O'Connor, 1952).

Histological

Preparing the hypothalamic blocks. All animals had their brains fixed by perfusion immediately after death, using the method described by Verney (1947). The fixatives used were 5% (v/v) formaldehyde in 0·9% (w/v) NaCl solution, 4% formaldehyde in 1·0% (w/v) $CaCl_2$ solution, a mixture of 40% (v/v) formaldehyde, glacial acetic acid and 80% (v/v) ethyl alcohol in the ratio 1 : 1 : 18, Bouin's fluid, Helley's fluid, and Susa. The brains were perfused at a hydrostatic pressure equivalent to 126 mm Hg. In some cases the brains were first washed through with hypo- or hypertonic saline; details are given in Table 1. In two animals, in order to inject the blood vessels, the fixation was followed by the infusion of an indian ink–plasma mass, made by adding 10% indian ink to human plasma reconstituted to three times normal strength. In one case the infusion was made at a pressure of 75 mm Hg, and in the other at 180 mm Hg. A block of tissue including the hypothalamus was cut from every brain; two blocks, one from an indian ink-injected specimen, were embedded in celloidin and sections were cut at 50 and 100 μ; the other blocks were embedded in paraffin and cut at between 7 and 20 μ. In every case serial sections of the hypothalamus and surrounding regions were made; with two of the paraffin blocks the sections were cut in the sagittal plane, all other blocks being cut in the frontal plane.

Staining procedures. The following stains were used: haematoxylin and eosin; Masson's trichrome; Weigert's haematoxylin and van Gieson; Heidenhain's azo-carmine (Bensley & Bensley, 1938); toluidin blue; Mallory's phosphotungstic acid–haematoxylin ammonia (Conn & Darrow, 1946); Wilder's silver nitrate–uranium method (Conn & Darrow, 1946); Bodian's silver proteinate method (Conn & Darrow, 1946); Gomori's chrome alum–haematoxylin–phloxin (Bargmann, 1950).

In the regions of the supraoptic and paraventricular nuclei the sections were drawn at a magnification of × 80 and the vesicles outlined in each section. In this way the vesicles could be traced from section to section and their number and size determined.

RESULTS

In the dog the supraoptic nucleus of each side is clearly divided into two parts by the optic tract, an anterior division lying dorsal and lateral to the optic chiasma, and a posterior division extending from the optic chiasma to the median eminence. Both these divisions of the nucleus have been found to contain vesicles in every normal animal examined. Moreover, in all but one of the animals, vesicles were present in the paraventricular nucleus.

The histological structure and size of the vesicles

Structure. The vesicles are basically spherical in shape, as can be seen in frontal and sagittal sections, but they frequently appear somewhat flattened, as, for example, when two vesicles are in close contact (Pl. 2, fig. 10) or when a vesicle is situated near the surface of the brain (Pl. 1, fig. 3). The vesicles are accommodated by the surrounding tissue in such a way as to show little distortion, and certainly no disruption, of nearby cellular elements. Glial cells and processes, the cell bodies of large neurones and nerve fibres and the cells of capillaries and blood vessels all lie in close proximity to the vesicles. Sometimes it may be observed that the tissue immediately surrounding a vesicle has a vacuolated appearance reminiscent of the typical histological appearance of the infundibular stalk and process.

A careful examination of the serial sections through which a vesicle may extend reveals in some quadrant a cell body which forms an integral part of the vesicle. This cell body is flattened over a segment of the spherical vesicle (Pl. 3, fig. 14) and at its periphery is seen to be continuous with the limiting membrane of the vesicle (Pl. 3, fig. 12). The cell body in question, apart from its attenuated form, appears quite similar to the cell bodies of neighbouring neurones. Pl. 2, fig. 6 shows a small vesicle in which the associated cell is particularly well seen. It is the same size as nearby neurones, has a large nucleus with clear nucleoplasm and distinct nucleolus, and Nissl material which is clumped together near the periphery of the cell (Pl. 2, fig. 9; Pl. 3, fig. 15). Moreover, in some instances, and in material stained by Bodian's method (Pl. 2, fig. 7), the origin of an axon process leaving the cell body can be seen. It is concluded that the vesicles are in fact large vesiculated neurones. It has been shown by Bargmann (1950) that the neurones of the supraoptic and paraventricular nuclei are heavily laden with a 'neurosecretory' material which is stained by Gomori's chrome alum–haematoxylin method. The stain also demonstrates clumps of this material in the axon processes of these cells, it is quite specific, and is not picked up by cells of other types in the hypothalamus. This being so, it seemed that the stain would give valuable information on the affinity between the vesiculated neurones and the other neurones of the region. It was found that all were stained in an exactly similar manner

(see Pl. 3). The vesiculated neurones are just as heavily laden with secretory granules as the normally appearing neurones. They show the same variability from deeply staining to lightly staining individual cells. (Pl. 3, fig. 15, shows a lightly staining and Pl. 3, fig. 12, a darkly staining vesiculated neurone.) Some particularly good examples of vesiculated neurones with axons arising from them are provided by this stain, and in these instances the axons may exhibit clumps of secretory material along their length in a comparable manner to the processes of the other neurones (see Pl. 3, figs. 11, 15). Such observations leave no doubt that the vesicles are intracellular expansions formed in the large neurones of the supraoptic and paraventricular nuclei.

In some sections the cell body of a vesiculated neurone can be seen to have broken away from the supporting tissue into the lumen of the vesicle and where this has happened the membrane of the vesicle can be seen in continuity with the cell and pulled into the lumen with it (see the small vesicle in Pl. 1, fig. 3). The limiting membrane can also be well seen when two vesicles are so close together as to exclude any tissue between them and are only separated by their contiguous membranes (Pl. 2, fig. 10). The membrane appears as a thin hyaline envelope which does not stain specifically with any of the methods used. Although it has a highly refractile appearance no reticular fibres could be detected in it by the use of Wilder's or Mallory's methods (see Pl. 2, fig. 8).

Size. The vesicles have a diameter ranging from 10 to 200 μ. In any one animal the means of the antero-posterior and transverse diameters have always fallen between 44 and 62 μ (uncorrected for shrinkage), and the greater number of vesicles have diameters clustered about the mean. A group of vesicles of varying sizes are seen in Pl. 1, fig. 4. At one extreme the vesicles grade down to a size no larger than the individual neurones of the nucleus, and there is nothing to indicate that the smaller intracellular vacuoles which can be seen in the cell bodies of some neurones are not small or developing vesicles. At the other extreme vesicles of diameter 100 μ are not uncommon, that is to say they may be ten or more times the diameter of the large nerve cells of the region. It is this great size which clearly distinguishes the vesiculated neurones from any previously described cellular elements of the hypothalamus.

Blood vessels. The supraoptic nucleus is the most richly vascularized group of nerve cells within the central nervous system: next to it, in richness of capillary bed, comes the paraventricular nucleus. These assertions are based on capillary counts (Finley, 1940). It is of particular interest, therefore, to inquire in what relation the vesicles stand to this dense vascular supply. The capillaries appear as an intricate network, the fibres of which twist in profusion between the closely packed cells (see Pl. 1, fig. 2). The capillaries lie so close to the vesiculated neurones as to appear to contribute to the structure of the vesicle wall, and the minute vessels may be found in equally intimate contact with them (Pl. 2, fig. 8). Some arteries of fair size enter the hypothalamus

through the region of the supraoptic nucleus, and the veins draining the paraventricular nucleus and anterior hypothalamic area emerge through the same region. Inevitably some vesicles lie extremely close to these vessels, they may protrude into the adventitia or may be distorted in shape by the vessel (Pl. 1, fig. 5). Nevertheless, they always retain their integrity and their lumina exhibit no direct connexions with any of these vessels. The structural independence of the vesicles from the blood vessels is established by the observation that injection mass does not appear in the cavity of any of the vesicles (Pl. 1, fig. 2), and again in a specimen in which the brain was not washed through, but was fixed by immersion, no erythrocytes were to be seen in the vesicular cavity.

Contents. The nature of the vesicular contents remains unsolved. The possibility of fat being present was investigated by Verney (1947) and the results were negative. It seemed particularly desirable to look for 'colloid' since the presence of substances described as such has frequently been observed in the neurohypophysis, and in particular Scharrer & Scharrer (1940) have described it as being present in vacuolated cells in the paraventricular and supraoptic nuclei of the dog. However, with all stains showing affinity for colloid, the vesicular cavity has remained clear. In other parts of the same sections colloid inclusions have been intensely stained, including colloid in the vacuoles of the pars intermedia, and colloid inclusions and droplets in the cells of the supraoptic nucleus and adjacent hypothalamic areas. So far histological methods have not revealed the nature of the vesicular contents.

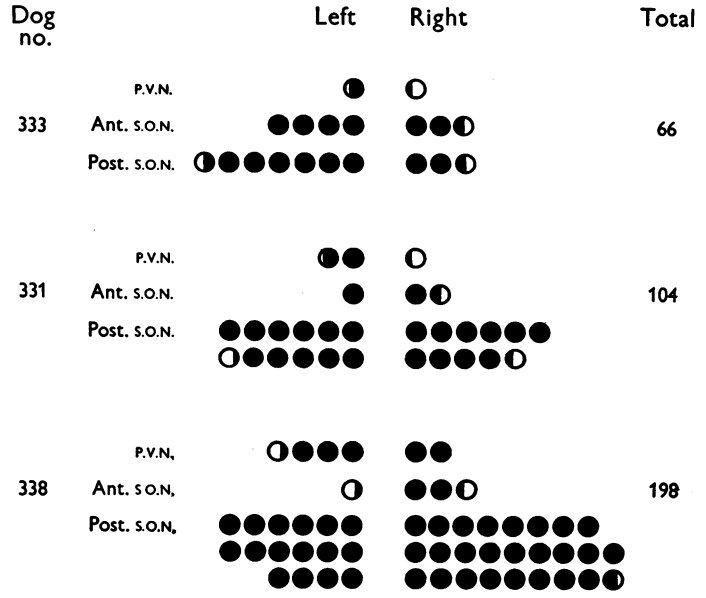
The numbers and distribution of the vesicles

Normal animals. The sections which have been prepared for the present investigation have been scrutinized to see whether vesiculated neurones could be found in other parts of the brain. The regions searched have included the entire hypothalamus and ventral thalamus, the preoptic and septal nuclei, the anterior third of the midbrain and the medial segment of the pyriform areas. A number of vacuoles are to be found grouped together in some of these regions. They are common in the supraoptic crest and neighbouring areas in the wall of the third ventricle, and in the infundibular bulb and stalk. These vacuoles did not appear to be intracellular and no vesiculated neurones were seen in areas other than the supraoptic and paraventricular nuclei.

It is usual to find that the vesicles are gathered together in the densest parts of the nuclei and are rarely found amongst the cells of the periphery. Occasionally a vesicle is to be seen outside the strict confines of the supraoptic nucleus in the anterior hypothalamic area or in the infundibular bulb, but cells of supraoptic type occasionally appear at similar sites. In the dog large groups of cells of a similar type are to be seen clustered about the veins which lie between the paraventricular and supraoptic nuclei, but although they stain

distinctively with Gomori's method none of them have been found in a vesiculated condition.

The numbers of vesicles observed in eight normal dogs are recorded in Table 1. The numbers for the right and left sides of the hypothalamus are separately recorded, and the figures for the supraoptic nucleus are divided into the numbers present in the anterior and posterior divisions. In addition, measurements are given for the average sizes of the vesicles in five of the animals; for the other animals measurements were not taken. The distribution



Text-fig. 1. The distribution of vesicles between the anterior supraoptic nucleus (ant. S.O.N.), posterior supraoptic nucleus (post. S.O.N.), and paraventricular nucleus (P.V.N.) of the right and left sides in three normal animals. One solid dot represents four vesicles.

of vesicles between the divisions of the nuclei are illustrated for three representative animals in Text-fig. 1. It will be seen that the distribution of vesicles is reasonably symmetrical between the right and left sides in most animals. However, with two of the animals, dogs 333 and 338, an elaboration was made in the fixing process. The heads of these animals were fixed by perfusion through the two common carotid arteries in the usual way, but the concentration of the solutions perfused into right and left sides was not the same. In dog 333, 1·0% NaCl was perfused into the left side followed by 5% formaldehyde in 1·0% NaCl, whilst the right side received, simultaneously, 0·8% NaCl followed by 5% formaldehyde in 0·8% NaCl. Dog 338 was treated similarly but here the concentrations of NaCl used were 1·2 and 0·6% on left and right

TABLE 1. The number of vesicles in the supraoptic and paraventricular nuclei of eight normal dogs and their mean diameters in μ. Antero-posterior diameter, a.p.; transverse diameter, tr.

					Supraoptic nucleus																
					Left						Right						Paraventricular nucleus		Total		
			Fixation process		Anterior division			Posterior division			Anterior division			Posterior division							
Dog	Sex	Weight (kg)	Fluid used to irrigate carotid vascular beds	Fixative	No.	a.p. (μ)	tr. (μ)	No.	a.p. (μ)	tr. (μ)	No.	a.p. (μ)	tr. (μ)	No.	a.p. (μ)	tr. (μ)	Left No.	Right No.	Total no.	a.p. (μ)	tr. (μ)
'Jetsam'* 282	F.	5	NaCl 0·9 %	Formaldehyde 5·0 % in NaCl 0·9 %	16	43	57	32	42	62	11	47	55	30	44	63	3	3	95	44	59
333	M.	22	Left carotid: NaCl 1·0 % Right carotid: NaCl 0·8 %	Left carotid: formaldehyde 5·0 % in NaCl 1·0 % Right carotid: formaldehyde 5·0 % in NaCl 0·8 %	16	46	53	26	49	49	10	45	53	10	54	48	3	1	66	48	51
338	M.	19·6	Left carotid: NaCl 1·2 % Right carotid: NaCl 0·6 %	Left carotid: formaldehyde 5·0 % in NaCl 1·2 % Right carotid: formaldehyde 5·0 % in NaCl 0·6 %	1	—	—	64	55	57	9	43	59	103	59	65	13	8	198	52	60
343	M.	19·7	NaCl 0·9 %	Formalin–acetic acid–alcohol	5	56	54	46	69	69	6	53	65	68	68	68	0	0	125	59	62
331	M.	17·9	NaCl 0·9 %	Formalin–acetic acid–alcohol	4	—	—	45	—	—	6	—	—	41	—	—	7	1	104	—	—
325	M.	13	NaCl 0·9 %	Formalin–acetic acid–alcohol	16	48	52	17	48	46	15	48	53	29	55	58	—	—	(77)	50	52
332	M.	18·2	NaCl 0·8 %	Formaldehyde 2·5 % in NaCl 0·8 %	89	—	—	399	—	—	67	—	—	396	—	—	—	—	(951)	—	—
'Nicky' 309	F.	20	NaCl 0·9 %	Formaldehyde 5·0 % in NaCl 0·9 %	14	—	—	30	—	—	11	—	—	32	—	—	—	—	87)	—	—

* Figures for the supraoptic nucleus taken from Verney (1947).

sides respectively. No correlation could be made between the tonicity of the washing and fixing solutions and either the numbers or size of the vesicles of the respective sides. Possibly such a correlation should not be expected from such brief periods of exposure to the differing tonicities. Further, great caution is needed in interpreting such experiments since the anastomoses between the carotid arterial beds in the dog are so extensive (Jewell, 1952) that admixture, or asymmetrical distribution of the two solutions, cannot be excluded.

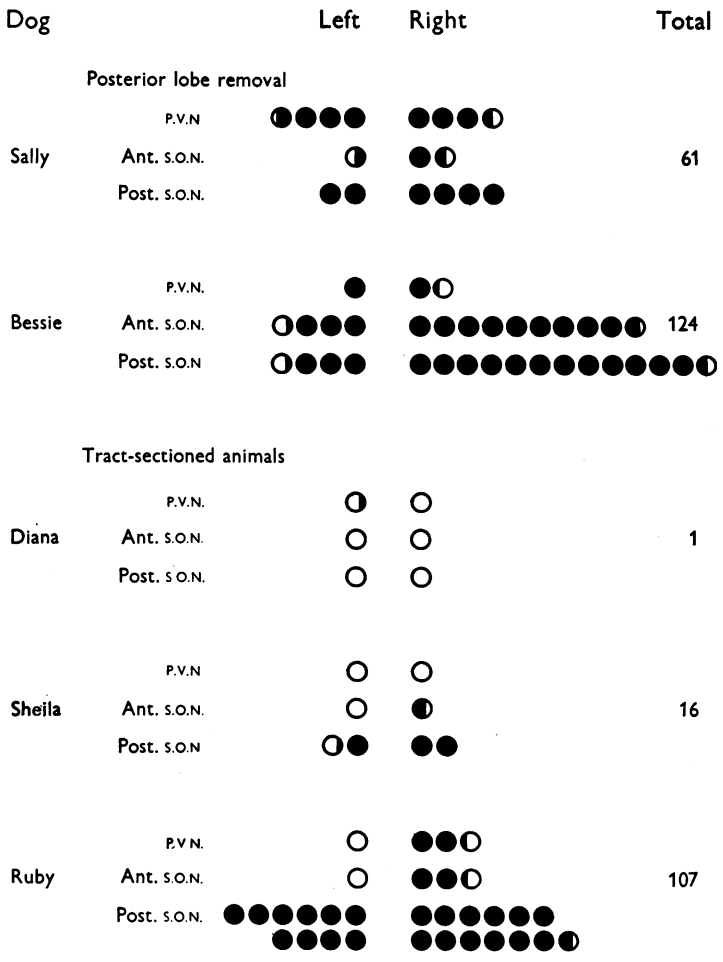
The total numbers of vesicles present in the neurohypophysial nuclei of the normal animals lay between 66 and 198, except for dog 332 in which 951 vesicles were counted in the supraoptic nuclei alone. It will be seen from the table that the numbers of vesicles present in the posterior divisions of the supraoptic nucleus are in every case considerably greater than the numbers present in the anterior divisions. And again the numbers present in the anterior divisions are usually greater than the numbers present in the paraventricular nucleus. This distribution is very different from the distribution of cell numbers between the three nuclear groups, and a consideration of this dissimilarity will be given in the discussion.

Operated animals. The lesion necessary to produce with certainty a permanent diabetes insipidus is the complete destruction of the pars nervosa, the pituitary stalk, and the median eminence. This can be achieved by high section of the supraoptico-hypophysial tracts. Less severe destruction of the neurohypophysis results in varying degrees of polyuria, whilst removal of the pars nervosa alone does not usually render the animal polyuric. A discussion of the correlation between the development of a permanent polyuria in the dog and the degree of damage to the neurohypophysis has been presented by O'Connor (1947).

The two post-hypophysectomized animals examined in the present study both proved to possess a number of vesiculated neurones, and neither had developed a permanent polyuria. In the case of 'Bessie' it may be supposed that the loss of cells from the neurohypophysis was not far removed from the average loss found in such animals—i.e. 76% from the supraoptic nuclei and 69% from the paraventricular nuclei. Despite this loss the number of vesicles present was well within the range for normal animals (see Text-fig. 2), the total being 124.

The case of 'Sally' is of particular interest since a number of relevant observations on this individual animal have already been published. Verney (1947) used the animal in survival experiments in which the effects of intracarotid injections of hypertonic solutions of sodium chloride were compared before and after removal of the posterior lobe of the pituitary. Although the animal did not develop a diabetes insipidus after the operation, its response to the injections, in terms of the amount of antidiuretic hormone released, was

reduced by 90%. The animal was sacrificed 51 days after the operation, and O'Connor (1947) has published counts of the numbers of cells present in the neurohypophysial nuclei. 21,100 cells remained in the supraoptic nuclei and



Text-fig. 2. As in Text-fig. 1, but showing the distribution of vesicles in two post-hypophysectomized animals, and in three animals with sectioned supraoptico-hypophysial tracts.

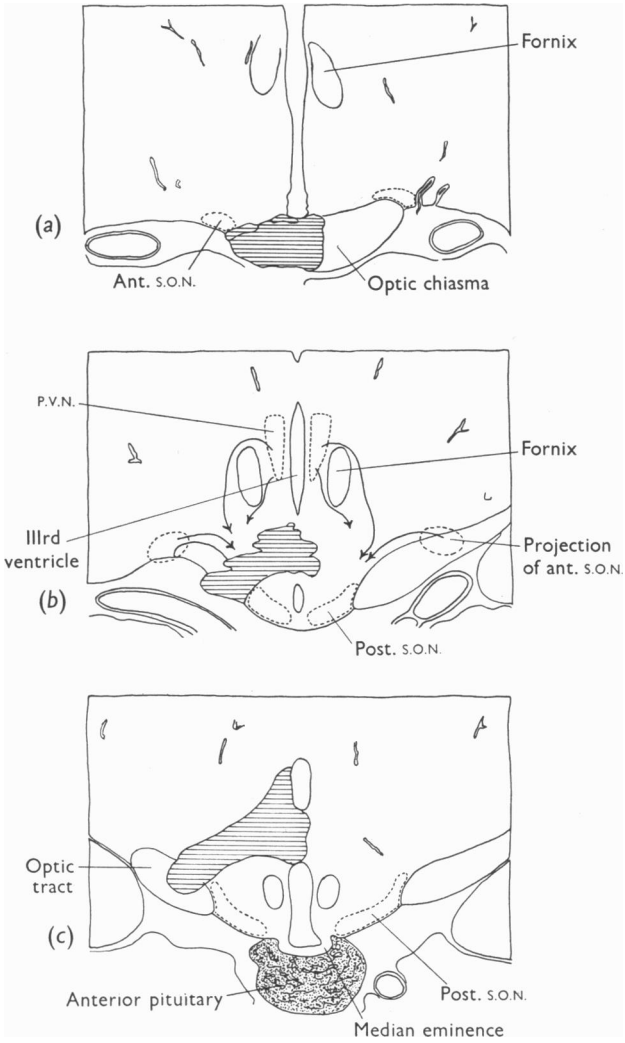
8000 in the paraventricular nuclei. These figures are a little higher than the average. Thirty-two vesiculated neurones were present in the supraoptic nuclei and twenty-nine in the paraventricular nuclei; a total of sixty-one. This is only slightly fewer than the total counted in any normal animal.

It appears, then, that despite the loss of cells in post-hypophysectomized

animals the number of vesiculated neurones present amongst the remaining cells is comparable with the number present in normal animals.

Of the three tract-sectioned animals two developed a permanent and high polyuria; one, however, failed to do so, and its daily output of urine returned to a normal level after the operation. Post-mortem examination of the two former animals revealed that the lesion had been accurately and symmetrically placed in both cases so as to section the supraoptico-hypophysial tracts. In each case the lesion had caused direct damage to part of the posterior divisions of the supraoptic nuclei and extended upwards and posteriorly through the anterior hypothalamic area. In these two animals the heavy loss of normally appearing neurones was paralleled by the disappearance of vesiculated neurones (see Text-fig. 2). Only one vesicle was present in 'Diana', and sixteen in 'Sheila'. The third animal, 'Ruby', in which the attempted tract section had failed to produce a permanent polyuria presented an extremely interesting post-mortem picture. The posterior divisions of the supraoptic nuclei appeared normal on both sides, with an apparently full complement of cells, and forty vesiculated neurones on the left side, and forty-nine on the right. Similarly, the anterior supraoptic nucleus and paraventricular nucleus of the right side appeared normal with nine vesiculated neurones in each. In contrast, these two latter nuclei of the left side showed a marked loss of cells and no vesicles (see Text-fig. 2). The stab wound had been asymmetrically placed and had entered the hypothalamus through the left side of the optic chiasma. From there it had passed dorsally and posteriorly through the anterior hypothalamic area involving the optic tract in its lateral extent and crossing the mid-line to involve the periventricular and adjacent areas of the right side in its medial extent (see Text-fig. 3). The lesion has passed dorsal to the left posterior supraoptic nucleus but has divided the descending tracts from the left anterior supraoptic nucleus and left paraventricular nucleus. The intrusion of the lesion into the right side of the hypothalamus might be suspected of dividing some of the descending fibres from the paraventricular nucleus of that side. That this would not be the case is demonstrated by reference to frontal sections stained with Gomori's method. As Bargmann (1950) has shown this stain is picked up by material present in the descending axons of the neurones. It therefore forms an extremely useful method of detecting the site of these descending tracts. In the case of the paraventricular nucleus its descending axons can be seen to pass laterally, dorsal to, as well as ventral to, the fornix, to join the fibres from the anterior supraoptic nucleus (presented diagrammatically in Text-fig. 3).

From these observations it may be concluded that the axons of the vesiculated neurones pass down the supraoptico-hypophysial tract in company with the axons of the normally-appearing neurones, and that all are affected in a similar way by lesions which involve these fibres.



Text-fig. 3. Projection drawings of frontal sections through the hypothalamus of 'Ruby' to show the extent of the lesion. The sections are 1 mm apart. (*a*) at the level of the optic chiasma; (*b*) anterior hypothalamic area; (*c*) median eminence. Lesion shown by horizontal lines. The arrows in *b* represent the descending fibre pathways from the paraventricular and anterior supraoptic nuclei. Abbreviations as in legend to Text-fig. 1.

DISCUSSION

Vacuolated cells were described in the supraoptic and paraventricular nuclei of the dog by Scharrer & Scharrer (1940), but they are depicted as containing colloid, and do not approach the vesiculated neurones in size, whilst their fibre connexions are not described. What relation such cells may bear to the

vesiculated neurones under discussion has not been elucidated. Green (1952) has stated that the vesicles are dilated or ruptured capillaries produced by perfusion at pressure. The results reported here refute such an assertion. Moreover, there seems no reason to suppose that the vesiculated neurones are vacuolated and degenerating cells, since with Gomori's stain they give evidence of being actively engaged in synthesizing 'secretory' granules. Evidence that the cells of the supraoptic and paraventricular nuclei are intensively engaged in an enzymic activity has been presented by Eränko (1951), and it is probable that Gomori's method selectively stains the products of this activity.

O'Connor (1947) has published complete counts of the numbers of cells present in the supraoptic and paraventricular nuclei of six normal dogs. The total cells of the supraoptic nuclei of the two sides varied between 64,200 and 93,000. The figures for one normal animal were published by Pickford & Ritchie (1945): the number of cells in the supraoptic nuclei of this animal was 72,320. Rasmussen (1940) made similar counts and found a range of 35,000–41,000 cells in the supraoptic nucleus of each side. Both reports give figures within the range found by O'Connor. It seems permissible, therefore, to use O'Connor's figures for the total numbers of cells in the supraoptic nuclei of normal animals for comparison with the present counts of vesiculated neurones. Figures for the numbers of cells normally present in the paraventricular nuclei will also be taken from O'Connor—a range of 11,800–20,100 cells. Pickford & Ritchie gave a figure of 44,500 for their animal, but this discrepancy will not compromise the argument.

There is no correlation between the weight of an animal and either the total number of cells or the total number of vesiculated neurones in its nuclei. Nor is there any correlation between the total numbers of cells present in the neurohypophysial nuclei, or the three divisions of the nuclei, and the numbers of vesiculated neurones. In Table 1, figures for the numbers of vesicles present in the three divisions of the nuclei are complete for five animals. It will be seen that by far the greatest number of vesicles (average 76%) are in the posterior division of the supraoptic nucleus; 18% are in the anterior division of the supraoptic nucleus, and only 6% in the paraventricular nucleus. This distribution is in contrast to the distribution of cells as can be seen from O'Connor's table where 44% of cells are in the posterior supraoptic nucleus, 39% in the anterior supraoptic nucleus and 17% in the paraventricular nucleus.

If the vesiculated neurones are concerned with an osmoreceptive function then these observations may indicate that the posterior divisions of the supraoptic nucleus exercise the greatest control over the secretion of antidiuretic hormone. The functions performed by the paraventricular and supraoptic nuclei are not precisely known, but the phylogenetic development of these two nuclei from the primitive nucleus preopticus may well reflect some divergence in their functional roles.

Verney (1947) has suggested that, if the vesicles are osmoreceptors, changes in their size might be registered by nerve processes attached to their surface as stretch receptors, thus transmitting impulses to the neurohypophysial cells. Using Bodian's silver-proteinate method it has not been possible to detect any specialized terminal processes on the vesicles (Pl. 3, fig. 13), and where isolated instances of a process projecting from the wall of a vesicle have been observed, they have been interpreted as arising from, and not running to, the vesicle. That is not to say that such terminations about the vesicles do not exist, and the application of more precise histological methods for this purpose will be one of the next steps to take. However, the present demonstration that the vesicles are in fact vesiculated neurones, with axons which probably traverse the infundibular stalk and terminate in the infundibular process, offers an alternative suggestion of the manner in which they might act. The vesiculated neurones may themselves be the ones which receive the osmotic stimulus and also themselves release antidiuretic hormone. Alanis & Matthews (1952) have shown that mechanical deformation of the surface of a neurone provokes a discharge and it could be imagined that the swelling or shrinking of a vesiculated neurone would provide an adequate stimulus to the cell. Such suggestions, however, go far beyond what can be demonstrated histologically, and considerably more experimental work will be necessary before it can even be asserted that the function of these structures is that of receiving or amplifying an osmotic stimulus.

Finally, these observations give no basis for assuming that the number of vesicles in the hypothalamus of any one animal is a static quantity. On the contrary, it is most probable that more neurones become vesiculated when the need for them arises, and, should some become redundant, that they will revert to the usual state. If this is so, and the number of vesiculated neurones present at any one time bears some relation to the previous history of the animal, and to the environment it has had to endure, then the wide range in the numbers observed would be in no way surprising.

SUMMARY

1. The vesicles which occur in the supraoptic and paraventricular nuclei of the hypothalamus of the dog have been examined histologically in normal animals and in animals subjected to operative interference with the neurohypophysis.

2. The vesicles are intracellular structures formed in the neurones of the nuclei. These vesiculated neurones give the same staining reactions as non-vesiculated ones, and have axon processes which are presumed to join the supraoptico-hypophysial tracts.

3. In post-hypophysectomized animals the number of vesiculated neurones

present was within the range found in normal animals. These animals did not develop a permanent polyuria.

4. In animals with sectioned supraoptico-hypophysial tracts very few vesiculated neurones were present. These animals developed a permanent polyuria.

I have to express my warmest thanks to Prof. E. B. Verney for first interesting me in this investigation, and for generously allowing me to use and examine material which he had prepared. I am also greatly indebted to Dr W. J. O'Connor for giving me material from his experimental animals together with much information about them; and to Mr A. W. Marrable, of the Department of Anatomy, Royal Veterinary College, for the great trouble and care he has taken in preparing the photomicrographs.

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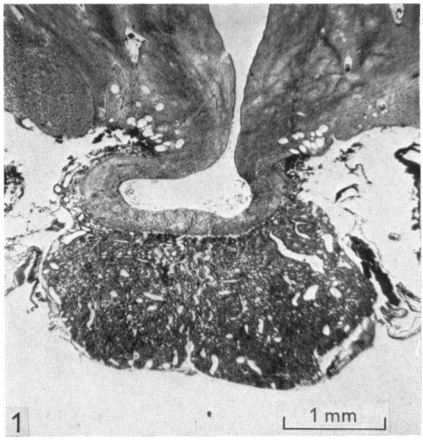
EXPLANATION OF PLATES

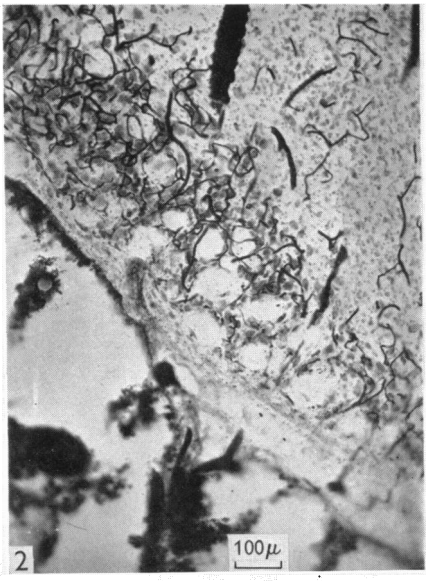
All photomicrographs are from paraffin sections through the posterior supraoptic nuclei of dogs, except Fig. 2 which is from a celloidin section, the celloidin being retained on the slide.

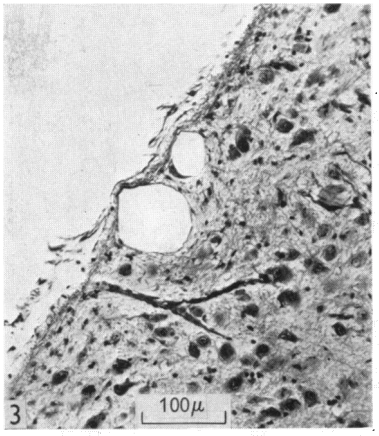
PLATE 1

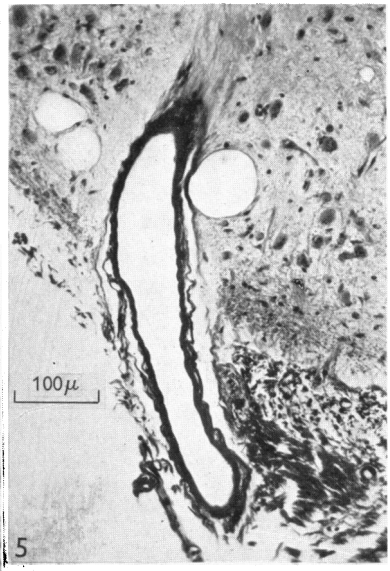
Fig. 1. Transverse section through the median eminence and pars distalis of a greyhound showing the distribution of vesicles in the posterior divisions of the supraoptic nuclei. Azan; 10μ thick.

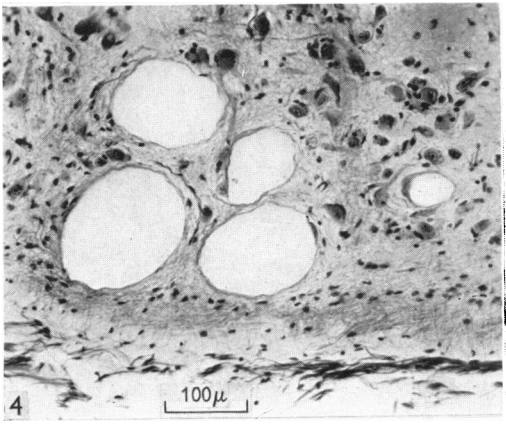
Fig. 2. Transverse section of the right posterior supraoptic nucleus of a dog in which the blood vessels had been injected with an indian ink–plasma mass. Numerous vesiculated neurones are present. Toluidin blue; 100μ thick.











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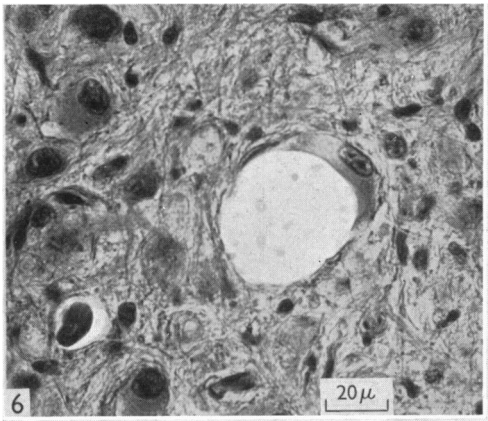
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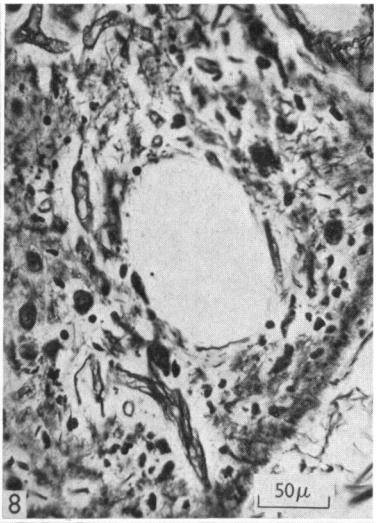
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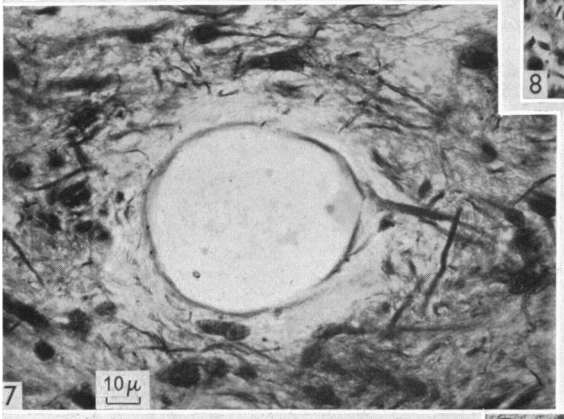
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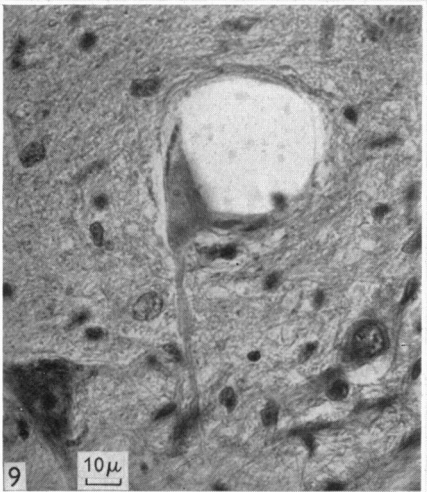
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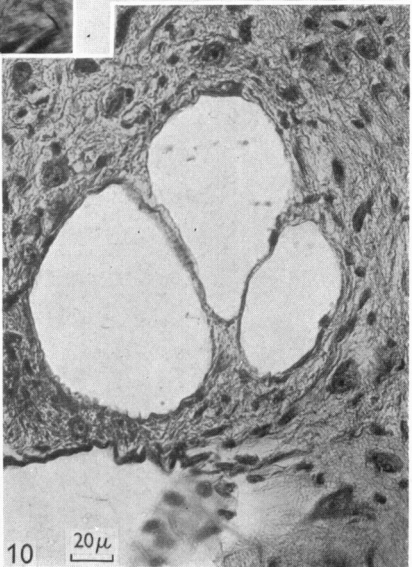
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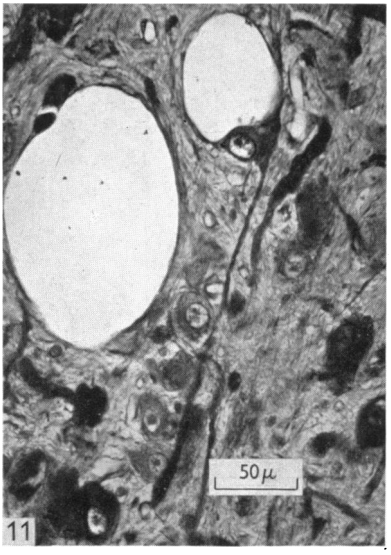
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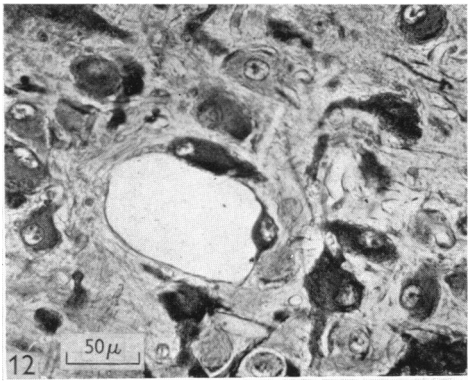
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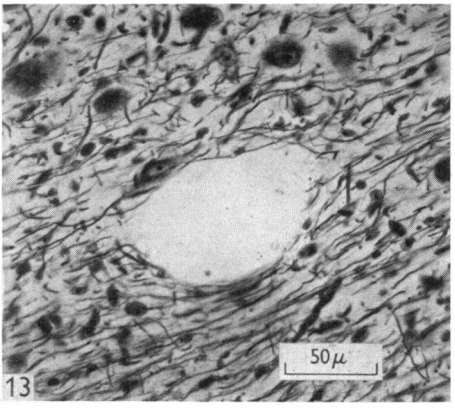
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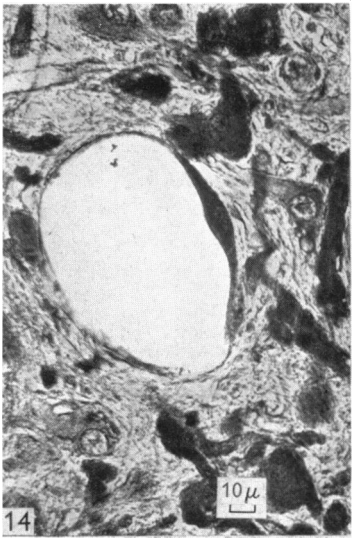
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14

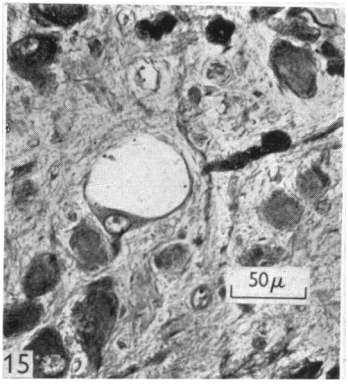
50μ
15

Fig. 3. Vesiculated neurones protruding from the surface of the tuber cinereum. Ventral aspect of the brain to the left of the photomicrograph. Transverse section; Masson's trichrome; 10μ thick.

Fig. 4. Group of vesiculated neurones of varying size and showing refractile borders. The associated neurone is clearly seen in the case of the small vesicle on the right of the field. Sagittal section; azan; 10μ thick.

Fig. 5. Vesicle protruding into the adventitia of an artery which penetrates the hypothalamus. Transverse section; Masson's trichrome; 10μ thick.

PLATE 2

Fig. 6. The crescentic form of the cell in a small vesiculated neurone. Note the clear nucleoplasm and cytoplasm. Nissl granules are not evident. Haematoxylin and eosin; 10μ thick.

Fig. 7. Vesiculated neurone stained with Bodian's method showing the axon process arising from the cell. 12μ thick.

Fig. 8. Wilder's method for reticular fibres showing the reticular network in blood vessels but the absence of fibres from the vesicle wall. 12μ thick.

Fig. 9. Vesiculated neurone showing the axon process leaving the cell and peripherally situated Nissl material. Haematoxylin and eosin; 10μ thick.

Fig. 10. A group of vesiculated neurones showing the walls between them. Azan; 10μ thick.

PLATE 3

Fig. 11. Application of Bargmann's modification of Gomori's method to vesiculated neurones. The smaller vesiculated neurone exhibits an axon process which runs for a considerable distance in the plane of the section. 'Secretory' material can be seen clumped along the axon. 15μ thick.

Fig. 12. Gomori's method revealing deeply staining and lightly staining cells. The vesiculated neurone is deeply staining but the amount of cytoplasm remaining about the nucleus is conspicuously less than that of neighbouring cells. 15μ thick.

Fig. 13. Bodian's method showing fine nerve fibres running in close proximity to a vesicle. No special endings about the vesicle could be discerned. 12μ thick.

Fig. 14. Gomori's method; large vesiculated neurone. The attenuated form of the neurone is well seen, and it is also heavily laden with 'secretory' granules. Note the axon process on the right of the field showing clumps of 'secretory' material. 15μ thick.

Fig. 15. Vesiculated neurone derived from a lightly staining cell. The origin of an axon process from this cell can be seen just below the nucleus. Gomori's method; 15μ thick.