AN EXPERIMENTAL ATTEMPT TO DETERMINE THE SITE OF THE NEUROHYPOPHYSIAL OSMORECEPTORS IN THE DOG

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The object of this investigation has been to define the site of the osmoreceptors, a term that has been applied to those hypothetical sensory elements that respond to changes in the osmotic pressure of their vascular environment, and through which the release of antidiuretic hormone from the neurohypophysis is physiologically regulated.

Confirmation is given to the cephalic localization of these receptors, and an attempt has been made to discover where, within the substance of the brain, they reside. The test of their activation has been the inhibition of urine flow by intracarotid infusions of hypertonic solutions during established water-diuresis. By surgical means the cephalic field of distribution of the carotid arteries in the dog has been restricted to defined regions of the brain, and information has thereby been acquired on the osmoreceptive status of these regions. In this way it has been contrived that the evidence for the localization of the osmoreceptors should rest solely on the use of a blood-borne physiological stimulus appropriate to the sensory elements in question.

The earlier sections of the paper present the results of the broader studies that this work has necessitated; first, an anatomical investigation of the arterial connexions of the circle of Willis and of the detailed vascular architecture of the diencephalon and hypophysis; and secondly, the devising of a method to trace the distribution of arterial blood and the application of this method to the demarcation of the cerebral fields of carotid and vertebral arteries.

The middle sections of the paper describe the surgical and experimental techniques employed, and the manner in which the posterior lobe of the pituitary gland, the posterior brain stem and the cerebral hemisphere have been excluded from being the site of receptors. Also described are experiments in which the permanent unilateral suppression of responses following intradural

ligation of an internal carotid artery has placed beyond cavil the assertion that the receptors lie within the substance of the brain.

The final sections present the evidence for the hypothalamic localization of the osmoreceptors and for the suggestion of a possible involvement of the thalamic paraventricular nucleus in the osmoreceptive process.

To trace the distribution of arterial blood two coloured suspensions have been employed. The suspensions are freely miscible with blood, cause no circulatory disturbance in the living animal during the period of their infusion, and tolerate histological procedures. The suspensions are infused into appropriate arteries at a terminal experiment in which the animal is killed before the suspensions have had time to recirculate.

In the dog the brain is normally supplied with blood from both carotid and vertebral arteries. The telencephalic field of the carotid arteries is that part of the hemisphere supplied by the anterior and middle cerebral arteries and includes the striate body; the vertebral arteries supply the hippocampus and posterior cerebral artery field, the midbrain, cerebellum and medulla. However, this strict partition is defied by two seemingly normal occurrences: common carotid blood may pass via the occipito-vertebral anastomosis to join the basilar stream, and vertebral blood may pass forward from the posterior communicating artery to mix with the carotid cerebral supply. The thalamus is supplied almost exclusively by vertebral blood that reaches it via branches of the posterior cerebral artery and the thalamic branch of the posterior communicating artery. The hypothalamus is divided in the sources of its supply: carotid blood irrigates the anterior nuclei via arterioles arising directly from the internal carotid and the immediate vicinity of its trifurcation; vertebral blood supplies the posterior nuclei via arteriolar branches of the posterior communicating artery, but, in addition, this blood may stream forward to supplement the carotid supply to the pre-infundibular nuclei. Situated near that part of the circle where the carotid and vertebral streams meet are the main nuclear groups of the neurohypophysis, the supraoptic nuclei. Particular attention has been paid to the volume partition of these nuclei according to the origins of their blood supply, in order to gain an index of blood distribution in the anterior hypothalamus. In the normal animal between 10 and 30 % of the total supraoptic nuclear material is supplied with blood of vertebral origin.

The naturally occurring asymmetry of the arterial supply to the posterior lobe of the pituitary gland has led to the elimination of this structure as a site of osmoreceptors. In each of a total of eight animals it was found that the posterior lobe was supplied with blood originating exclusively from one carotid, yet an osmotic release of antidiuretic hormone had been obtained from infusions of hypertonic solutions into the other carotid.

The exclusion of carotid blood from the posterior brain stem has been achieved by (*a*) the ligation of the two occipital arteries, and, on one side, by (*b*) the ligation of the occipital and posterior communicating arteries. Osmotic responses to carotid infusions were retained after these procedures and thus made secure the assignment of the receptors to the prosencephalon.

Of prosencephalic structures the greater part of the telencephalon has been eliminated from being the receptor site by demonstrating the ipsilateral retention of responses after total hemispherectomy. This conclusion was supported by evidence from animals in which the carotid bloods, while evoking osmotic responses, were asymmetrically distributed within the telencephalon.

The heavy degenerative cell loss in all thalamic nuclei of the hemispherectomized animal, excluding some cell groups of the midline, substantiated earlier indications that the receptors were not in the dorsal diencephalon. Alternative and equally compelling evidence for this conclusion was obtained from experiments in which carotid blood was directly excluded from the thalamus. Such exclusion was attained by tying the posterior communicating and occipital arteries of one side together with the middle cerebral or anterior cerebral artery. It was also attained in experiments in which the internal carotid of one side was ligated intradurally, this resulting in a redistribution of blood from the circle such that the thalamus of one side was deprived of any carotid flow. These same experiments afforded, too, unequivocal evidence for the exclusion of the posterior nuclear groups of the hypothalamus as a possible site for the receptors.

The collation of the several evidences summarized above has led to the inference that the osmoreceptors are situated somewhere in the anterior hypothalamus or preoptic areas, that is, in the region comprised by the medial and lateral preoptic areas, the suprachiasmatic nucleus, the nucleus

supraopticus diffusus, the anterior hypothalamic area, the paraventricular and supraoptic nuclei, the dorsomedial and ventromedial nuclei, and the dorsal and lateral hypothalamic areas. This region has always received carotid blood when there have been osmotic responses to intracarotid infusions.

The restriction of the cephalic distribution of the carotid of one side of this receptive zone was successfully achieved by ligature of the anterior cerebral, middle cerebral and posterior communicating arteries just beyond the carotid trifurcation, together with occlusion of the ipsilateral occipital artery. The sequel, however, was disappointing in that osmotic responses were lost on that side; but the experiment provoked conjecture upon the possible neuronal organization of the osmoreceptive apparatus, since it was found that in this animal the operation had caused cystic destruction of the thalamic paraventricular nucleus together with all other anterior and medial thalamic nuclei. In all previous animals, including the hemispherectomized one, in which osmotic responses had been retained, the thalamic paraventricular nucleus and its connexions with the hypothalamus had remained intact.

The conclusion is drawn that the osmoreceptors are situated in the anterior hypothalamus. There are indications that they are not of unvarying sensitivity, and that their functioning may be dependent upon the integrity of nervous connexions with the thalamic paraventricular nucleus.

I. INTRODUCTION

Recent work (Verney 1946, 1947) has shown that the release of antidiuretic hormone from the pars nervosa of the pituitary gland is physiologically determined by the osmotic pressure of the arterial blood. Alterations in the osmotic pressure of the blood were produced by the injection or infusion of hypertonic solutions into the common carotid arteries of unanaesthetized bitches provided with carotid loops, and the output of antidiuretic hormone was measured by the effect of the injection or infusion upon a concurrently established water-diuresis. It was shown that when two solutions with such diverse properties as those of sodium chloride and sucrose were injected so as to give the same calculated percentage increase in the osmotic pressure of the carotid blood, then the amounts of antidiuretic principle released were the same. The osmotic determination of the output of this hormone was thus established. The increase in osmotic pressure of the carotid blood in these experiments was large—being of the order of 50 %—but it was restricted to a short period, e.g. 10 s. Further experiments were undertaken to determine the effects of smaller changes in osmotic pressure maintained over longer periods; and intracarotid infusions were made for periods up to 40 min. By these means it was found that a maintained increase of some 2 % only in the osmotic pressure of the blood in one common carotid trunk was sufficient gradually to reduce the rate of urine flow from a water-diuresis maximum to the sort of rate that prevailed at the beginning and end of a normal response to ingested water, i.e. a reduction of some 90 %; this reduction was effected through the intermediate release of post-pituitary antidiuretic substance at an average rate of 1 μU/s. ($0{\cdot}5 \times 10^{-9}$g/s in terms of the standard powder*). On the basis of these investigations it was postulated that the neurohypophysis was functionally linked with sensory elements sensitive to changes in the osmotic pressure of their vascular environment. Such elements were termed osmoreceptors, and these were envisaged as

* The unit (U) is the specific antidiuretic activity corresponding to that yielded by 0·5 mg of the International Standard Preparation of Dry Pituitary (Posterior Lobe) Powder, when extracted by the prescribed method. The quantity here mentioned, viz. 1 μU/s, in view of the recent achievement of du Vigneaud and his colleagues (1953) in isolating as an octapeptide and determining the chemical structure of vasopressin, can now be expressed as 2×10^{-12} g/s of the pure substance, i.e. about 12×10^{8} molecules/s (Verney 1954).

being continually engaged in transmuting osmotic pressure changes in their environment into appropriately effective messages to the neurohypophysis. The present paper is concerned with experiments designed to discover the site of these osmoreceptors.

Some evidence has been advanced in a paper already referred to (Verney 1947) to indicate where the osmoreceptors may be situated. It was shown that they are neither in the carotid sinus nor in the carotid body. Further, observations on the effects of increases in the osmotic pressure of the common carotid blood before and after ligation of the ipsilateral internal carotid near its origin led to the conclusion that the osmoreceptors lay in the vascular bed normally supplied by the internal carotid artery. This conclusion was based on the evidence of a series of observations on each of two animals. In the one ('Sally') the pars nervosa of the pituitary had been removed, and the small residual inhibition of urine flow then elicited by injections of hypertonic solutions into a common carotid artery was absent when the same injections were made after the animal's internal carotid branch had been tied near its origin. In the second animal ('Pat') the early effect of such ligation was to suppress the ipsilateral and osmotically determined release of antidiuretic hormone. Further experiments on this animal showed, however, that occasionally quite large releases occurred; these were attributed to the presence of the short anastomotic vessels which in the dog connect the internal maxillary branch of the external carotid with the 'ophthalmic'* branch of the internal carotid; and the prediction was made that were one internal carotid tied intradurally, i.e. beyond the 'ophthalmic' anastomosis, the responses to a raised osmotic pressure in the common carotid trunk of the same side would be consistently and permanently suppressed.

The purpose of the work now to be described has been to test the validity of this inference and, in the light of the results, to define the anatomical region in which the osmoreceptors lie. Unequivocal evidence was early forthcoming that the receptors are situated somewhere in the prosencephalon; and our working hypothesis has been that they lie in the anterior part of the hypothalamus, i.e. in or near the main neurohypophysial nucleus (supraoptic) with which they are supposedly in functional linkage. Our reasons for adopting this hypothesis have been, first, that the diencephalon is known to be the region in which many autonomic activities are integrated; secondly, that so far as can be judged by the completely unconcerned attitude of the animal during the antidiuretic response to a raised osmotic pressure of the carotid blood, the response is devoid of perceptive accompaniment; thirdly, that it would seem judicious to begin the search for the osmoreceptors in or near the final path through which the osmotic release of antidiuretic hormone is believed to be effected, viz. the supraoptic nuclei and neurohypophysis; and fourthly, that one of us (Verney 1947) has described, within the field of the supraoptic nucleus, vesicles which appear to be admirably suited to an osmoreceptive function, and has discussed their possible mode of action in this regard. More recently one of us (Jewell 1953) has shown that these vesicles are intracellular structures formed in the neurones of the nuclear masses, such vesiculated neurones having axon processes that presumably join the supraoptico-hypophysial tracts. We have, therefore, in the course of the work now to be described, watched particularly for evidence for and against this nucleus containing the receptors we seek. In order to become furnished with the information and conditions

* The nomenclature of the vessels forming this anastomosis is discussed by one of us elsewhere (Jewell 1952).

needed for the ultimate test of our hypothesis, we have found it necessary (1) to determine routes, additional to the 'ophthalmic' anastomosis, through which, in the dog, external carotid blood may reach the cerebrum; (2) to examine in detail the vascular supplies to the hypothalamus and thalamus, and to the hypophysis; (3) to devise a method for tracing the cerebral distribution of arterial blood from carotid and vertebral sources; and (4) to contrive by surgical procedures, on the one hand to prevent carotid blood from reaching the brain, and in particular the anterior hypothalamus, and on the other to restrict the field of distribution of one internal carotid to the anterior hypothalamic region.

We would here emphasize two points in connexion with our attempts to localize the osmoreceptors. First, we have used a blood-borne physiological stimulus, viz. a raised osmotic pressure in the capillary blood, to determine whether the receptive elements lie within the vascular field of a particular vessel; and secondly, the positive defining of the neuroreceptive field depends upon retention of response with restriction of the vascular bed that carries the osmotic stimulus. Clearly the emplacement of destructive, e.g. electrolytic, lesions in certain areas of the brain, and their association with disappearance of the antidiuretic response to a raised osmotic pressure in the capillary blood of these areas, could give information only on the efferent path between receptors and neurohypophysis, and none on the localization of the receptors themselves.

II. EXPERIMENTAL

A. Routes by which external carotid blood may reach the cerebrum

Information in connexion with our first need has already been supplied by one of us (Jewell 1952), and to this we must now briefly refer. It was shown that in the dog there were five routes through which external carotid blood might reach the cerebrum, viz. (1) the occipito-vertebral anastomosis, (2) the anastomosis between the ascending pharyngeal artery and the internal carotid artery, (3) the anastomotic artery, (4) the ophthalmic anastomosis and (5) the ethmoidal anastomosis. It was considered that routes (2) and (5) were unlikely to be involved in the active transference of carotid blood to the circle of Willis, and our subsequent work gives no reason to revise this view other than that route (5) may operate under conditions of extreme cerebral demand. Accordingly, these anastomotic pathways will not be considered further here. General confirmation has been given to the carotid circulation in the dog by the work of Daniel, Dawes & Prichard (1953), and we have added to the material examined three neoprene casts of the arteries of the dog's head prepared in the manner described by these authors.

The anatomical disposition of the occipito-vertebral anastomosis is seen in figure 1. It is probable that, unlike the conditions in man, blood frequently passes from carotid to basilar by this route in the dog; as we shall show later in experiments in which tracer substances have been injected into the common carotid arteries, these substances have often appeared in the hind- and midbrain regions supplied by the basilar artery. Moreover, when there is operative reduction in the carotid inflow to the circle of Willis with consequential increase in the basilar inflow, then, from a similar site of injection, much tracer substance is invariably distributed in the field usually referred to the vertebral supply. The most

ready and, as we shall show later, the proper explanation of this is that the occipito-vertebral anastomosis is now carrying a larger amount of blood from the external carotid to the ventral spinal artery. This anastomosis, then, is an important route by which external carotid blood may reach the circle of Willis. Moreover, neoprene casts have revealed a number of secondary anastomotic routes in the occipital region between carotid and vertebral fields. Muscular branches of the occipital artery, sometimes taking origin very close to the external carotid, were joined by twigs of both ipsilateral and contralateral vertebral arteries, and the auricular artery had long anastomotic connexions with the

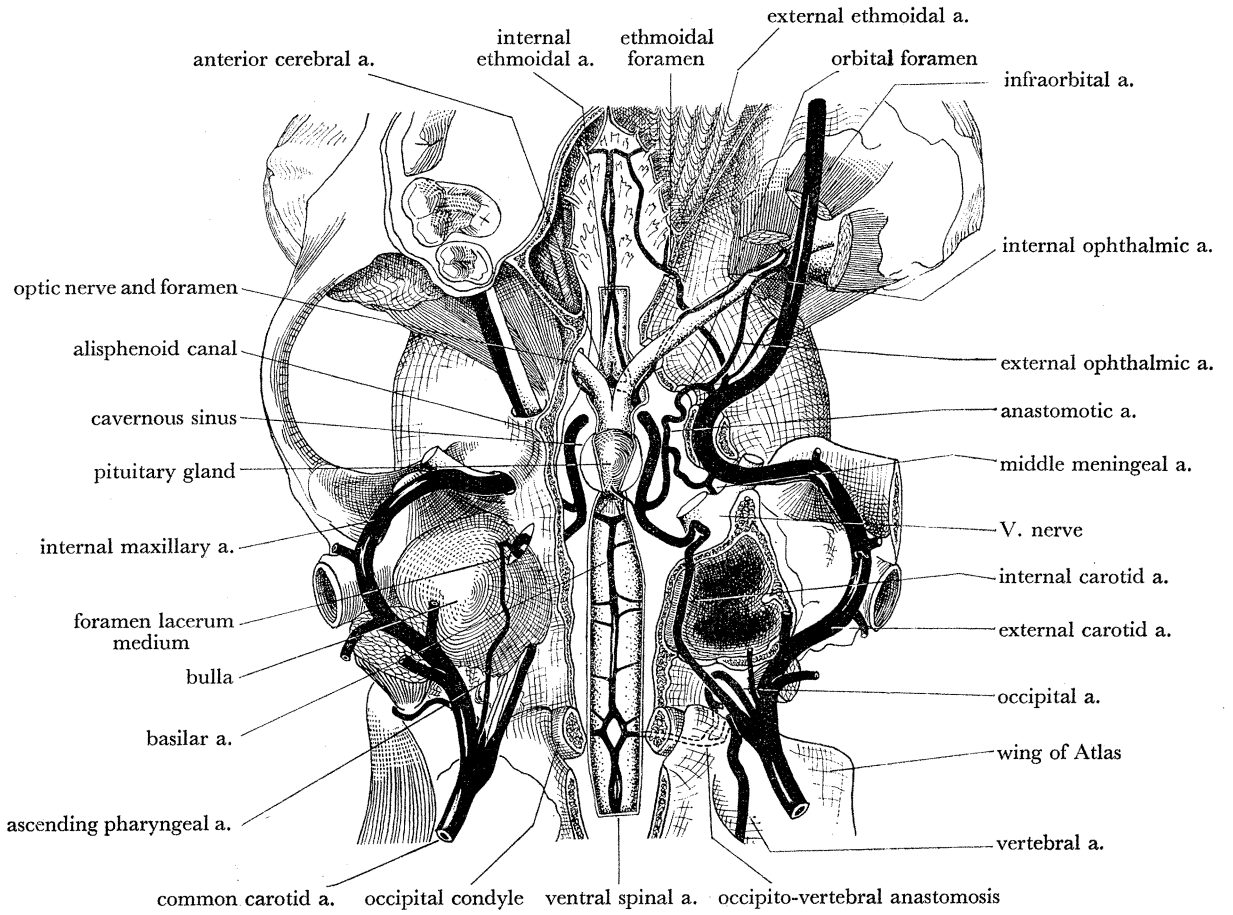


FIGURE 1. Semi-diagrammatic illustration of anastomoses which do or may conduct external carotid blood to the cerebrum.

ipsilateral vertebral. The existence of such anastomoses in the neck of the dog was demonstrated by Astley Cooper (1836). In experiments in which we have attempted to exclude carotid blood from the basilar flow by ligation of the occipital artery, our intention has sometimes been frustrated by the active transference of blood through these secondary anastomotic channels.

The second and largest route by which external carotid blood may reach the cerebrum is through the anastomosis between the internal maxillary artery and the internal carotid as it runs forward in the cavernous sinus (figure 1). This anastomotic artery undoubtedly contributes to the internal carotid inflow to the circle of Willis under normal conditions in

the dog, a contribution which will of course be entirely suppressed by intradural ligation of the internal carotid.

The ophthalmic anastomosis is formed by the junction in the orbit (figure 1) of the external ophthalmic artery which arises from the internal maxillary artery or its orbital branch, and the internal ophthalmic artery which, arising from the anterior cerebral artery soon after its origin, runs through the optic foramen with and along the optic nerve, to which it supplies nutrient twigs. The internal ophthalmic artery is a small vessel, much smaller than the external ophthalmic, and the direction of blood flow within it under normal conditions is not known. As we shall see later, however, there is evidence that after the internal carotid has been tied intradurally the direction of flow in the internal ophthalmic artery is towards the cerebrum.

With this information on the arterial links between the extracranial and intracranial fields we come now to our second need, viz. detailed anatomical knowledge of the vascular supplies to the hypothalamus and thalamus, and to the hypophysis.

B. The vascular supplies to the hypothalamus and thalamus, and to the hypophysis

A precise description of the arterial supply to the hypothalamus of the dog has not been found in the literature, although it is possible to infer some general idea of its pattern from the existing descriptions of the supply in other mammals (Clark 1938; Wislocki & King 1936), and from information incidentally given in descriptions of the vascular supply to the hypophysis of the dog (Dandy & Goetsch 1910; Basir 1932; Green & Harris 1947). Such descriptions, however, were inadequate for our purpose, as it was necessary to know the origin and course of the arterioles supplying the various nuclei of the hypothalamus, and, in addition, the trunk source from which they receive their blood. Accordingly, a careful study has been made of the vascular pattern of the dog's hypothalamus and thalamus, and the results of these observations will now be presented.

(1) *Methods and materials*

(*a*) *Macroscopic*

Three neoprene casts of the arteries of the brain were prepared in the manner described by Daniel *et al.* (1953). Two of these were examined when partially macerated to note the relation of the vessels to cerebral structures, and the maceration was then taken to completion. Further, the base of the brain of a dog in which the arteries had been injected with Indian ink, and in which the vessels of the circle of Willis were kept intact, was cleared in methyl benzoate and examined for general relationships of the hypothalamic and thalamic vessels.

(*b*) *Microscopic*

Reconstruction of the arterial supply from serial sections. Reconstruction drawings of the arterial supply to the ventral hypothalamus were prepared from two animals. The brains of these animals had been fixed by perfusion with a mixture of 50 ml. 40% formaldehyde, 50 ml. glacial acetic acid and 900 ml. of 80% ethanol, using the method described by

Verney (1947). A block of tissue including the hypothalamus, and with the meninges attached, was removed from the brain and embedded in celloidin. From these blocks serial sections were cut at 150μ thickness and stained with van Gieson's fluid. One block was cut in a horizontal plane and the other in a transverse plane. The sections were then projected at a magnification of $\times 50$ on to tracing paper, and each one was drawn. The drawings of the horizontal sections were reassembled into a single ventral perspective of

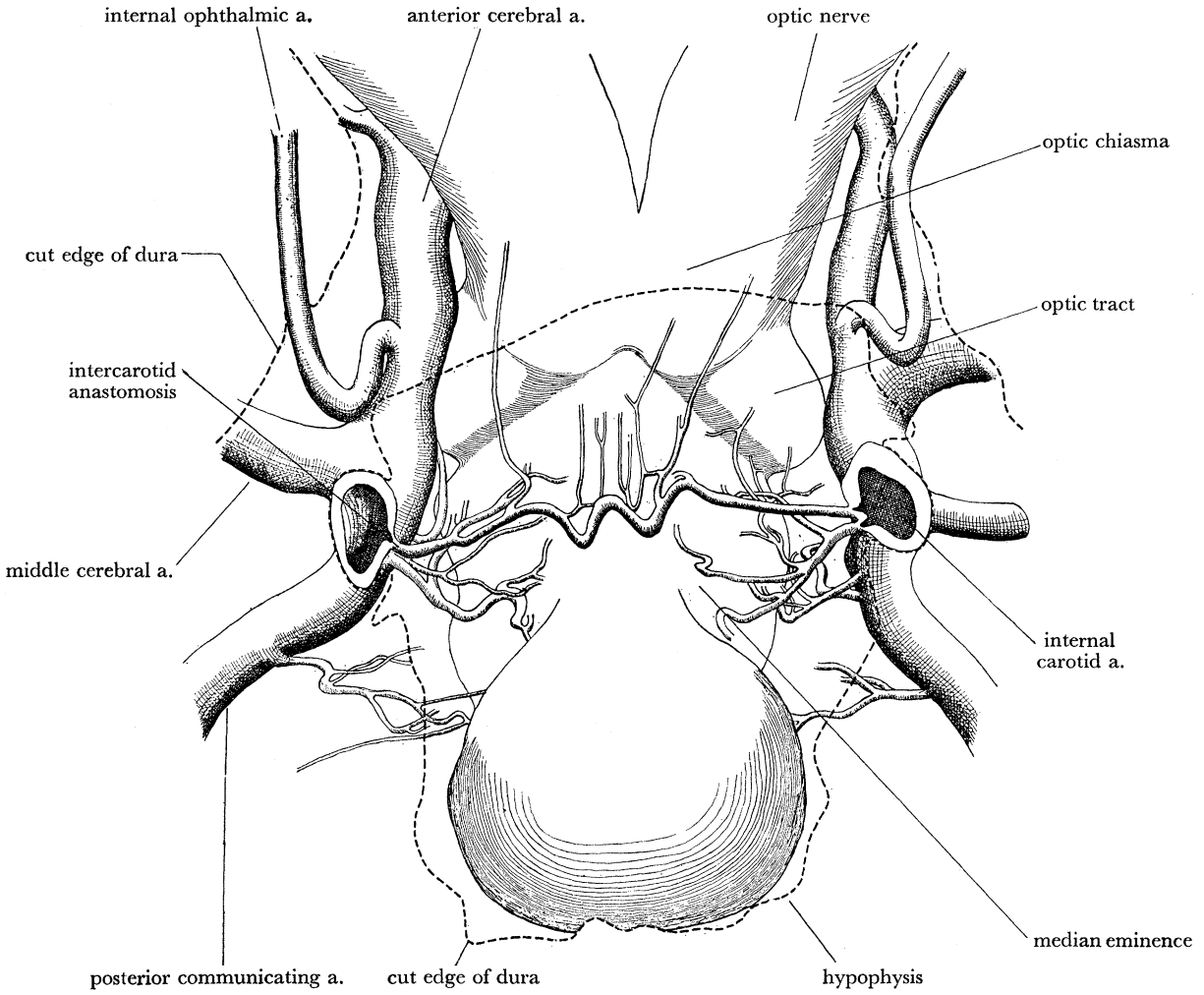


FIGURE 2. Reconstruction drawing from serial sections of the hypothalamic region of dog 367*a* cut at 150μ thickness in the horizontal plane. To show the intercarotid anastomosis and the arteries of supply to the glandular hypophysis, the median eminence and the posterior division of the supraoptic nucleus (Magn. $\times 12$).

the vessels (figure 2). The drawings of the transverse sections were assembled into five blocks involving the whole hypothalamic region, and each of these was drawn in perspective.

In a third animal a hypothalamic block was prepared in a similar manner except that in this case the basisphenoid bone and structures of the sella turcica were retained *in situ* on the block, and the whole was decalcified by Kristensen's (1948) method before being embedded. Serial sections of this block were cut at 200μ in the transverse plane (see figure 4, plate 10).

Injected preparations. Two brains were prepared for sectioning in which the blood vessels had been injected with an Indian-ink plasma mass. The apparatus and technique were a modification of those described by Verney (1947). Dried human plasma—kindly supplied by the Cambridge Regional Blood Transfusion Centre—was reconstituted at 3 times normal strength. To it was added 10 to 15 % (v/v) of black waterproof drawing ink ('Indian ink'). Cannulae were inserted into the carotid arteries, and the head was washed through with warm saline followed by the warm fixative—4 % formaldehyde to which had been added calcium chloride to 1 %. At this point a stout ligature was tied round the neck—excluding the carotid arteries and jugular veins—the vertebral arteries were ligated, and the neck was severed on the cardiac side of the ligatures. The vertebral canal was plugged with Plasticine and the warm injection mass switched in. Cannulae in the jugular veins were attached through a Y-piece to an upright tube in which the mass was allowed to rise to a height of 70 cm. After the pressure had been maintained for some time the arterial cannulae were clamped, and, it being ensured that there was no leak, the whole head was immersed in the formaldehyde-acetic acid-ethanol fixative. After fixation, a block of tissue, with meninges intact, was removed from the ventral part of the brain and embedded in celloidin. Serial sections at a thickness of 500μ were made of two such blocks, one cut in the transverse plane and the other in the sagittal plane. Photomicrographs were prepared from the transverse sections, and these give a clear picture of the arterial supply to the hypothalamus (figure 3, plate 9). The arterial supply to the thalamus was reconstructed by superposition of appropriate portions of these photomicrographs (figure 6, plate 11).

A third brain was prepared in an exactly similar way, using as the injection mass a warm gelatin solution coloured with Boston red. The whole brain was blocked in celloidin and cut serially at a thickness of 750μ.

Whilst the description which follows is based upon an examination of this material, there has been opportunity to confirm the observations on a much larger number of sectioned brains that had been prepared for other purposes.

(2) *Results*

(*a*) *The vascular supply to the hypothalamus*

The hypothalamus of the dog is clearly defined in sections; laterally it extends to the medial forebrain bundle which is sharply bounded by the internal capsule and cerebral peduncles; posteriorly it ends with the mamillary bodies; only anteriorly, where the cell groups which form the anterior and lateral hypothalamic areas merge imperceptibly into the preoptic areas and these in turn into the septal region, may difficulty be experienced in determining its limit.

The main features of the arterial supply are illustrated in the photomicrographs of figure 3, plate 9, which are taken from successive transverse sections. A brief description will be given of the origin of the arterioles supplying the various nuclei of the hypothalamus, and the supply to the supraoptic nuclei will be considered in greater detail. The paper of Rioch (1929) has provided the chief source of information on the nuclear configuration of the carnivore hypothalamus, and the nomenclature for the hypothalamic nuclei here used is that of Rioch, Wislocki, O'Leary, Hinsey & Sheehan (1940).

Preoptic region. The most anterior extensions of the hypothalamus receive their arterial supply from the anterior cerebral artery. The preventricular region, i.e. the lamina terminalis and the septal nuclei, is supplied by vessels from the anterior cerebral arteries where these unite over the optic nerves. The vessels run dorsad and caudad to supply the whole of this region. As the anterior cerebral arteries run lateral to the optic chiasma they give rise to arteries which penetrate the brain floor on either side of the optic chiasma, to supply the medial preoptic area. The lateral preoptic area may be supplied by the same group of vessels or by small arteries which arise from the primary branches of the anterior cerebral arteries in this region—that is, the branches which run to supply the frontal and olfactory lobes. Similarly, the anterior commissure receives vessels that arise from the anterior cerebral arteries, curl over the optic tracts where these originate, and run dorso-mediad to reach the commissure (figure 3*a*, plate 9). The anterior part of the stria terminalis and the antero-medial part of the internal capsule are also supplied from the anterior cerebral arteries, although some of the vessels arise in the angle between anterior and middle cerebral arteries. In other words, these are the vessels of the anterior perforated substance. In this region the veins run closely parallel with the arteries, and large numbers of small veins are to be seen passing in a ventro-lateral direction from regions near the ventricle to curl out and over the optic tracts to join the large and irregular veins of the ventral brain surface (figure 3*a*, *b*).

Optic chiasma and suprachiasmatic nucleus. The arterial supply to the optic chiasma is quite distinct. The vessels supplying it arise entirely from the intercarotid anastomosis, a vessel which is peculiarly well developed in the dog (figure 2). It is a sinuous vessel which arises at either end from the internal carotid artery where this is actually surrounded by the dura (figure 4, plate 10), and then arches up to lie just caudal to the optic chiasma and just below the median eminence. Its most caudal undulations approximate to the pars tuberalis of the hypophysis. This is shown in figure 3*b*, plate 9. Arising from the intercarotid anastomosis are two series of vessels, one of which runs forward on the optic chiasma whilst the other turns back into the pars tuberalis. In this latter function the intercarotid anastomosis behaves exactly like the rest of the important group of hypothalmic arteries that arise near it from the internal carotid or posterior communicating arteries; their role will be considered a little later. The forward-running branches are closely applied to the chiasma and give rise to a mass of small arteries that penetrate perpendicularly into its substance (figure 3*a*). There they disperse, though some do reach and supply the suprachiasmatic nucleus; and the immediately adjacent parts of the optic nerves and optic tracts receive vessels from the same source. One of the primary branches of the intercarotid anastomosis may curl round in the groove between the two optic nerves and disperse in the lamina terminalis. In the monkey, Finley (1940) has described an anastomotic vessel with similar relations to the optic and anterior tuberal region. In this animal, however, in contrast to the dog, the vessel arises from the ophthalmic arteries and not directly from the internal carotids.

Paraventricular nucleus. The region of the paraventricular nucleus can readily be seen in figure 3*d*, plate 9, where its intense vascularity picks it out amongst less densely supplied areas. Its arterial supply has not been easy to trace, though, by contrast, its venous drainage is most conspicuous. The veins run ventrally and anteriorly to emerge through

the anterior divisions of the supraoptic nucleus (figure 3*a*). It will be seen from figure 3*d* that the branches of the posterior cerebral artery come sufficiently close to the paraventricular nucleus to be a possible source of supply to it. However, it is probable that its main supply is in conformity with the rest of the hypothalamus and comes from the ventral vessels. In figure 3*e* can be seen sections of a large branch of the posterior communicating artery which describes a semicircular course. It ascends through the medial forebrain bundle, circumscribing the ventral hypothalamus and dispersing into the thalamus. In this section the main trunk is just posterior to the most posterior extent of the paraventricular nucleus and to this it sends small twigs.

Anterior hypothalamic area. Figure 3*d* shows the typical features of the vascularity of this region. It is supplied principally by small arteries that arise from the posterior communicating artery, and ascend through the posterior division of the supraoptic nucleus to terminate in the anterior hypothalamic area. Some of these small arteries are the more posterior members of an important group of arteries that supply the glandular hypophysis, the median eminence, and the posterior division of the supraoptic nucleus. The lateral boundaries of the hypothalamus are here still receiving small branches from the middle cerebral artery or its primary branches, and small vessels from the same source curl back over the optic tract to form another route of supply to the anterior hypothalamic area.

Middle, dorsal and caudal hypothalamic regions. Apart from the supraoptic nucleus the rest of the hypothalamus receives its arterial supply from the posterior communicating artery. These regions are the ventro-medial and dorso-medial nuclei, the arcuate periventricular nucleus, the dorsal hypothalamic area, the posterior hypothalamic area and the mamillary complex. Arising from the posterior communicating artery in its course lateral to the hypophysis are one or more large vessels that penetrate the brain just medial to the now divergent optic tract, and ascend in an arc closely following the lateral boundary of the hypothalamus (figure 3*e*, plate 9). These vessels supply the more lateral and the dorso-medial regions. The ventro-medial region, which includes the mamillary complex, is supplied by small arteries that arise directly from the posterior communicating. They are clearly seen in figure 3*e*. The more anterior region of the dorsal hypothalamic area may also receive blood from a small branch of the choroidal artery (arising from the middle cerebral) that pierces the floor of the diencephalon medial to the internal capsule and chiefly supplies the thalamus.

Supraoptic nucleus: anterior division. The supraoptic nucleus is the most conspicuous nuclear group of the hypothalamus. It is conspicuous in toluidin-blue sections by virtue of its dense population of large, richly staining cells; it is conspicuous in injected sections because its profuse capillary network contrasts with the neighbouring less vascular regions. The same may be said of the paraventricular nucleus but to a less degree. The supraoptic nucleus is divided by the diverging optic tracts into an anterior and a posterior division. The most anterior extensions of the supraoptic nucleus are to be found just lateral to the optic chiasma, where they are situated above the anterior cerebral arteries. However, as the optic tracts diverge, the supraoptic nucleus is forced into a more lateral position, and is, in fact, carried out dorsal to the middle cerebral artery. This can be clearly seen in figure 3*a* and *b*, plate 9. This division of the supraoptic nucleus thus receives its supply directly from these two cerebral arteries. A great many of the vessels supplying this

division arise in the angle between anterior cerebral and middle cerebral arteries, and then curl forward over the optic tract to reach the nucleus. This is well illustrated in figure 3*b*. Here, on the left side (right side of the figure), the section is so cut that the wall of the middle cerebral artery is retained in the section, and this makes visible a number of small arteries arising in the wall and behaving as above described. Despite its density, the supraoptic nucleus is still able to accommodate many arteries and veins that supply or drain more dorsal regions, and in particular the veins from the paraventricular nucleus leave by this route.

Supraoptic nucleus: posterior division. The posterior division of the supraoptic nucleus commences at the level of the median eminence just dorsal to the pars tuberalis, and, as the optic tracts diverge and make room for it, it spreads out on the floor of the tuber cinereum lateral to the infundibular recess. Its arterial supply is from a complex of vessels that are best considered together as the vessels of the glandular hypophysis, the median eminence and the posterior supraoptic nucleus. Most members of this group arise from the internal carotid artery immediately upon its emergence from the dura; the rest originate in the medial wall of the posterior communicating artery where this joins the internal carotid. The intercarotid anastomosis is the most conspicuous member of the group and its relationships have already been described. The other members of this group of arteries are equally striking in appearance. They are stout vessels which, whilst they originate close together, radiate and fan out over the tuberal area (figure 2). They branch several times before reaching the brain substance, and some of the branches may directly penetrate the brain floor—particularly in more lateral positions—to supply the supraoptic nucleus and adjacent medial nuclei. Most of the vessels, however, twist and turn before penetrating any tissue and, although they wind over the surface of the pars tuberalis and median eminence, they never insinuate themselves into this tissue substance. Rather they give rise to a large number of small vessels, which either loop up into the median eminence or connect directly with the sinusoids of the pars tuberalis. These features are seen in figure 3*b* and *c*, plate 9. These same features also characterize the more posterior vessels of the group that arise from the posterior communicating artery. Here again some branches rise directly into the floor of the hypothalamus, and in this way the most posterior and lateral extents of the supraoptic nucleus are supplied (see figure 3*d*). Other branches enter the cleft between the posterior aspect of the median eminence and the posteriorly directed infundibular process. Here they lie parallel to the tissue surface and give rise to small branches that either join the sinusoids of the pars tuberalis of this region, or loop down on to the dorsal surface of the infundibular stalk.

A little farther posteriorly there arise from the posterior communicating artery the vessels already referred to, which supply the posterior and lateral hypothalamic nuclei (figure 3*e*). It will be seen that particular interest attaches to the direction of blood flow in the posterior communicating artery, for, if the flow is from the internal carotid artery towards the posterior cerebral artery, it is possible that the whole hypothalamus will be supplied from the internal carotid arteries. Alternatively, if vertebral blood flows forward, the vertebral arteries may form the exclusive source of supply to at least the more posterior hypothalamic nuclei. The method, to be described later, of tracing the cerebral distribution of arterial blood has been applied to this question; but before we turn to these

experiments, a few remarks must be made upon the vascular supply to the thalamus and to the pituitary gland; knowledge of the supply to the infundibular process has formed, as we shall see, an essential component in the experimental exclusion of this part of the neurohypophysis from being the site of the osmoreceptors.

(*b*) *The vascular supply to the thalamus*

The thalamus is supplied from two primary sources—branches of the posterior communicating artery and branches of the posterior cerebral artery. There is usually present one large thalamic artery arising from the posterior communicating on each side and taking origin 3 to 5 mm posterior to the internal carotid artery (figure 5, plate 10; and figure 6, plate 11). The vessel crosses the medial aspect of the internal capsule and rises through the substance of the subthalamus and thalamus within a few millimetres of the midline. In the neoprene casts the artery terminates as a tuft of fine vessels occupying the territory of the anterior and middle thalamus. No branches cross the midline. In the serial sections the nuclear groups supplied by this vessel could be discerned (figure 6). Its most ventral branches supply the reticular nucleus laterally and the dorsal hypothalamic area and nucleus reuniens medially. Long parallel-lying branches run into the ventral nucleus, whilst terminal branches run into all the anterior nuclei and also turn to the midline to supply the parataenial and paraventricular thalamic nuclei. In specimens showing a very large posterior communicating thalamic vessel its domain was seen to include the more anterior intralaminar nuclei, the nucleus submedius and the dorso-medial group of nuclei.

The thalamic vessel described above was found on both sides in all the specimens examined, but it was noticed that a supplementary supply might arise from either of two sources. In one instance the choroidal branch of the middle cerebral artery gave rise to a vessel that formed an anastomosis with a small branch of the posterior communicating artery and supplied the anterior thalamus, whilst in several instances a small branch of the posterior cerebral artery was seen to pursue a recurrent course to gain the medial aspect of the cerebral peduncle and to supply structures extending from the posterior hypothalamic area to the dorso-medial thalamic nucleus. The anterior cerebral arteries were not seen to make any substantial contribution to the thalamic supply, but branches from them, running posteriorly into the septal region, made contact through minute vessels with the capillary bed of the anterior thalamus (see figure 5, plate 10). Such vessels might form an important alternative route of supply to the thalamus were the primary arterial pathways to be occluded.

The other main source of supply to the thalamus comes in a postero-medial direction from branches of the posterior cerebral artery. These branches are large and run in the groove between the lateral geniculate body and the anterior colliculus (see figure 5). Their number may be augmented by branches of the anterior cerebellar artery. The vessels penetrate the thalamus to permeate the lateral and posterior nuclei; some branches run forward superficially on the dorsal thalamic surface to supply mid-thalamic regions, and they may reach the anterior tubercle.

Finally, it should be noted that a number of small arteries (the thalamo-perforate arteries) arise directly from the posterior limbs of the circle of Willis, penetrate

the substance of the midbrain, and supply postero-ventral thalamic regions (see figure 5).

A general feature of the supply to the hypothalamus and thalamus is that arteries tend to rise through their substance in a dorso-medial direction. The more dorsal an area or nuclear group the more lateral will be the origin of the vessels that supply it. This characteristic was noted by Clark (1938) in relation to the blood supply to the hypothalamus of the monkey. Foix & Hillemand (1925), observing a similar phenomenon in human material, described the vessels supplying the diencephalon as schematically divisible into paramedian, short circumferential and long circumferential, a schema that finds a counterpart in the dog in the perforate hypothalamic vessels, the posterior communicating thalamic arteries, and the posterior cerebral branches respectively. Some of these arteries are end-arteries without alternative anastomotic sources of supply, so that when their origins are occluded, as in the experimental procedures we shall describe later, cystic lesions may appear in the thalamus.

(c) The vascular supply to the posterior lobe

The vascular architecture of the hypophysis of the dog has been described by Dandy & Goetsch (1910), Basir (1932) and Green & Harris (1947). The posterior lobe of the pituitary is supplied by a small artery which arises from the internal carotid in the cavernous sinus. It is thus an extradural vessel and is in no way connected with the vessels of the circle of Willis. It pierces the meninges where these are closely applied to the posterior pole of the posterior lobe.

From observations at dissection, and confirmation of these in serial sections, we have found the origin of this vessel in the dog to be variable. The artery may be a single vessel arising from the right or from the left carotid. Alternatively, it may be formed from the anastomosis of a similar artery from each carotid (figure 7, plate 10); this is described by Dandy & Goetsch (1910) as typical, and they do not give other variants; and Wislocki (1937) gives this as the typical mode of supply in the cat. In the dog, again, the two vessels that form this anastomosis may arise from the anastomotic arteries in the cavernous sinus on either side, instead of from the carotid arteries; and yet again, the posterior lobe artery may be a single vessel arising from one anastomotic artery.

These anatomical investigations, in giving information both on the routes—other than the direct one through the internal carotid artery—by which blood of common carotid origin may reach the cerebrum, and on the origins of the vessels supplying the hypothalamus and thalamus, have also suggested operative procedures by which blood of common carotid origin—i.e. in the infusion experiments, blood of raised osmotic activity—might be on the one hand excluded from the prosencephalon, and on the other excluded from or restricted to that part of the diencephalon in which we suspect the osmoreceptors to lie. In any experimental interference, however, with the inflows or outflows of the circle of Willis, it is impossible to foretell, on anatomical and haemodynamic considerations alone, what will be the quantitative effect of such interference on each of the remaining inflows and outflows and *a fortiori* on the extracranial source of supply to the capillary bed of a particular region of the cerebrum. The supraoptic and paraventricular nuclei, for example, are very near the midline of the hypothalamus, the nuclear groups of one side

being separated from those of the other by only one or two millimetres, and it may be important to know whether, under the conditions of our physiological observations, the blood of one internal carotid artery is supplying only the nuclei of the same side or is reaching those of the other side as well. Clearly a method is needed by which the field of distribution of the blood, and the cellular elements in this field, can be examined and identified histologically, and with the assurance, moreover, that the blood-tenancy of the field is the same as it was when the test of a physiological response was being applied.

By the means which we shall describe, it has been possible to map in the brain the normal fields of distribution of blood from the common carotid trunks, and so to determine which structures have received blood of raised osmotic activity in the injection and infusion experiments. In addition, after operative interference with the blood supply to the brain, those structures that still receive blood of carotid origin and, conversely, those from which it has been excluded, can be precisely demarcated. To a description of the method that we have used and of the preliminary information thereby acquired, we now propose to turn.

C. Tracing the cerebral distribution of arterial blood

For the reasons that have just been given, a substance was sought which might be added to the circulating blood and later identified in sections. The properties ideally required for such a substance are that it is innocuous and so inert as to cause no change in the calibre of the blood vessels. If particulate, it should offer no obstruction to capillary flow; it should be fully miscible with blood, and the particles should not aggregate when added to it. The substance must be readily demonstrable in any tissue, and must endure histological procedures. Moreover, it would be of great advantage to have more than one substance so that the blood distribution of several arteries could be simultaneously determined.

Now the time required in the living dog for an element of blood to pass from about the middle of the common carotid trunk to the retina is some 3 s (Verney 1947). If, then, a substance capable of demarcating the capillary bed into which it is to be carried be infused into the common carotid, and the animal suddenly killed at the sixth second after the substance first enters the carotid blood, there will be no risk of the substance's reaching the general circulation in the period of survival (some 3 s) after the substance first enters the cerebral capillaries. Moreover, if two such substances are infused, the one into the one carotid, the other into the other, and the clearance of the one from the cerebral capillaries is less immediate than that of the other, there will be little risk in so short a period as 3 s of any appreciable movement of the fringes of capillary beds supplied from different sources, or that the capillary beds so demarcated do not accurately represent those severally supplied by the two carotid bloods during life.

(1) *Methods*

(*a*) *Selection of suitable 'tracer' substances*

Of twenty-six substances investigated, only three approached the ideal requirements sufficiently nearly to be acceptable for use. The substances examined are listed in table 1, together with notes upon a number of their properties, and indications as to why some of the substances are unsuitable. From these preliminary investigations it emerged that it would be best to direct attention to fine particulate dispersions.

The two most readily available particulate dispersions, Indian ink and a suspension of carmine, could not be used because they aggregate grossly in blood. Drinker & Churchill (1927) used a graphite suspension made by de Haën and known as Hydrokollag 300.

TABLE 1. SOME PROPERTIES OF SUBSTANCES TESTED WITH A VIEW TO THEIR POSSIBLE USE AS TRACERS OF BLOOD DISTRIBUTION

substance	particulate appearance	appearance in blood	effect of histological reagents	final appearance in tissue
azo-carmine	suspension of fine rod-like particles	unchanged	extracted by 'Susa'	diffuse, particles no longer discernible
Congo red	soluble, no particles	unchanged	dye is extracted	sections not made
trypan blue	soluble, no particles	unchanged	dye is extracted	sections not made
Evans's blue	soluble, no particles	unchanged	dye is extracted	readily discernible but diffuse
chlorazol sky blue	soluble, some fine particles	unchanged	some extraction	stains the vessel walls
chlorazol fast pink	soluble, some fine particles	unchanged	some extraction	stains the vessel walls
lithium carmine	soluble with heat	unchanged	some extraction	sections not made
methylene blue	soluble	unchanged	little extracted	diffuse, and fades
neutral red	soluble, some deposit	unchanged	extracted by formaldehyde, and diffuses into celloidin	diffuse
toluidin blue	soluble, forms a precipitate with chlorazol sky blue, the particles being much larger than erythrocytes	unchanged	not tried	not tried
solway blue	suspension, with particles larger than erythrocytes	unchanged	not tried	not tried
Prussian blue reagents	soluble	unchanged	no detectable extraction	diffuse
hydrocollargol	not homogeneous, some aggregates	unchanged	no effect	clearly defined distribution
Indian ink	homogeneous, particles in Brownian movement	aggregates		clearly defined distribution when used in plasma
carmine	not homogeneous, fine suspension	aggregates	not tried	not tried
stained erythrocytes	resuspension difficult	detectable	not tried	not tried
colloidal graphite ('Dag') (1)	homogeneous particles *ca.* 2 μ, aggregation on keeping	unchanged	no effect	clearly defined distribution
colloidal graphite ('Dag') (2)	homogeneous particles 1 to 2 μ, no aggregation on keeping	unchanged	no effect	clearly defined distribution
colloidal calcite ('Dag')	homogeneous particles, *ca.* 1–2 μ	unchanged	no effect	not visible
Boston red	homogeneous particles 1 to 2 μ, settles on keeping	unchanged	extracted by chloroform	avoiding chloroform, clearly defined distribution though somewhat faint
soluble starch	soluble	unchanged	no effect	with iodine visible in larger vessels only
carbon black (I.C.I.)	fairly homogeneous, particles in Brownian movement	some aggregates: smaller than erythrocytes	no effect	clearly defined distribution
Monastral fast blue (I.C.I.)	fairly homogeneous, particles in Brownian movement	some loose aggregates	no effect	clearly defined distribution
corvic latex (I.C.I.)	barely visible	blood clumps immediately	not tried	not tried

Later, Field & Drinker (1936) described experiments in which colloidal graphite and a suspension of calcite were used. Messrs Acheson Colloids ('Dag') Ltd kindly supplied us with two such suspensions, but in use both these initial preparations proved disappointing. The graphite was a potent depressor agent when tested upon the cat's blood

pressure, whilst the calcite, although excellent in all other respects, proved to be in such fine division that it was impossible satisfactorily to follow its distribution with a polarizing microscope or to stain it with histochemical reagents. Finally, amongst some substances kindly supplied by Messrs Imperial Chemical (Pharmaceuticals) Ltd, were two that approached the ideal requirements sufficiently closely to make them reliable indicators of blood distribution during life. They are a suspension of carbon black (VS) and a suspension of Monastral fast blue (BNVS). They are both fine suspensions—the makers give $<0{\cdot}3$ to $1{\cdot}0\mu$ as the particle size of the former, $<0{\cdot}3$ to $0{\cdot}3\mu$ as that of the latter, and 7·8 and 6·2 as the corresponding pH—in which all the particles are to be seen in Brownian movement. Suitable specimens mix freely with blood, and aggregation of the particles either does not occur or is insignificant; when tested upon the cat's blood pressure 1·0 ml. of the undiluted blue suspension given intravenously caused no change, and the same volume of the black suspension had only a slight depressor effect. We have found the degree of aggregation in blood to vary with different specimens of the suspensions, and the degree may increase with keeping; the specimens should therefore be tested for the absence of gross aggregation before they are used. The formation of small loose aggregates does not impugn the reliability of the suspensions as tracers of blood distribution under the conditions in which we have used them, as the following experiments will show. A specimen of Monastral fast blue (M.F.B.) and one of carbon black (C.B.) contained respectively 23·7 and 10·7 % total solids. The M.F.B. was diluted with an equal volume of sterile 0·85 % NaCl. On examination of a drop of each suspension, all the particles were in Brownian movement. Into a 1 ml. syringe containing 0·1 ml. M.F.B. was withdrawn, from an animal with a carotid loop, 0·9 ml. of arterial blood. The contents of the syringe were immediately ejected into a glass pot, and a drop was transferred to a slide and examined forthwith under a cover-slip. Thirty-two seconds elapsed between the appearance of blood in the syringe and its examination under the microscope. There was no rouleaux-formation by the red cells, but small loose aggregates of the M.F.B. particles had occurred which were easily broken up by pressing lightly on the cover-slip. Many of the particles were unaggregated. In a similar experiment with the C.B. suspension the appearances were much the same. The suspensions were then tested for their ability to pass through a capillary bed in the living animal by the method used for measuring the time of passage of blood from the common carotid trunk to the retina (Verney 1947). The left pupil of a bitch which had been provided with a left carotid loop was fully dilated by means of a lamella of homatropine (*B.P.* 1948) and one of cocaine (*B.P.* 1948); and while the retinal vessels were being watched with an ophthalmoscope, 0·4 ml. of the M.F.B. suspension was injected into the carotid artery in 2 s. The blue suspension swept through the retinal vessels and cleared immediately. The injection was twice repeated with the same results. Similar observations were then made with the C.B. suspension; the suspension cleared from the retinal vessels, but not so rapidly as did the M.F.B. suspension. Further injections of the C.B. suspension were made, and when a total of 2·6 ml. had been given little clumps of black suspension could be seen oscillating with the pulse in the retinal arterioles. This quantity is far in excess of that used for demarcating a cerebral vascular bed; in such experiments, as we shall see, the suspension was allowed to enter the cerebral vessels over a period of 3 s only, and there can be, we feel, no serious risk that its distribution does not correspond with that of the

arterial stream into which it is infused. Moreover, we would emphasize that none of the animals, in the short period of survival during which the suspensions were entering the brain, showed any abnormality in behaviour. Nor when the suspensions were infused, one into each carotid, for a period of 7 sec was there any change in arterial pressure (figure 8). This last experiment was made on an anaesthetized animal; and the preparation of the suspensions, and the conditions under which each was given, were the same as when the suspensions were being infused for the purpose of demarcating the carotid vascular beds in the living animal (figure 9, plate 10). The two substances are unaffected by any of the solutions used by us in histology; the tissue blocks can be decalcified with impunity, and left for long periods in celloidin without the appearance of the suspensions being affected. Both are readily detected in tissue sections, and are readily differentiated from one another.

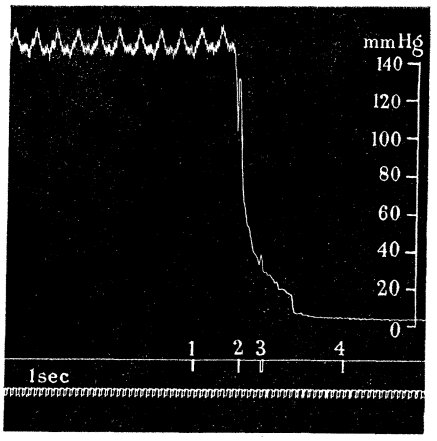


FIGURE 8. Bitch no. 436. Wt. 12·5 kg. Pentobarbitone (0·67 ml. 5 % solution in 10 % ethanol i.v.) anaesthesia. Record of femoral arterial pressure. The common carotid trunks were exposed, and the tracing gives the arterial pressure during the intracarotid infusion of the coloured suspensions, each at a rate of 0·2 ml./s. The carbon black was infused into the right carotid, the Monastral fast blue into the left. The first signal marks the time when the dispersions appeared at the carotid needles, the second when chloroform was injected into the heart, the third when the heart was incised, and the fourth when the infusion was stopped. Time marker: 1 s. There is no change in arterial pressure during the 6 s when the suspensions were entering the carotid streams. The fall of pressure at the intracardiac injection of chloroform is precipitous.

Quite recently, and through the courtesy of Messrs Acheson Colloids Ltd, Product Development Department, we have been supplied with a colloidal graphite (E.B. 1015B 'Dag') suspension containing about 7 % of solids in water. This, too (table 1, colloidal graphite ('Dag') 2), has proved an excellent indicator of blood distribution, and we have used it in conjunction with Monastral fast blue for the final demarcation of the carotid vascular beds of two ('Root', p. 264 and 'Juno', p. 299) in our series of animals subjected to surgical restriction of the cerebral carotid supply. The majority of particles are 2μ and less in size, with an occasional larger particle. When mixed with freshly drawn whole blood, or with blood diluted with heparinized saline, the graphite suspension stays perfectly in dispersion and no aggregates appear. Moreover, the substance appears quite inert in the vascular stream. The suspension was infused for 8 s into a carotid artery of the anaesthetized dog (one animal under chloralose; a second under pentobarbitone), whilst

respiratory movements and blood pressure were recorded; the infusions caused no disturbance in these records. From the trials and histological examinations so far made this colloidal graphite would appear to be the substance that most nearly fulfils the requirements of an ideal demarcating agent that we have earlier enumerated.

(b) Infusion technique

The aim of the technique adopted was to add the suspensions at a constant rate to the blood passing along the carotid arteries in the living animal, and to cause as little disturbance as possible to the normal flow. The animal was to be killed before the suspension had had time to recirculate into the proximal ends of the arteries, and the head immediately removed and placed in fixative. It was found that the suspensions were easily detected when present in a concentration of 1 % solids in the blood. Now the blood flow through the carotid artery of a dog of medium size is of the order of 2 ml./s; thus, if each suspension is brought to a concentration of 10 % total solids and infused at a rate of 0·2 ml./s, a final concentration of approximately 1 % solids in the blood will result.

The infusion apparatus and technique were similar to those described elsewhere (Verney 1947), and suitably modified for infusion of the two suspensions. To this end two 10 ml. all-glass syringes of equal bore were inserted side by side in a holder which gripped their barrels (see figure 9, plate 10). A brass plate was arranged across the ends of the two plungers so that when pressure was applied to the plate both plungers were propelled down the barrels at the same speed. To prevent any rotation of the plate, it carried at each end, and at 90°, a cylindrical rod which slid through a horizontal channel in a stationary block of brass. The apparatus was adjusted to deliver 0·2 ml. of each suspension per second. A three-way tap was closely attached to the nozzle of each syringe by means of transparent plastic tubing. The direct arm of the tap led to a length of catheter or plastic tubing, into the other end of which was tied the infusion needle—external diameter 0·6 mm—and a 20 ml. syringe was attached to the side arm of the tap by means of inexpansible plastic tubing. These syringes were to contain 0·85 % NaCl; they were worked by hand, and their purpose was to keep the needles clear of blood once these had been inserted. A small piece of glass tubing was interpolated in the catheter tube, near the needle, to act as a window, first to indicate when the artery had been successfully punctured, and secondly to allow observation of the exact moment at which the coloured suspensions reached the needles. The volumes of the catheter tubing were adjusted so as to be the same on each side. The suspensions were kept in the refrigerator, and asepsis precautions were taken when withdrawing quantities for use.

The procedure was as follows. The fluid-containing parts of the apparatus and the solutions of sodium chloride were sterilized by boiling, and the syringes filled with the appropriate suspensions and saline. The animal, before and after it had been brought to the special room (Verney 1947), received the same treatment and lay on the table in the same position as in experiments in which the effects of infusing hypertonic solutions into the common carotids had previously been measured. After the skin in the area of the apex beat, and the tissue in the underlying interspace, had been anaesthetized with procaine, the apparatus was placed on a platform at a convenient height above the animal. The catheter tubing and infusion needles were then filled with saline from the 20 ml. syringes,

and each needle was gently pushed through the skin of the carotid loop and into the blood stream. As soon as one needle had been inserted its bore was kept free by the slow injection of saline. When both needles were satisfactorily in position a metronome, beating seconds, was started, the two three-way taps were simultaneously turned to the syringes containing the suspensions, and the infusion motor was instantly switched on. A few seconds elapsed while the saline was being cleared from the catheter tubing by the advancing suspensions, and these then suddenly appeared at the windows by the needles. This moment was taken as zero time, and the suspensions were allowed to infuse into the circulating blood until, at the sixth second, the heart was arrested by injecting chloroform into it through the anaesthetized chest wall. Thereupon the infusion motor was stopped, the thorax and heart were cut open with an amputation knife, and the head was removed and covered with fixative, for which purpose formaldehyde-acetic acid-ethanol (p. 205) has proved most suitable.

(*c*) *Histological*

The dissection of the head was carried inward so as always to be one step behind the slowly penetrating fixative. Eventually the brain was removed with the meninges intact on its ventral surface and, to guard against a remainder of small spicules of bone, the whole was decalcified with formic acid by the method described by Kristensen (1948), and blocked up in celloidin. Several embedding techniques were tried, and finally a modification of the one described by Chesterman & Leach (1949) was adopted; this included infiltrating with low viscosity nitrocellulose but embedding in celloidin. Blocks of any size were first dehydrated in the usual way and then infiltrated with 5, 10 and 20% low viscosity nitrocellulose in succession; the block spent a few days in each strength, and was finally embedded in 14% celloidin. Serial sections of the whole brain were cut in the transverse plane, and one 200μ section from every 2 mm of tissue was cleared and mounted. In the hypothalamic region, however, every section was mounted, and one section from each 1 mm of tissue was stained with toluidin blue for nuclear groups. The sections were taken up to 95% alcohol, passed into absolute alcohol and chloroform, cleared in benzene, and were mounted in benzene balsam. The dura was retained in all blocks in order to avoid any disturbance to the vessel pattern in the sub-arachnoid space.

The black and blue suspensions can always be distinguished in the capillary bed of the tissue, but when they are mixed it is not possible to gather more than a rough idea of their relative concentrations. Here is revealed one of the weaknesses of the tracer method, in that, while the coloured suspensions indicate the field of distribution of the arterial blood, only a crude index is given of the relative amounts that have flowed to different regions. The blue suspension gives in our experience a more complete injection of the cerebral capillary bed than does the carbon black (see figures 13, 14 and 15*A*, plates 10 and 12), and this conforms with the observations reported earlier on the rates of their clearances from the retinal vessels. It is sometimes to be observed that one colour or the other appears at some small and apparently anomalous site in the tissue. When this occurs it is usually to be noticed that the colour in question is present nearby in one of the larger arteries or veins, and one may reasonably doubt whether the suspension has circulated there in the living animal. It seems probable that the suspension has been carried there by admixture

after death, or during the agonal changes in venous and cerebrospinal-fluid pressures when the heart is arrested with chloroform. In recording the distributions of the suspensions, therefore—and this has been done by making projection drawings at a magnification of some 5 to 10×, supplemented by observation of the sections under a stereoscopic microscope—the presence or absence of the suspensions in the capillaries and minute vessels alone has been plotted. We shall first give in general terms the cerebral distribution of carotid and of vertebral blood as disclosed by the method we have described, and then proceed to a more detailed analysis of the distribution of these bloods in the hypothalamus and thalamus and in the pituitary gland. Reference to figure 5, plate 10, will show the topography of the origin of the major cerebral vessels from the circle of Willis.

(2) *Results*

(*a*) *Cerebral distribution of common carotid blood*

This has been determined in nine animals. Three of these were anaesthetized with chloralose (0·1 g/kg body wt. intravenously) and three with pentobarbitone (33 mg/kg body wt. intravenously). The remainder had been provided with carotid loops; and in one of these animals the skin of the loops was anaesthetized with procaine HCl, and in the other two no local anaesthetic was given. With these last two animals the conditions and circumstances under which the suspensions were infused were the same as those under which hypertonic solutions had earlier been given. The pattern of distribution is illustrated in figure 10, which gives the appearances in the brain of an animal in which blue suspension was infused into the right, and black into the left carotid while the animal was under chloralose anaesthesia. The carotid blood is found to be distributed throughout the fields of the anterior cerebral and middle cerebral arteries, that is to say, it is supplied to the greater part of the two hemispheres, including the striate body, to the extreme anterior thalamus and to the anterior hypothalamus. In most instances there was a more or less marked asymmetry of distribution of the carotid blood of the two sides between anterior cerebral structures. One carotid usually dominated and supplied not only the structures of the ipsilateral side but the medial region of the contralateral side as well (figure 10). The line of demarcation between the two distributions always remained sharp, however, and the coloured suspensions did not overlap in the tissue by more than one or two millimetres. In general our observations confirm those of Krammer (1912), who injected methylene blue into the carotid artery of an anaesthetized dog and followed the distribution by macroscopic examination; but there is one feature of the carotid distribution which was not mentioned by him, and which is of considerable importance in connexion with our subsequent experiments. In four of our animals the brain stem posterior to the hypothalamus was entirely devoid of the coloured suspensions, these regions being supplied with blood from the vertebral arteries, but in three other animals these same regions carried a noticeable quantity of the suspensions, and in the remaining two they were quite heavily laden with them. It is evident that the blood flowing in the basilar artery was receiving a contribution from the common carotid arteries in these latter animals, and it seemed most likely that the blood was reaching the basilar through the occipito-vertebral anastomosis. This anastomosis is, as we have seen, well developed in the dog and is an important route additional to that of the internal carotid artery by which blood of common

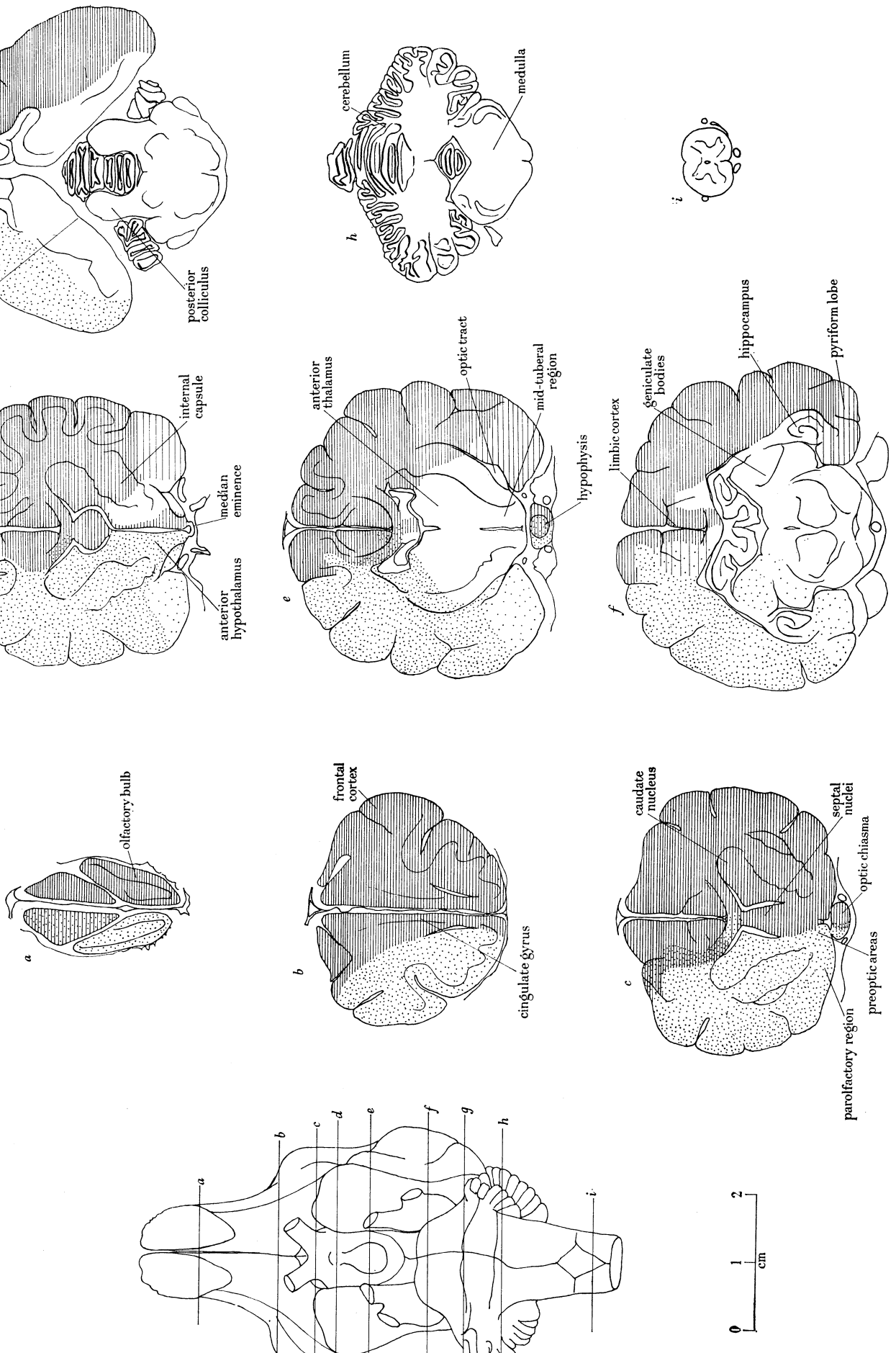


FIGURE 10. The distribution of suspensions in the brain of dog 378 mapped in selected sections as indicated in the key diagram. Blue suspension infused into right carotid—dots; black suspension infused into left carotid—lines. All sections seen from the anterior surface.

carotid origin may reach the brain (Jewell 1952). This interpretation was supported by later experiments in which the occipito-vertebral anastomosis had been tried; under these conditions no blood of common carotid origin reached, as a rule, the vertebral fields (see p. 244).

In the dorsal regions of the hemisphere an asymmetry, seen in the carotid supply at anterior levels, persists nearly to the occipital poles, as is shown in figure 10, where black suspension occupies the medial and lateral gyri of the right side throughout almost their whole extent. These gyri, however, are supplied by the anterior cerebral arteries, so that just such a distribution would appear whenever one carotid flow dominated in supplying these arteries. In general, the third ventricle seemingly forms (figure 10*d*) a barrier to the two carotid distributions, so that the supply to the hypothalamus is usually neatly divided between the two suspensions. This division extends dorsally through the massa intermedia, so that when the thalamus is receiving carotid blood each side contains only suspension from the ipsilateral carotid. The suspensions may be entirely absent from the thalamus, however, or, when present (as on the right side in the instance illustrated, figure 10*d*), the suspension is much less concentrated, and rarified patches appear in its distribution. Similarly, at about the level of the anterior thalamus, the suspensions are much less concentrated in many of the ventral regions, including the piriform lobes, the optic tract, the lateral hypothalamus and the medial fibres of the internal capsule. Evidently these regions have received blood from another source as well as the marked blood from the carotids. Apart from its most anterior nuclei, the thalamus is devoid of any suspension, and, as more posterior sections are examined, the area that has received no carotid supply expands to include the whole brain stem.

(*b*) *Cerebral distribution of vertebral blood*

The cerebral distribution of vertebral blood has been determined in five animals, three of them being under chloralose anaesthesia and two under pentobarbitone anaesthesia. In one of the latter animals and in one of those under chloralose anaesthesia, the vertebral and common carotid arteries of the same side were simultaneously injected; whilst in the other animal under pentobarbitone anaesthesia the two vertebrals were simultaneously injected with monastral fast blue, one common carotid artery with colloidal graphite and the other carotid with Boston red (but see notes in table 1 regarding this latter substance). As had been expected, a vertebral field of supply complementary to that of the carotid arteries was found; but two less-predictable features call for particular comment. One is that although the vertebral 'tracer' suspensions were largely concentrated in regions posterior to the hypothalamus, nevertheless, small amounts were found to be dispersed, in varying concentration and of rather patchy distribution, throughout the rest of the brain. It was evident that some vertebral blood was passing forward along the posterior communicating artery to be mixed with internal carotid blood and distributed with it in the anterior and middle cerebral arteries. The other noticeable feature of the vertebral supply was the ipsilateral distribution of each vertebral artery. In one animal in which only one vertebral artery was injected, the suspension was restricted almost exclusively to one side of the brain. A slight trace of the suspension appeared in the thalamus and geniculate bodies of the contralateral side, and had presumably been diverted there at the

posterior limbs of the circle of Willis. Stream-lining is, then, a conspicuous feature of the vertebral flow in the basilar artery of the dog, and, despite the fact that the blood from the two vertebral arteries runs for a considerable distance in a common vessel, little admixture may occur between the two flows. The phenomenon has recently been recorded in the rabbit by McDonald & Potter (1948, 1949, 1951); but neither its occurrence, nor the fact that vertebral blood may, under seemingly normal conditions, be admixed with carotid blood and distributed through the brain, was mentioned by Krammer (1912). We wish, however, not to associate any rigid concept of normality with this forward extension of vertebral blood into the anatomical carotid field; as has been already stressed, the large arterial anastomosis comprised in the circle of Willis gives to the sources of its distributed blood a lability under which variation from animal to animal, and in the same animal under differing circumstances, may well be expected.

The opportunity was taken to examine the nature of the cerebral vascular tree in the neoprene casts earlier described (p. 205). Here the cerebral fields supplied by the middle cerebral, anterior cerebral and posterior cerebral arteries occur as adjacent tufts deriving from the parent vessels. Their territories correspond with those as found in the infusion experiments, although the isolation of the territories is, as might be expected, not complete. Quite large vessels lying on the orbital surface of the frontal lobe connect anterior and middle cerebral arteries, whilst connexions between anterior and posterior cerebral arteries in the limbic cortex may also be of considerable calibre.

From these general observations on the cerebral distribution of carotid and vertebral bloods we pass to a detailed description of their distribution in the thalamus, hypothalamus and pituitary body.

D. The distribution of carotid and vertebral blood in the thalamus, hypothalamus and hypophysis

In all the animals in which the cerebral distribution of the carotid and vertebral blood has been traced by the infusion of coloured suspensions, the thalamic and hypothalamic regions have been carefully scrutinized in order to map out the detailed distribution of the suspensions within them.

(1) *The distribution in the thalamus*

Most commonly the thalamus was found to derive its blood supply exclusively from the vertebral arteries. In most animals in which the carotid distributions had been demarcated the thalamic nuclei were completely devoid of suspension, whilst in animals that had received vertebral infusions the thalamus was well and heavily injected from its most posterior to most anterior pole (see dog 376, figure 12). In these cases a clear line of demarcation between the vertebral thalamic field and the carotid middle cerebral field was seen following the line of the reticular nucleus. However, on the right side of one animal (dog 378, figure 11), that had received carotid infusions, the anterior thalamic nuclei and the reticular nucleus were seen to be carrying suspension, and this injected zone in the periphery of the anterior thalamus was in continuity with the fully injected caudate nucleus, stria terminalis and internal capsule. Evidently blood had reached the thalamus from the middle cerebral artery.

The above conditions would only be expected to obtain where carotid blood was *not* flowing posteriorly in the posterior communicating artery. Were the flow in a posterior direction, 'marked' carotid blood would enter first the vessels supplying the anteroventral thalamus and then the posterior communicating thalamic branch to reach middle and lateral thalamic nuclei. Thus varying proportions of the thalamic nuclei could become

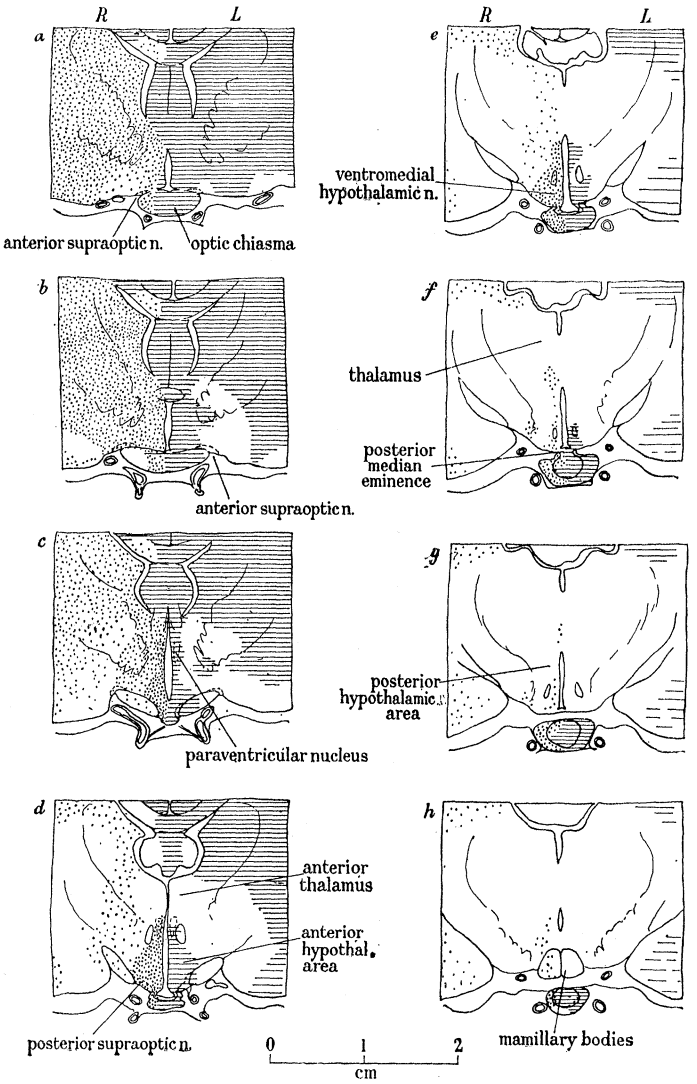


FIGURE 11. The distribution of suspensions in the hypothalamic region of dog 378. Blue suspension infused into the right carotid—dots; black suspension infused into the left carotid—lines. Selected frontal sections. The distances between the anterior surfaces of sections *a* and *b*, *b* and *c*, etc. are 1, 1, 1, 1·5, 1, 1, and 2·3 mm respectively.

injected with suspension. Such a nice balance of inflow into the circle of Willis was not found in the animals under examination, but an instance was found in which a more exaggerated extension of the carotid field had occurred such that the blood of one side was flowing posteriorly as far as the origin of the anterior cerebellar artery. As a result the posterior cerebral artery was carrying this marked carotid blood (see figure 5, plate 10) and the entire thalamus of that side was injected with suspension.

(2) *The distribution in the hypothalamus and glandular hypophysis*

The blood supply to the hypothalamus has as a rule proved to be divided between the carotid and vertebral arteries in a rather striking manner. When the black and the blue suspensions have been infused into the carotid arteries the suspensions have appeared in the supraoptic, paraventricular and adjacent anterior nuclei of the hypothalamus, whilst

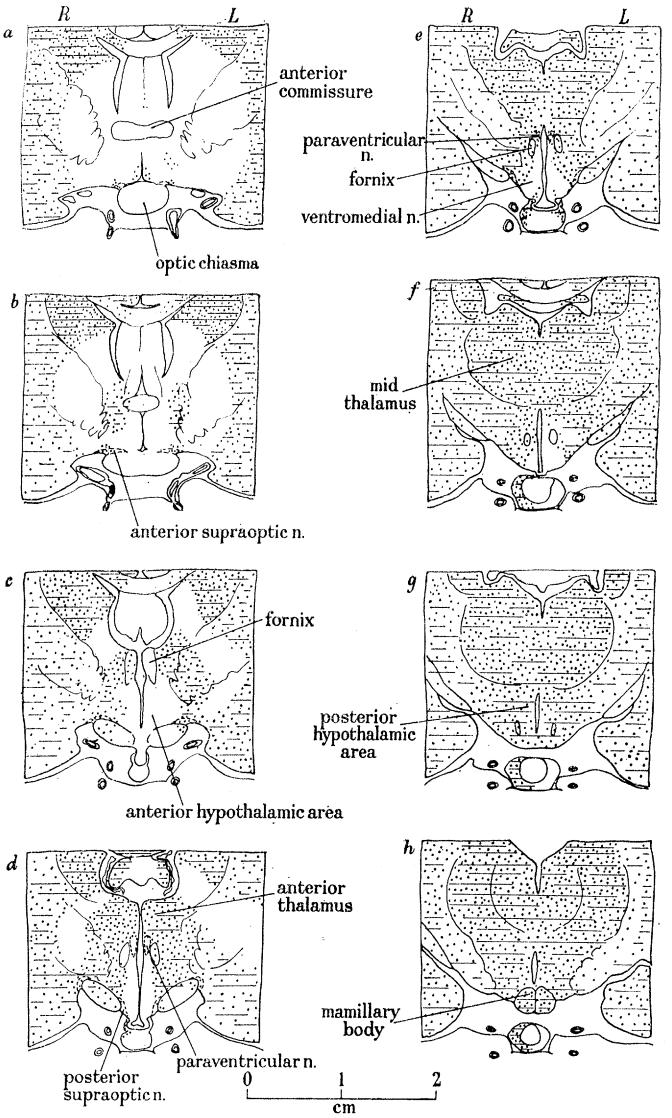


FIGURE 12. The distribution of suspensions in the hypothalamic region of dog 376. Blue suspension infused into the right vertebral—dots; black suspension infused into the left vertebral—lines. The black and blue suspensions have mixed. Selected frontal sections. The distances between the anterior surfaces of sections *a* and *b*, *b* and *c*, etc. are 0·8, 0·5, 0·7, 1, 2, 1 and 1 mm respectively.

these same regions have been as a rule practically devoid of suspension when the vertebral arteries have been so infused. Exactly the converse is true of the posterior hypothalamic nuclei.

These arterial distributions are illustrated in figures 11 and 12. Figure 11 shows the distribution of 'tracer' suspension in dog 378 in which, when under chloralose anaesthesia,

black suspension was infused into the left carotid and blue suspension into the right. The symmetry of distribution of the two suspensions in the hypothalamus and glandular hypophysis is evident from the figure. Figure 12 shows the distribution in dog 376 (under chloralose anaesthesia) in which black suspension was infused into the left vertebral artery and blue into the right. In this particular animal there has been considerable crossing of black and blue from one side to the other, so that wherever the suspensions do appear in the cerebrum they are mixed together. This admixture must have occurred where the basilar divides to form the posterior limbs of the circle of Willis, because the more posterior portions of the brain—the medulla and posterior folds of the cerebellum—were divided almost symmetrically down the midline into black-injected and blue-injected halves. Moreover, in this animal the anterior and middle cerebral fields are contaminated with vertebral blood, and this vertebral contribution has apparently streamlined into the larger branches of the anterior and middle cerebral arteries seeing that their anatomical hypothalamic fields are practically devoid of suspension.

Comparison of these two series of maps (*a* to *h* in figures 11 and 12) will show that the field of distribution of the carotid arteries in the hypothalamus of dog 378 is almost exactly coincident with the field that is *not* injected following the infusion of suspensions into the vertebral arteries of dog 376. In dog 378 the whole of the preoptic region carries suspension from the carotid infusions, but at more posterior levels patches of tissue devoid of suspensions appear in the lateral hypothalamus and ventral thalamus, so that, at the level of the median eminence, a 'cone' of tissue in the medial hypothalamus has been isolated as alone receiving carotid blood (figure 11*d*, see also figure 15*A*, plate 12). This isolated 'cone' of injected tissue is finally obliterated at the level of the posterior median eminence (figure 11*f*). In dog 376 exactly the reverse state obtains. At the level of the posterior median eminence a patch of uninjected tissue appears in the medial hypothalamus (figure 12*f*) and expands at more anterior levels until, at the level of the optic chiasma, the whole of the preoptic area is seen to be practically devoid of suspension (figure 12*a*). It is thus possible to describe precisely, in these two animals, the sources from which the various hypothalamic nuclei have received their blood.

The medial and lateral preoptic areas and the anterior hypothalamic area are supplied with carotid blood, as also is the suprachiasmatic nucleus, although the latter may receive a trace of vertebral blood (figure 12*a*). The anterior division of the supraoptic nucleus is supplied mainly with carotid blood, but it is interesting to observe that in dog 378 there are patches devoid of suspension in this division of the nucleus (figure 11*a*, *b*), whilst in dog 376 a conspicuous amount of suspension is to be seen in it (figure 12). It has thus received some vertebral blood. The median eminence, antero-medial part of the posterior division of the supraoptic nucleus, and the paraventricular nucleus are supplied almost exclusively with carotid blood, whereas the postero-lateral part of the posterior division of the supraoptic nucleus is supplied predominantly with blood of vertebral origin. The dorso-medial and ventro-medial nuclei are supplied with carotid blood only in their anterior extents, the rest of these nuclei being supplied with vertebral blood; and the supply to the lateral hypothalamic area is also divided. The posterior hypothalamic area and the whole mamillary complex are supplied with vertebral blood.

This pattern of the blood supply to the hypothalamus finds, in general, a rational

explanation in terms of the vascular anatomy previously described. In particular, the 'cone' of tissue in the ventro-medial hypothalamus which receives carotid blood seems most likely to be the region supplied by the group of vessels named the arteries of the glandular hypophysis, the median eminence, and the posterior supraoptic nucleus (p. 210). These vessels, as we have seen, arise directly from the internal carotid arteries and adjacent portion of the posterior communicating arteries, and they have been observed filled with suspension in animals that have been given black and blue intracarotid infusions. The most posterior members of this group of vessels arise directly from the posterior communicating arteries, and supply the more lateral extents of the posterior divisions of the supraoptic nucleus. It is these parts of the supraoptic nucleus which receive a considerable proportion of vertebral blood, or may be supplied entirely from this source.

While, then, the main features in the distributions of carotid and vertebral blood to the hypothalamus are explicable in terms of static vascular anatomy and the meeting of these bloods at the anterior origins of the posterior communicating arteries, there are others which show that vertebral blood may reach the anatomical hypothalamic field of the carotids. We have already drawn attention to the fact that in dog 376 (figure 12) some blood of vertebral origin had reached the anterior divisions of the supraoptic nucleus. A similar observation was made in dog 377, in which the suspensions were infused the one into a carotid the other into the vertebral of the same side. Again, in dog 369 in which black suspension had been infused into the right vertebral artery, there was more suspension in both right divisions of the supraoptic nucleus than in the corresponding position in dog 376 (figure 12), and there was also suspension in the optic chiasma, whilst in both animals suspension was to be seen in the glandular hypophysis. It is evident that some vertebral blood may be carried even into the mouths of those small vessels which we have described as arising from the internal carotid just before its trifurcation, though it is not clear how this is effected. It may be that the pulsatile movement of blood at the site of the arterial anastomosis between internal carotid and posterior communicating is sufficient —and especially so should the meeting pulse waves be out of phase—to cause some blood of vertebral origin to be transferred to the terminal segment of the internal carotid. Be this as it may, the relative concentration of the suspension in the supraoptic nuclei (with the exception of the postero-lateral parts of the posterior divisions) and in the paraventricular nuclei following its infusion into the carotid arteries is so much greater than after its infusion into the vertebral arteries, that it is justifiable to conclude that the main supply to these nuclei is from the carotids. The appearances of the suspensions in the supraoptic nuclei are illustrated in figures 13, 14 and 15, plates 10 and 12. Figure 13 gives the appearance in the anterior division of the right nucleus in a dog ('Doris', p. 270), in which black suspension had been infused into the right carotid, and figure 14 gives the appearance in the corresponding field in a dog ('Toby', p. 242), in which blue suspension had been infused into the right carotid. Figure 15*A* illustrates the appearance and distribution of the suspensions in the posterior divisions of the nuclei after the infusion of black suspension into the right carotid and blue into the right vertebral.

The test of whether or no the osmoreceptors are in the region of the anterior hypothalamus demands a clearer representation of the distribution of suspensions within it than can be given in the merely descriptive terms hitherto employed. For comparison of

the pattern of distribution under normal conditions with that subsisting when this pattern has been upset by surgical interference, a form of representation in which the distribution can be portrayed in a roughly quantitative way becomes imperative. Now the most conspicuous and self-limited histological feature of the anterior hypothalamic region is the supraoptic nucleus; its outline is well defined, usually even in unstained sections, and the distribution of suspension within it can be portrayed more accurately than that in any other part of this region. We decided therefore to use such portrayal as a provisional index of the distribution of the blood supplied to this region of the diencephalon, in the hope that we should thereby be furnished continually during the course of this investigation with evidence for or against our working hypothesis that the receptors are in the anterior hypothalamus. The method and the results obtained in two living and in two anaesthetized animals, in none of which had there been any intentional interference with the cerebral inflows, will now be given.

(3) *Partition of the supraoptic nuclei according to the origins of their blood supply*

The first animal ('Molly', no. 341) had been perineotomized and provided with two carotid loops. It was killed during the infusion of suspensions into the carotids under conditions and circumstances which were the same as those under which intracarotid infusions of hypertonic solutions of sodium chloride had earlier been given. The brain was fixed, embedded and sectioned in the manner already described. Projection drawings of the serial sections through the hypothalamus were then made at a magnification of 30 × on tracing paper, and the outlines of the supraoptic nuclei and the distribution of the suspensions within them carefully recorded. There was as a rule no difficulty in this with the anterior divisions and with the antero-medial part of the posterior divisions, as owing to the profuse blood supply to the nuclei (Finley 1940) the suspensions in the capillary beds are much denser (see figures 13, 14 and 15*A*, plates 10 and 12) than elsewhere. With the postero-lateral part of the posterior divisions (the vertebral field), the mapping of the boundaries of the nucleus was difficult, and the difficulty was only partly overcome by the facts that the vertebral blood was carrying suspension from the occipito-vertebral anastomosis and that the staining of each fourth section with toluidin blue allowed the course of the nucleus in the intervening sections to be roughly assessed. Over the extent of the posterior divisions, therefore, the distribution of the suspensions in and in the neighbourhood of the nuclei in the unstained sections was first recorded on the drawings. The sections were then dismounted, stained with toluidin blue, remounted and projected on to the original drawings, and the outline of the nuclei mapped thereon. A record was thus obtained both of the extent of the nuclei and of the distribution of the suspensions within them. This distribution was then checked by examination of each section under the microscope. The same method was also adopted with any unstained sections through the anterior divisions where difficulty was encountered in determining the borders of the nuclear material. Each nuclear area, with a control area of 10000 sq.mm was then cut from the drawings and weighed. In twenty-four such control weights the maximum percentage deviation from the mean was 9. In those instances in which the nuclear area was partitioned by the nature of the injection of its vascular bed, the paper replica was divided in conformity with this partition and each part was weighed. From these weights and the known

thickness of the sections the volume of each block of nuclear material was computed, on the assumption that the area as drawn represented the average extent of the nuclear material at all levels in the section. This will almost certainly give an overestimate of the volume, but the error is likely to be about the same with all the brains which have been dealt with in this way. The volume so computed was then represented as a rectangular block; the transverse and antero-posterior dimensions of this were the transverse width of the nuclear material and the depth of the section respectively, and the dorso-ventral extent of the block was calculated from these dimensions and the previously computed volume. The information previously obtained on the nature and distribution of the suspensions within the nuclear areas was then transferred to the drawings of the blocks. In the figures obtained in this way (see, for example, figure 16) the projection of each rectangle in a dorsal direction is at an arbitrary angle and is isometric in the sense that the length of the projection is such as to give the block the required volume.*

In the animal 'Molly' black suspension had been infused into the right, and blue into the left carotid; and the carotid blood distribution as disclosed by projection maps of the brain sections, was complicated by two factors: (1) the anterior cerebral area of the left carotid dominated over that of the right, and (2) each occipito-vertebral anastomosis was carrying suspension into the basilar blood. However, examination of the prepared projection drawings made it evident that the major part of the nucleus on each side was well supplied with blood from the ipsilateral carotid, whilst the remainder—the postero-lateral part of the posterior division—was supplied with vertebral blood contaminated with anastomotic blood. Because of this contamination it was not possible accurately to determine the proportion of the nucleus that was supplied with blood of essentially vertebral origin; the proportion was approximately 10 % of the whole nucleus and 14 % of the posterior divisions. The left paraventricular nucleus carried predominantly the blue suspension, the right carried predominantly the black. In the posterior hypothalamic area, including the mamillary nuclei, the suspensions were very sparse; since well-marked releases of antidiuretic hormone were being obtained from intracarotid injections of hypertonic solutions in this animal (see figure 19, p. 238, the osmoreceptors can hardly be in this, the posterior hypothalamic, area. The correctness of this inference will be established later.

With the second animal (no. 377) black suspension was infused into the right carotid artery and blue into the right vertebral after the animal had been anaesthetized with chloralose. Chloroform was injected into the heart at the sixth second after the suspensions had reached the infusion needles; and the brain was fixed, embedded in celloidin and sectioned in the way previously described. As with 'Molly', projection drawings of the serial sections through the nucleus were made, and the same method was used in constructing a representation of the nuclear volume and of the distribution of suspensions within it. In this animal, however, only the right nucleus was treated in this way; and since the borders of the nucleus were well defined by the intensity of the injections within its bed (see figure 15*A*, plate, 12), there was no need to dismount the unstained sections and stain them with toluidin blue. The results are given in figure 16. The demarcation

* In the finished sections the linear measurements are such that they must be increased by 6·6 % to equal those of the original tissue. The volumes as given in the figures (and elsewhere in this paper) must therefore be increased by 21 % to give the true volumes during life.

between the carotid and vertebral fields in the posterior division was quite sharp, and there was no difficulty in determining the partition of the nucleus between its two sources of blood supply. The vertebral field occupies 47 % of the posterior division and 33 % of the whole nucleus of the one side.

With the third animal ('Taffy', no. 368) a precisely similar procedure was adopted as with the first ('Molly'), but blue suspension was infused into the right carotid and black into the left. The pattern of distribution of the suspensions in the supraoptic and para-

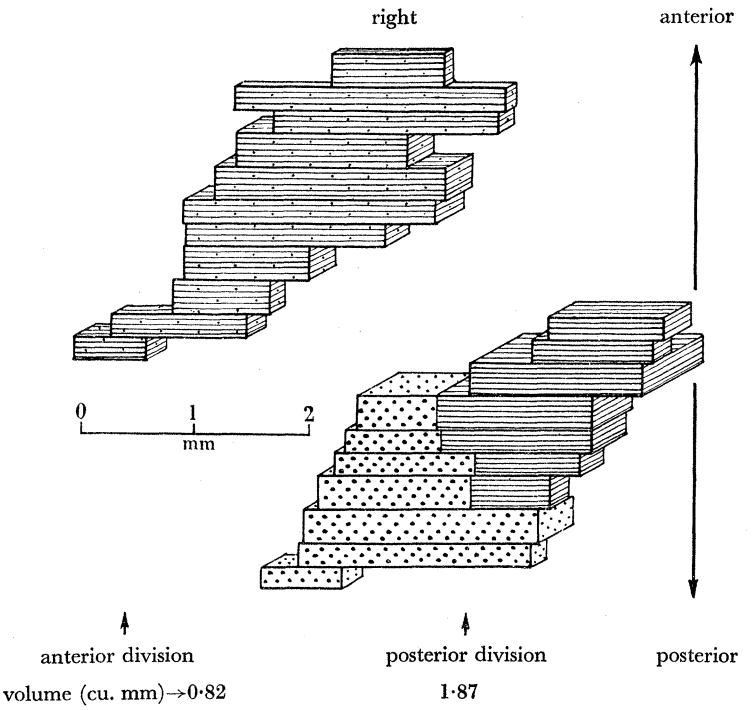


FIGURE 16. Dog 377. Plan and isometric projection of the series of blocks of nuclear material composing the supraoptic nucleus, and the distribution of the suspensions within them. Constructed from serial frontal sections of the hypothalamus. The anteroposterior width of each rectangle is the thickness of the section, the lateral width is the horizontal extent of nuclear material in the section. The volume of nuclear material in each section is computed from the product of the area of the nucleus as measured from drawings of the section, and the thickness of the section: the length of the projection in this figure is such as to give the rectangular block a volume equal to that so computed. Black suspension had been infused into the right carotid and blue into the right vertebral artery. Black suspension—thin parallel lines. Blue suspension—dots.

ventricular nuclei was almost exactly the same as in 'Molly', but with the colours reversed. The treatment of the fourth animal (no. 437) was similar to that of no. 377 (figure 16); but in this instance, as well as in 'Taffy', the contribution that the occipito-vertebral anastomosis was carrying to the circle was so large that it was not possible to express quantitatively the partition of the posterior division of the nucleus between the carotid and vertebral sources of its blood supply. From the figures with 'Molly' and animal no. 377, however, it would seem that between 10 and 30 % of the total supraoptic nuclear material is supplied with blood of predominantly or exclusively vertebral origin.

Before considering the implications of this work on the vascular supply and blood distribution to the anterior thalamus and to the hypothalamus and its nuclei in the design of experiments to localize the osmoreceptors, mention must be made of observations on the origin of blood distributed to the posterior lobe.

(4) *The distribution of carotid blood in the posterior lobe*

As we have seen, the arterial supply to the posterior lobe, i.e. the pars nervosa and pars intermedia, derives entirely from the internal carotid as it passes forward in the cavernous sinus; and the origin of this supply may be unilateral or bilateral. In conformity with this we have observed that coloured suspensions infused into the vertebral arteries do not reach the posterior lobe (see figure 12*f*, *g* and *h*). When, on the other hand, they are infused into the carotids the posterior lobe always contains solely the one or the other suspension or a mixture of the two (see figure 11*f*, *g* and *h*).

The variation in the origin of the posterior lobe artery with corresponding variation in the carotid source of the blood distributed to it is, for our purposes, a fortunate circumstance, in that it gives the opportunity of determining whether or no the osmoreceptors lie in this part of the hypophysis; and this question will be the first to engage attention in our attempts by surgical, physiological and histological procedures to define the site of these receptors. Consideration must now be given to the implications of the work we have so far described in the design of experiments to preclude carotid blood from, or to restrict it to the anterior hypothalamus.

(5) *The design of experiments to preclude carotid blood from, or to restrict it to the anterior hypothalamus*

We mentioned early in this paper the prediction, from the effects of ligation of the internal carotid near its origin on the osmotic release of antidiuretic hormone, that were this vessel tied intradurally the responses to a raised osmotic pressure in the blood of the common carotid trunk of the same side would be consistently and permanently suppressed (Verney 1947). While, on the hypothesis that the osmoreceptors are located in the anterior hypothalamic region, our present studies would hardly diminish the likelihood of this prediction proving correct, there are three points in connexion with the anatomy of the vascular supplies to the hypothalamus and the sources of their blood that might theoretically detract from the absoluteness of the expected post-operative findings. First, the origin of the intercarotid anastomosis (figure 4, plate 10) is such that the chance of this vessel being included in a ligature applied intradurally to the internal carotid is remote; if it escapes it will presumably continue to function in carrying carotid blood to the pars distalis of the hypophysis and the antero-medial part of the posterior division of the ipsilateral supraoptic nucleus. Secondly, the volume flow through the ipsilateral occipito-vertebral anastomosis would be expected to increase on intradural ligation of the internal carotid because of the increase in pressure drop along the anastomosis coincident with the fall in pressure in the circle of Willis and the increase in vertebral arterial flow. [But whether this would be associated with an increased percentage of carotid blood in the

forwardly directed stream in the posterior communicating artery, and if so whether the increased percentage would be sufficient to cause a measurable release of antidiuretic hormone from osmotic stimulation of structures in the anterior hypothalamic region when hypertonic solutions were injected into the common carotid artery, are indeterminable by theory.] Thirdly, the direction of flow in the ipsilateral internal ophthalmic artery might be reversed on intradural ligation of the internal carotid, and a small amount of external carotid blood be thereby carried into the anterior cerebral stream. The absolute exclusion of blood in one common carotid from the cerebrum, and in particular from the anterior hypothalamus could, then, be theoretically assured only by combining intradural ligation of the internal carotid and intercarotid anastomosis with ipsilateral ligation of the occipito-vertebral anastomosis and, possibly, of the internal ophthalmic artery as well. On the other hand, after simple intradural ligation of an internal carotid, and on the hypothesis that the osmoreceptors are in the anterior hypothalamus, a very small residual response to a raised osmotic pressure in the common carotid blood might or might not be encountered; at all events traces of blood of common carotid origin would be expected still to reach this part of the brain and possibly to be detected by our technique of infusing coloured suspensions. But whatever the outcome of such experiments may be, more than merely negative or, at most, suggestively positive evidence as to the site of the receptors can hardly be expected from them alone. Localizing evidence of a positive nature would seem to be attainable only by a release of antidiuretic hormone when blood with a raised osmotic pressure is experimentally restricted to some finite region of the brain; and we must now consider what surgical procedures would be needed for the definitive distribution of such blood to the anterior hypothalamic region.

This part of the brain is, as we have seen, vascularized by small vessels that arise intradurally from the internal carotid trunk and its anterior cerebral, middle cerebral and posterior communicating branches in the neighbourhood of its trifurcation. The blood carried to this region is mainly of carotid origin. To this may be added, through the posterior communicating vessel, a small amount of blood from a vertebral source; and the postero-lateral part of the posterior division of the supraoptic nucleus is regularly supplied with such blood. If, then, it were possible under surgical conditions to tie the anterior cerebral, middle cerebral and posterior communicating arteries of one side a short distance from their origins from the internal carotid, this vessel would be left to supply only the tiny hypothalamic arteries that arise in the region of the trifurcation. The cerebral distribution of carotid blood on the operated side would thus be restricted to the anterior hypothalamic region; and if the osmoreceptors were located there we should expect the release of antidiuretic hormone in response to a rise in the osmotic pressure of the common carotid blood to persist. That such an operative procedure would be calculated to cause restriction of carotid blood to the anterior hypothalamic region has been demonstrated upon a cadaver in the following way. The skull was trephined and the carotid trifurcation exposed on the left side. The anterior cerebral, middle cerebral and posterior communicating arteries were ligated a little way beyond their origins, and in addition the left occipito-vertebral anastomosis was tied. Coloured gelatins were then infused into the common carotid arteries under constant and equal pressure, Prussian-blue gelatin into the left, and carmine gelatin into the right carotid. Serial sections of the celloidin-embedded

brain were then made. The blue gelatin was restricted in its distribution to the hypothalamic region of the left side, and to a narrow strip passing ventro-laterally from between the left caudate nucleus and anterior thalamus to the ventro-lateral border of the internal capsule, the vascular bed of the rest of the brain being well injected with the red gelatin; and it was encouraging to see that the extent of the blue-injected region was very like that which we have learned to be the hypothalamic zone of distribution of the carotid artery. In figure 17 are given maps to illustrate the extent of the blue-injected region. It began to

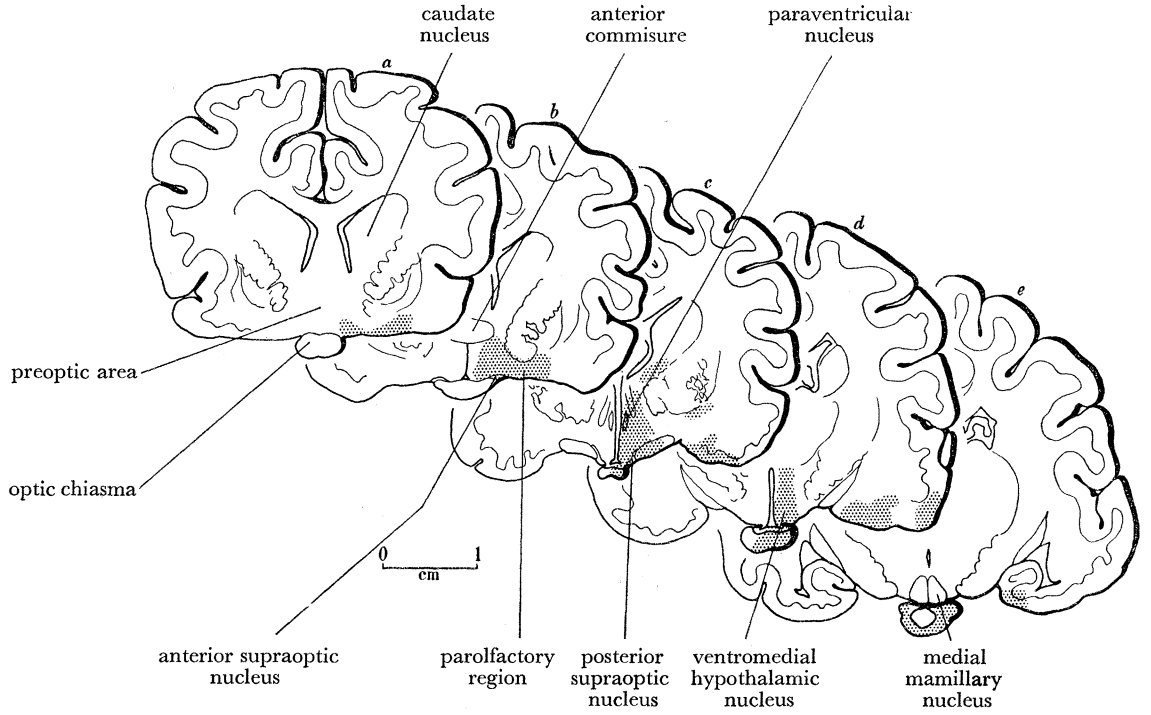


FIGURE 17. Dog D 14. Bled to death under chloralose anaesthesia. Left anterior cerebral, middle cerebral, posterior communicating and occipital arteries ligated. Gelatin masses infused into the common carotid trunks; carmine gelatin into the right, Prussian-blue gelatin into the left; distribution of Prussian-blue gelatin indicated by stipple. Projection drawings of five selected sections through the serially sectioned brain to show the restricted zone of distribution of the left internal carotid blood. *a* shows the anterior limit of the zone. This zone expands in the lateral hypothalamus (*b*) and comes to occupy the whole extent of the anterior hypothalamus (*c*); it contracts in the mid-tuberal region (*d*) and disappears just anterior to the mamillary bodies (*e*). The distances between the anterior surfaces of sections *a* and *b*, *b* and *c*, etc., are 1, 2·1, 2 and 4 mm respectively.

the left of the anterior part of the chiasma, stretching laterally and dorsally from the ventral surface of the brain (figure 17*a*). As the sections were traced posteriorly the blue-injected region expanded dorsally and medially to include finally the whole of the medial part of the left hypothalamus (figure 17*c*). The region then contracted and disappeared just anterior to the left mamillary body (figure 17*d*, *e*). The thalamus was entirely outside the blue-injected territory. The extreme anterior part of the anterior division of the left supraoptic nucleus was red-injected, a little more posteriorly the injection was mixed, and the remaining and major portion of this division was blue-injected. The anterior part of the

vascular bed of the left paraventricular nucleus was red, the intermediate part a mixture of red and blue, and the posterior part was blue. The anterior part of the posterior division of the left supraoptic nucleus was blue-injected and remained so for most of its extent as it was traced backwards; only in its most posterior and lateral part did it receive the red injection. The results suggest that valuable information of a localizing nature would be forthcoming if the needed operative procedures could be made compatible with the animal's survival. We now turn to a description of experimental work which, on the basis of these preliminary observations and theoretical considerations, has been undertaken with a view to localizing the osmoreceptors in the living animal.

E. Experiments to localize the osmoreceptors

(1) *Methods*

(a) Surgical

All the animals that were used for tests of antidiuretic response to intra-carotid infusions were prepared by perineotomy and the formation of two carotid loops. The operations were performed under ether anaesthesia by techniques as described in previous papers (Verney & Vogt 1938; Verney 1947). Other surgical procedures to which the animals were subjected during the period of observation will be described later, each in its appropriate context.

(b) The care and training of the animals, their preliminary treatment, and the circumstances of the experimental procedures

Each bitch was maintained during the whole period of observation (except when this was interrupted by a particular surgical operation) on a standard diet of minced horseflesh, biscuit and milk. It was fed each evening at 5 p.m., and drinking water was withheld after the first 'hydrating' dose of water had been given. Comparable experiments were, as a rule, done at the same time of day; and when not otherwise employed during the daytime, the animals were in the open air on the flat roof of the building.

They were carefully trained to lie quietly on their right side for long periods, and all observations were made in the special chamber described by one of us elsewhere (Verney 1947). The temperature of this room was only roughly controlled, and lay between the limits of 19·5 and 22° C during the periods in which the observations reported in this paper were made.

On the day of observation the animal was given 300 ml. of warm water (temp. 37° C) by stomach tube at 9 a.m., and allowed to run free on the roof till 11 a.m., when it was returned to its kennel; and $1\frac{1}{2}$ h later it was given a second and smaller dose (usually 250 ml.) of water. At 2 p.m. the animal was brought to the experimental room, placed in a Pavlov stand, catheterized, given the test dose of water—300 to 400 ml. (temp. 37° C), according to size of animal—and then laid on its right side on a warmed table, the urine thereafter being collected continuously into a series of graduated glass tubes. In a series of cognate observations on any one animal, the first doses, the second and the test doses were each kept severally the same. Before an intravascular injection the local area of skin was shaved and cleaned with surgical spirit.

(c) The technique of intravascular infusion

The preparation of the solutions and the technique of their intracarotid or intravenous infusion have been described in an earlier paper by one of us (Verney 1947), and we would emphasize the following points in connexion with the technique. The bevel of the needles used for intracarotid infusion was shortened and the tip carefully sharpened under the microscope, so that when it was introduced through the upper wall of the artery the lumen of the needle was free in the lumen of the vessel and no significant damage resulted from

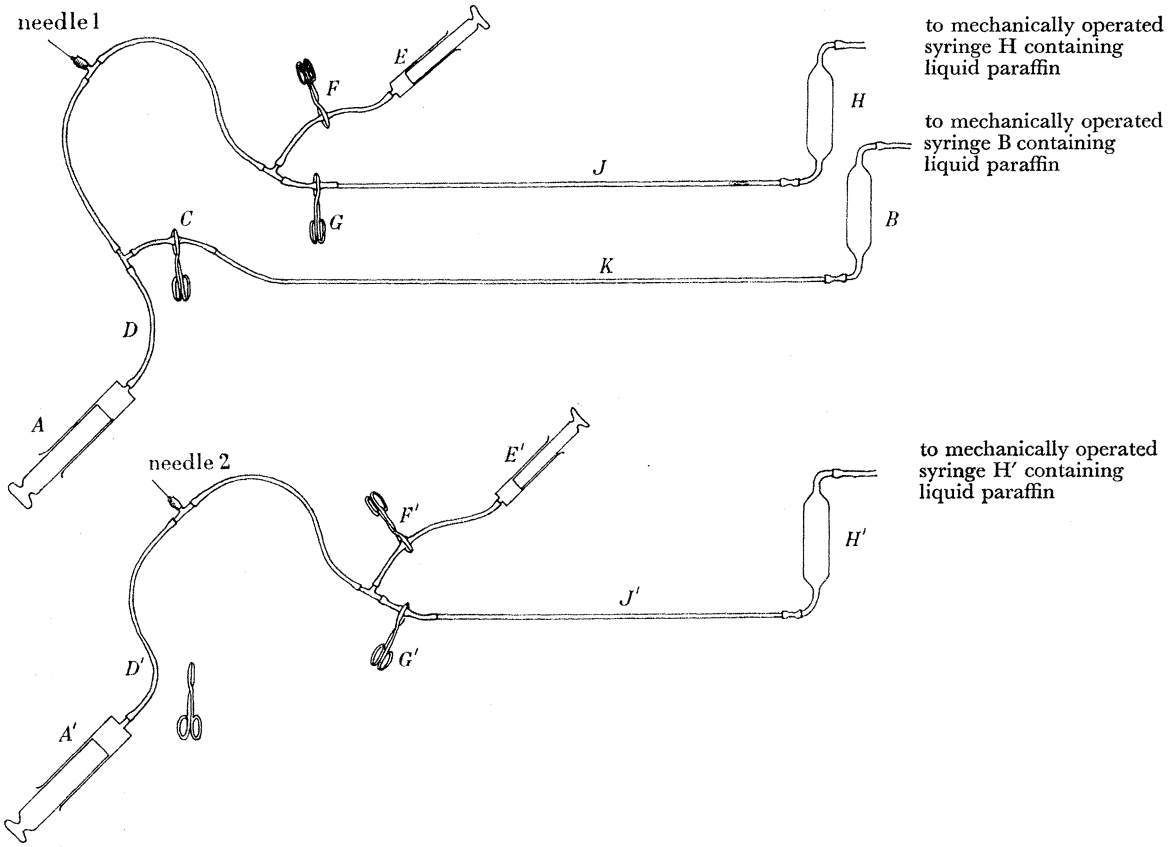


FIGURE 18. Diagram of the apparatus used for simultaneous infusion of hypertonic solutions into two vessels. Syringes *E* and *E'* are each of 5 ml. and *AA'* of 20 ml. capacity. The bulbs *H*, *H'*, the syringes *E*, *E'* and their attached tubes are filled with the chosen hypertonic solution as far as the glass T-pieces to which the needles are attached; and the bulb *B*, syringes *A*, *A'* and their connexions are similarly filled with 0·85 % NaCl. The lower part of the figure also shows the arrangement when single infusions only are to be given. For description of the procedure see text.

contact of the tip with the under-wall of the artery. Immediately the needle had been introduced the artery at the site of puncture was lightly compressed between thumb and finger for a minute or so while the slow infusion of physiological saline was being maintained. With these precautions there was, on release of the compression, no macroscopic evidence of damage to the arterial wall; and the infusion was switched from physiological to hypertonic saline at a later and appropriate time.

The infusions were given when diuresis was established, and were as a rule begun either 40 or 45 min after the test dose of water had been administered. In a few instances

simultaneous infusions either into one carotid and the malleolar vein or into the two carotids have been given. The apparatus for this technique is given diagrammatically in figure 18, and the procedure is as follows. Needle 1 is first introduced into the saphenous vein or into a carotid, and the manually controlled infusion of 0·85 % NaCl (syringe *A*) changed to the automatic infusion of the same solution (syringe *B*) by transferring the Spencer–Wells forceps *C* to the tube *D*. The plunger of the syringe *B* is moved by a constant-speed motor and the rate of infusion is 0·26 ml./min. Needle 2 is then introduced into the one or the other carotid, and at the appropriate time the manually controlled infusion of 0·85 % NaCl (syringe *A'*) is changed to the automatically controlled infusion of the hypertonic solution (syringe *H'*) by removing clamp *F'*, withdrawing the plunger of syringe *A'*, replacing the clamps *D'* and *F'*, removing clamp *G'* and immediately starting the motor which operates syringe *H'*. The infusion through the needle 1 is then changed to the hypertonic solution by stopping the motor which operates syringe *B*, transferring the clamp from *D* to *C*, removing clamp *F*, withdrawing the plunger of syringe *A*, replacing clamp *F*, transferring clamp *G* to position *D* and immediately starting the motor which operates the syringe *H*. Syringes *E* and *E'* also contain the hypertonic solution. The tubes *J*, *J'* and *K* are of Polythene, and the remaining flexible tubes are of rubber-catheter tubing. By this technique the hypertonic solutions begin to enter the two vessels within about 2½ min of each other and the two infusions are then maintained for so long as may be desired. In order to ensure constancy in output of the mechanically operated syringes each has been filled with sterile liquid paraffin and connected with the upper end of a vertical glass bulb which itself contains the solution to be infused; to the lower end of the bulb is attached the tube *J*, *K* or *J'* (figure 18).

In the experiments to be reported there was no technical hitch with either the single or the double infusion, and, unless otherwise stated, the animals gave no sign of recognition of the procedure.

(2) *The animals and their operation histories*

The following animals have been used in tests for the osmotic release of antidiuretic hormone, and to facilitate reference the operative procedures to which they were subjected are here given in brief. All, except when otherwise stated, were performed under ether or pentobarbitone-sodium anaesthesia and with full asepsis precautions.

'Whitethroat', no. 303. Wt. 15 kg. 2 December 1942: perineotomy. 8 January 1943: carotid loop, left side, the sinus being denervated. 30 April 1943: carotid loop, right side. 22 May 1946: left kidney removed. 24 October 1947: right kidney denervated, abdominal splanchnic nerves excised along with the sympathetic chains in their renal segment. 28 January 1949: left internal carotid tied intradurally. 4 August 1949: killed during intracarotid infusion of coloured suspensions.

'Brandy', no. 336. Wt. 11·3 kg. 4 December 1946: exposure of right vertebral artery, and attempt to enclose it in a tunnel of skin so as to form a vertebral artery loop. 11 December 1946: an area of gangrene had appeared on the loop, so this was excised and the vertebral artery tied above and below its looped zone. 28 March 1947: carotid loop, left side.

'Molly', no. 341. Wt. 12 kg. 13 March 1947: carotid loop, left side. 9 October 1947:

perineotomy. 27 October 1947: carotid loop, right side. 3 May 1950: killed during intracarotid infusion of coloured suspensions.

'Daphne', no. 364. Wt. 22·1 kg. 30 November 1948: perineotomy. 7 March 1949: carotid loop, left side. 23 March 1949: carotid loop, right side. 5 July 1951: under chloralose anaesthesia and without asepsis precautions, left anterior and middle cerebral arteries and left posterior communicating artery tied. Killed during intracarotid infusion of coloured suspensions.

'Doris', no. 379. Wt. 14·1 kg. 4 April 1950: perineotomy. 19 April 1950: carotid loop, left side. 7 June 1950: carotid loop, right side. 2 February 1951: left internal carotid tied intradurally. 19 April 1951: left occipital artery tied. 7 May 1951: killed during intracarotid infusion of coloured suspensions.

'Vesta', no. 380. Wt. 17·8 kg. 2 March 1951: carotid loop, left side. 19 March 1951: perineotomy. 2 April 1951: carotid loop, right side. 21 May 1951: left occipital artery divided between ligatures. 27 June 1951: left anterior and middle cerebral arteries and left posterior communicating artery tied. 30 June 1951: animal died; head tissues perfused through carotids with saline and formaldehyde-saline at room temperature, followed (at temp. 39° C) with saline and finally prussian-blue gelatin (left carotid) and carmine gelatin (right carotid).

'Linda', no. 385. Wt. 20·5 kg. 19 March 1951: perineotomy. 1 May 1951: carotid loop, left side. 25 May 1951: left occipital artery tied, and carotid loop made on right side. 19 August 1951: left anterior and middle cerebral arteries and left posterior communicating artery tied. 21 January 1952: killed during intracarotid infusion of coloured suspensions.

'Paris', no. 393. Wt. 14·6 kg. 4 January 1952: carotid loop, left side. 25 January 1952: perineotomy. 1 February 1952: carotid loop, right side. 3 April 1952: both occipital arteries tied. 30 April 1952: killed during intracarotid infusion of coloured suspensions.

'Rita', no. 394. Wt. 16·8 kg. 9 January 1952: carotid loop, left side. 25 January 1952: perineotomy. 3 March 1952: carotid loop, right side. 29 May 1952: killed during intracarotid infusion of coloured suspensions.

'Toby', no. 395. Wt. 11·8 kg. October 1951: perineotomy. November 1951: carotid loop, left side. 15 February 1952: both kidneys denervated. 24 April 1952: carotid loop, right side. 31 October 1952: right occipital artery tied. 19 November 1952: right posterior communicating artery tied. 23 March 1953: killed during intracarotid infusion of coloured suspensions.

'Regan', no. 400. Wt. 23·5 kg. 7 March 1952: carotid loop, right side. 1 April 1952: perineotomy. 21 April 1952: carotid loop, left side. 25 September 1952: left internal carotid tied intradurally. 22 March 1953: killed during intracarotid infusion of coloured suspensions.

'Clio', no. 401. Wt. 18·3 kg. 19 March 1952: carotid loop, left side. 1 April 1952: perineotomy. 21 April 1952: left occipital artery tied, and carotid loop made on right side. 9 October 1952: left middle cerebral artery tied, but during the tying of the posterior communicating artery the vessel was ruptured and the animal died.

'Jink', no. 405. Wt. 12 kg. 15 October 1952: carotid loop, left side. 29 October 1952: perineotomy. 10 November 1952: carotid loop, right side. 2 December 1952: left occipital artery divided between ligatures. 16 February 1953: left posterior communicating and

left middle cerebral arteries tied. 24 March 1954: killed during intracarotid infusion of coloured suspensions.

'Brindle', no. 409. Wt. 11·2 kg. Left hemispherectomy by Dr F. Howarth: first stage, 27 October 1952; second stage, 21 November 1952; third stage, 6 January 1953. 26 February 1953: carotid loop, left side. 12 March 1953: perineotomy. 1 July 1953: left occipital artery tied, and carotid loop made on right side. 22 March 1954: killed during intracarotid infusion of coloured suspensions.

'Girl', no. 416. Wt. 13·5 kg. 22 March 1953: carotid loop, left side. 13 April 1953: perineotomy. 4 May 1953: carotid loop, right side. 3 June 1953: left occipital artery divided between ligatures. 18 November 1953: left anterior cerebral and posterior communicating arteries tied. 23 March 1954: killed during intracarotid infusion of coloured suspensions.

'Root', no. 432. Wt. 15·0 kg. 18 May 1954: perineotomy. 5 November 1954: carotid loop, left side. 30 December 1954: carotid loop, right side. 31 March 1955: right internal carotid tied intradurally. 5 July 1955: killed during intracarotid infusion of coloured suspensions.

'Juno', no. 439. Wt. 12·7 kg. 3 November 1954: carotid loop, left side. 19 November 1954: perineotomy. 9 December 1954: carotid loop, right side. 16 March 1955: left anterior and middle cerebral arteries and left posterior communicating artery tied. 16 May 1955: left occipital artery divided between ligatures. 6 July 1955, killed during intracarotid infusion of coloured suspensions.

(3) *The exclusion of the posterior lobe from being the site of the receptors*

(*a*) *Evidence from the arterial supply to the posterior lobe*

In previous observations on a series of eight animals in all of which a carotid loop had been made on each side and the effect of intracarotid injections of hypertonic solutions determined, osmotic release of antidiuretic hormone occurred in all the animals irrespective of whether the injections were made into the right or into the left common carotid trunk. In view of the variation which we have described in the origin of the artery to the posterior lobe, it would seem extremely improbable that in all these animals the artery derived from both internal carotids. Indeed, in one of the animals ('Nicky'), which has meantime been killed, dissection showed that the arterial supply to the posterior lobe was exclusively from the left internal carotid; responses to increases of osmotic pressure of the blood in the right common carotid trunk had, however, been not only present but also greater than those to similar increases in the left common carotid trunk (Verney 1947, table 7). The correctness of the inference that the osmoreceptors are not in the posterior lobe has been established by mapping the distribution of carotid blood in an animal in which the responses to intracarotid injection of hypertonic solutions had previously been determined; and we shall now present the evidence obtained from experiments on this animal.

(*b*) *Evidence from the distribution of carotid blood to the posterior lobe*

In this animal ('Molly', no. 341), in which the responses to intracarotid injections of hypertonic solutions of sodium chloride and of sucrose had been measured, we decided to trace the distribution of carotid blood to the posterior lobe by infusing coloured suspensions

into the carotids by the method previously described (p. 217; figure 9). The animal lay in the same position and had received the same preliminary treatment as in experiments in which the responses to increases in the osmotic pressure of the carotid blood had been obtained, and the time after the test dose of water at which the suspensions were infused was the same as that at which the hypertonic solutions had been injected. The responses to such injections are illustrated in figures 19; definite osmotic releases of antidiuretic hormone followed injections into either the right (*a*, figure 19) or the left (*b*, figure 19) common carotid trunk. In the final experiment black suspension was infused into the right and blue into the left carotid; and the animal was suddenly killed at the sixth second after the infusions had been started. Examination of the sections of the fixed and celloidin-embedded brain showed that the posterior lobe contained black dispersion only (figure 20, plate 12). Since responses to increases of osmotic pressure had been obtained from injections into either carotid trunk, yet blood from the left carotid was not reaching the posterior lobe, it is clear that the posterior lobe, i.e. the pars nervosa and pars intermedia, cannot be the site of the osmoreceptors. Similar facts which confirm this conclusion have since been obtained from other animals, viz. 'Whitethroat', p. 261; 'Paris', p. 242; 'Regan', p. 264; 'Brindle', p. 252; 'Rita', p. 253; Root', p. 267; and 'Juno', p. 298.

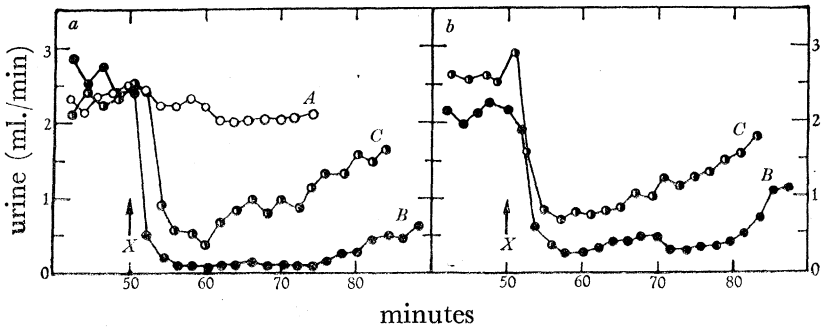


FIGURE 19. 'Molly', no. 341. Responses to intracarotid injections, at the arrows *X*, of 4·0 ml. in 10 s. *a*, right carotid: *A*, 0·144M-NaCl; *B*, 0·514M-NaCl; *C*, 0·892M-sucrose. Similar injections of 0·342 and 0·427M-NaCl gave no response. *b*, left carotid: *B*, 0·603M-NaCl; *C*, 1·05M-sucrose. A similar injection of 0·556M-NaCl gave a very small response, and of 0·514M-NaCl no response. Abscissae: time after the test dose (350 ml.) of water. This animal was killed during the infusion of coloured suspensions into the carotids: blue into the left, black into the right. The posterior lobe contained black dispersion only (see figure 20, plate 12).

(4) *The assignment of the receptors to the prosencephalon*

We have seen that in the dog the vertebral and carotid streams meet at the trifurcation of the internal carotids, the anterior and middle cerebral supplies thus becoming contaminated with blood of vertebral origin. This was so, for example, in 'Molly' and 'Taffy', the two animals in which the distribution of the carotid bloods were determined under living conditions, the forward movement of blood in the posterior communicating artery here being disclosed by the carriage of 'marked' carotid blood into the basilar artery through the occipito-vertebral anastomoses. Moreover, the postero-lateral parts of the posterior divisions of the supraoptic nuclei are vascularized from the posterior communicating arteries and regularly receive blood of vertebral origin. Although it would

seem unlikely that, in the many animals in which tests of the osmotic release of antidiuretic hormone by intracarotid injections and infusions have been made—and the results of the tests have, with two unilateral exceptions, always been positive—carotid blood was invariably being carried by the occipital arteries into the vertebral streams, and that the responses were being initiated from some region behind the anterior diencephalon and hypothalamus, yet an unequivocal answer to this question could seemingly be obtained only by testing the effect of tying the occipital arteries on the responses to a raised osmotic pressure in the common carotid blood. That such responses are indeed present after an occipital artery has been tied is shown by the observations recorded in figure 21. These results were obtained from an animal in which the left occipital was tied when the right

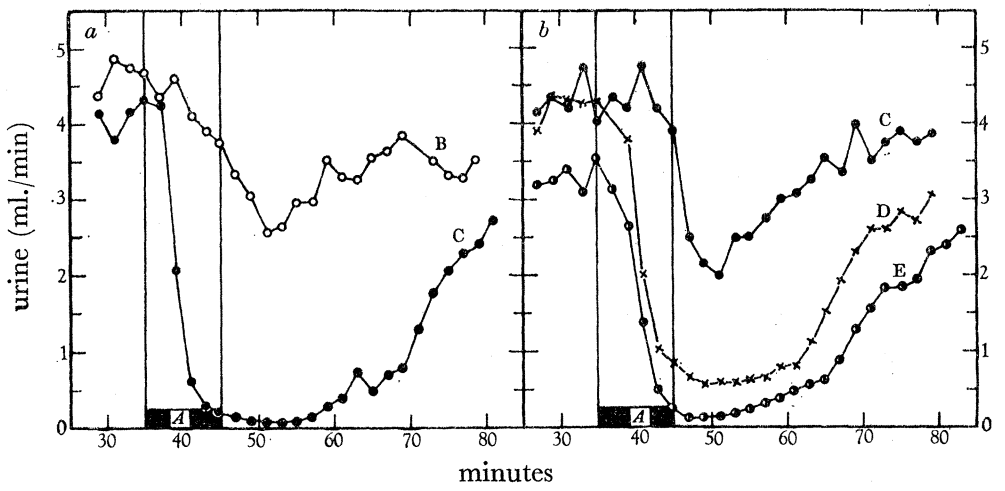


FIGURE 21. 'Vesta', no. 380. Responses to intracarotid infusions of hypertonic solutions of sodium chloride during established water-diuresis. The left occipital artery had previously been tied. The infusions were made at 1·05 ml./min during the ten-minute periods shown by the rectangles *A*. *a*, infusions into the right common carotid: 0·86M, graph B; 1·28M, graph C. *b*, infusions into the left common carotid: 1·28M, graph C; 1·50M, graph D; 1·71M, graph E. Abscissae: time after the test dose (350 ml.) of water.

carotid loop was made. They are confirmed by those obtained from another animal (see figure 52*a*, *b*, p. 306) submitted to similar surgical procedures. In the one instance (figure 21) the response to infusion into the left common carotid was smaller than that to the same infusions into the right, in the other instance (figure 52*a*, *b*) the opposite obtained. In both instances further operative procedures on the animals concerned had been planned, so it was not possible to determine what effects, if any, ligation of an occipital artery had produced on the cerebral distribution of the ipsilateral carotid blood. In a third animal, therefore, it was decided simply to test the effects of bilateral ligation of the occipital arteries on the osmotic release of antidiuretic hormone, and thereafter to map the distribution of the carotid bloods in the usual way. The results of these procedures will now be described.

(*a*) *Experiments with 'Paris', no.* 393

Responses before ligation of both occipital arteries. During the 6 weeks before operation fifteen intracarotid infusions of hypertonic solutions of sodium chloride were given, each at

the rate of 1·05 ml./min. over a period of 10 min. This animal, for some unknown reason, gave exceptionally small diuretic rates of urine flow in response to water; and the inhibitions produced by the infusions were unusually irregular, so that it was impossible to 'calibrate' the animal with satisfactory precision. Nevertheless, the inhibitions were

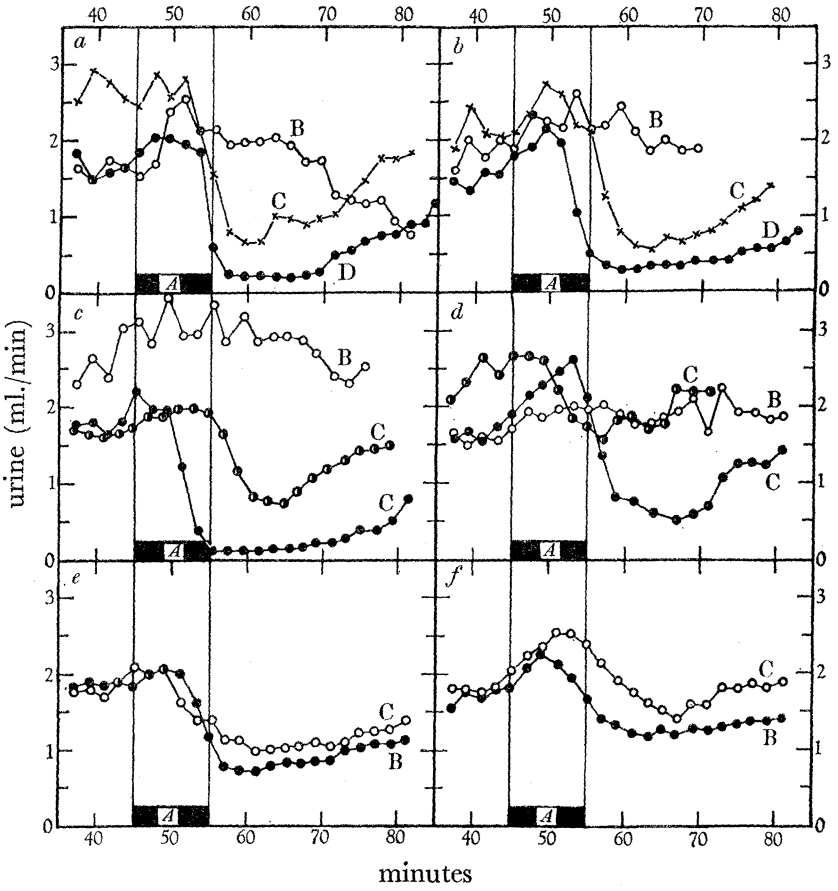


FIGURE 22. 'Paris', no. 393. Responses to infusions of hypertonic solutions of sodium chloride during established water-diuresis. The infusions were made at 1·05 ml./min during the 10 min periods shown by the rectangles *A*. *a* and *b* before, *c* and *d* after ligation of both occipital arteries. *a*, infusions into the right carotid: 0·60M, graph B; 0·86M, graph C; 1·03M, graph D. *b*, infusions into the left carotid: 1·03M, graph B; 1·20M, graph C; 1·37M, graph D. *c*, infusions into the right carotid: 0·86M, graph B; 1·37M, graphs C. *d*, infusions into the left carotid: 1·37M, graph B; 1·71M, graphs C. In *e* and *f* are given the means of the positive responses to infusions into the right and the left carotid respectively, the graphs B being the means before operation, and C after operation. In *e* the mean strength of the infused sodium chloride was 0·86M, graph B, and 1·20M, graph C. In *f* the mean strength was 1·20M, graph B, and 1·46M, graph C. Abscissae: time after the test dose (300 ml.) of water. Osmotic responses to intracarotid infusions are present after ligation of the occipitals, but are smaller than before.

sufficiently definite, and sufficiently graded with strength of infusion, to allow any gross change that might result from ligation of the occipital arteries, to be detected. The responses are illustrated in figure 22*a* and *b*, *a* giving the responses to infusions into the right carotid, and *b* those to infusions into the left. The graphs B, C and D show the effects of infusions, during the period *A*, of 0·60, 0·86 and 1·03M-NaCl respectively into the right carotid, and

of 1·03, 1·20 and 1·37M-NaCl into the left carotid, the weakest solution in each of the two series having no antidiuretic action. On one occasion 0·69M-NaCl infused into the right carotid produced a larger response than that shown in the figure to 0·86M (figure 22*a*, graph C); and on one occasion 1·20M-NaCl infused into the left carotid failed to give an antidiuretic response. Otherwise the graphs in the figure give a fair representation of the responses to intracarotid infusions of hypertonic solutions of sodium chloride. Both occipital arteries were then tied. Each was carefully identified as it crossed the lateral side of the internal carotid artery, and was ligated about 3 mm from its origin. Observations on the release of antidiuretic hormone were resumed 14 days after operation.

Responses after operation. Over a period of 14 days eight intracarotid infusions of hypertonic solutions of sodium chloride were given, four into the right and four into the left carotid, under the same conditions as those pertaining before operation. The effects are illustrated in figure 22*c* and *d*, *c* giving the responses to infusions into the right carotid, and *d* those to infusions into the left. The graphs B and C show the effects of infusions, during the period *A*, of 0·86 and 1·37M-NaCl respectively into the right carotid, and of 1·37 and 1·71M-NaCl into the left carotid. Comparison of these with the effects obtained before operation (figure 22*a*, *b*) will show that a given infusion now causes a smaller release of antidiuretic hormone. This is again shown in figure 22*e* and *f*, where the mean of all responses to infusions into the right (figure 22*e*) and that to infusions into the left carotid (figure 22*f*) before operation (graphs B) are compared with the means of the responses obtained after operation (graphs C); and this is further emphasized by the fact that the mean strength of the infused solution was greater on both right and left sides after operation (1·20M, right; and 1·46M, left) than it was before (0·86M, right; and 1·20M, left). In this animal, then, osmotic responses to intracarotid infusions were still present, though diminished, after both occipito-vertebral anastomoses had been occluded; and it was important to know the parts of the cerebrum which were now being supplied by carotid blood and with which the retention of the responses was associated. The animal was therefore killed during the intracarotid infusion of the black and blue suspensions, and the cerebral distribution of the carotid bloods determined by the methods previously described.

Tracing the cerebral distribution of the carotid blood. The black suspension was infused into the right carotid, the blue into the left, and chloroform was injected into the heart 6 s after the suspensions had reached the carotid needles. Examination of the sections of the celloidin-embedded brain revealed that the anterior and middle cerebral fields were well injected, but that the spinal cord, medulla, posterior part of the cerebellum and the ventral part of the pons were free from both black and blue suspensions. The occipito-vertebral anastomoses had, therefore, been effectively occluded. But on the right side the field of the anterior cerebellar artery was sparsely injected and that of the posterior cerebral artery was for the most part well injected with the black suspension, while on the left side both these fields were sparsely injected with the blue suspension and contained a trace of the black suspension as well. Evidently the normal forward direction of flow in the posterior communicating vessels had become reversed as a result of tying both occipital arteries, and the carotid streams were now mixing with unmarked vertebral blood in the region of the posterior limbs of the circle of Willis, a little right carotid blood being carried even into the mouths of the left

anterior cerebellar and posterior cerebral arteries. The appearances in the rest of the brain were in conformity with these findings. The paraventricular nucleus, the anterior division of the supraoptic nucleus and the *whole* of the posterior division were, on the left side, exclusively blue-injected, and on the right side exclusively black-injected. The right half of the pars distalis of the pituitary had received only the black, and the left half only the blue suspension, while the posterior lobe was exclusively blue-injected, its artery being readily traced from its origin from the left internal carotid in the cavernous sinus to its entry into the posterior pole of the lobe. This last finding confirms the conclusion reached from the experiments on 'Molly', p. 237.

The backward extension of carotid blood as far as the posterior limbs of the circle of Willis was a disappointing accompaniment of ligation of the occipital arteries, in that the results with this animal failed to exclude the mesencephalon unequivocally from being the site of the receptors. On the left side, however, the blue suspension in this region was so sparse (the predominant supply being unmarked vertebral blood), and yet osmotic releases of antidiuretic hormone were being obtained from left intracarotid infusions, that it would seem very unlikely that this part of the brain was involved in the responses. Nevertheless, it had now become clear that the most hopeful way of excluding the blood of one carotid from both hind- and midbrain regions would be to combine occlusion of one occipito-vertebral anastomosis with ligation of the ipsilateral posterior communicating artery. This was done in one animal, and the results will now be given.

(*b*) *Experiments with 'Toby'*, no. 395

This animal was prepared in the usual way by perineotomy and the formation of two carotid loops.

Responses before ligation of right occipital and posterior communicating arteries. Over the period of 6 months before operation, six infusions of hypertonic solutions of sodium chloride, at the rate of 1·05 ml./min, were made into the right carotid, and six into the left. The responses to infusions into the right carotid were consistently greater than those to infusions into the left. The latter are illustrated in figure 23*b*. The response shown in figure 23*a* is that to the infusion of 1·20M-NaCl into the right carotid; actually this was obtained 11 days after the right occipital artery had been tied. Eight days later the right posterior communicating vessel was ligated; the vessel was about 0·5 mm in diameter, and it was tied where it crossed the lateral face of the pars distalis at the level of the mamillary bodies and 4 mm posterior to the internal carotid. Description of the operative procedure for tying this vessel or the internal carotid or other of its branches will more conveniently be given later (p. 255 and figures 31*a*, and 31*b*, plate 12). In this instance it was interesting to observe that when the ligature was applied to the posterior communicating artery the part of the vessel visible posterior to the ligature increased in diameter, thus showing that the flow in the vessel had hitherto been in an anterior direction. The animal made an uninterrupted recovery, and experiments were resumed 4 weeks after the operation.

Responses after operation. Over a period of 10 months six infusions were made into the right carotid and five into the left. The responses are illustrated in figure 23*c* and *d*. Those from left carotid infusions were greater than before operation (cf. *d* with *b*), and on the right side the response to a given infusion was at least as great as and probably greater than

that obtained before the ipsilateral posterior communicating vessel was tied (cf. *c*, graph C with *a*). The response shown in figure 23*c*, graph C, was obtained a month after operation, and when the same infusion was given 10 months later a very similar effect was seen. Thus it was now of importance to know the distribution of the carotid blood that was carrying this effective osmotic stimulus to the brain.

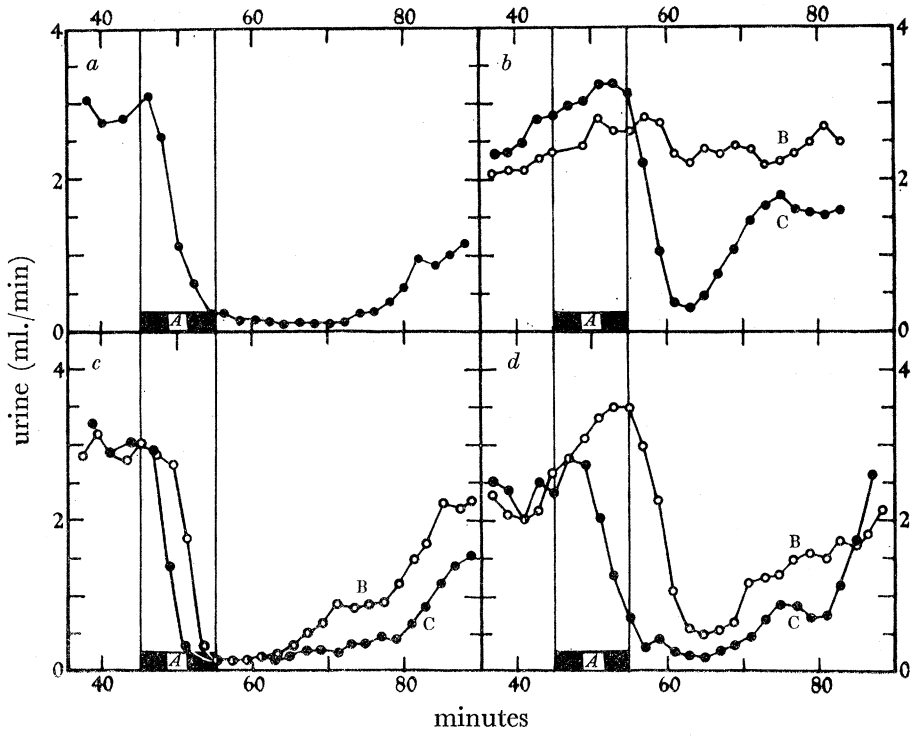


FIGURE 23. 'Toby', no. 395. Responses to infusions of hypertonic solutions of sodium chloride during established water-diuresis. The infusions were made at 1·05 ml./min during the ten-minute periods shown by the rectangles *A*. *a* and *c*, infusions into the right carotid; *b* and *d* infusions into the left. *a*, infusion of 1·20M-NaCl 11 days after the right occipital artery had been tied, and 8 days before the right posterior communicating artery was tied. *b*, infusion of 1·71M-NaCl, graph B, and of 2·05M-NaCl, graph C, before the right occipital artery had been tied. *c* and *d* give responses after the right occipital and right posterior communicating vessels had been tied. *c*, infusion of 0·52M-NaCl, graph B, and of 1·20M-NaCl, graph C. *d*, infusion of 1·71M-NaCl, graph B, and of 2·05M-NaCl, graph C. Abscissae: time after the test dose (300 ml.) of water. When the right occipital had been tied, osmotic responses to ipsilateral carotid infusions were not diminished as a result of further tying of the right posterior communicating artery.

Tracing the cerebral distribution of the carotid blood. The procedure was that already described. The Monastral fast blue suspension was infused into the right carotid, the carbon black into the left, and the animal was suddenly killed at the seventh second after the suspensions had reached the carotid needles. The distribution of the suspensions in the brain sections was carefully mapped, but here attention need only be directed to those parts of the brain from which blood of right-sided origin had been specifically excluded. The anterior thalamus of the right side was well injected with the blue suspension, but immediately behind the site of the ligature on the posterior communicating vessel the blue

injection of the thalamencephalon suddenly became scattered and sparse and quickly disappeared almost entirely from this region of the brain. The whole of the midbrain and hindbrain on the right side were, as well, practically free from all injection. The corresponding regions of the brain on the left side had received only left-carotid or vertebral blood. Thus the ligation of the right occipital and posterior communicating vessels had successfully excluded the ipsilateral carotid blood from the whole of the hind- and midbrain, and indeed from most of the posterior nuclei of the diencephalon as well. The conclusion we wish to draw for the moment, however, is that the receptors are situated somewhere in the prosencephalon. It is true that minute traces of the blue suspension were found at scattered points in the mid- and posterior brain stems—presumably owing to some tiny and unidentified anastomosis between the right carotid and vertebral blood streams (p. 204)—but the presence of these traces could not possibly invalidate the conclusion we have just drawn.

Incidentally, it was of interest to observe that after this animal had recovered from the tying of the right posterior communicating artery, digital occlusion of both carotid loops was unassociated with any detectable change in conscious behaviour. During such occlusion the forward flow of vertebral blood in the left posterior communicating vessel, supplemented, may be, by anastomotic flow through connexions between branches of the posterior and anterior cerebral arteries in the limbic cortex (p. 222), was, apparently, now solely sufficient to give adequate supply to the fields of both the left and the right anterior and middle cerebral arteries.

(c) Confirmatory evidence from other animals

In all the animals (eleven in number, excluding 'Paris' and 'Toby') in which osmotic responses were measured and the carotid distributions traced with suspensions, the sections of the brains have been scrutinized in order to eliminate those regions which could not be directly concerned in the responses. The most secure criterion for such deduction is that the region under examination should be devoid of suspension on a side on which satisfactory responses had been obtained from ipsilateral carotid infusions. The converse correlation, absence of response with presence of suspension in the region under examination, is not a reliable index of this region's not being essentially concerned, since dilution of its carotid supply with blood from other sources may have occurred in a degree sufficient to prevent an osmotic response but insufficient clearly to affect the final microscopic picture. Using the former criterion we have found that in six of the eleven animals satisfactory responses were being obtained when none of the corresponding carotid blood was reaching the hindbrain; and in one of these animals *both* black and blue suspensions were absent from this region, although osmotic responses had followed infusions of hypertonic solutions into either common carotid trunk. In the same way confirmatory evidence for the exclusion of the midbrain from being the site of the receptors has emerged. In two of the eleven animals all midbrain structures were free from suspension on a side on which ipsilateral carotid infusions had been osmotically effective. In two other animals only the ventral and posterior regions of the midbrain satisfied the adopted criterion, whilst in a third, by some vagary of blood supply, only the tectum was so excluded. The regions excluded by this collected evidence are illustrated in figure 24 (p. 246); they comprise the

whole of the hind brain and mid brain. These results verify the conclusion reached in the previous section from the experiments on 'Paris' and 'Toby'.

The receptors having thus been assigned to the prosencephalon, it will be convenient at this stage, first, to give any incidental evidence, that has been forthcoming in the course of our investigations, for the exclusion of certain regions of the telencephalon from being the site of the receptors; and secondly, to describe the results of experiments on one animal in which almost the whole of one cerebral hemisphere was removed.

(5) *The exclusion of the telencephalon from participation in the osmotic release of antidiuretic hormone*

(*a*) *Evidence from the dominance of one anterior cerebral field and from the absence of carotid contamination of the posterior cerebral field*

Extensive regions of a hemisphere may receive no carotid blood from the ipsilateral side for two reasons: first, the anterior cerebral arteries, which anastomose or are joined by a short and large anterior communicating artery, may receive blood exclusively or dominantly from one side alone (see, for example, figure 10); and secondly, the posterior cerebral artery may receive blood of exclusively vertebral origin.

The frontal pole and medial surface of the hemisphere. In four animals in which osmotic responses were given by infusions into either carotid trunk, an asymmetry of distribution of carotid blood in the anterior part of the brain was demonstrated. In 'Girl' (see later, p. 288) the suspension infused into the left carotid was absent from both frontal poles, whilst in 'Toby' (p. 242) that infused into the right carotid was similarly deficient at these sites. In the other two animals ('Paris', p. 239; 'Molly', p. 228) the suspension from one side was here so sparse as to be negligible. The frontal pole forms a narrow anterior extension of the hemisphere, and where the brain broadens behind it, posterior to the presylvian sulcus, the cortex of the lateral surface is supplied by the middle cerebral artery and hence is not involved in the asymmetry under discussion. On the other hand, the medial surface of the hemisphere, bordering the longitudinal fissure, is supplied by the anterior cerebral artery; and the same four animals afford evidence for the exclusion of this region from being concerned in the osmotic response. The region is continuous with the medial surface of the frontal poles and includes the cingulate gyrus and the whole depth of the hemisphere medial to the anterior extension of the lateral ventricle. Dorsal to the corpus callosum a longitudinal segment of the hemisphere, including the whole limbic cortex and the cortex of the dorsal surface medial to the ectomarginal (or collateral) sulcus, is similarly involved. This segment may extend to the extreme posterior pole of the hemisphere to merge with the gyri of the tentorial surface.

The asymmetry of distribution of carotid blood is also reflected in the region ventral to the cingulate gyrus, embracing the parolfactory area and the septal nuclei. Thus in both 'Girl' (p. 288) and 'Paris' (p. 239) suspension from the left side is very sparse in these regions. The differentiation is not so sharp here as elsewhere, however, and in other animals there has evidently been sufficient admixture of the carotid bloods to make interpretation somewhat equivocal.

The tentorial surface. The gyri of the tentorial surface are readily excluded from further

consideration since, when carotid blood has not joined the basilar flow, this region is usually found to be devoid of suspension. Four of the animals presented unequivocal evidence of this nature, suspension being absent here from a side from which osmotic responses had been obtained.

The region involved includes the gyrus dentatus and the hippocampus extending dorsally to the floor of the lateral ventricle, the crura and body of the fornix, and the fimbria. The rhinencephalon is continued ventrally as the pyriform lobe, and it is noticeable that suspension is often sparse in this region, presumably owing to much of its blood supply coming from the vertebral arteries. Moreover, in three animals ('Toby', p. 242; 'Girl', p. 290; 'Jink', p. 285) the pyriform lobe of the operated side was found to be damaged and cystic in part, yet good osmotic responses had been obtained from ipsilateral carotid infusions. It thus seems justifiable, on this evidence alone, to include this region with the structures of the tentorial surface as not being essentially concerned in the responses.

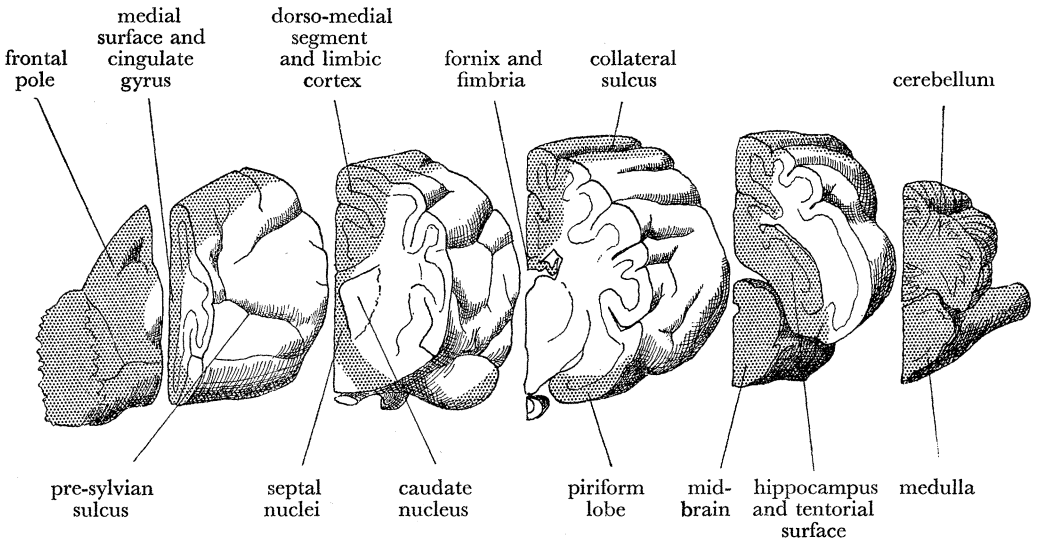


FIGURE 24. Perspective of blocks of one side of the dog's brain to illustrate the regions (stippled) which have been excluded from essential participation in the osmotic response by the fact that, although osmotic responses were being obtained from infusions into a carotid, these regions were free from suspension when this was later infused into the same carotid.

The parts of the telencephalon which have thus been excluded from participation in the osmotic release of antidiuretic hormone are shown by the stippled regions in figure 24. Also shown are the midbrain and hindbrain; these have already been excluded by the evidence presented in the previous section (p. 238) of this paper. In all the animals, as is clear from the figure, there has remained a considerable area of the lateral hemisphere, in the parietal and temporal regions, that has still received blood, as has the underlying corpus striatum, from the ipsilateral carotid. It became of importance, therefore, to determine whether these regions were essentially involved in the responses by seeing whether responses were present after operative removal of a cerebral hemisphere. The operation was kindly done for us by Dr F. Howarth, and we shall now describe the procedures undertaken and the results obtained with the animal 'Brindle'.

(*b*) *Experiments with 'Brindle', no.* 409

Operative procedures. The left cerebral hemisphere was removed in three stages. Under pentobarbitone-sodium (40 mg/kg body wt. i.v.) anaesthesia, a left temporal flap of scalp was turned down over the zygoma, and the temporal muscle similarly treated. A wide area of bone was then removed and the opening extended medially with rongeurs until the superior sagittal sinus became visible. A flap of dura was reflected over the temporal muscle, and the exposed cortex was removed deeply by suction after occlusion by diathermy of vessels just outside the removal area. Much of the corpus striatum was similarly taken away. Further removal of tissue included, posteriorly, the occipital cortex except its medial surface and the part beneath the lateral venous sinus, and, ventrally, the superior temporal gyrus. The frontal pole could not be safely reached owing to the overhanging frontal air sinus. The dura was then reconstructed and the wound closed in layers. Ten hours after the end of the operation the animal drank water freely, and further recovery was uneventful.

Three and a half weeks after this operation the second stage of the procedure was undertaken, viz. occlusion of the left frontal air sinus. Under pentobarbitone-sodium (40 mg/kg body wt. i.v.) anaesthesia, a skin flap was turned down over the face and the roof of the air sinus removed. The sinus communicated with its fellow through a large opening. The endosteum was scraped away and the cavity and its communication were plugged with bone chips and muscle pulp. Strips of muscle from the undersurface of the skin flap were then turned into the floor of the cavity, the flap was replaced and the wound closed by two layers of sutures. Recovery was uneventful.

Six and a half weeks later the third stage was undertaken, viz. removal of the remaining parts of the left hemisphere. Under pentobarbitone-sodium (30 mg/kg body wt. i.v.) anaesthesia, the original opening in the skull was extended in an anterior direction by nipping away the floor of what had been the left frontal air sinus. Removal of the remaining cortex was then proceeded with as in the first stage. When this had been done, the falx from the cribriform region to its junction with the tentorium, the edge of the tentorium, and the base of the middle cranial fossa were all visible. Half an hour later, haemostasis being satisfactory, the dura and temporal muscle were replaced, and the scalp wound was closed with layered sutures. Over the last hour of the operation the animal received 500 ml. of citrated dog blood. Recovery was uneventful.

Three weeks later preparation was made for intracarotid infusion experiments by a series of three operations comprising (1) the formation of a left carotid loop, (2) perineotomy and (3) ligation of the left occipital artery and formation of a right carotid loop. At this last operation the occipital artery looked to us abnormally large; it was double ligatured and divided about 4 mm from its origin. Infusion experiments were started 3 months later.

Responses after removal of the left cerebral hemisphere. Three infusions were made into the right carotid (1·37M, 1·71M and 2·06M-NaCl), each at the rate of 1·05 ml./min, and in neither instance was there any clear indication of an osmotic release of antidiuretic hormone. The effects of one of these infusions (2·06M) are shown in figure 25*a* (graph B) and they are very similar to those of the same infusion given into the arm vein (graph C). When, however, the same and weaker infusions were made into the left carotid,

well-marked responses were consistently seen. Five such infusions were made, and the effects of two of them (0·86M and 1·37M) are shown in figure 25*b*, graphs B and C, respectively. We then decided to determine the cerebral distributions of the two carotid bloods.

Tracing the cerebral distribution of the carotid blood. This was done in the usual way, the Monastral fast blue suspension being infused into the left carotid, the carbon black into the right, and the animal suddenly killed at the seventh second after the suspensions had reached the carotid needles. The operation site was quite clean post-mortem; and maps of the distribution of the suspensions in the sections of the celloidin-embedded brain are given in figure 26. The ablation is practically a total hemispherectomy. The left olfactory bulb remains but in a shrunken state, and a posterior fragment of the ventro-medial part of the frontal lobe is present as also is the ventro-medial tip of the piriform lobe, but this last is cystic. The head of the caudate nucleus is extensively damaged, and the only part of the

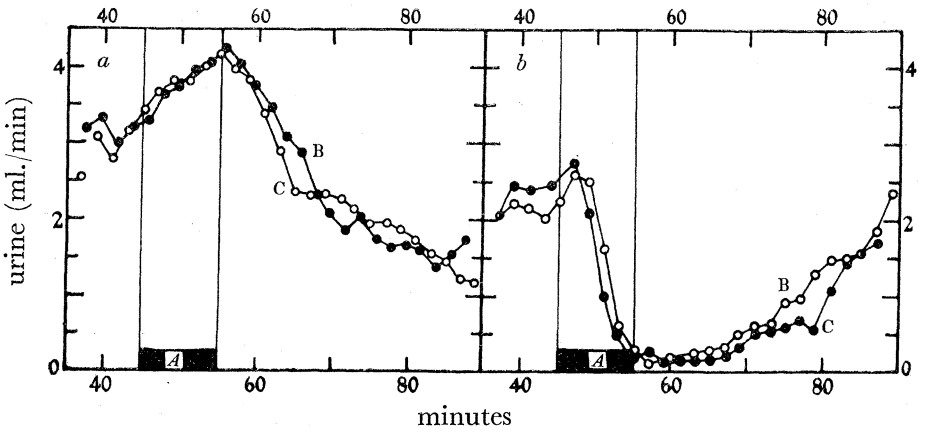


FIGURE 25. 'Brindle' no. 409. Effects of infusions of hypertonic solutions of sodium chloride during established water-diuresis. The infusions were made after left hemispherectomy and were given at 1·05 ml./min during the 10 min periods shown by the rectangles *A*. *a*, 2·06M infused into the right carotid, graph B, and into the right arm vein, graph C. *b*, infusions into the left carotid: 0·86M, graph B; 1·37M, graph C. Abscissae: time after the test dose (300 ml.) of water.

corpus striatum and internal capsule that remains is the antero-medial part of the head of the caudate nucleus, but this is isolated and fibres passing lateral from it will have been severed. Moreover, this is just the region that was almost free from suspension in 'Girl' (see later, p. 289 and figure 46, p. 289), an animal in which well-marked responses had earlier been forthcoming. The corpus striatum on the right side in 'Brindle' is well injected with the black suspension, a mere trace of the blue being detectable at scattered points, and this applies also to the cortical areas on this side with the exception of the field of the anterior cerebral artery, i.e. the medial surface of the hemisphere, bordering the longitudinal fissure, where the injection is predominantly blue. Now it will be recollected that well-marked osmotic responses had occurred from infusions into the left carotid. The findings in this animal, therefore, in conjunction with those of the preceding section of this paper, exclude the whole of the telencephalon,* with the exception of the preoptic region, and with the possible exception of the parolfactory region, from being essentially

* Although the extreme anterior part of the hypothalamus (optic chiasma and lamina terminalis) is not strictly a part of the diencephalon we shall use the latter term as embracing the whole of the hypothalamus.

involved in the osmotic response, and give experimental justification for focusing attention on the diencephalon* as containing the receptors we seek. In narrowing down their site within the diencephalon we shall, then, look particularly for evidence for the exclusion of areas and nuclei in the thalamus and hypothalamus from being essentially concerned in the osmotic response; and in this connexion we would again emphasize that the most secure

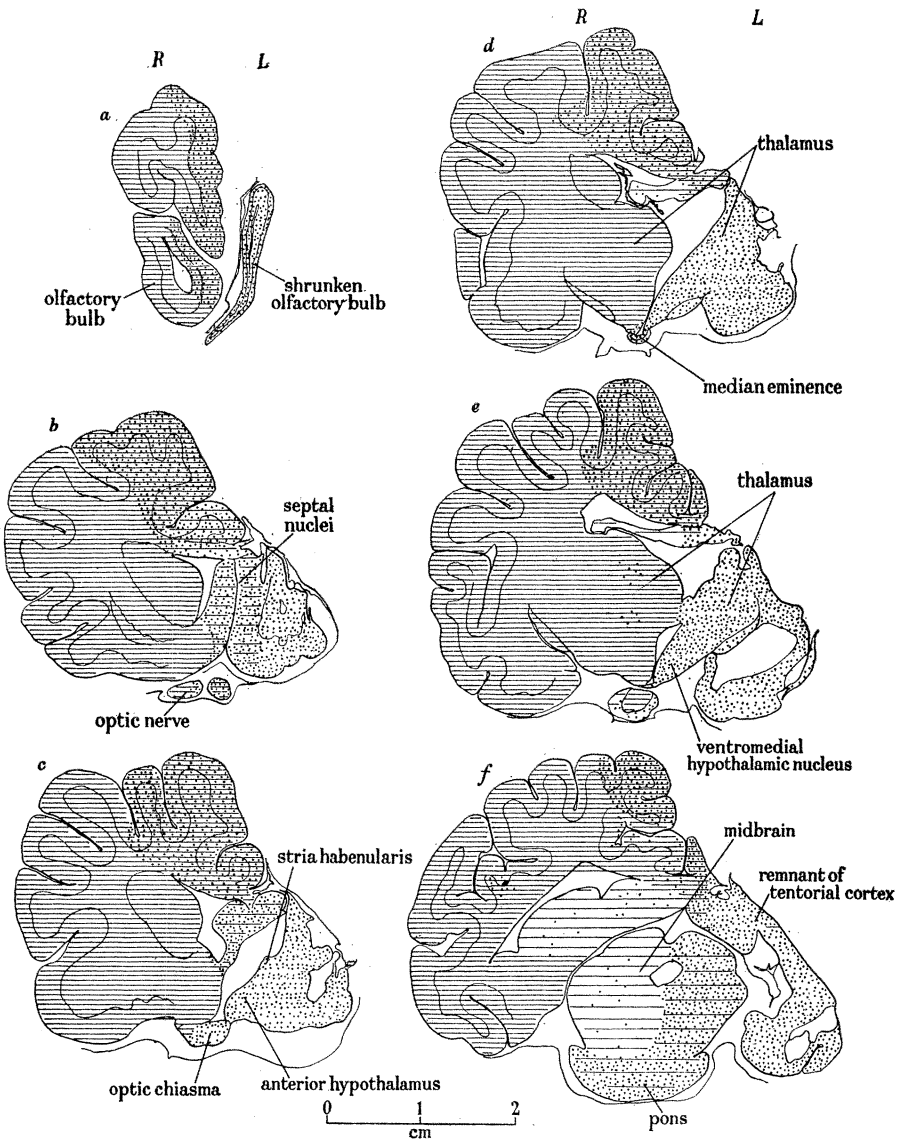


FIGURE 26. 'Brindle', no. 409. Maps of selected sections to show the cerebral distribution of the suspensions. Blue suspension infused into the left carotid—dots; black into the right—lines. The distances between the anterior surfaces of sections *a* and *b*, *b* and *c*, etc., are 16, 4, 2, 3 and 9 mm respectively.

criterion of such exclusion is the presence of the response with either absence of suspension from, or post-operative degenerative changes in, the restricted region under review.

Evidence for the exclusion of parts of the diencephalon. From post-operative degenerative changes and from distribution of suspensions, certain areas and nuclei in the diencephalon of this animal can be excluded from being the receptors' site. In order to facilitate

* See footnote p. 248.

description of such relevant findings figure 27 is now presented to show the topography of the nuclei and areas in the dog's diencephalon.

In preparing the diagram use was made of serial frontal sections through the celloidin-embedded diencephalon of each of two dogs. One block was cut at 100μ and every fifth section stained with thionin. The second block was cut at 75μ and every fourth section stained with thionin and the next adjacent section stained by Weil's technique. Projection drawings were made from both series at an enlargement of ten times. On these the distinctive anatomical structures were outlined, the finer structures, and the boundaries of the nuclei, being determined by the use of the microscope. In defining the extent and configuration of the nuclei the papers of Rioch (1929, 1931) have been closely followed, and his illustrations of toluidin-blue stained sections have been compared with our

KEY TO FIGURE 27

Thalamic nuclei

n. anterodorsalis	*AD*
n. anteromedialis	*AM*
n. anteroventralis	*AV*
n. parataenialis	*PT*
n. medialis dorsalis	*MD*
n. submedius	*SM*
n. tractus habenulo-peduncularis lateralis	*HPL*
n. tractus habenulo-peduncularis medialis	*HPM*
n. parafascicularis	*PF*
n. subparafascicularis	*SPF*
n. paracentralis	*PC*
n. centralis lateralis	*CL*
n. commissuralis interparataenialis	*Com*
n. commissuralis interanterodorsalis	*Com*
n. commissuralis interanteromedialis	*Com*
n. rhomboidales	*RH*
n. centralis medialis	*CE*
n. paraventricularis anterior	*PA*
n. paraventricularis posterior	*PP*
n. reuniens	*RE*
n. lateralis pars anterior	*LA*
n. lateralis pars intermedia	*L*
n. lateralis pars posterior	*L*
n. suprageniculatus	*SG*
n. limitans	*LIM*
n. posterior	*P*
n. reticularis	*R*
pulvinar	*PUL*
area pretectalis	*Pre Tect*
n. ventralis pars anterior	*VA*
n. ventralis pars medialis	*VM*
n. ventralis pars externa	*VE*
n. ventralis pars arcuata	*V Arc*
n. ventralis pars commissuralis	*VC*
n. geniculatus lateralis	*GL*
n. geniculatus medialis	*GM*
substantia grisea pregeniculata	*PreG*
n. habenularis lateralis	*HL*
n. habenularis medialis	*HM*

Hypothalamic nuclei

n. suprachiasmaticus	*Ch*
n. supraopticus	*SO*
n. paraventricularis hypothalamicus	*PV*
n. supraopticus diffusus	*D*
dorsal hypothalamic area	*DH*
lateral hypothalamic area	*LH*
anterior hypothalamic area	*AH*
perifornical area	*FP*
n. ventromedialis	*VMH*
n. dorsomedialis	*DMH*
n. periventricularis arcuatus	*PeA*
posterior hypothalamic area	*PH*
premamillary area	*PM*
medial mamillary nucleus	*MM*
lateral mamillary nucleus	*ML*
supramamillary area	*MS*

Associated tracts and areas

septal nuclei	*S*
stria terminalis	*St*
fornix	*F*
anterior commissure	*AC*
bed nucleus of the anterior commissure	*NCA*
medial preoptic area	*MPre*
lateral preoptic area	*LPre*
Medial parolfactory area	*MParol*
optic chiasma	*OC*
optic tract	*OT*
stria medullaris (habenularis)	*Sm*
periventricular system	*Pe*
inferior thalamic peduncle	*ITP*
caudate nucleus	*C*
mamillo-thalamic tract	*MT*
n. subthalamicus	*STh*
n. rubra	*NRUB*
n. commissuralis posterior	*NPC*
cerebral peduncle	*CP*
internal capsule	*IC*
posterior commissure	*PCom*

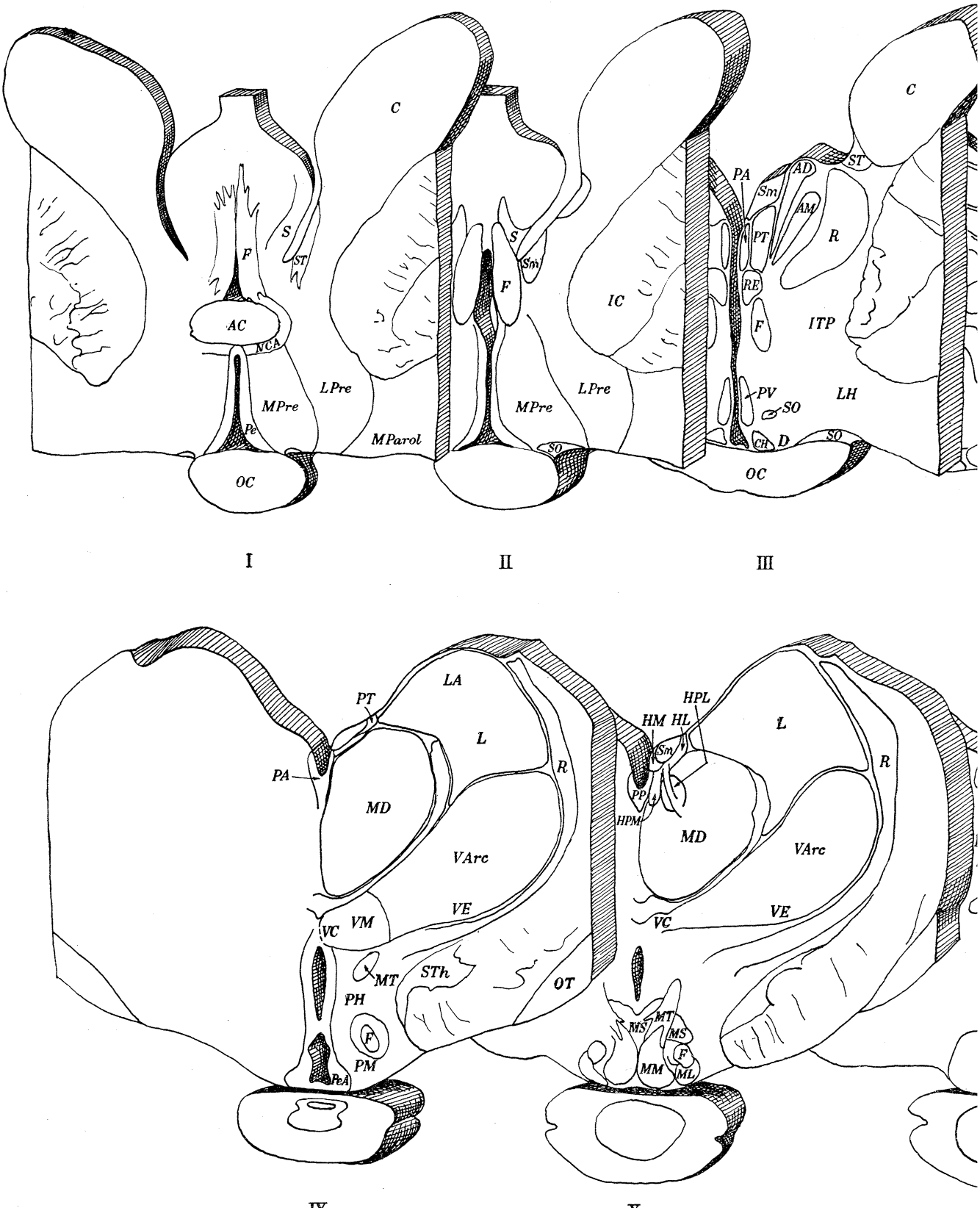
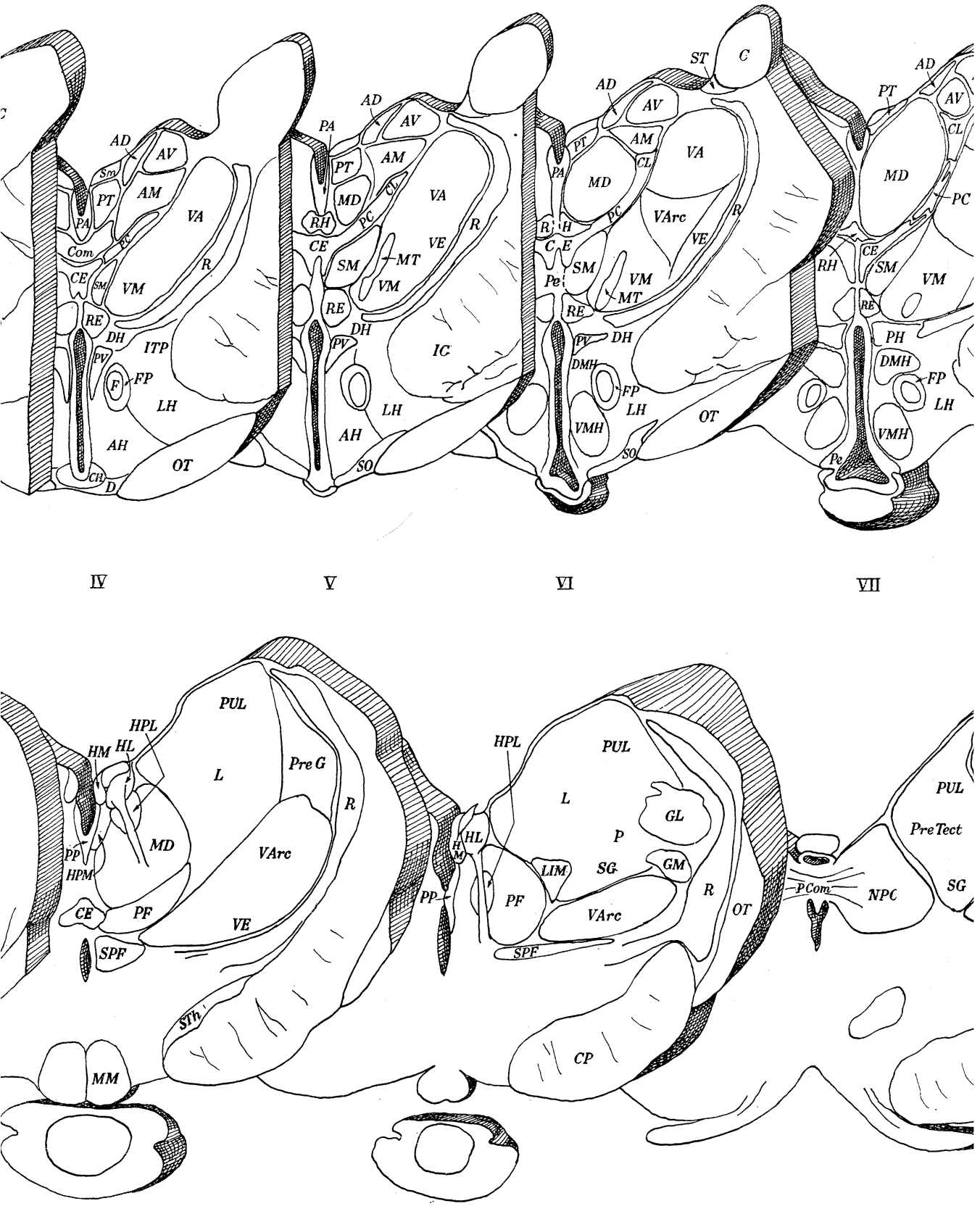
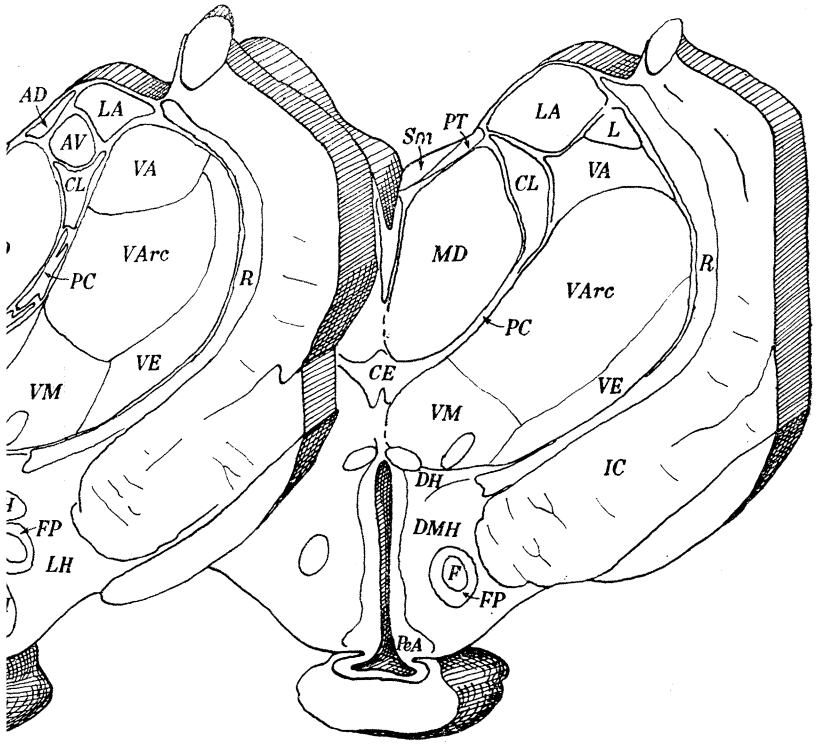


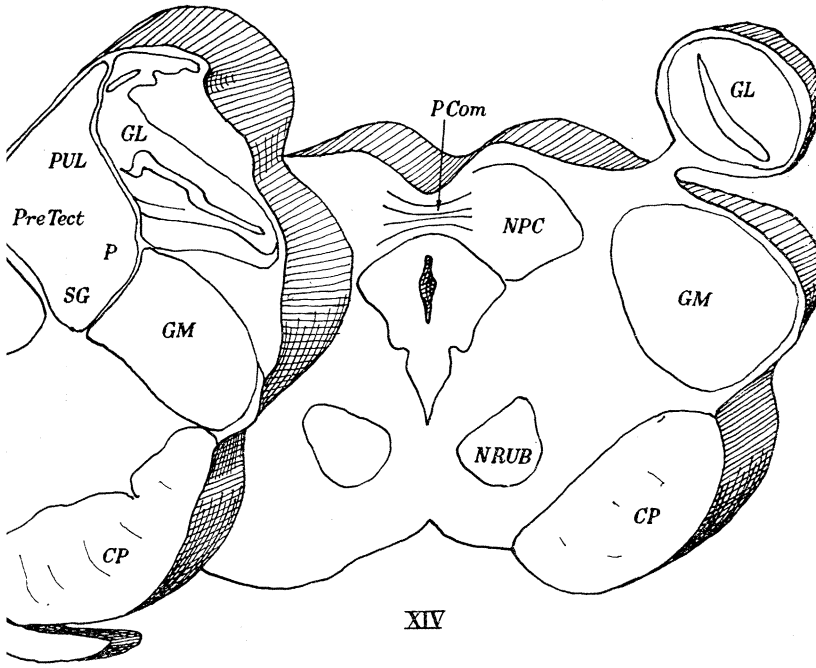
FIGURE 27. Serial frontal sections of the diencephalon to show the topography of the nuclei and areas in th room temperature, and when the effluent from the external jugular veins was fairly clear the perfusi were made of the celloidin-embedded material. The sections shown form a complete series, and t description see text. The key to nomenclature is given opposite.



as in the thalamus and hypothalamus of the dog. The heads of two animals under chloralose anaesthesia
perfusing fluid was changed to the formalin-acetic acid-ethanol fixative. Blocks were cut from the brains so
s, and the projected thickness of each is isometric in the sense that the length of the inclined lines of shading



VIII



XIV

0 1 2 3 4 5
mm

esthesia were perfused through the carotids with 0·9 % NaCl at
ains so as to include the whole diencephalon, and serial sections
shading represents the actual depth of the section. For further

(*Facing p.* 250)

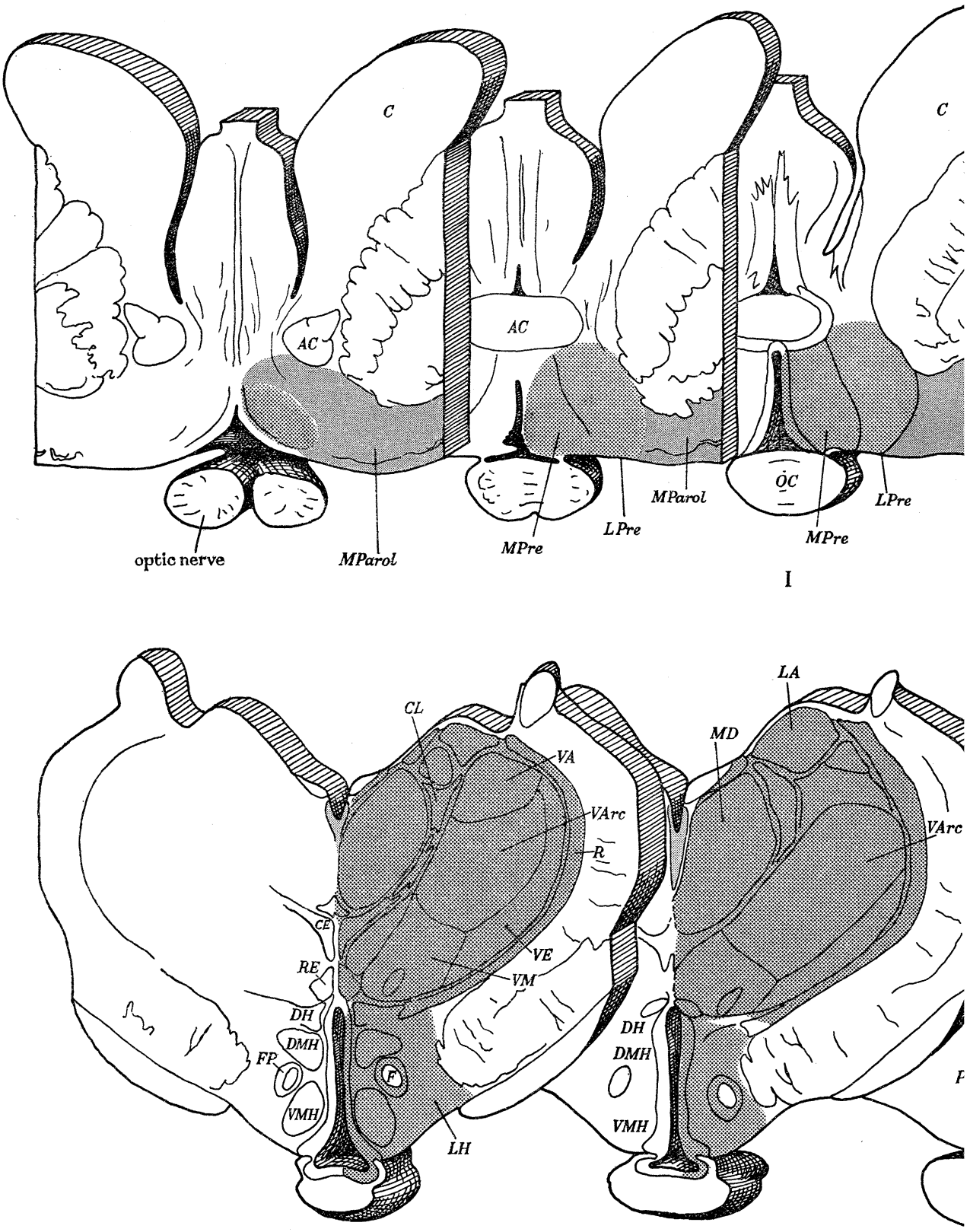
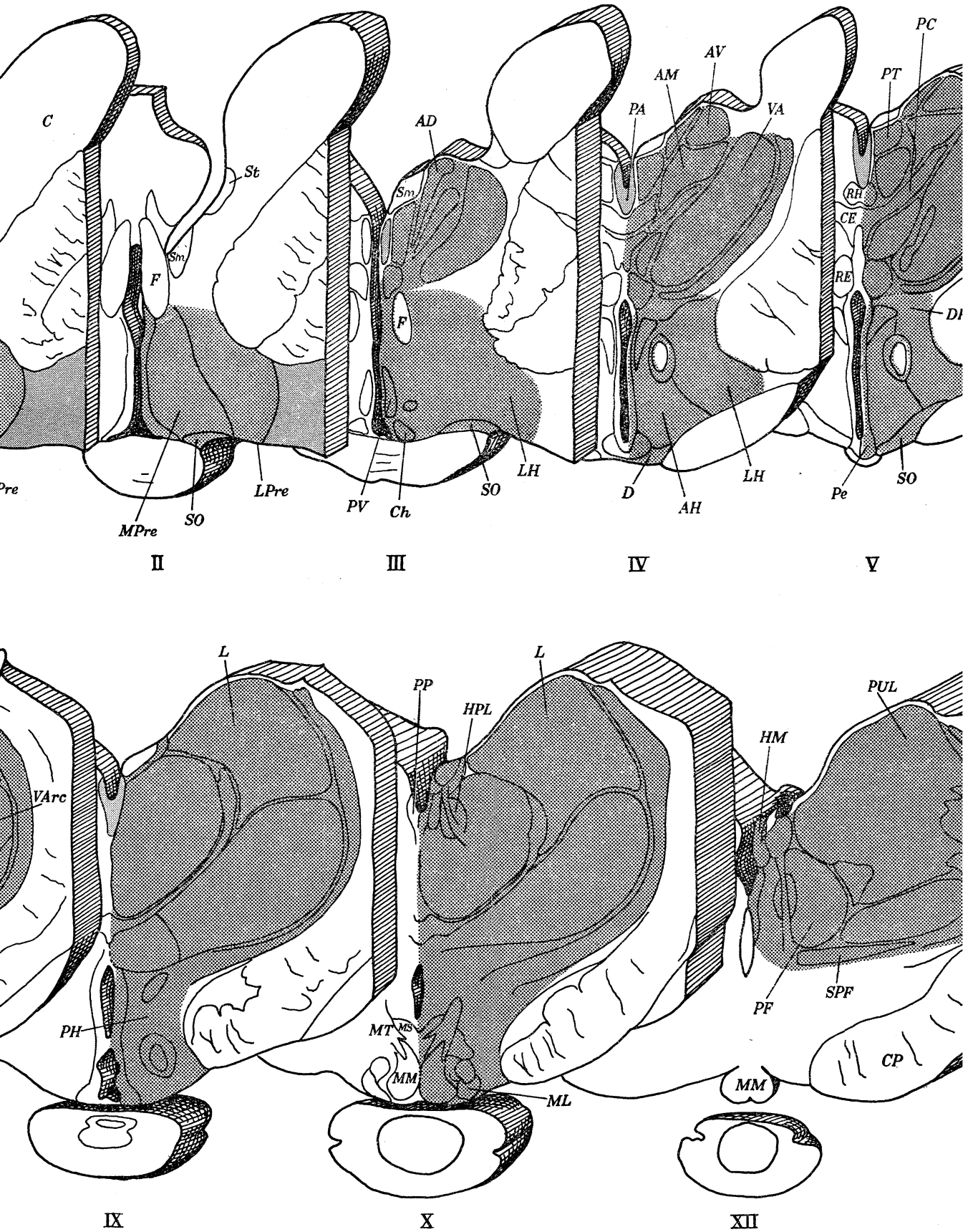
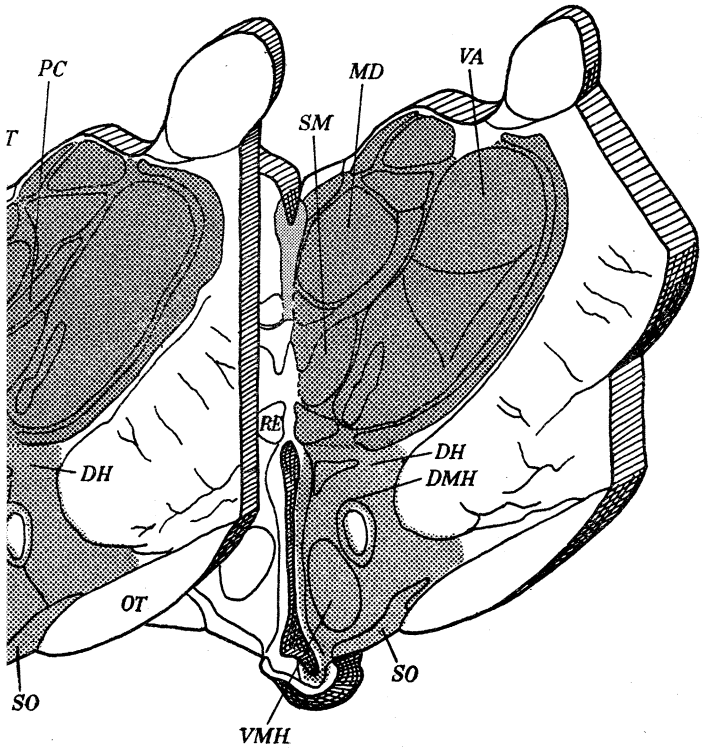


FIGURE 48. Diagrammatic representation of the parts of the diencephalon excluded as sites for the osmc lie. The heavy black stipple indicates nuclei and areas excluded by evidence from blood distribution o evidence is desirable. Red stipple indicates the region in which the receptors lie and which has alwa the nuclei is given; corresponding sections carry the same numeral.



he osmoreceptors by collected evidence from animals in which responses were retained after operation, and sh
oution or degenerative cell loss or both. Light black stipple indicates regions not completely excluded by evidenc
has always received carotid blood where responses have been present. The figure should be studied in conjun



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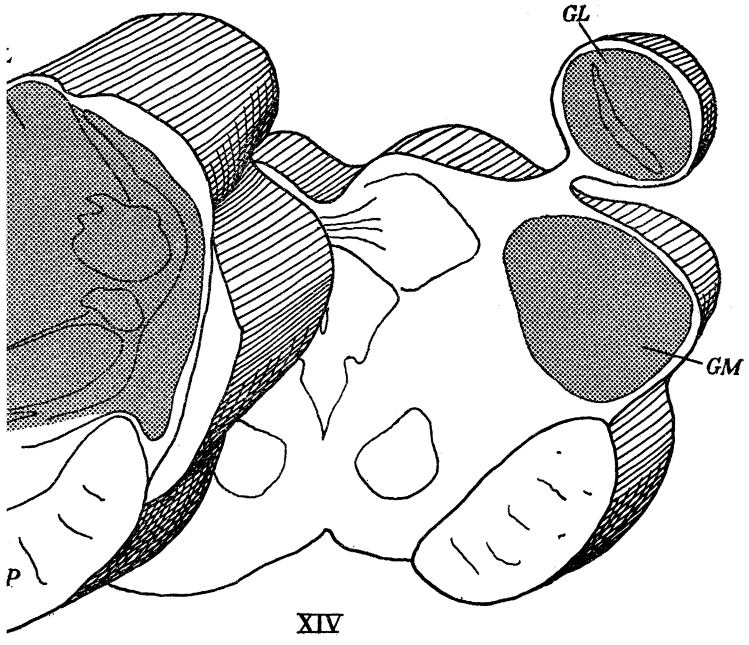

XIV

0 1 2 3 4 5
mm

ı, and showing the region in which, by inference, the receptors
ı evidence from blood distribution, and for which confirmatory
ı conjunction with figure 27 in which the nomenclature of all

preparations. The Weil-stained sections give valuable supplementary information, since some nuclei are characterized by the manner in which fibres run through them. The illustrations of Weil-stained sections through the thalamus of the cat presented by Ingram, Hannett & Ranson (1932) have also proved useful for comparative purposes.

The nomenclature of Rioch (1929) for the thalamic nuclei has been employed, but subdivision of some of the nuclei has not been attempted. The nuclei commissuralis interparataenialis, interanterodorsalis and interanteromedialis have not been separately indicated. The subdivisions of the ventral nucleus are somewhat arbitrary, and only the nucleus lateralis pars anterior has been separately indicated as a subdivision of the lateral nucleus.

In 'Brindle' the left thalamus is much shrunken, and all the dorsal thalamic nuclei have degenerated with the exception of the anterior and posterior paraventricular nuclei, which are well preserved, and of cell groups in some other medial nuclei. The greater part of the nucleus parataenialis persists, and a considerable population of cells remains in the region of the commissural nuclei of the anterior group. Similarly the nucleus ventralis pars commissuralis is still extant, and groups of cells belonging to the paracentral nucleus can be seen between the completely degenerate nucleus medialis dorsalis and the ventral nuclei. The nucleus paraventricularis anterior now forms a promontory into the third ventricle from the medial wall of the shrunken thalamic tissue. Some cells probably persist in the nucleus reuniens and nucleus rhomboidalis, and the nucleus reticularis has a few remaining cells. These features are indicated in figure 28. Langley & Grünbaum (1890) noted the persistence of nerve cells in the 'central, grey substance of the third ventricle' in the thalamus of a hemidecorticate dog in which the brain, given them by Professor Goltz, had evidently suffered more severe damage than in 'Brindle'. Papez (1938), on the other hand, has reported on the thalamus of a hemidecorticate dog in which the damage appears to have been less extensive. He noted the persistence of the nucleus ventralis commissuralis, but in his animal, in contrast with 'Brindle', more of the medial and ventro-medial nuclei appear to have been preserved including the nucleus medialis centralis and paracentralis. Probably in 'Brindle' there was a greater involvement of the striatum and pallidum in the lesion; it is to these structures that the midline and intralaminar nuclei project (Powell & Cowan 1954; Cowan & Powell 1955). The stria habenularis (medullaris) is intact, and the habenular nuclei do not show cell loss. The left hypothalamus is distorted and shrunken but the nuclei are well preserved, none showing any obvious cell loss.

With respect to the distribution of the suspensions (figure 26): on the left side the whole of the thalamus remainder is well injected with the blue; left carotid blood has been flowing backwards in the posterior communicating artery to mix with vertebral blood in the region of origin of the left anterior cerebellar artery. The left hypothalamus is distorted and is carrying exclusively the blue suspension, both divisions of the supraoptic nucleus, the paraventricular nucleus and the mamillary nuclei being well injected. On the right side the anterior thalamus is well injected with the black suspension, but more posteriorly there is intrusion of a little blue, the basilar blood being contaminated, in spite of ligation of the left occipital artery, with blood of left carotid origin. The right hypothalamus and its nuclei, while well and predominantly injected with the black suspension, carry a little blue as well, and this blue contamination is especially evident in the antero-medial part of the anterior division of the supraoptic nucleus (cf. the dominance of the field of the left

anterior cerebral artery over that of the right). A measure was made, by the method previously described, of the distribution of the suspensions in the supraoptic nuclei; the results will be given later (table 2, p. 280). The intercarotid anastomosis is receiving blood mostly from the left internal carotid as is shown by the fact that the chiasma and pars

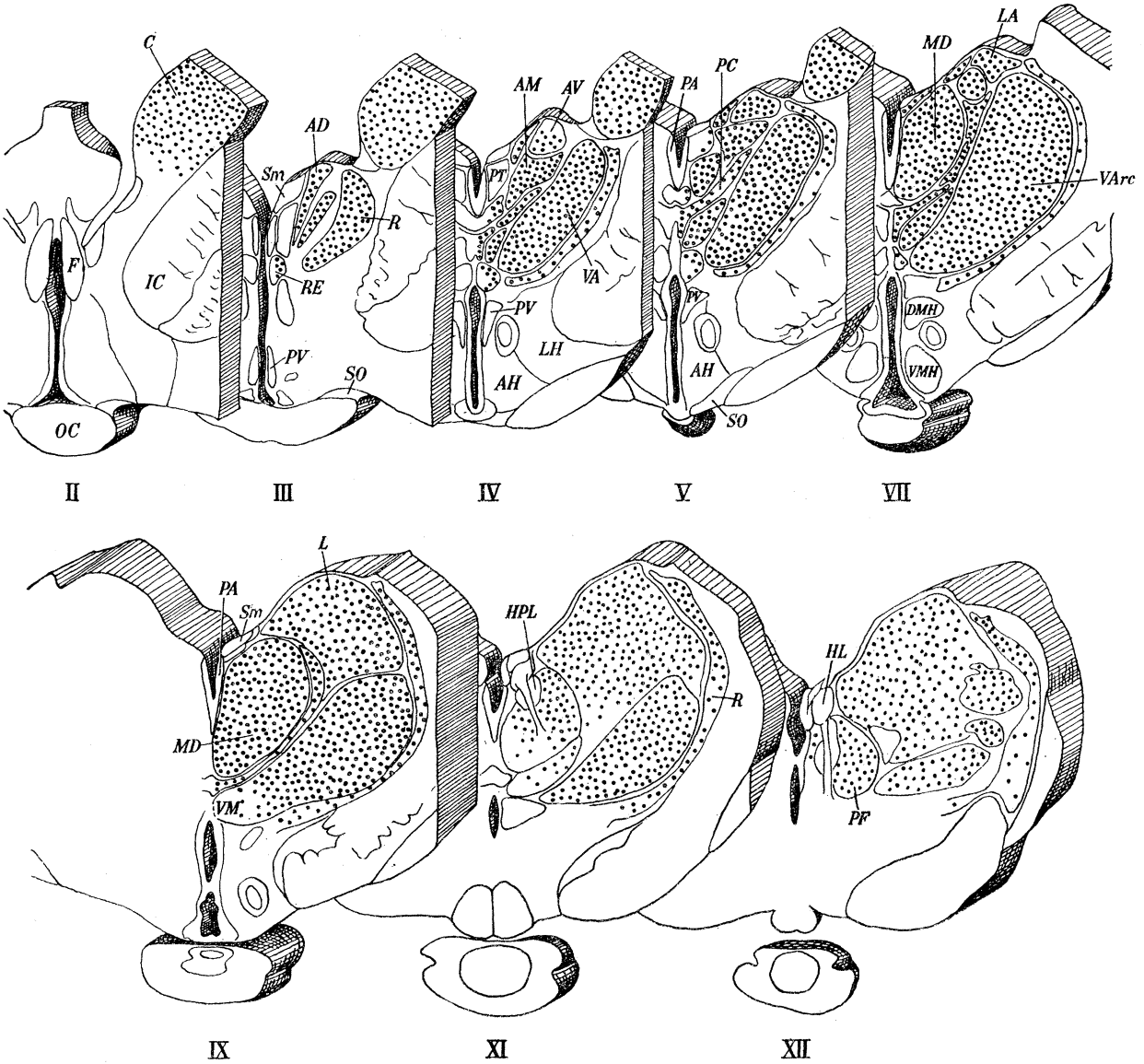

FIGURE 28. 'Brindle', no. 409. Nuclei of the diencephalon which have suffered degenerative lesion. The sections are selected from those shown in figure 27 and carry the same numerals. The black dots indicate severe cell loss. Abbreviations as in figure 27.

distalis are, except at their right margins, exclusively injected with the blue suspension. The posterior lobe carries black suspension only, a fact that confirms the conclusion previously reached with 'Molly' (p. 237).

On the evidence from this animal and on that from the two animals in the previous section it is possible, then, to exclude the greater part of the thalamus from being the site of the receptors. For in 'Brindle' most of the anterior thalamic nuclei that were included in the field of distribution of the left carotid (from infusions into which osmotic responses

were being obtained) had become degenerate; and in 'Toby' (p. 243) the posterior part of the right thalamus (behind the level of the mamillary nuclei and the ligature on the posterior communicating artery) was only very sparsely and patchily injected with suspension from the right carotid, although there had been well-marked osmotic responses from carotid infusions on this side. This is strong evidence against the posterior thalamic nuclei being primarily concerned in the response, and this inference is supported by the observations on 'Paris' (p. 241) in which the corresponding field on the left side was receiving blood mainly of vertebral origin.

We conclude, therefore, from the results so far obtained, that the receptors are somewhere in the anterior part of the diencephalon. Little more evidence of a localizing nature could be expected from any further experiments on the lines already described; but before turning to the effects of tying an internal carotid, or one or more of its primary branches, on the osmotic response and on the diencephalic distribution of the two carotid bloods, we propose to describe the findings in one of the two animals in which no response was obtainable from infusions into one of the carotid arteries. And we do so at this stage because correlation of the responses, absent from infusions into one carotid and present from infusions into the other, with the distributions of the carotid bloods, gives evidence which, on the background of that already presented, is consistent with the view that the receptors are in the anterior part of the diencephalon. Incidentally, the evidence obtained from this animal ('Rita', no. 394) showed that the absence of response to infusions into the one carotid was not related to the posture of the animal.

(6) *Observations on an animal ('Rita', no. 394) in which no osmotic responses could be obtained from intracarotid infusions on one side*

This animal was prepared in the usual way by perineotomy and the formation of two carotid loops. While infusions of hypertonic sodium chloride into the right common carotid trunk during established water-diuresis gave well-marked antidiuretic responses, the effect of the same or even stronger infusions into the left was negative. These results are illustrated in figure 29. On the left-hand side of the figure are given the responses to infusions, each at 1·05 ml./min for 10 min, of 1·20M (graph B) and of 1·37M (graph C) into the right carotid. A small but definite response was also obtained to a similar infusion of 1·03M-NaCl; and when (figure 29*c*) 0·68M-NaCl was infused at 0·2 ml./s for 35 s a definite inhibitory response followed, unaccompanied by any perceptive manifestation. The effects of infusions into the left carotid are shown in figure 29*b*, the graphs B and C giving the effects of 1·37M and D the effect of 1·71M-NaCl, and no antidiuretic response was seen when the strength of the infused solution was raised to 2·58M. No response, either, was given to 0·86M-NaCl infused at 0·2 ml./s for 20 s (figure 29*d*); when a stronger solution (1·03M) was similarly infused, but for 10 s only, a small response followed, but as the infusion was associated with whining and head movement, the response was evidently of emotional origin. The results in figure 29*b*, graph B, were obtained with the animal lying in the usual position, viz. on its right side, those in graph C with the animal lying on its left side; posture, then, is not the factor on which this absence of response depends.

As such negative effects have been encountered only rarely, we thought it would be of value to determine at once the distributions of the two carotid bloods in this animal. The

usual procedure was adopted, the blue suspension being infused into the left, the black into the right carotid, and the animal was suddenly killed by the intracardiac injection of chloroform at the seventh second after the suspensions had reached the carotid needles; this was 1 h after the 'test' dose of water. The animal remained perfectly quiet while the suspensions were being infused. Examination of the sections of the celloidin-embedded

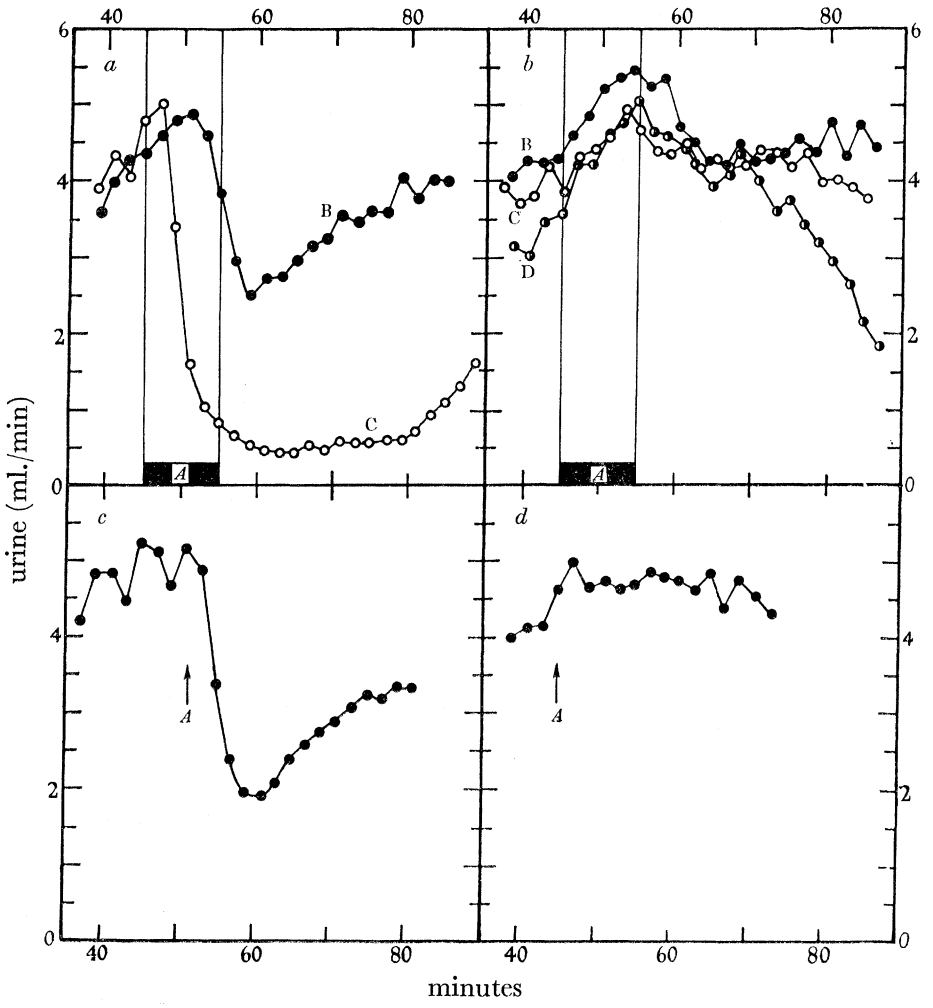

FIGURE 29. 'Rita', no. 394. Responses to intracarotid infusions of hypertonic solutions of sodium chloride during established water-diuresis. *a*, infusions into the right carotid: 1·20M, graph B, and 1·37M, graph C. *b*, infusions into the left carotid: 1·37M, graphs B and C, and 1·71M, graph D; in the experiment of graph C the animal was lying on its left side, and in that of graph B on its right side, this latter position being the one regularly adopted. In *a* and *b* the infusions were at 1·05 ml./min during the 10 min period shown by the rectangles *A*. *c*, infusion, at the arrow *A*, of 0·68M at 0·2 ml./s for 35 s. *d*, infusion, at the arrow *A*, of 0·86M at 0·2 ml./s for 20 s. Abscissae: time after the test dose (350 ml.) of water.

brain showed that the cerebral supply of the right carotid dominated strongly over that of the left; the blue suspension in the left anterior cerebral field was heavily contaminated with black, and the right carotid blood had apparently passed posteriorly to join with basilar blood at the back of the circle of Willis, the mixture then diluting the left carotid blood in the left posterior communicating vessel. Thus the right thalamus and

hypothalamus (including both divisions of the supraoptic nucleus) are well injected, and exclusively so, with the black suspension; and, as we have seen, well-marked responses had previously been given to infusions into the carotid on this side. On the left side, however, while the dorsal thalamic nuclei are well injected with, predominantly, the blue suspension, the rest of the thalamus and the whole of the hypothalamus carry a mixture of black and blue, and it is noticeable that the injection here is sparse and patchy in contrast with the heavy and uniform injection with the black suspension in the corresponding regions on the right side. The physiological and post-mortem findings in this animal, therefore, while in themselves of no localizing value, nevertheless concur with and find reasonable interpretation in the conclusion enunciated just now, viz. that the receptors are somewhere in the anterior part of the diencephalon.

We turn now to the effects of tying an internal carotid, or one or more of its primary branches, on the osmotic response and on the diencephalic distribution of the two carotid bloods.

(7) *Ligation of the internal carotid intradurally, and its effects on the osmotic release of antidiuretic hormone*

(*a*) *Experiments with 'Whitethroat', no.* 303

'Whitethroat' was an animal that had been provided with two carotid loops; and before the observations that we are about to describe were made the left kidney had been removed and, later, the abdominal lumbar sympathetic chains with the splanchnic nerves and left suprarenal gland had been excised.

Responses before intradural ligation of the left internal carotid artery. Over the period of 8 months before operation the renal responses, during water-diuresis, to the intracarotid infusion of hypertonic solutions of sodium chloride were determined on seventeen occasions. Those illustrated in figure 30*a* and *b* are typical. All the infusions were made at a constant rate of 1·03 ml./min and for a period of 10 min. In figure 30*a* is shown a big inhibitory response to the infusion of 1·71 M-NaCl into the right common carotid, and in figure 30*b* are shown the responses to the infusion of 1·71 and 1·37 M-NaCl into the left common carotid. The infusion of weaker solutions showed that bigger responses were obtained from the left than from the right carotid, e.g. 1·03 M-NaCl gave a definite response when infused into the left but no response or a mere trace of one when infused into the right carotid. Satisfactory responses from infusions into the right and left carotid were obtained 4 and 3 days before intradural ligation of the left internal carotid. To a description of the technique of this operation we now turn.

The surgical procedure adopted for ligation of the artery. As the surgical procedure for intradural ligation of the internal carotid is very similar to that for ligation of the anterior or middle cerebral or the posterior communicating artery, or of a combination of these, it will be described at this stage in general terms, and any significant variations therefrom will be mentioned in the context of individual experiments.

The day before the operation the top and the side of the head, including the ear, and the area of the right malleolar vein are shaved. The following morning the animal is anaesthetized with pentobarbitone sodium given intravenously in 5% solution in a dose of 33 mg/kg body wt. The animal is then placed in a prone position on the operating

theatre table, and the head fixed by a special clamp containing two horizontal rods that pass between the jaws and the distance between which can be adjusted; the degree of opening of the jaws can thus be varied during the operation. Further procedures are carried out with full asepsis precautions. After a cannula, attached to a 10 ml. burette, has been tied into the right malleolar vein, a midline sagittal incision is made through the

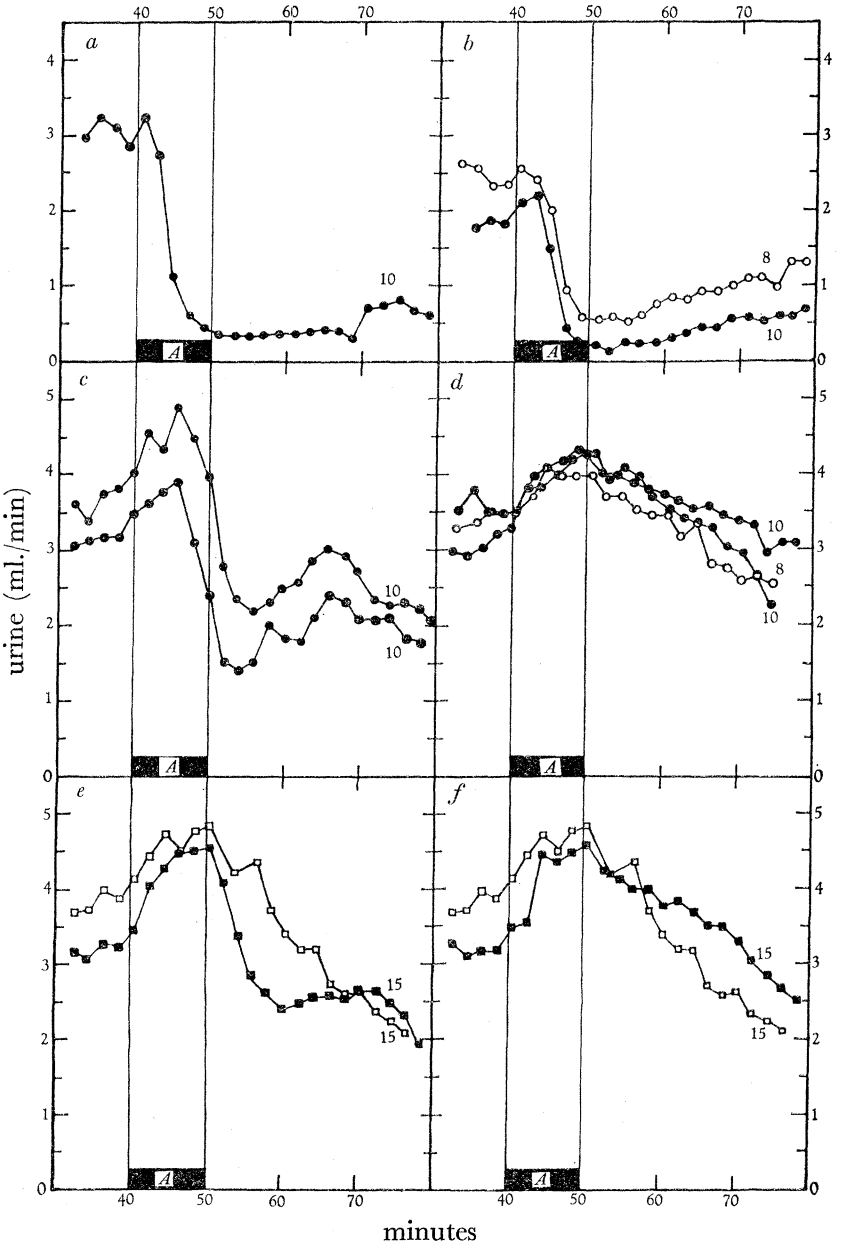

FIGURE 30. 'Whitethroat', no. 303. Responses to infusions of hypertonic solutions of sodium chloride during established water-diuresis. The infusions were made at 1·03 ml./min during the 10 min periods shown by the rectangles *A*. *a* and *c*, infusions into the right common carotid; *b* and *d*, infusions into the left common carotid. *a* and *b*, before the operation of intradural ligation of the left internal carotid; *c*, *d*, *e* and *f*, after operation. The figures on the graphs are the percentage strengths of the infusions. In *e* and *f* the black squares give the responses to infusions into the right and left carotid respectively, the open squares the response to an intravenous infusion. Abscissae: time after the test dose (350 ml.) of water.

skin of the vertex from the base of the nose (the stop) to the base of the occiput, and another incision at right angles to the first and passing over the anterior part of the zygomatic arch. The periosteum is then elevated from the zygoma, and four holes are drilled through the anterior and posterior ends of the arch in such positions that the ensuing saw-cuts lie between each of the two pairs of holes. The arch is cut through in two places so as to sever the middle three-fifths from the temporal and maxillary fifths (figure 31*a*). The temporal fascia is then divided about 1 cm from its attachment to the orbital fascia and to the frontal, sagittal and nuchal crests, and the muscle is reflected from the temporal fossa through the gap in the zygoma. This is assisted by full opening of the jaws, a movement which, at the same time, carries the coronoid process of the mandible ventrally and away from a position in which it was shielding the wing of the sphenoid. A small trephine hole is then made in

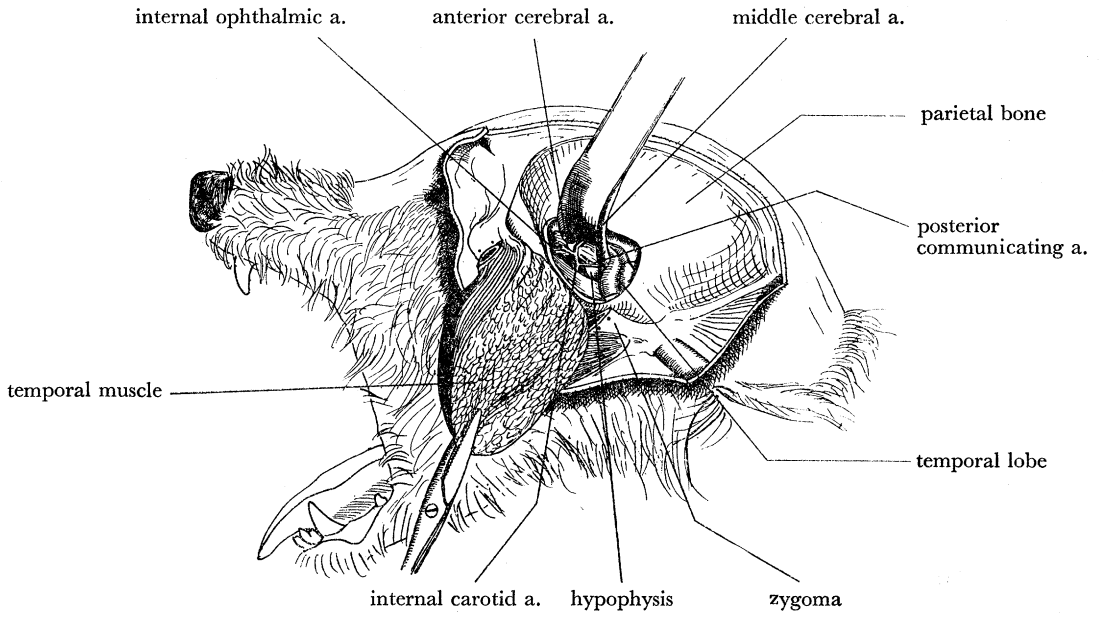

FIGURE 31*a*. To illustrate the intradural approach to the left internal carotid artery and its trifurcation. A photograph of the exposure is presented as figure 31*b*, plate 12.

the convexity of the parietal bone just anterior to the intracranial course of the middle meningeal artery, and the hole enlarged with rongeurs in anterior and ventral directions so as to form an oblong opening stretching forward to the orbital ridge and almost to the optic and orbital foramina, and with its lower edge at the level of the sella turcica. At this stage some 10 to 15 ml. of 30 % NaCl are slowly given intravenously in order to shrink the brain. The dura is pierced in the middle of the opening and divided by radiating incisions into several flaps which are then everted over the bone edges. In order to expose the region of the trifurcation of the internal carotid one of a series of silver-plated retractors shown in figure 32*a*, *b*, *g*, *h*, plate 12, is used. These, on the kind recommendation of Professor B. A. Houssay, had been specially shaped on a cadaver with the object of avoiding damage to the brain and, at the same time, of not obstructing the view of the pituitary region when the piriform lobe is lifted. By these means, and with the aid of suitable lighting (for this purpose a motor cycle head-lamp focused through a solution of

cupric sulphate, the whole being mounted on an adjustable stand, has proved very satisfactory), an excellent exposure and view of this region is obtained (figure 31*a* and *b*, plate 12). A blunt hook (figure 32*d*) is then carefully passed around the medial surface of the internal carotid just after its emergence from the dura, and is replaced by a tiny aneurysm needle (figure 32*f*) through which has been threaded a length of no. 000 silk. This instrument was made by mounting a fine sewing needle on a metal handle and filling most of the eye with solder so that a small hole was left near the blunt end of the needle. When the aneurysm needle has been removed, the thread is firmly tied by means of long and fine dissecting forceps, and the ends of the silk are cut short.

When one or more of the three primary branches of the internal carotid are to be tied, the procedure is as follows. An assistant occludes the ipsilateral common carotid by placing a 'bulldog' clip on the loop; and by means of the sharp hook shown in figure 32*e*, the pia-arachnoid membrane is pierced at the chosen site on either side of the vessel which is to be tied, and a track made behind and around it with the blunt hook *d* (figure 32). This is then replaced by the threaded aneurysm needle, the needle carefully withdrawn, and the small U-shaped instrument (figure 32*c*) is placed across the vessel in such a way that the silk thread lies in the grooves at the ends of the limbs of the U; the short end of the thread can then be withdrawn to a convenient distance without the thread rubbing against the medial surface of the vessel and incurring the risk of its rupture. The thread is then tied and the 'bulldog' clip removed from the carotid loop.

After the vessel has been tied in this way a little sterile saline is ejected by syringe over the surface of the brain and into the subdural space; and the dura flaps are replaced, but no attempt is made to sew them together. Sterile penicillin-sulphadimidine powder (1000 units penicillin to 1 g sulphadimidine) is then sprinkled on to the brain and dura and over the surface of all the other tissues exposed in the wound. The jaw is closed, and the temporal fascia repaired anteriorly and dorsally; posteriorly it is sewn to the thin layer of subcutaneous muscle so as to avoid undue pressure on the brain. The middle segment of the zygomatic arch is then replaced and fixed by means of silver wire sutures, the skin edges sewn together and the cannula is removed from the malleolar vein. In the next 48 h 40000 units of penicillin are given intramuscularly at intervals of 6 h. In our experience it is better not to give fluids parenterally in the immediate post-operative period, but rather to wait until with recovering consciousness the animal can take them by mouth and gradually satisfy its fluid needs.

'Whitethroat', whose left internal carotid was tied on 28 January 1949 by the method above described, was the first animal in which we had attempted such surgical procedures. Recovery was uneventful, and experiments on the osmotic release of antidiuretic hormone were resumed on 17 February 1949, i.e. 20 days after operation. The results of these experiments will now be described.

Responses after operation. Over the period of 4 months after operation the renal responses to infusions of sodium chloride, given under the same conditions as in the pre-operative period, were determined on eleven occasions. They are illustrated in figure 30*c* and *d*. Definite inhibitions were produced by the infusion of 1·71 M-NaCl into the right carotid (*c*, figure 30), but, as was expected, they were smaller than the pre-operative response to the same infusion because of the increase in blood flow through the right

internal carotid when the left internal carotid had been tied. Infusions into the left carotid however—infusions which, before operation, were producing big inhibitions of urine flow —now produced no inhibition whatsoever (*d*, figure 30). It is to be noted in addition that from the beginning of infusion the urine flow increased; this has been observed before during *intravenous* infusions of sodium chloride and of sucrose (Verney 1947; figure 34, graph B, and figure 39*a*, graph B) and seemingly derives from osmotic inhibition of the reabsorption of fluid in the proximal tubule, possibly coupled with an increase in glomerular filtrate flow associated with an increase in blood and extracellular fluid volumes; but its rapidity of onset is remarkable, an increase in urine flow being observed when as little as 1·5 mmoles have entered the circulation.This diuresis effect is to be seen during the early stage of the infusions into the right carotid (*c*, figure 30), then to become reversed by the accumulating secretion of antidiuretic hormone. When much stronger solutions (2·57 M-NaCl) were used, the release of antidiuretic hormone was still detected in the response to infusions into the right carotid (*e*, figure 30), whereas with the left carotid the course of urine flow was indistinguishable from that during and following an intravenous infusion (*f*, figure 30). We shall have occasion later to refer to this diuresis effect under conditions in which its extinction becomes a major index of the release of antidiuretic hormone. The point on which we wish to lay emphasis just now is that in this animal simple intradural ligation of one internal carotid artery completely and permanently suppressed the big inhibitions of water diuresis that were previously being produced by increase in the osmotic pressure of the common carotid blood of the same side. The results make unequivocal the conclusion reached by one of us elsewhere (Verney 1947) that the osmoreceptors lie in the vascular bed normally supplied by the internal carotid artery.

The cerebral distribution of the blood of the right and of the left common carotid was then determined, and to the technique and results of this investigation we now turn.

Tracing the cerebral distribution of the carotid blood, 4 *August* 1949. The method was similar to that already described, but, since this was one of the earliest experiments in which it was used, some measures were included in the procedure which were dropped from later experiments. We were at that time concerned at the possibility of a movement of blood in the brain, after death, distorting the true picture of the distribution of the suspensions; and so it was decided to try and fix the fluids in the head, immediately after the suspensions had been infused, by freezing it solid. For this purpose a mixture of ethanol and solid carbon dioxide was prepared with a temperature of −54° C. An apparatus was devised by means of which the nasopharynx and basisphenoid region were flooded with the freezing mixture immediately after the infusion of the coloured suspensions. The head was preserved at −10° C in 8% formaldehyde in 70% ethanol. Dissection showed that the site of operation was quite clean, and that there had been perfect healing of the zygomatic arch. The whole brain was embedded in celloidin and sectioned as previously described.

There can be no doubt that the technique was highly successful in fixing all the fluids of the head in the position occupied by them at death. Unfortunately, however, the brain tissue was so distorted and broken up by the freezing process that it was difficult to identify the nuclear groups in the hypothalamus. For this reason the freezing process was not used in further experiments. Despite this distortion the gross distribution of the suspensions in the brain could be quite well followed, and is illustrated in figure 33.

Blue suspension had been infused into the left carotid (the operated side) and black into the right carotid. As might have been expected, black has occupied the entire anterior and dorsal segments of the brain. Evidently the black suspension has been carried by the right carotid blood into the anterior and middle cerebral arteries of the left as well as of the right side and has been dispersed throughout their fields of distribution. The blue suspen-

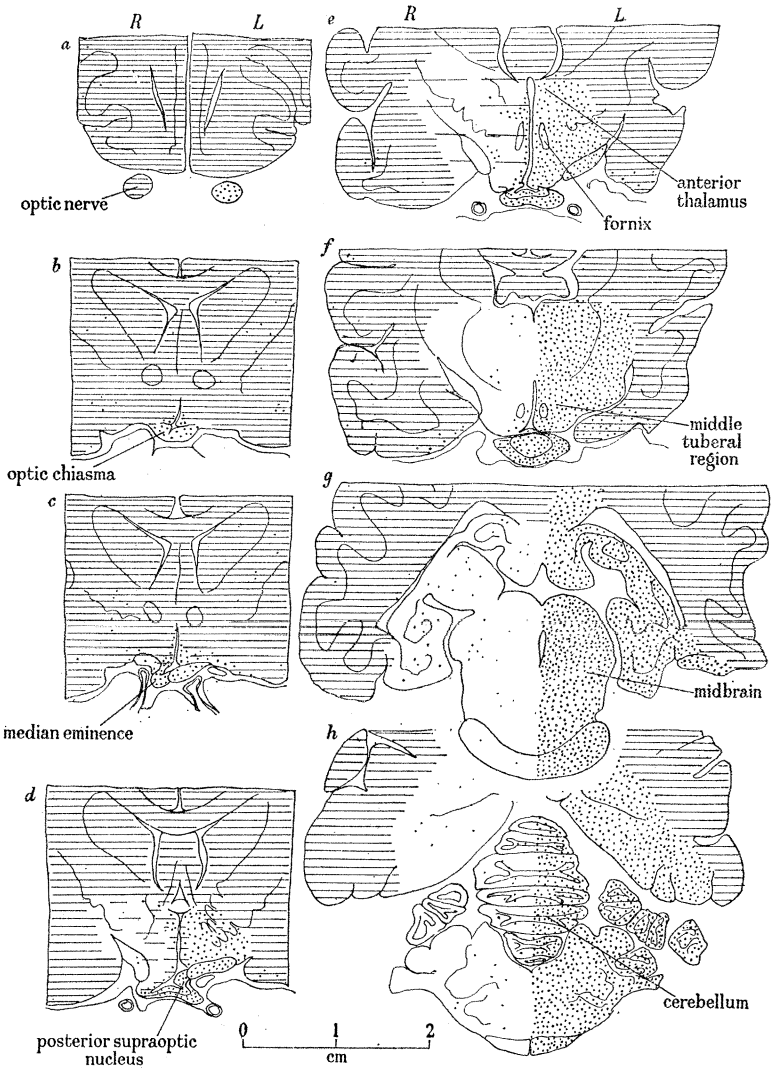

FIGURE 33. 'Whitethroat', no. 303. Maps of selected sections to show the cerebral distribution of the suspensions. Blue suspension infused into the left carotid—dots; black into the right—lines. The distances between the anterior surfaces of sections *a* and *b*, *b* and *c*, etc., are 9, 1·6, 1·6, 1, 1, 10 and 8 mm respectively.

sion (except in rare and minute traces) has been excluded from these fields, but it has not been entirely excluded from the brain. Blue suspension occupies the left half of the posterior brain stem. Its most anterior extent is in the optic chiasma, whence its field of distribution widens out posteriorly to include the posterior hypothalamus, thalamus, midbrain, medulla and cerebellum. These latter areas are those normally supplied by vertebral blood. The blue suspension is also dispersed in the gyri of the left hemisphere that rest on the tentorium osseum. This blue-injected field is closely matched on the right side by a region almost entirely devoid

of suspension (figure 33*d–h*). The explanation of the appearance of the blue suspension in these areas of 'Whitethroat's' brain is, as is apparent from the maps, that it has passed from carotid to vertebral trees through the occipito-vertebral anastomosis. It will be observed that no blood was passing from the right carotid through its occipito-vertebral anastomosis; the changes in pressure-flow relationships in the carotid and vertebral arteries resulting from intradural ligation of the left internal carotid would favour a relative enhancement of the left anastomotic flow. Incidentally, the absence of right carotid blood from the normal vertebral field and the presence of antidiuresis responses to infusions into the right carotid is in keeping with the findings in 'Paris' (p. 241) and 'Toby' (p. 244), findings which indicated that the receptors lay in the *anterior* region of the prosencephalon. Moreover, the facts that the vertebral field in 'Whitethroat' includes the anterior thalamus and its nuclei—on the left side this region was well injected with the blue suspension, on the right side sparsely so—and that right carotid blood is absent from this field, affords strong evidence against the thalamus being the part of the anterior region of the diencephalon in which the receptors lie. There are three more points of interest in the gross distribution of the suspensions. First, only blue suspension is present in the posterior lobe; this fact again confirms our earlier conclusion that the osmoreceptors are not in the posterior lobe. Secondly, it will be noticed that the left optic nerve carries blue suspension only, suggesting that the direction of flow in the left internal ophthalmic artery is now towards the brain. Before this animal was killed the retinae were observed with an ophthalmoscope when injections of 1 ml. 1 % Evans's blue in 0·85 % NaCl were rapidly made into the left common carotid trunk; and it is of interest that the dye was seen quite clearly to sweep through the left retinal vessels, but that none crossed to reach the right retina. When, however, the right common carotid was occluded, then the dye reached the right retina from the left common carotid. The results are the same as those obtained in animals (Verney 1947) in which there had been no permanent interference with the carotid circulation. This is readily explicable by the anatomy of the external and internal ophthalmic arteries and the ophthalmic anastomosis from which the arteria centralis retinae arises; when the right common carotid was occluded the dye was carried by the left ophthalmic anastomosis into the left anterior cerebral artery and thence through the anterior communicating vessel into the right internal ophthalmic artery and so to the right retina. Thirdly, the optic chiasma carries blue suspension only. As we have seen, it derives its blood supply from the intercarotid anastomosis; and examination of the serial sections showed quite clearly that the origin of the anastomosis from the left internal carotid was on the cardiac side of the ligature and that the vessel was carrying blue suspension from left to right. The medium eminence, too, carries blue suspension only; and this suspension is present throughout the pars distalis, in the right lateral quarter of which there is a little black suspension as well. Moreover, the blue suspension in the left hypothalamus has extended to the ventro-medial region of the right hypothalamus (cf. the 'cone' of hypothalamic tissue supplied with carotid blood and illustrated in figures 11, 12 and 15*A*). The appearances show, therefore, that the blood flow from the left carotid into the intercarotid anastomosis, and into the other members of its group having a similar origin, is preponderating over the flow from the right carotid into its corresponding branches; this would be expected from haemodynamic considerations.

The minute distribution and partition of carotid and vertebral blood to the nuclear groups of the hypothalamus of 'Whitethroat' was, however, difficult to determine, first (as already stated) because of the derangement of structure produced by the freezing process, and secondly because of the carriage of blue suspension through the occipito-vertebral anastomosis into the left vertebral field. The indications from the sections are: (1) that the anterior divisions of both supraoptic nuclei carry mainly black suspension and that blue suspension is present as well in the more posterior parts of the nuclei; (2) that the right posterior division contains a mixture of black and blue suspension in its antero-medial extent and that as it spreads laterally with the optic tract it contains sparse blue suspension only; and (3) that the left posterior division is well injected with the blue suspension. There is, however, much uncertainty in these inferences. It was not possible to determine the position of the paraventricular nucleus.

Discussion. These indications of the distribution of the suspensions in the supraoptic nuclei become intelligible in terms of: (1) the supply of right carotid blood to both right and left anterior and middle cerebral arteries; (2) the supply to the intercarotid anastomosis (the left origin of which, as will be remembered, we failed to occlude) being predominantly from the left carotid; and (3) the carriage forward by the left posterior communicating artery of vertebral blood to contaminate the middle cerebral supply to the anterior division of the left nucleus, this vertebral blood itself containing blue suspension that has been conveyed to it by the left occipito-vertebral anastomosis. The physiological effects of simple intradural ligation of 'Whitethroat's' left internal carotid on the responses to a raised osmotic pressure of the carotid blood, viz. a big dimunition in the response to right-sided infusions and extinction of the response to left-sided infusions, are compatible, on the basis of the cerebral distribution pattern of the carotid and vertebral bloods, with the hypothesis that the site of the receptors is the anterior hypothalamic region. On the other hand, if the receptors are in or in the region of the supraoptic nuclei it seems strange that, with the posterior division of the left nucleus carrying blue suspension only, no antidiuresis response was obtainable from infusions into the left carotid artery; though it is impossible to say how much of this division was being supplied with vertebral blood and the degree to which this was being contaminated with carotid blood reaching it through the occipito-vertebral anastomosis. Now 'Whitethroat' was an oldish bitch—she was fully grown when admitted to the Department and had been here nearly $6\frac{1}{2}$ years when her internal carotid was tied—and infusions of rather high concentration were needed to elicit antidiuresis responses. It seemed, then, on the basis of the observed cerebral distribution of the suspensions, that were the experiment repeated with a younger animal the contamination of the vertebral blood with carotid blood passing through the occipito-vertebral anastomosis might be sufficient to elicit a small residual response when hypertonic solutions were infused on the operated side, and that this response would disappear when the anastomosis had been tied. Incidentally, it was hoped that the results of such an experiment would also provide evidence for or against the inference from 'Whitethroat' that the receptors are not in the thalamus.

Owing to uncertainty and imprecision in the mapping of the distribution of the suspensions in the hypothalamic region of 'Whitethroat's' brain it was planned to make an experiment similar to that on 'Whitethroat', and to see whether a small residual

response was detectable from infusions into the left common carotid trunk after the left internal carotid had been tied intradurally; if such response was found, the distribution of the carotid bloods was to be determined with the omission of the freeze technique. Another animal could then be taken, and the program extended to include the effect of occlusion of the left occipito-vertebral anastomosis. Thereafter the distributions of the carotid bloods in the two animals were to be compared. We shall now describe the results obtained from the following of this program. Actually two animals ('Regan' and 'Root') were used in the first part of the investigation and one ('Doris') in the second. All were prepared in the usual way by perineotomy and the formation of two carotid loops.

(*b*) *Experiments with 'Regan', no.* 400

Responses before intradural ligation of the left internal carotid artery. During the 3 months before operation six infusions of hypertonic sodium chloride were made into the right carotid and nine into the left. The responses to infusions into the right carotid were much greater than those to infusions into the left. They are illustrated in figure 34. Each infusion was at the rate of 1·05 ml./min over a period of 10 min. The responses shown in figure 34*a* are to infusions into the right carotid of 0·68M-NaCl (graph B) and 1·37M-NaCl (graph C), and the response shown in figure 34*b* is to an infusion into the left carotid of 2·06M-NaCl.

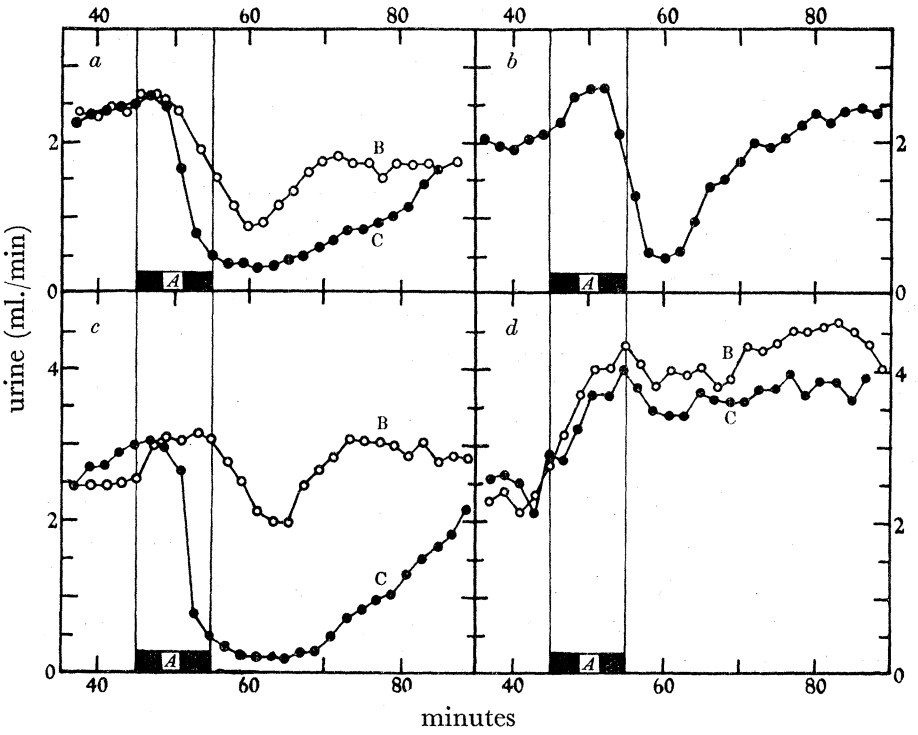

FIGURE 34. 'Regan', no. 400. Responses to infusions of hypertonic solutions of sodium chloride during established water-diuresis. The infusions were made at 1·05 ml./min during the 10 min periods shown by the rectangles *A*. *a* and *b*, before intradural ligation of the left internal carotid; *c* and *d*, after operation. *a*, infusions into the right carotid: 0·68M, graph B; 1·37M, graph C. *b*, infusion into the left carotid, 2·06M. *c*, infusions into the right carotid: 1·37M, graph B; 1·71M, graph C. *d*, infusions of 2·57M into the left carotid, graph C, and intravenously, graph B. Abscissae: time after the test dose (400 ml.) of water.

The responses to left carotid infusions were unfortunately so small that it seemed likely they would disappear whichever internal carotid were tied. We felt, however, more concerned at this stage with evidence for or against a thalamic site for the receptors than with evidence for a residual response to an intracarotid infusion when the ipsilateral internal carotid had been tied, so the left internal carotid was tied intradurally by the technique previously described. Infusion experiments were started again $3\frac{1}{2}$ weeks after the operation.

Responses after operation. Infusions into the right carotid now gave, as was expected, responses much smaller than those obtained before operation. They are illustrated in figure 34*c*, where the graph B is the response to an infusion of 1·37 M-NaCl (cf. figure 34*a*, graph C) and graph C is that to an infusion of 1·71 M-NaCl. The small response obtained before operation to an infusion of 2·06 M-NaCl into the left carotid is now completely abolished (figure 34*d*, graph C), the effect of the infusion being indistinguishable from that of the same infusion given intravenously (figure 34*d*, graph B). Later, and by the technique previously described, the animal was killed during the intracarotid infusion of the coloured suspensions, the blue suspension being infused into the right carotid, the black into the left; and sections were made of the celloidin-embedded brain.

Tracing the cerebral distribution of the carotid blood. The occipito-vertebral anastomoses appeared to be small vessels, and they were carrying no appreciable amount of carotid blood into the vertebral streams, as is shown by the fact that the basilar and anterior cerebellar arterial fields are practically free from suspensions. The right cerebral hemisphere is liberally supplied with blue suspension whilst the thalamus of this side is sparsely so injected. Examination of the ligature on the left internal carotid showed that while it constricted the vessel it did not occlude it. The supply to the left side of the brain had thus come to be shared between left carotid and vertebral flows. The results from this animal, although affording no diencephalic evidence of an exclusion character (except to suggest, from the somewhat sparse injection of the right thalamus, that the receptors are not in this region), are, nevertheless, consistent with the view that the receptors are in the hypothalamus. For this region was, on the right side, well and exclusively supplied with right carotid blood and responses to right-carotid infusions were retained, while on the left side the supply to this region was by no means confined to the ipsilateral carotid. Both vertebral and right-carotid bloods had reached the left hypothalamus, and no responses were obtained from infusions into the left carotid. Incidentally, the posterior lobe was injected exclusively with the black suspension, a fact which again confirms the conclusion initially reached with 'Molly', p. 237. In the hope of obtaining information of a more defining character we tried another experiment of similar nature, and the results obtained with 'Root', no. 432, will now be given.

(*c*) *Experiments with 'Root', no.* 432

Responses before intradural ligation of the right internal carotid artery. During the three weeks before operation two infusions of hypertonic sodium chloride were made into the right carotid and four into the left. The responses to infusions into the right carotid were much greater than those to infusions into the left. They are illustrated in figure 35*a* and *b*. Each infusion was at the rate of 1·05 ml./min over a period of 10 or 15 min. The response shown in figure 35*a* is to an infusion into the right carotid of 1·20 M-NaCl; a similar infusion of

0·86M-NaCl also gave a well-marked response. On the left side no response was given to one infusion of 0·86M-NaCl, and only minimal responses to infusions of 1·20M, 1·71 and even 2·05M-NaCl. The response to an infusion of 2·05M-NaCl is given in figure 35*b*. We then decided to tie the right internal carotid intradurally. If the well-marked response from right-sided infusions was thereby suppressed and the small response from left-sided infusions also vanished, the theory of specific receptors in the anterior diencephalon (carotid field) would clearly be jeopardized. The previously described technique for ligating the internal carotid intradurally was adopted. The vessel had a short trunk and it was successfully tied just proximal to its junction with the posterior communicating artery. The animal's complete return to health was uneventful—up to the third day after operation when, in walking, it decided to turn round it did so by wheeling to the right—and infusion experiments were started again 3 weeks after the operation and continued over the next 10 weeks.

Responses after operation. No response could now be obtained to infusions into the right carotid. Tests were made with 1·20 M and 1·71 M-NaCl, each solution being infused at the rate of 1·05 ml./min for 15 min; and the result of the latter infusion is shown by the graph B in figure 35*c*; it is not distinguishable from that to the intravenous infusion of the same solution (figure 35*c*, graph C). Tests were also made with more rapid infusions for a much shorter period. The result of one of these is shown in figure 35*e*, where at the arrow *A* 0·51M-NaCl was infused for 20 s at the rate of 0·45 ml./s; no inhibition of urine flow followed the infusion. When this experiment was repeated with 0·68M-NaCl a small inhibition followed (similar to that produced by an intravenous injection of posterior pituitary extract of the order of 0·5mU), but as the animal began to whine 9 s after the start of the infusion and persisted in doing so till just after its end, the accompanying release of antidiuretic hormone was evidently caused by emotional stress and not specifically by the rise in the osmotic pressure of the carotid blood. As in the two previous animals ('Whitethroat' and 'Regan'), therefore, intradural ligation of the internal carotid completely suppressed the responses that were being obtained beforehand to hypertonic infusions into the ipsilateral carotid trunk. On the left side, however, the responses which, before operation, were minimal (figure 35*b*), *now became definitely larger.* A small response was sometimes seen even to 0·86M-NaCl, and quite large responses were given by 1·20 and 1·71M-NaCl. The response to the infusion of this last solution is given in figure 35*d*; it should be compared with the minimal effect of similar infusions before operation (figure 35*b*). Moreover, the rapid infusion of 0·51M-NaCl (0·45 ml./s for 20 s) which, as we have seen (figure 35*e*), produced no response when given into the right carotid, caused a definite inhibition of urine flow when given into the left (figure 35*f*); there was no emotional disturbance associated with the infusion and the animal was apparently quite unaffected by the procedure. Briefly, then, the effects of intradural ligation of the right internal carotid in this animal have been to suppress the responses to ipsilateral infusions and to increase markedly those to contralateral infusions. On this animal we also made, by the technique described earlier (p. 235), a few experiments involving simultaneous infusion on the one hand into each carotid, and on the other into one carotid and the saphenous vein. We shall refer to these in the final discussion (p. 314).

Tracing the cerebral distribution of the carotid blood. Four days after the last of the above observations had been made, the animal was killed by the usual technique during the

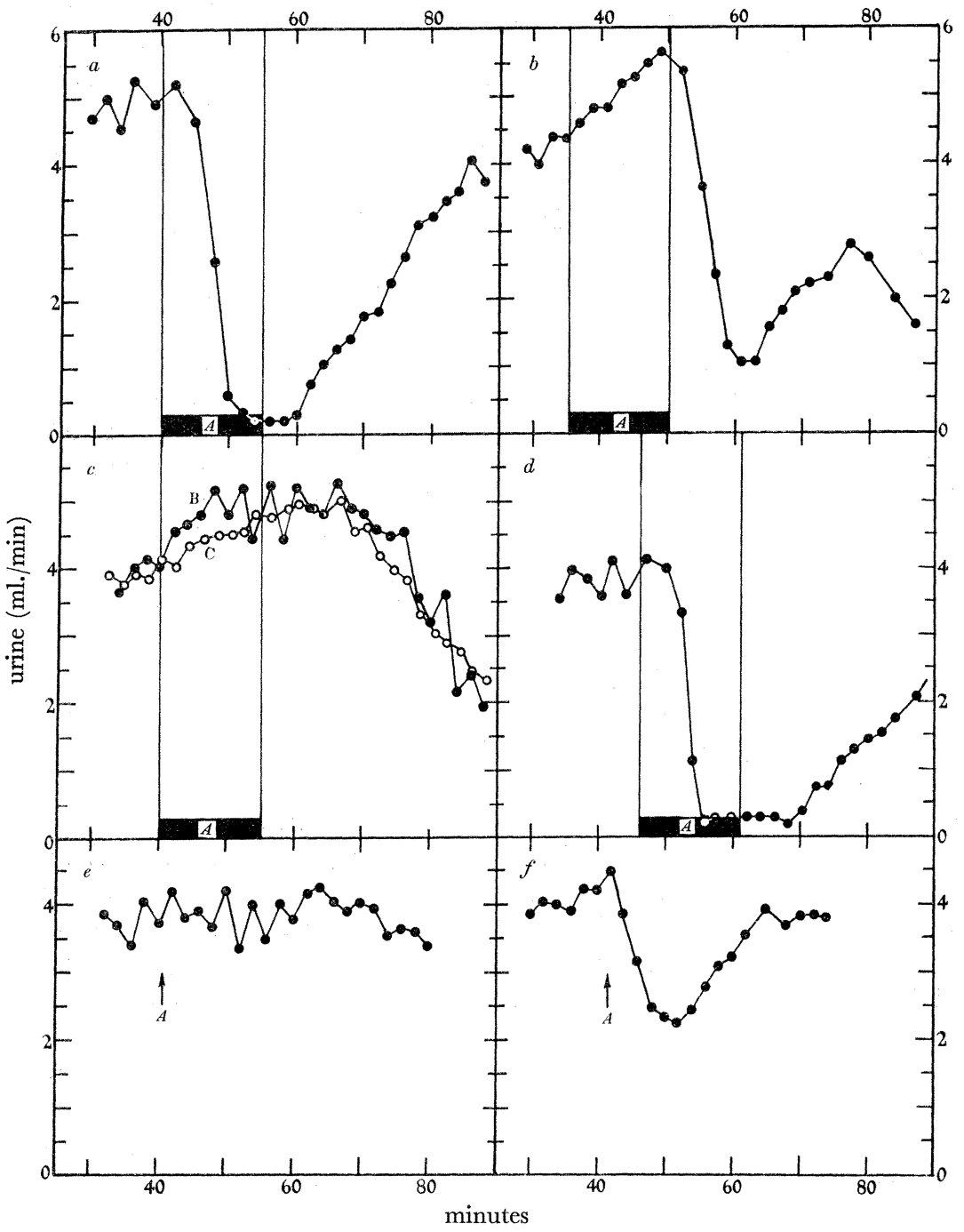

FIGURE 35. 'Root', no. 432. Responses to infusions of hypertonic solutions of sodium chloride during established water-diuresis. The infusions during the 15 min periods shewn by the rectangles *A* were made at 1·05 ml./min. *a* and *b* before, *c*, *d*, *e* and *f* after intradural ligation of the right internal carotid. *a*, infusion into right common carotid, 1·20M. *b*, infusion into left common carotid, 2·05M. *c*, infusion of 1·71M into right common carotid (graph B) and into malleolar vein (graph C). *d*, infusion into left common carotid, 1·71M. *e*, infusion into the right common carotid at the arrow *A* of 0·51M at the rate of 0·45 ml./s for 20 s. *f*, the same infusion as in *e*, but given into the left common carotid. Abscissae: time after the test dose (*a*, *b*, *c* and *d*, 450 ml.; *e* and *f*, 400 ml.) of water.

intracarotid infusion of coloured suspensions (figure 9 plate 10). In this instance we used as the black suspension Messrs Acheson Colloids' colloidal graphite (solid content about 7 % in water). The blue suspension (Monastral fast blue) was infused into the left carotid, the black into the right, and the animal was killed by the intracardiac injection of chloroform at the eighth second after the suspensions had reached the carotid needles. The brain-sections revealed the following features.

The wall of the internal carotid within the ligature is very thin, but the lumen has not been completely occluded, there being a residual channel about 100μ in diameter. The origin of the intercarotid anastomosis on the right side could not be identified.

With respect to degenerative changes in the cerebrum, there is slight superficial damage to the right pyriform lobe and distortion of its cortex with some cell loss. No cystic lesion is present, but in the diencephalon there is, on the right side, distinct cell loss in the nuclei parataenialis, centralis medialis, paracentralis, centralis lateralis, reuniens and dorso-medialis. There is no such loss in the thalamic paraventricular nucleus.

Examination of the general distribution of the suspensions shows that neither the black nor the blue suspension has been carried by the occipital arteries into the vertebral fields. The basilar blood is therefore unmarked. Black suspension (from right carotid) is present in the middle cerebral arterial field (lateral hemisphere and striate body) of the right side in quantity, having reached this site through the small ligature-escaping lumen in the right internal carotid, and through the right internal ophthalmic artery, the lumen of which contains this black suspension. The black suspension is present, too, but as a mere trace, in the lateral hemisphere of the left side, and has probably reached there through the posterior-lobe anastomotic artery (the posterior lobe is exclusively black-injected) from right to left internal carotid. There is marked left-carotid dominance in the cerebral carotid distribution, the blue suspension alone occupying both anterior cerebral fields, and this suspension has been carried posteriorly in the left posterior communicating vessel to mix with unmarked basilar blood at the level of the left posterior cerebral artery, the field of which is sparsely blue-injected.

In the diencephalon (figure 36) the distribution is as follows. On the right side the entire thalamus (except for a trace at its anterior limit) and the posterior hypothalamus (from the mid-infundibular plane) are devoid of suspension, but more anteriorly the medial part of the hypothalamus is blue-injected. On the left side the posterior thalamus, at the level of the habenular nuclei, is very sparsely injected with the blue suspension and the injection is only a little denser at the level of the mid-dorso-medial nucleus. Anterior to this the left thalamus is patchily but more heavily injected with the blue suspension slightly contaminated with the black. The left middle and posterior hypothalamic regions carry the blue contaminated with the black suspension, but more anteriorly the hypothalamus is well injected, and exclusively so, with the blue suspension.

We may now enumerate in more detail the structures in the anterior hypothalamus (see figure 36) reached by the black and the blue suspensions, it being recollected that after operation no responses were obtainable from infusions into the right carotid (black-injected), while the responses to infusions into the left (blue-injected) were increased. On the right side the lateral preoptic area, the lateral hypothalamic area (anterior), the dorsal hypothalamic area (anterior) and the perifornical area are black-injected. The anterior

division of the supraoptic nucleus is well injected with the black suspension and contains a trace of the blue in its antero-medial aspect, and the paraventricular nucleus has black suspension in its postero-dorsal part only. The posterior division of the supraoptic nucleus and the anterior hypothalamic area are devoid of black suspension. On the left side all

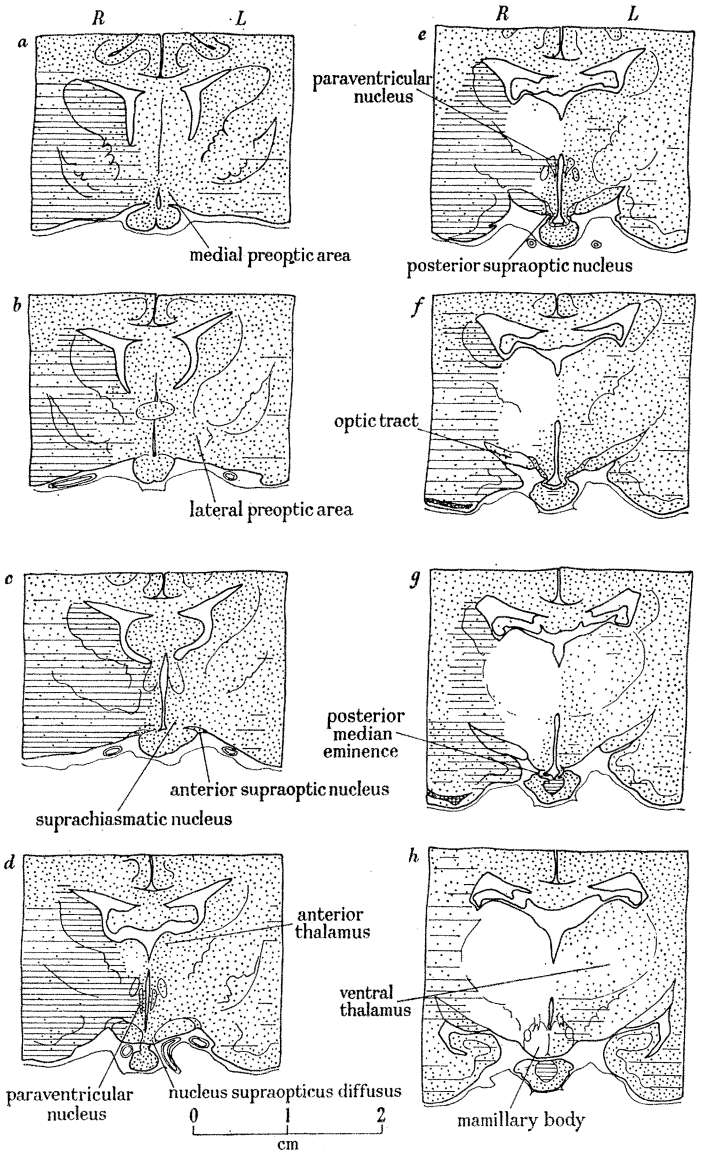

FIGURE 36. 'Root', no. 432. Maps of selected sections to show the diencephalic distribution of the suspensions. Blue suspension infused into the left carotid—dots; black into the right—lines. The distance between the anterior surfaces of the sections *a* and *b*, *b* and *c*, etc., is 1 mm except between *g* and *h* where it is 3 mm.

nuclei and areas of the anterior hypothalamus have received almost exclusively the blue suspension. The supraoptic nucleus (both anterior and posterior divisions) and the paraventricular nucleus are well and exclusively injected with the blue suspension. In addition, and on the *right* side, the posterior division of the supraoptic nucleus, the anterior hypothalamic area and periventricular system and the antero-ventral part of the

paraventricular nucleus are also well and exclusively injected with the blue suspension, and a trace of this extends forward into the suprachiasmatic nucleus. The supraoptic nuclear volume and the partition of the suspensions within it were measured by the method previously described. The results are given in figure 37; as much as 76% of the total volume was occupied exclusively by the blue suspension (see later, table 2, p. 280).

Discussion. The marked left-carotid dominance in the cerebral fields of distribution of the two internal carotids after the right internal carotid had been tied implies a post-operative increase in the volume flow of blood in the left internal and common carotid arteries. As a consequence of this the receptors in the left internal carotid field would receive from a given left-carotid infusion a smaller stimulus than before operation, and the antidiuretic response would be expected to diminish. Such a phenomenon was, it will be remembered, seen with 'Regan' and 'Whitethroat', the two previous animals subjected

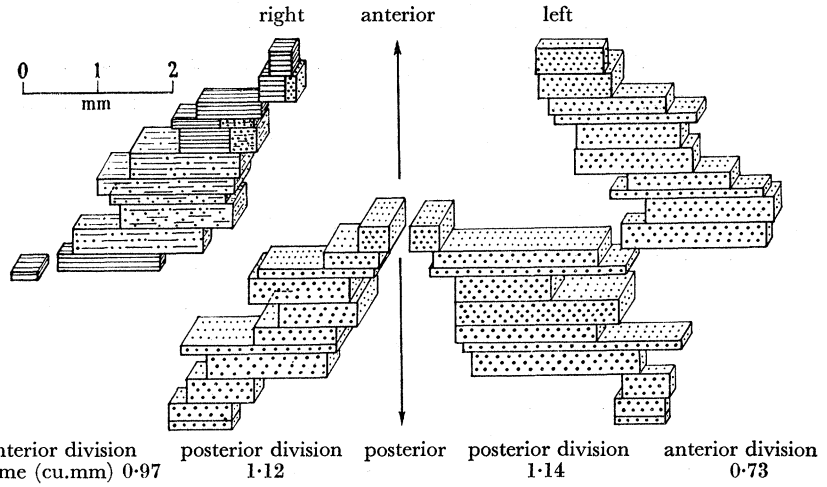

FIGURE 37. 'Root', no. 432. Plan and isometric projection of the series of blocks of nuclear material comprising the supraoptic nucleus, and the distribution of the suspensions within them. Black suspension—thin parallel lines. Blue suspension—dots.

to intradural ligation of one internal carotid artery. With the present animal ('Root'), however, the small response to left-sided infusions did not vanish when the contralateral internal carotid was tied; on the contrary, the response became much bigger. The only reasonable inference from this finding is that there has been a concurrent and large encroachment of the osmoreceptive field of the left internal carotid into that of the right. Now the maps of the diencephalic distributions of the suspensions (figure 36) show a conspicuous trespass of the left carotid blood into the right anterior hypothalamus, and it is difficult to resist the conviction that this trespass gives the structural basis for the observed post-operative increase in osmotic response to left-sided intracarotid infusions and concurrent disappearance of response to right-sided infusions.

It is of interest, too, to compare the effects seen in this animal with those seen in 'Paris' (p. 241). In 'Paris', it will be recollected, ligation of both occipital arteries was followed by a diminution of response to infusions into each common carotid trunk, and this was associated with a backward extension of the blood of each internal carotid artery to the posterior limb of the circle of Willis. Each carotid, then, had largely usurped the vertebral

territory of the diencephalon (see p. 222), and the observed decrease in osmotic response from infusions on either side is explicable in terms of the associated increase in carotid flows unaccompanied by any appreciable change in the carotid domain of the osmoreceptive field. In 'Root', on the other hand, an increase in the one carotid flow following ligation of the contralateral internal carotid was associated with a big increase in osmotic response to ipsilateral infusions. The proportion of total osmoreceptive field supplied by the ipsilateral internal carotid, therefore, was then increased; and we have already given facts that point to this extension being in the contralateral anterior hypothalamus. The evidence from this animal, then, is not only consistent with, but strongly supportive of the view that the receptors are in the anterior hypothalamus.

In none of the three animals ('Whitethroat', 'Regan' and 'Root') subjected to intradural ligation of one internal carotid was a residual response to ipsilateral infusions into the common carotid trunk detected. In conformity with our program, a fourth animal ('Doris', no. 379) was therefore taken, and, as we shall see later, after intradural ligation of an internal carotid a small residual response to ipsilateral intracarotid infusions was detected. To the results of experiments made on this animal we shall now turn.

(d) Experiments with 'Doris', no. 379

Responses before intradural ligation of the left internal carotid artery. During November and December 1950 the renal responses to intracarotid infusion of hypertonic solutions of sodium chloride over a period of 10 min. were measured. The responses are illustrated in figure 38. During the period *A* on the left-hand side of the figure 0·93 M-NaCl was infused

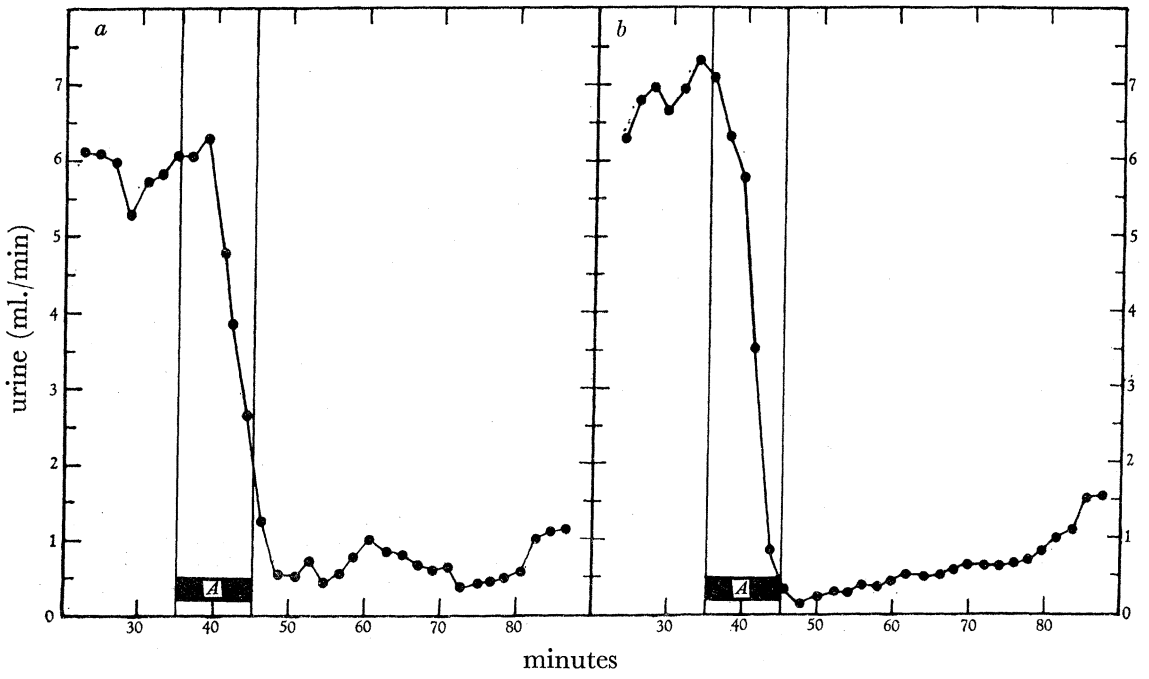

FIGURE 38. 'Doris', no. 379. Responses to infusions of hypertonic solutions of sodium chloride during established water-diuresis and before intradural ligation of the left internal carotid. The infusions were 0·93 M and were made at 1·05 ml./min during the 10 min periods shown by the rectangles *A*. *a*, infusion into the right common carotid; *b*, infusion into the left common carotid. Abscissae: time after the test dose (350 ml.) of water.

into the right carotid at the rate of 1·05 ml./min., and during the period *A* on the right-hand side of the figure the same infusion was made into the left carotid. In both instances there is a big and long-lasting inhibition of urine flow; and effects very like these were obtained in another and identically conducted pair of experiments. The constancy of these effects having been established, we proceeded to the intradural tying of the left internal carotid. This was done on 2 February 1951, the procedure closely following that adopted with 'Whitethroat'. Recovery was rapid and uneventful. Experiments were resumed a fortnight after operation, and nineteen observations were made over the ensuing 2-month period.

Responses after operation. These are illustrated in figure 39. Comparison of the responses given in *a* and *b* with those in figure 38 will show that the effect of the operation has been to reduce the response to an infusion of 0·93M-NaCl into the right carotid (*a* in the figures), and to abolish that to the same infusion into the left (*b* in the figures); after intradural ligation of the left internal carotid, the effects of an infusion of this strength into the left carotid trunk are indistinguishable from those of an intravenous infusion (*b*, figure 39). The results are similar to those obtained with 'Whitethroat', 'Regan' and 'Root'. When, however, the strength of the solution was increased to 1·28M a difference was detected between the effects of its infusion into the left carotid and into the malleolar vein (*d*, figure 39). During the period of the intravenous infusion the urine flow continuously increased; but during that of the intracarotid infusion, if any increase occurred at all, it was quickly followed by a progressive decline. And there remained a suggestion of such difference when the strength of the infusion was raised yet further to 1·71M (*f*, figure 39). We suspected that this difference between the effect of an intravenous and that of a left intracarotid infusion was owing to contamination of the vertebral blood with carotid blood reaching it through the left occipito-vertebral anastomosis. It was decided, therefore, to tie the left occipital artery and to redetermine the responses afterwards.

On 19 April 1951 the bifurcation of the left common carotid was exposed through a midline incision over the thyroid cartilage; and the sinus nerve, the internal carotid with the carotid sinus, and the occipital artery were all carefully identified. The internal carotid and the carotid sinus seemed to be much smaller than usual, and the occipital artery much larger than usual, and larger than the normal internal carotid. The occipital artery was freed for a distance of 10 to 15 mm from its origin and double ligatured midway along the freed portion. Recovery from the operation was rapid and uneventful, and observations on the animal were resumed a week later.

Responses after ligation of the left occipital artery. The results of intracarotid and intravenous infusions are illustrated in figure 40. In *a* is shown the response to the infusion of 1·28M-NaCl into the right carotid; it is not appreciably different from that to the same infusion before operation (*c*, figure 39). When, however, this infusion is made into the left carotid the course of urine flow has now become indistinguishable from that produced by the intravenous infusion (*b*, figure 40), and this indistinguishability still holds when the strength of the infusion is raised to 1·71M (*c*, figure 40). The effects of tying the left occipital artery, therefore, has been to suppress the small difference which was previously detected between the response to left intracarotid and that to intravenous infusion (*d*, figure 39); and the inference follows that, before operation, some carotid blood with

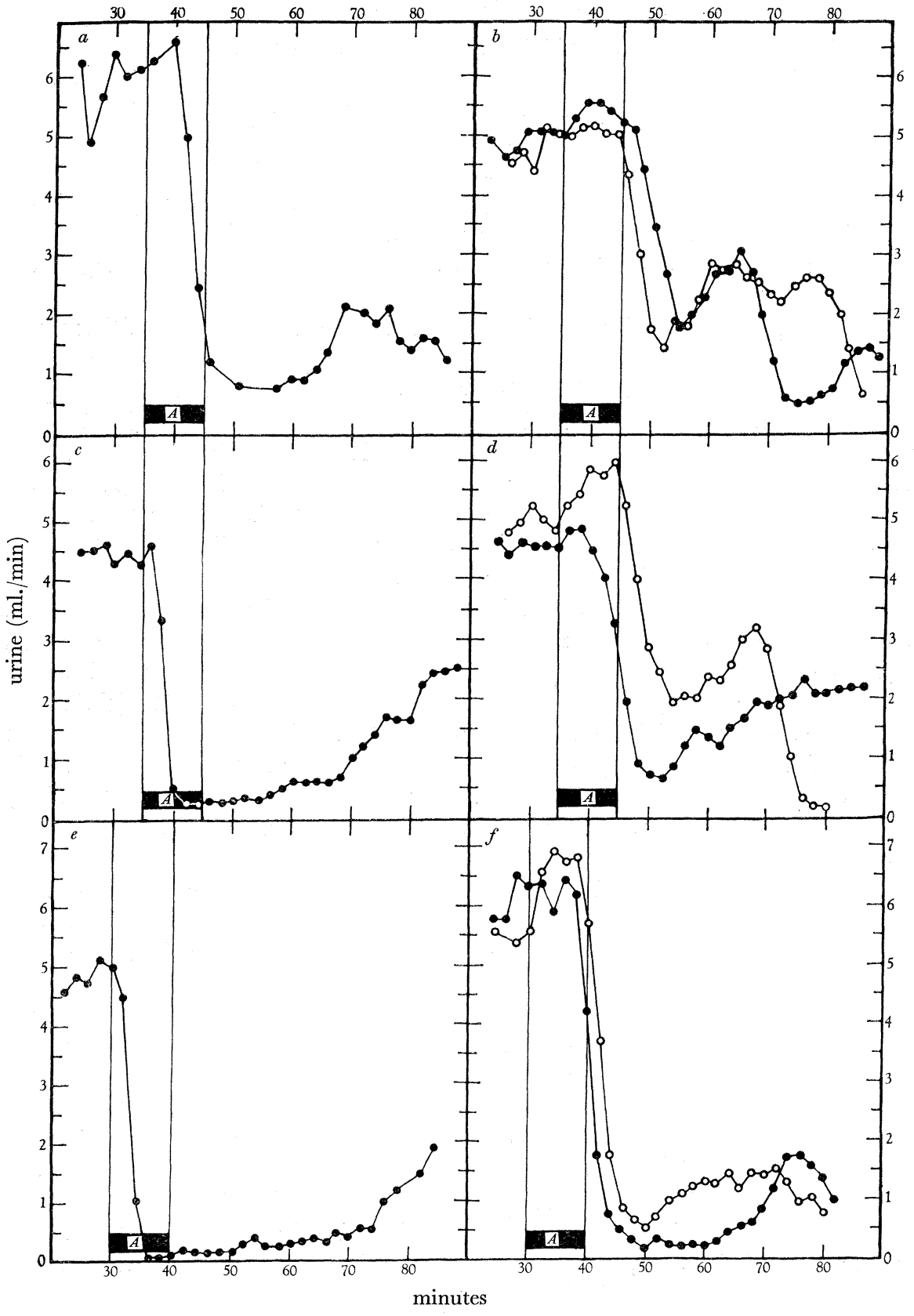

FIGURE 39. 'Doris' no. 379. Responses to infusions of hypertonic solutions of sodium chloride during established water-diuresis and after intradural ligation of the left internal carotid. The infusions were made at 1·05 ml./min during the 10 min periods shown by the rectangles *A*. *a*, *c* and *e*, infusions into the right common carotid; *b*, *d* and *f*, infusions into the left common carotid (black circles) and into the malleolar vein (open circles). The strengths of the infusions in *a* and *b* were 0·93M, in *c* and *d*, 1·28M, and in *e* and *f*, 1·71M. Abscissae: time after the test dose (350 ml.) of water.

raised osmotic properties was being carried to the osmoreceptors by the vertebral arterial stream. It was now decided to trace, in this animal, the cerebral distribution of the bloods in the two carotid trunks.

Tracing the cerebral distribution of the carotid blood. The method was as described on p. 217, the animal having received the same preliminary treatment and being placed in the same position as in an experiment in which the responses to intravascular infusions had been measured (see figure 9, plate 10). Blue suspension was infused into the left carotid and black into the right. The animal was killed at the seventh second (30 min after the last dose of water) by the intracardiac injection of chloroform, the heart rapidly incised, and the head was removed and placed in ethanol-formaldehyde-acetic acid fixative. On

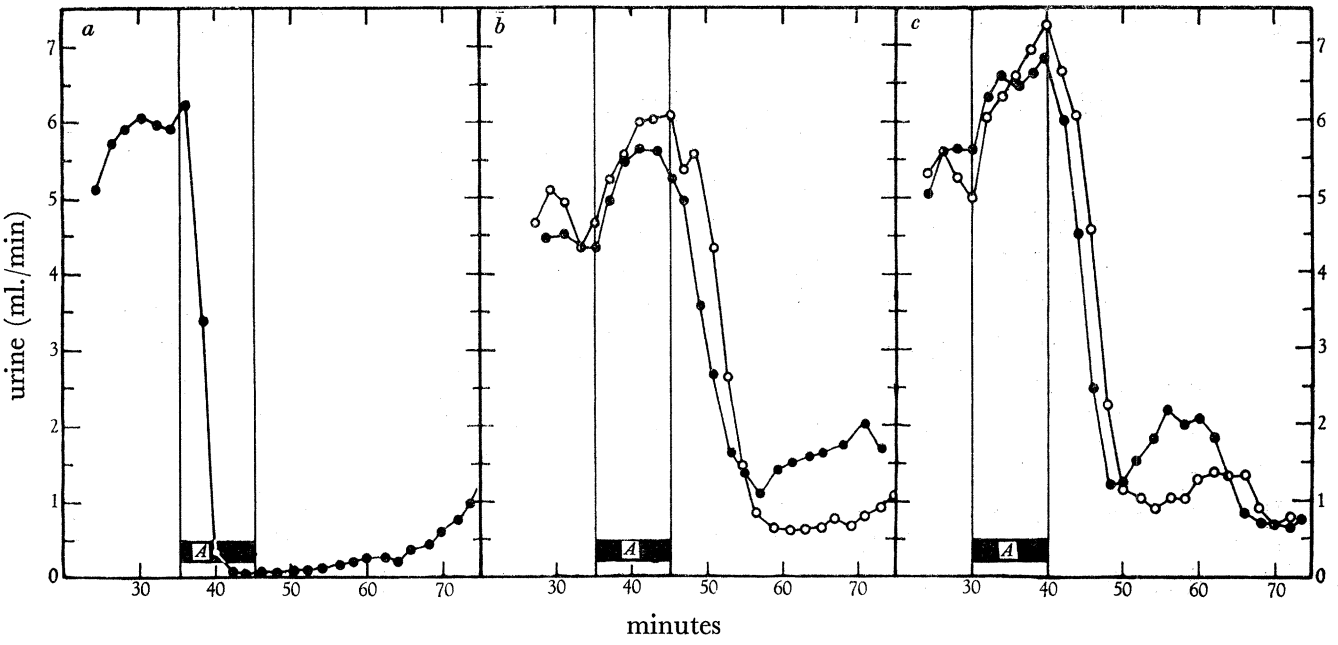

FIGURE 40. 'Doris', no. 379. Responses to infusions given as in figure 39, but after ligation of the left occipital artery. *a*, infusion into the right common carotid; *b* and *c*, infusions into the left common carotid (black circles) and into the malleolar vein (open circles). The infusions in *a* and *b* were 1·28M-NaCl, and in *c*, 1·71M-NaCl. Abscissae: time after the test dose (350 ml.) of water.

dissection later the field of operation was quite clean, and there had been perfect bony union of the zygomatic arch. After fixation, the whole brain was embedded in celloidin and sectioned as described earlier in this paper (p. 218). Maps of selected sections to show the distribution of the suspensions made clear that no blue suspension was entering the vertebral fields and that, as only a trace of black suspension was present in the medulla, the flow through the right occipito-vertebral anastomosis was minimal; the blood in the basilar artery, therefore, was unmarked blood. But black suspension was present in *both* posterior cerebral artery fields and in the dorsal cerebellar areas of *both* sides. Blood in the right carotid artery, therefore, was passing not only across the front of the circle of Willis to supply the left anterior and middle cerebral artery fields, but also across the back of the circle, this latter phenomenon being one which did not occur in either 'Whitethroat', 'Regan' or 'Root'. It appears, therefore, that this swing of blood across the back of the

circle in 'Doris' was owing to the drop in the vertebral contribution caused by the occlusion of the left occipito-vertebral anastomosis. As the right carotid blood passed the back of the circle it became diluted with unmarked vertebral blood; and observation showed, as would be expected, that the posterior cerebral and dorsal cerebellar fields on the left side were less intensely injected with the black suspension than were the corresponding fields on the right side. Thus the whole thalamus on the right side was well injected with the black suspension, whilst on the left it was sparsely so injected, except at its extreme anterior end.

Left carotid blood, carrying the blue suspension, has, however, not been entirely excluded from the brain. It has reached the antero-ventral region of the hypothalamus where it occupies a small half-cone of tissue to the left of the third ventricle. As will be seen from the maps (figure 41), the anterior part of the chiasma carries blue suspension exclusively, and as the chiasma widens posteriorly, first the right and then the left tip become occupied by the black suspension. Just before it divides into the optic tracts its central blue zone becomes continuous with the beginning of the blue-injected half-cone of tissue in the antero-ventral hypothalamus. The base of the half-cone trespasses a little to the right of the midline, and its apex occupies the ventral part of the left paraventricular nucleus; posteriorly it disappears at the level of the posterior median eminence. The pars distalis carries anteriorly both blue and black suspensions, but more posteriorly it carries only the black suspension, and the posterior lobe is exclusively black-injected. Now this blue-injected region of the hypothalamus is the region which, as we have seen, is definitively supplied by the group of vessels which we have called the arteries of the glandular hypophysis, the median eminence and the posterior supraoptic nucleus. Indeed, examination of the sections showed that the intercarotid anastomosis had, in this animal too, escaped inclusion in the carotid ligature, and was carrying blue suspension from left to right: the left internal carotid in the cavernous sinus and just proximal to the ligature had a very much reduced lumen, but the vessel was carrying exclusively blue suspension right up to the point of occlusion. Other than the hypothalamic region just described, the only parts of the brain that carried blue suspension were the lateral side of the left olfactory bulb, the beginning of the left olfactory tract and the optic nerves. The optic nerves, over the 4 mm through which they were followed anterior to the chiasma, carried suspension which was predominantly blue, and the left nerve became exclusively blue just before it joined the chiasma.

It now became important to know the distribution of the suspensions in the supraoptic and paraventricular nuclei (see figure 42). Examination showed that on the right side the whole extent of the supraoptic nucleus, with the exception of the antero-medial tip of the posterior division, was well injected with exclusively black suspension. On the left side the whole of the anterior division of the supraoptic nucleus was well injected with almost exclusively black suspension, there being a little blue in the medial tip of the nucleus near its anterior extremity. The anterior part of the posterior division carried blue suspension only and was well injected; but as one traced the nucleus caudad and laterally, the ventral extent of the nucleus appeared uninjected and, more laterally, the blue suspension became replaced by black. Only an insignificant part of the anterior division and a part of the posterior division of the left supraoptic nucleus, therefore, were receiving blood of left

carotid origin. The left paraventricular nucleus was reached by the extension dorsally of the blue-injected region of the hypothalamus, but most of the vascular bed of the nucleus contained black suspension only. A measure of the supraoptic nuclear volume and of the distribution therein of the blue and of the black suspension was then obtained by the method previously described. With the anterior divisions of the nucleus there was no difficulty in accurately mapping their extents, but with the left posterior division the

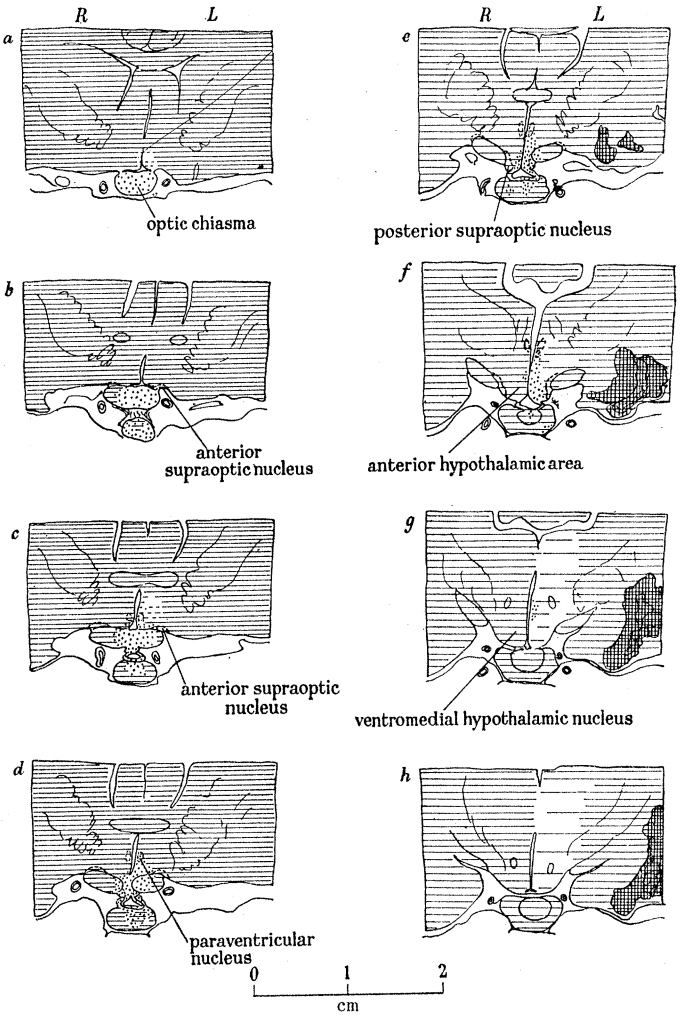

FIGURE 41. 'Doris', no. 379. Maps of selected sections to show the diencephalic distribution of the suspensions. Blue suspension infused into the left carotid—dots; black into the right—lines. The distances between the anterior surfaces of the sections *a* and *b*, *b* and *c*, etc., are 1, 0·7, 0·5, 0·7, 1, 1 and 1 mm respectively. The cross-hatched areas represent cystic lesion.

sparseness of the injection in certain regions precluded, in the unstained sections, an accurate representation of the course of the nuclear material. So with these sections the modification of staining them after the distribution of the suspensions had been marked on the drawings, reprojecting the sections and mapping the outlines of the nuclei, was adopted. The results are shown in figure 42. The proportion of the left posterior division which is exclusively supplied with left carotid blood is 42 %. Of the total nuclear volume, viz. 5·75 cu.mm, the right carotid exclusively supplies 3·39 cu.mm, i.e. 59 %, and the left

carotid 0·99 cu.mm, i.e. 17 %. If, therefore, the receptors are in the supraoptic nuclei one must conclude that when the ipsilateral carotid supply to the nuclear bed of one side is confined to about a third of that nuclear bed, osmotic releases of antidiuretic hormone are no longer detectable by the methods used.

Discussion. If we assume that before the occipital artery was tied most of the anterior diencephalon on the side on which the internal carotid had been tied was deriving its blood supply, as in 'Whitethroat' (figure 33) and 'Root' (figure 36), from the basilar artery, and that, as in 'Whitethroat', this supply was heavily contaminated, through the occipito-vertebral anastomosis, with blood of left carotid origin, then it is evident that the occlusion of this anastomosis has led in 'Doris' (figure 41) to a material reduction in the

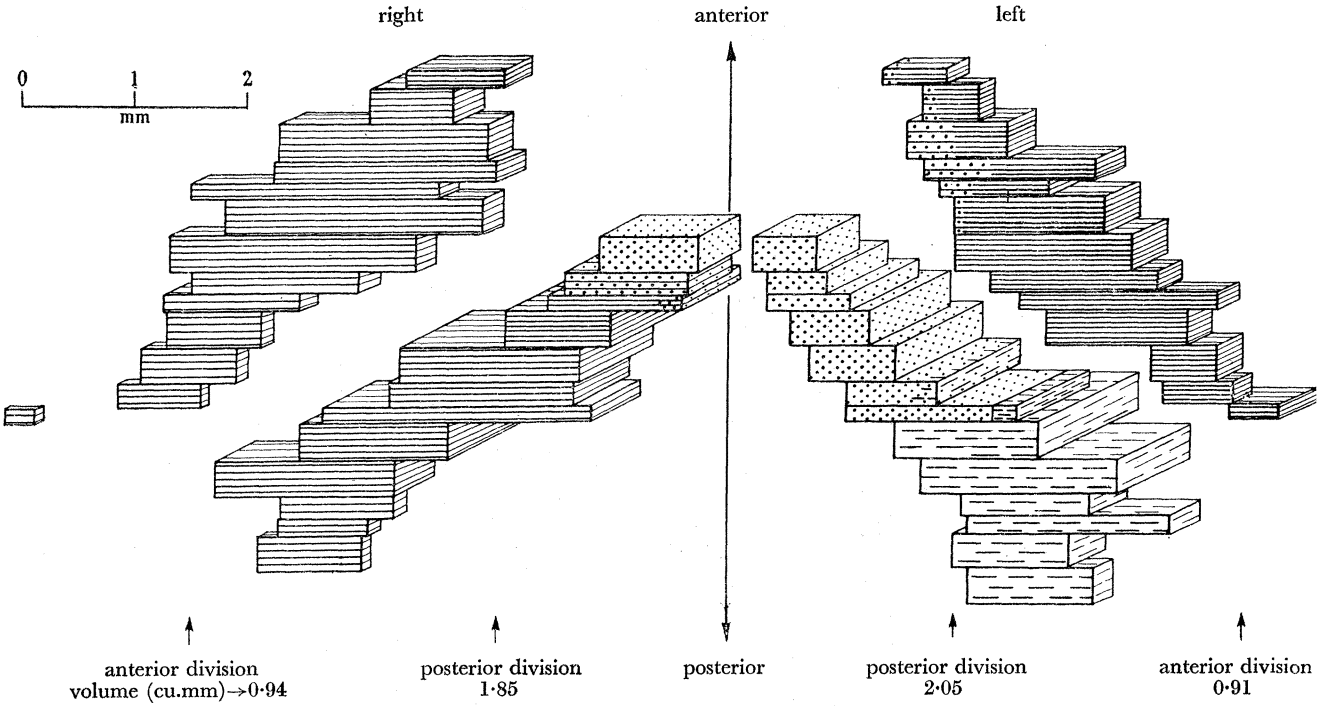

FIGURE 42. 'Doris', no. 379. Plan and isometric projection of the series of blocks of nuclear material comprising the supraoptic nucleus, and the distribution of the suspensions within them. Black suspension—thin parallel lines. Blue suspension—dots.

diencephalic field previously reached by the left carotid blood. The occipital artery in 'Doris', as was noticed when it was tied, was abnormally large, and had presumably been making a weighty contribution to the vertebral stream. It is possible, then, that before the occipital was tied the postero-lateral part of the posterior division of the left supraoptic nucleus was being supplied predominantly with blood of vertebral origin but highly contaminated with left carotid blood. Such supply would, on the hypothesis that the receptors are in, or in the region of the nuclei, fit with the small release of antidiuretic hormone that was obtained from left carotid infusions in the interval between intradural ligation of the left internal carotid and ligation of the left occipital artery. When, however, the left occipital was tied there was a swing of right carotid blood (as the maps clearly reveal) across the back of the circle of Willis, supplemented maybe by an increased flow through

the right occipito-vertebral anastomosis, and the postero-lateral part of the posterior division of the left supraoptic nucleus now became supplied with blood of right carotid and of vertebral origin. These considerations fit with the observed disappearance in 'Doris', when the left occipital artery was tied, of the residual response to left carotid infusions, and accord with the hypothesis that some of the receptors are in, or in the region of, the posterior divisions of the supraoptic nuclei.

The increased cerebral field of distribution of right carotid blood resulting from ligation of the left occipital artery signifies an increased right carotid volume flow; and from this alone a still further diminution in the response to a given right intracarotid infusion from that encountered after intradural ligation of the left internal carotid would have been expected. But such an effect would be opposed by the extended field—should this extension involve the osmoreceptive field—on which the smaller increase in osmotic pressure was now operating. Although no series of comparisons between the responses to infusions into the right carotid before and after ligation of the left occipital artery was pursued, it is evident from figure 39*c* and figure 40*a* that the response after ligation of the left occipital is at least no smaller than that before this operation. This, then, is strong evidence that the increase in the right carotid field when the left occipital artery was tied included an encroachment into the osmoreceptive field. But from the diencephalic distribution of the suspensions no certain indication of the site of such encroachment is given by the findings in this animal alone. The right carotid blood has, for example, taken over the supply to the whole of the right thalamus and to the anterior tip of the left thalamus, a result that would be consistent with a thalamic location of the receptors. On the other hand, the supply of right-carotid blood to the anterior hypothalamus has dominated over that of left-carotid blood, and a measure of this dominance is given by the fact that 59 % of the total supraoptic nuclear volume is being supplied exclusively by the right carotid. In view of the evidence from previous animals against a thalamic location of the receptors we prefer, therefore, to link the retention of, and possible increase in, osmotic response to right intracarotid infusions in this animal with the increased hypothalamic field of the right internal carotid, and to consider this association as evidence that the receptors are in the anterior region of the hypothalamus. This argument does not, of course, conflict with the usual diminution in the response to an infusion into one common carotid when the contralateral internal carotid is tied; it may well be that the ratio between increased encroachment into the osmoreceptive field (when such increase occurs) and increase in ipsilateral carotid flow is, in this instance, less than in the instance we have just been considering.

(8) *Recapitulation of current localizing evidence and conclusions*

It may at this stage be profitable to recapitulate the results so far obtained in the localization of the osmoreceptors. The posterior lobe of the hypophysis has been excluded from being their site (experiments with 'Molly', p. 237; 'Paris', p. 242; 'Brindle', p. 252; 'Rita', p. 253; 'Whitethroat', p. 261; 'Regan', p. 264 and 'Root', p. 267); and the persistence of the osmotic release of antidiuretic hormone from intracarotid infusions when both occipital arteries or one occipital and the ipsilateral posterior communicating arteries have been tied has given strong evidence that the receptors lie in the anterior region of the

prosencephalon, i.e. anterior to the coronal plane of the mamillary bodies (experiments with 'Paris', p. 241; and with 'Toby', p. 243; supported by observations on 'Molly', p. 228). This evidence is reinforced by the facts first, that in 'Whitethroat' (figure 33), after intradural ligation of the left internal carotid, responses were being obtained from infusions into the right carotid, and yet none of this blood was reaching the posterior diencephalon, and secondly that in 'Root' (p. 267, see also figure 36) this part of the diencephalon was receiving, after ligation of the right internal carotid artery, only a trace of left carotid blood and yet well-marked responses were being elicited by infusions into the carotid of this (the left) side. The conclusion is justified, therefore, that the receptors are in the anterior part of the prosencephalon. From this region the telencephalon has been excluded, partly from the results in animals that showed a dominance of one anterior cerebral field and an absence of carotid contamination of the posterior cerebral field (figure 24), and entirely (with the possible exception of the parolfactory region) from the results in 'Brindle' (p. 248 and figure 26) in which, in spite of left hemispherectomy, well-marked osmotic responses were obtained from left intracarotid infusions without intrusion of left carotid blood into right telencephalic areas that had not otherwise been excluded. We infer, therefore, that the receptors are in the anterior part of the diencephalon. And in this region all dorsal thalamic nuclei except the thalamic paraventricular nucleus, and a few persisting cell groups in some other medial nuclei, have been excluded from being the receptors' site by the observations on 'Brindle' (figure 28). Moreover, in 'Whitethroat' (figure 33) no right carotid blood was detected in any part of the thalamus, although well-marked responses were being obtained from infusions into the right common carotid trunk. The evidence, then, is wholly in favour of the receptors being in the hypothalamic region of the diencephalon.

Further evidence as to the site of the receptors has arisen in the course of experiments that were undertaken with the primary object of excluding from the brain the blood in one common carotid trunk, experiments which in their very failure completely to exclude such blood have afforded evidence which is not only compatible with, but also supportive of the view that it is in the anterior part of the hypothalamus that the receptors are situated. For in 'Root' (p. 264 and figures 35 and 36), ligation of the right internal carotid, while causing complete suppression of the large response previously elicited by right intracarotid infusions, and absence of right carotid blood from almost the whole of the hypothalamus, led to a marked increase in response to left intracarotid infusions, an increase which was associated with a conspicuous trespass of left carotid blood into the right hypothalamus. This trespass was especially evident in the antero-ventral region and disappeared just behind the posterior division of the supraoptic nucleus, so that the mamillary complex on the right side was quite free from left carotid blood and on the left side was only sparsely so supplied. In 'Molly' (p. 228), too, it was observed that the mamillary complex on each side was only sparsely injected with the suspensions, although osmotic responses had been obtained from infusions into either carotid trunk.

In connexion with the hypothesis at which we have now arrived, viz. that the receptors are in the antero-ventral region of the hypothalamus, two additional observations are of seeming relevance. First, with 'Toby' (p. 242) it will be recollected that, the right occipital artery having been already tied, subsequent tying of the right posterior

communicating artery was associated with a slight increase in the response to right intracarotid infusions (figure 23*a*, *c*). Now it was noticed at operation that before the posterior communicating vessel was tied the vessel was carrying blood forwards. The ligation, then, probably caused an increase in right carotid flow; and in spite of this the response to right intracarotid infusions was afterwards slightly increased. Thus it is difficult to resist the inference that this slight increase in response was caused by a caudad expansion of the diencephalic carotid field involving supplementary encroachment into the osmoreceptive field, and that some at least of the receptors lie in the fringe of carotid and vertebral hypothalamic beds. Secondly, with 'Doris' (p. 271), when the left internal carotid had been tied, no diminution in the response to an infusion into the right carotid occurred as a result of occluding the left occipital artery, and this in spite of an undoubted increase in the volume flow through the right carotid artery. Here, again, one is led to infer that the observed increase in the cerebral field of distribution of right carotid blood included encroachment into the osmoreceptive field; and in this instance the diencephalic expansion of the right carotid vascular bed involved not only a caudad movement beyond the right anterior hypothalamic region—right carotid blood was actually crossing the back of the circle—but also a movement into the front of the left anterior hypothalamic region, as the maps (figure 41) have revealed. The evidence, then, wholly testifies to some, at least, of the receptors being at the posterior end of the anterior hypothalamic region, and suggests in addition that some of them may be at the anterior end of this region.

Now the most conspicuous nuclear groups in the anterior hypothalamic region are the two divisions of the supraoptic nucleus, and we have throughout this work paid particular attention to this nucleus with respect to the possibility of correlation between the volume of nuclear material supplied with blood of raised osmotic activity, and the magnitude of antidiuretic response. It will be convenient now to bring together the facts that bear upon this question. They are given in table 2, and it is with the results obtained from the first eight animals that we are now primarily concerned. All were killed during the intra-arterial infusion of coloured suspensions by the technique previously described, only no. 377 being under a general anaesthetic (chloralose) during the procedure. In this animal the infusions were made into one carotid and the ipsilateral vertebral artery; with the other animals they were made into the two carotid arteries. The results with the first two animals in the table show that in the absence of any surgical interference with the inflows into the circle of Willis the proportion of *total* nuclear material supplied exclusively by each internal carotid is between 33 and 46%; and we shall assume this figure as a basis from which the effects of various surgical procedures upon it may be assessed, noting at the same time any accompanying change in the antidiuretic responses to intracarotid infusions. The surgical procedures with whose effects on these two values we are at present concerned are, first, ligation of the left occipital after left hemispherectomy; secondly, ligation of both occipital arteries; thirdly, ligation of a posterior communicating artery after earlier ligation of the ipsilateral occipital; fourthly, intradural ligation of one internal carotid artery; and fifthly, ligation of an occipital after earlier intradural ligation of the ipsilateral internal carotid.

Ligation of the left occipital, after left hemispherectomy ('Brindle', table 2), was associated with an increased response to left intracarotid infusions, and the percentage of total

TABLE 2. THE EFFECTS OF VARIOUS OPERATIONS ON THE PERCENTAGE OF TOTAL NUCLEAR MATERIAL (I.E. VOLUME OF BOTH DIVISIONS OF BOTH SUPRAOPTIC NUCLEI) CARRYING EXCLUSIVELY SUSPENSION FROM EACH INTERNAL CAROTID ARTERY, AND ON THE ANTIDIURETIC RESPONSES TO INFUSIONS OF HYPERTONIC SOLUTIONS INTO EACH COMMON CAROTID ARTERY

The upper of the two figures for right and left nuclear volumes is the volume of the anterior division, the lower is the volume of the posterior division. With the animals marked with an asterisk the unstained sections were dismounted and then stained with toluidin blue in order to increase the accuracy of measurement of the volumes of the nuclei.

animal	nature of operation	nuclear volume (cu.mm.)			percentage of total nuclear material carrying exclusively, after operation, suspension from		volume (cu.mm.) of nuclear material carrying exclusively, after operation, suspension from		response after, compared with that before, each operation to infusion into	
		right	left	total	right carotid	left carotid	right carotid	left carotid	right carotid	left carotid
*'Molly', no. 341	nil	0·9 2·2	1·4 2·3	6·80	44·0	46·0[1]	3·00	3·10[1]	(response present)	(response present)
no. 377[2]	nil	0·82 1·87	—	5·38	33·5	33·5	1·80	1·80	—	—
*'Rita', no. 394	nil	—	—	—	50[3]	0	—	—	(response present)	(response absent)
'Brindle', no. 409	left hemispherectomy	—	—	—	—	—	—	—	no information	no information: response present
	ligation of left occipital artery	0·85 1·02	0·96 1·34	4·17	25	55	1·04	2·30	no information: response absent	increased
'Paris', no. 393	ligation of both occipital arteries	0·50 1·26	0·49 1·44	3·69	48	52	1·76	1·93	diminished	diminished
'Toby', no. 395	ligation of right occipital artery	—	—	—	—	—	—	—	not diminished	no information
	ligation of right posterior communicating artery	1·12 1·21	1·09 0·89	4·31	54	0[4]	2·33	0[4]	increased	increased as compared with the response before the occipital was tied
'Root', no. 432	intradural ligation of right internal carotid artery	0·97 1·12	0·73 1·14	3·96	5·5	75·8	0·22	3·00	abolished	increased
*'Doris', no. 379	intradural ligation of left internal carotid artery	—	—	—	—	—	—	—	diminished	much diminished
	ligation of left occipital artery	0·94 1·85	0·91 2·05	5·75	59	17	3·39	0·98	not further diminished	abolished
'Jink', no. 405	ligation of left occipital artery	—	—	—	—	—	—	—	no certain change	increased
	ligation of left middle cerebral and posterior communicating arteries	1·53 1·57	1·55 1·55	6·19	0[5]	50	0[5]	3·10	the small response was abolished	increased
*'Girl', no. 416	ligation of left occipital artery	—	—	—	—	—	—	—	no information	slightly diminished
	ligation of left anterior cerebral and posterior communicating arteries	0·67	0·43	3·47	52	22	1·80	0·76	not diminished	no certain change
'Juno', no. 439	ligation of left anterior and middle cerebral arteries and left posterior communicating artery	—	—	—	—	—	—	—	appeared, having been absent before	abolished
	ligation of left occipital artery	1·14 1·92	0·69 0·74	4·48	44·9	45·5[6]	2·01	2·04[6]	no change	no change
*'Linda', no. 385	ligation of left occipital artery	—	—	—	—	—	—	—	no information	no information
	ligation of left anterior and middle cerebral arteries and left posterior communicating artery	1·05 1·88	0·98 2·02	5·93	37·5	35·0	2·22	2·08	slightly diminished	abolished

(1) The nuclear material here carried a trace of suspension from the right carotid, this having reached it through the right occipito-vertebral anastomosis.
(2) Measurement was made of the right nucleus only. The total nuclear volume has been taken as double that of the one side, and the total nuclear material supplied by the one carotid has been assumed to be the same as that supplied by the other.
(3) No volume measurements were made, but the whole of the right nucleus carried exclusively the suspension of its own side while the left nucleus carried a mixture of the two suspensions.
(4) The whole left nucleus contains both suspensions, that from the ipsilateral carotid dominating.
(5) The whole right nucleus contains both suspensions, that from the contralateral carotid dominating.
(6) In 55 % of the nuclear material to which these figures refer the cells were very sparse indeed.

nuclear material carrying exclusively suspension from the left carotid was then found to have risen to 55. On the other hand, the percentage carrying exclusively suspension from the right carotid had fallen to 25, and no antidiuretic responses to right intracarotid infusions were then being recorded. These results are clearly compatible with the supra-optic-nuclear localization of the receptors.

Ligation of both occipital arteries ('Paris', table 2) was followed by a diminution in the antidiuretic response to intracarotid infusion, and the proportion of nuclear material supplied by each carotid rose, so that the whole of each supraoptic nucleus was being supplied exclusively by ipsilateral carotid blood. We have already referred to the big increase in the cerebral distribution of the internal carotid bloods in this animal, this distribution extending as far as the back of the circle of Willis, and it may be that there was an accompanying increase in carotid blood flow, and hence restriction to the rise in osmotic pressure from a given intracarotid infusion, and that this now more than compensated for increased involvement of the osmoreceptive field. The results are consistent with the hypothesis that the receptors are in the supraoptic nuclei, but give no positive support thereto. We have no evidence on the effect of simple tying of one occipital artery on the distribution of carotid blood in the supraoptic nucleus, but the effect on the antidiuretic response to an intracarotid infusion has been mostly an increase with ipsilateral infusions ('Jink', 'Girl', 'Brindle' and 'Toby', table 2) and, in the one animal ('Jink') to which this test was applied, no certain change with contralateral infusions. These results, again, while consistent with our hypothesis, afford no positive evidence in its favour.

The same deduction applies to the findings in 'Toby' (table 2) after ligation of the right posterior communicating artery when the right occipital had earlier been tied. Here the response to right intracarotid infusions was, if anything, increased as a result of tying the right posterior communicating artery, and then as much as 54% of the total nuclear volume was shown to be carrying exclusively suspension from the right carotid. None, however, of the nuclear volume was carrying exclusively suspension from the left carotid, yet the responses to infusions on this side were greater than before the right occipital artery was tied. Although this last finding threatens the tenability of our hypothesis, it does not prohibit it seeing that the whole of the left nucleus carried predominantly the suspension from the left carotid, and that it is impossible to say how large a proportion of the left nuclear flow derived from the carotid of that side. The only conclusion justified by the results we have just given on the volumes of nuclear material supplied by each carotid and the associated responses to intracarotid infusions is that the results are compatible with the view that the receptors are in or in the region of the supraoptic nuclei.

When, however, we consider the findings in 'Root' and 'Doris' (table 2), positive evidence in favour of our hypothesis is forthcoming. In 'Root', it will be remembered, the right internal carotid was tied intradurally, and the effect of this was to abolish the large osmotic response previously obtained from right intracarotid infusions, and to increase markedly the previously obtained small response from left intracarotid infusions. The table shows that whereas the proportion of total supraoptic nuclear volume supplied exclusively by right carotid blood was only 5%, the proportion supplied exclusively by left carotid blood was 76%. And with 'Doris', ligation of the left occipital artery 11 weeks

after the left internal carotid had been tied, while abolishing the small residual response to left carotid infusions, did not further diminish the response to right carotid infusions, and this in spite of the concomitant increase in right common carotid blood flow as revealed by the increased field of distribution of the right internal carotid blood (figure 41). These results imply a diminution in the proportion of the osmoreceptive field supplied by the left and an increase in that supplied by the right carotid blood; and when we come to measure the percentages of total nuclear material exclusively supplied by each carotid we find that the implied changes in blood supply to the osmoreceptive field are factually reflected in these percentages; the percentage carrying exclusively suspension from the left carotid was 17, and that carrying exclusively suspension from the right was 59.

Lastly, in an animal ('Rita', table 2) in which there had been no experimental interference with the carotid supplies, and in which well-marked osmotic responses were obtained from infusions into the right but no responses from infusions into the left carotid trunk, the whole of the right nucleus carried exclusively suspension from the right carotid and was well injected, and the whole of the left nucleus carried a mixture of both suspensions and was sparsely and patchily injected.

Although the evidence which has been adduced in connexion with the localization of the osmoreceptors testifies to their being placed in that region of the ventral hypothalamus which lies between the preoptic areas in front and the junctional fringe between the ventral hypothalamic fields of the carotid and vertebral vascular beds behind, and is consistent with the view that within this region their definitive site is the supraoptic nuclei, nevertheless we have felt it imperative to test these inferences through other and more direct experimental channels. Clearly there are two ways in which this might theoretically be done; first, through the circumscribed preclusion of the blood of one carotid from the antero-ventral hypothalamic region, and secondly, through the partial or complete restriction of the blood of one carotid to this same region. Since it seemed probable that experimental procedures with the former objective would entail anoxic dissolution of the supraoptic cytons and so render equivocal the interpretation of any subsequently found suppression of response, we have given attention only to experiments of the latter nature. We planned, then, initially to see what the effects would be of tying a posterior communicating and middle cerebral artery in one animal and a posterior communicating and anterior cerebral artery in another. Our first experiment ('Clio', no. 401) of this nature was unsuccessful in that when we had reached the stage of tying the vessels there was a mishap with the posterior communicating artery. The middle cerebral had been successfully ligated, but during the tying of the posterior communicating the vessel was ruptured and the consequence was fatal. With the next two animals, however, we achieved what we set out to do, and we shall now give the results that were obtained.

(9) *Partial restriction of the blood of one carotid to the diencephalon by unilateral ligation of the posterior communicating artery with either the middle or the anterior cerebral artery, and the effects of these procedures on the osmotic release of the antidiuretic hormone*

(*a*) *Experiments with 'Jink', no.* 405

This animal was prepared in the usual way by perineotomy and the formation of two carotid loops. Two infusions of sodium chloride were then made into each common

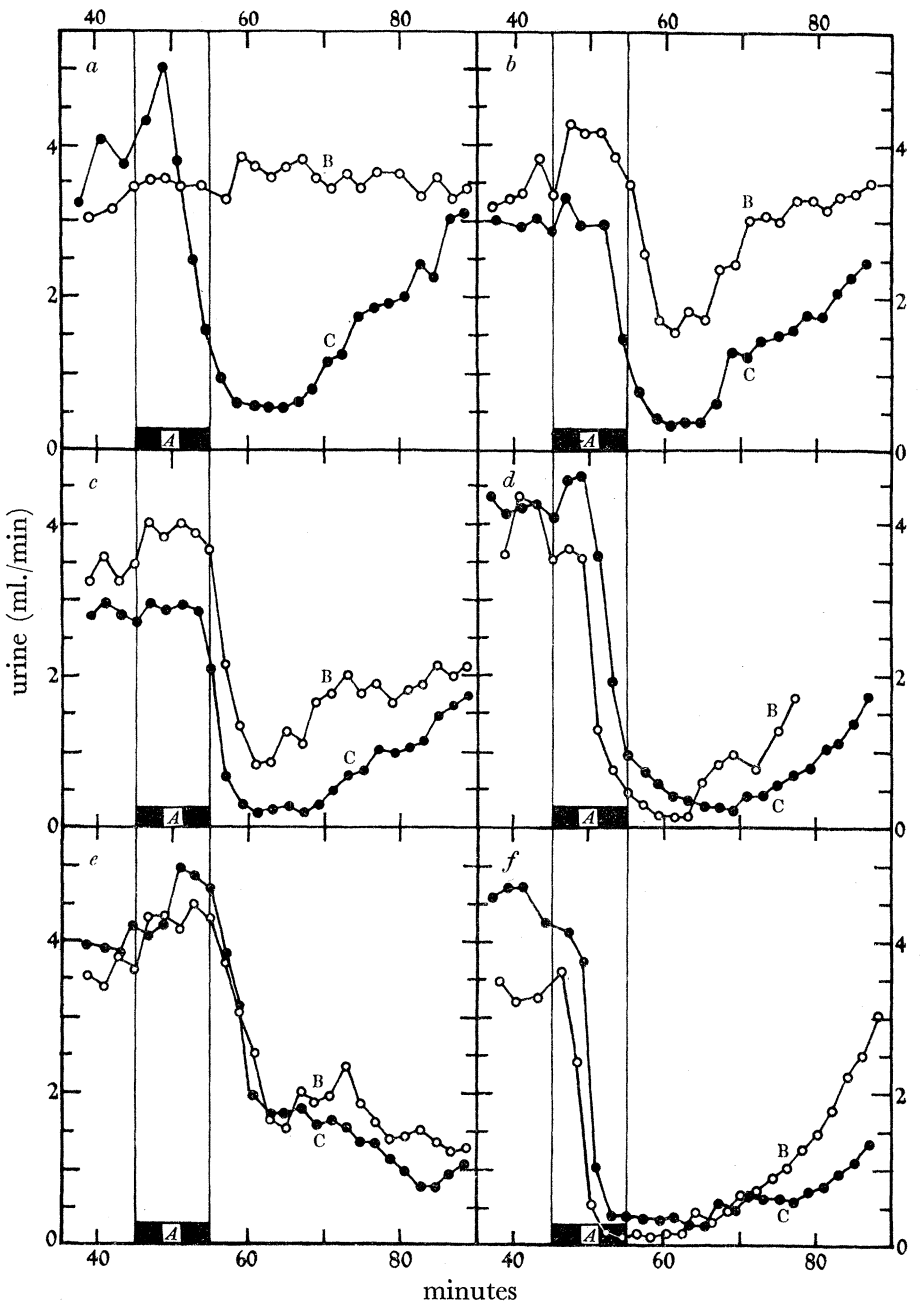

FIGURE 43. 'Jink', no. 405. Effects of infusions of hypertonic solutions of sodium chloride during established water-diuresis. The infusions were made at 1·05 ml./min during the 10 min periods shown by the rectangles *A*. *a* and *b* before, *c* and *d* after ligation of the left occipital artery; *e* and *f*, after subsequent ligation of the left posterior communicating and left middle cerebral arteries. *a*, infusions into the right carotid: 1·28M, graph B; 2·05M, graph C. *b*, infusions into the left carotid: 1·28M, graph B; 1·71M, graph C. *c*, infusions 2·05M into the right carotid, 7 days (graph B) and 13 days (graph C) after operation. *d*, infusions into the left carotid: 1·28M, graph B; 1·71M, graph C. *e*, infusions of 2·05M into the right carotid, graph C; and intravenous, graph B. *f*, infusions of 1·28M into the left carotid 4 weeks (graph B) and 33 weeks (graph C) after the left posterior communicating and left middle cerebral arteries had been tied. Abscissae: time after the test dose (300 ml.) of water.

carotid trunk, and the results are shown in figure 43*a* and *b*. Each infusion was at the rate of 1·05 ml./min for a period of 10 min. Figure 43*a* (right intracarotid infusions) shows that 1·28M (graph B) has no appreciable action on the course of urine flow, whereas 2·05M (graph C) gives a definite antidiuretic response. Infusions into the left carotid (*b* in the figure) were more effective than those into the right; the graph B shows the response to 1·28M and graph C that to 1·71M. The left occipital artery was then divided between ligatures 2 to 4 mm from its origin, and the responses to intracarotid infusions were again tested in order to see what would be the effect on these of subsequent ligation of the left middle cerebral and posterior communicating arteries.

Responses before ligation of the left middle cerebral and posterior communicating arteries. These are illustrated in figure 43*c* and *d*. There was no certain change in the response to an infusion into the right carotid as a result of tying the left occipital (cf. *c* and *a*, figure 43), whereas this procedure was followed by an increased response to infusions into the left carotid (cf. *d* and *b*, figure 43). The responses in *c* and *d* were obtained during the 5-week period after the left occipital had been tied. The left middle cerebral and posterior communicating arteries were then ligated by the technique previously described. The posterior communicating was first dealt with; this was tied about 3 mm from its anterior origin. The middle cerebral was then similarly treated, and the ligature here, too, was placed about 3 mm from the origin of the vessel. No bleeding was encountered, but there was a little damage to the piriform lobe by the posterior edge of the retractor. The whole operative technique was completed in 7 h, recovery was uneventful, and the wound healed by first intention. The animal, during the first week after the operation, always circled to the left in walking, and for about a month her behaviour was a bit more 'excitable' than usual. We shall now describe the effects, after operation, of intracarotid infusions of hypertonic solutions of sodium chloride.

Responses after operation. The first infusion was made 10 days after operation, and during the ensuing 8 months four infusions were made into the right carotid and three into the left. The results of these are illustrated in figure 43*e* and *f*. The response that was previously given to a right intracarotid infusion of 2·05M-NaCl (*c*, figure 43) was now suppressed, there being no difference between the effects of an intracarotid (figure 43*e*, graph C) and an intravenous (graph B) infusion. On the left side, however, the responses were greater than those before operation, as will be seen by comparison of the graphs B and C in figure 43*f* with the graph B in figure 43*d*, all these being responses to infusions of 1·28M-NaCl. The disappearance of the small response to infusions into the right carotid was seemingly owing, at least in part, to an increased right carotid flow when the left middle cerebral and posterior communicating arteries had been tied. Conversely, the increase in response to infusions into the left common carotid trunk, associated presumably with a decreased volume flow through that vessel, showed that at least part of the total osmoreceptive field was still being reached after operation. It now became important to know the distribution of the carotid blood, and this was determined by the method previously described.

Tracing the cerebral distribution of the carotid blood. The blue suspension was infused into the left, the black into the right carotid, and the animal was suddenly killed at the seventh second after the suspensions had reached the carotid needles. On dissection of the head the operation field was quite clean, and the blue colour was conspicuous in left-sided extra-

cranial structures. Examination of the sections of the celloidin-embedded brain showed that there was admixture of the suspensions in the anterior cerebral fields, but that each middle cerebral field was well injected with the suspension of its own side and this in spite of the ligature on the left middle cerebral artery. This vessel had, however, not been completely occluded; the sections showed a very small remaining lumen through which blood, well marked with the blue suspension was leaking. The left posterior communicating was completely closed by its ligature at the level of the posterior median eminence. The brain stem and cervical cord carried blue suspension on both sides; clearly some vessel other than the left occipital (see p. 204) was carrying left carotid blood into the vertebral stream. For this reason, and because of the small leak through the left middle cerebral trunk, interpretation of the distribution picture was difficult, but the indications were that right carotid blood was flowing backwards in the right posterior communicating artery and mixing with vertebral blood at the origins of the right posterior cerebral and right anterior cerebellar arteries, and that the left posterior cerebral artery was carrying vertebral blood (contaminated with blue suspension) well forward to the anterior thalamic region. The left piriform lobe was extensively damaged and was cystic.

The distribution of the suspensions in the hypothalamus was as follows. In the preoptic region the septal nuclei of both sides were almost exclusively blue-injected, there being a slight trace of black on the right side. Ventral to them the preoptic areas were dominantly blue on both sides, the left side showing a trace, and the right side a considerable amount of black, and as one passed back to the supraoptic areas a clearer demarcation became evident, the right side being dominantly black-injected and the left side blue-injected. The mid- and posterior-hypothalamic areas were, on the left side, exclusively blue-injected, and on the right these areas carried both suspensions. These distributions in the hypothalamus were well reflected in the nuclei, the paraventricular nucleus and both divisions of the supraoptic nucleus being, on the left side well injected, and exclusively so, with the blue suspension, while on the right side, although dominantly injected with the blue suspension, they carried an evident amount of the black as well. Measurement of the supraoptic nuclear volumes showed (table 2) that 50% of the total nuclear material carried exclusively the suspension that had been infused into the left carotid artery. In the stained sections all the hypothalamic nuclei appeared normal histologically with the exception of the dorso-medial nucleus and medial mamillary nucleus, of the left side. In the dorsal tuberal region there was a small cyst extending for about 1·6 mm in an antero-posterior direction, and having a maximum vertical height of 1·9 mm and transverse diameter of 0·5 mm (figure 44). In addition to direct destruction of the dorso-medial nucleus this cyst has involved the descending column of the fornix, which is shrunken and shows severe fibre loss. This loss has resulted in a compacting of the cells in the lateral part of the medial mamillary nucleus, which is the primary site of termination of the fornix (Allen 1944), and, more posteriorly, the left mamillary body (posterior medial mamillary nucleus) is smaller in size than the right and appears to have suffered cell loss.

Examination of the thalamus showed on the right side that while the ventral nuclei carried, in addition to the black suspension, a considerable amount of the blue, the anterior and medial nuclei and the dorsal part of the lateral nucleus were well and exclusively injected with the black suspension; this changed to a sparse admixture of both suspensions

at more posterior levels. On the left side the ventral nuclei and the ventral midline nuclei were fairly well injected with the blue suspension, but in the anterior, medial and lateral nuclei and in the habenular nucleus this injection was sparse and patchy. Examination of the stained sections (see figure 44) showed that some of the thalamic nuclei had suffered cell loss, to be ascribed to damage in the region of the pallidum and pyriform lobe, as well as to damage accompanying the small cystic lesion in the hypothalamus. Cell loss has occurred mainly from the anterior and intralaminar nuclei including the nuclei antero-medialis and anteroventralis, centralis medialis, paracentralis and centralis lateralis, with less severe loss from the nuclei submedius, ventralis medialis and reticularis (see figure 44). The thalamic paraventricular nucleus was intact, as were all other dorsal thalamic nuclei.

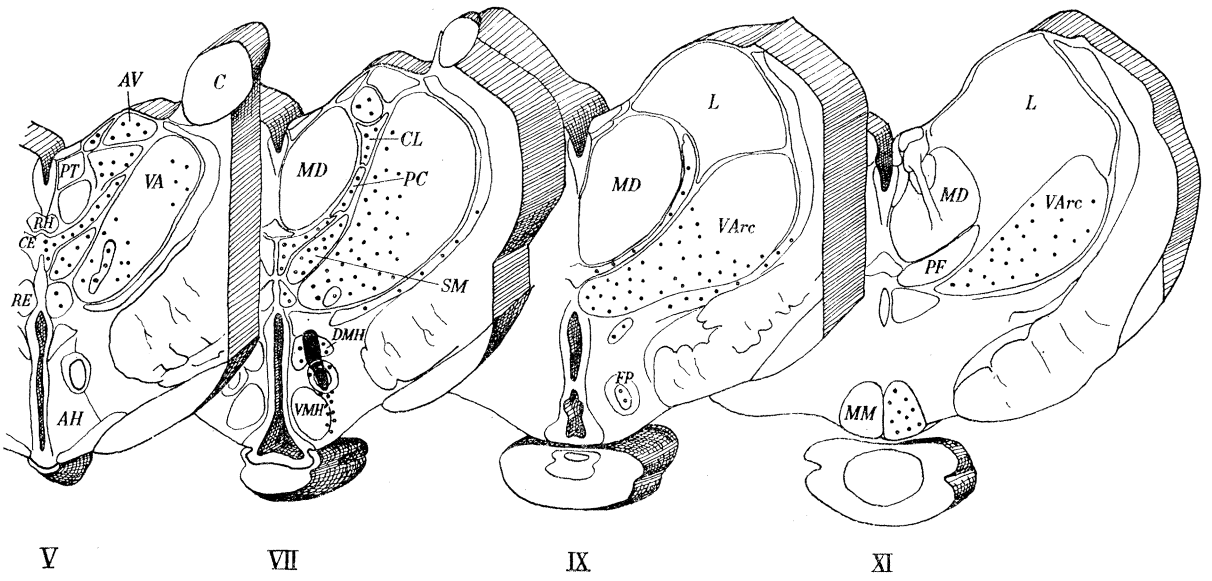

FIGURE 44. 'Jink', no. 405. Nuclei of the diencephalon which have suffered degenerative lesion. The sections are selected from those shown in figure 27 and carry the same numerals. Cystic lesion in the dorsomedial hypothalamic nucleus shown black; nuclei showing cell loss indicated by black dots. Abbreviations as in figure 27.

Now well-marked antidiuretic responses had, as we have seen (figure 43*f*), been obtained from left intracarotid infusions in this animal. The sparse injection of the left dorsal thalamus, along with the nuclear degenerative changes described above, is evidence, therefore, against the thalamus playing a primary role in these responses; and this is in harmony with the fact that although the antero-dorsal region of the right thalamus was well and exclusively injected with suspension from the ipsilateral carotid, no response was obtained from infusions on that side. On the other hand, the fact that the whole of the left supra-optic nucleus was well and exclusively injected with suspension from the left carotid (table 2) is consistent with the view that the receptors are in this, the antero-ventral, region of the hypothalamus. In conformity with our plan, the next investigation was on an animal in which the left anterior cerebral and posterior communicating arteries were eventually tied, and the effects of this procedure on the osmotic responses and the distribution of carotid blood determined.

(*b*) *Experiments with 'Girl', no.* 416

This animal was prepared in the usual way by perineotomy and the formation of two carotid loops. A month after the second carotid loop had been made the left occipital artery was divided between ligatures about 4 mm from its origin. Before this was done an infusion of 1·71 M-NaCl into the left carotid gave a response a little greater than those given afterwards by 1·71 M and 2·06 M (figure 45*b*).

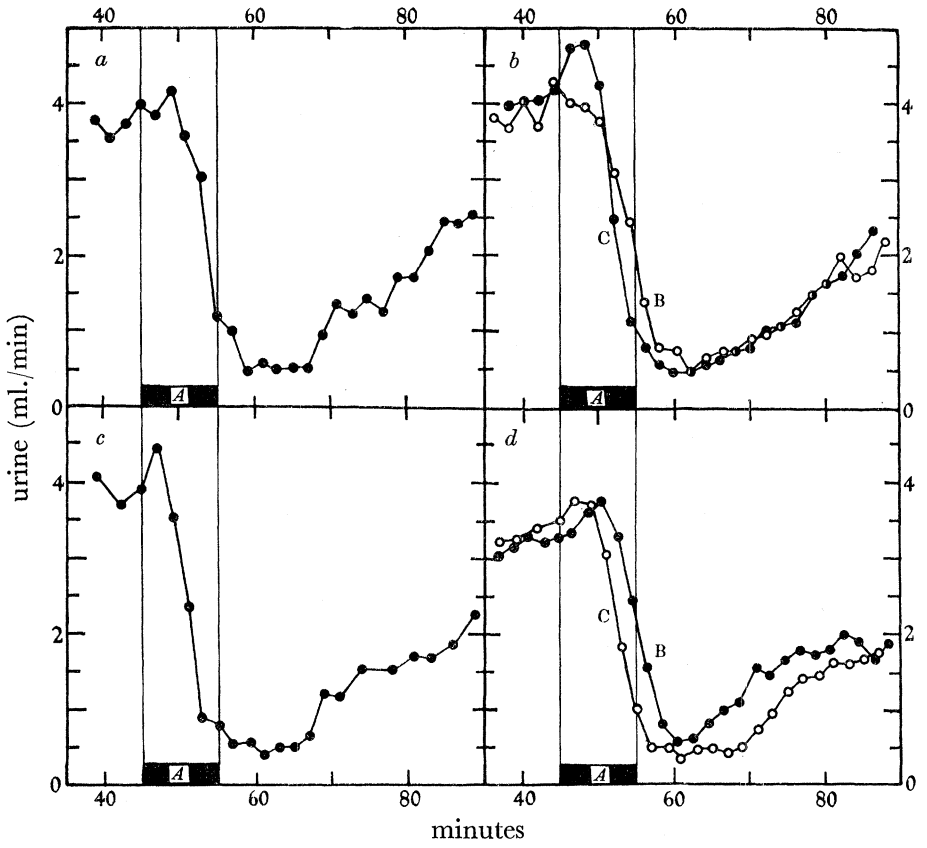

FIGURE 45. 'Girl', no. 416. Responses to intracarotid infusions of hypertonic solutions of sodium chloride during established water-diuresis. The infusions were made at 1·05 ml./min during the 10 min periods shown by the rectangles *A*. *a* and *b*, after ligation of the left occipital and before ligation of the left anterior cerebral and posterior communicating arteries; *c* and *d*, after operation. *a*, infusion into the right carotid: 1·71 M. *b*, infusions into the left carotid: 1·71 M, graph B; 2·06 M, graph C. *c*, infusion into the right carotid: 1·71 M. *d*, infusions into the left carotid: two responses to 2·06 M; 4 weeks after operation, graph B; 5 weeks after operation, graph C. Abscissae: time after the test dose (300 ml.) of water.

Responses before ligation of the left anterior cerebral and posterior communicating arteries. These are illustrated in figure 45*a* and *b*. Each infusion was at the rate of 1·05 ml./min for a period of 10 min. In *a* is shown the response to 1·71 M-NaCl infused into the right carotid trunk, and in *b* are shown the responses to 1·71 M (graph B) and 2·06 M (graph C) infused into the left. The left anterior cerebral and posterior communicating arteries were then tied by the technique previously described. As the anterior cerebral artery rose towards the dorsal surface of the optic nerve it gave origin to its internal ophthalmic branch, and

the anterior cerebral was tied just beyond this. Attention was then given to the posterior communicating vessel, and this was ligated about 3 mm behind its junction with the internal carotid. Recovery was uneventful, and the wound healed by first intention. During the first week after operation the animal always circled to the left when walking. Tests of the effects of intracarotid infusions of hypertonic solutions of sodium chloride were begun 3 weeks after operation, and the results of these will now be given.

Responses after operation. These are illustrated in figure 45*c* and *d*. The responses to infusions into the right carotid (*c*) were certainly not diminished as compared with those obtained before operation (figure 45*a*); and there was no certain eventual change in the response to infusions into the left carotid (cf. figure 45*d* with *b*), there being an indication that the response was increasing with time; B and C (figure 45*d*) give the effects of the same intracarotid infusion (2·06M) 4 and 5 weeks respectively after the operation. It was then decided to determine the distribution of the carotid blood; this was done by the technique previously described.

Tracing the cerebral distribution of the carotid blood. The blue suspension was infused into the left, the black into the right carotid, and the animal was suddenly killed at the seventh second after the suspensions had reached the carotid needles. The field of operation was quite clean, and there had been complete union of the temporarily resected zygomatic arch. Examination of the sections of the celloidin-embedded brain showed that the general distribution was such as would have been expected from the position of the ligatures. Thus the whole of the right telencephalon, including the tentorial gyri, is well injected, and exclusively so, with the black suspension, and the right carotid blood which carries it has extended to supply the left anterior cerebral field. The cervical cord, the hindbrain, with the whole of the cerebellum, and the midbrain contain the minutest trace of suspension, and that of the black only; the transfer of left carotid blood to the vertebral artery has therefore been effectively suppressed by the ligature on the left occipital artery, and the basilar blood is contaminated only to an insignificant degree with right carotid blood. Evidently the right carotid blood has not only spread into the left anterior cerebral field but also flowed backwards in the right posterior communicating artery to a point between the origins of the right posterior cerebral and anterior cerebellar arteries. The ligature on the left posterior communicating artery has, however, prevented a similar transfer of left carotid blood towards the back of the circle of Willis, with the result that the field of the left posterior cerebral artery is supplied entirely by basilar blood and is therefore practically free from all suspension; the appearances of the tentorial gyri (those on the right being well injected with and those on the left practically devoid of black suspension) are most striking. The left olfactory bulb is, in its anterior region, exclusively blue-injected, having received its supply from the external ethmoidal artery. As the bulb passes along the base of the frontal lobe it becomes predominantly black-injected, and here the left middle cerebral field begins as a blue-injected region of cortex immediately dorsal to the olfactory bulb. As one proceeds posteriorly this blue-injected region expands with the expansion of the cortex to involve the whole neopallium, including the piriform lobe, but excluding the tentorial gyri (which are practically free from suspension, being supplied by unmarked vertebral blood) and the gyri of the medial surface of the hemisphere (which are well and exclusively injected with the black suspension, their blood supply being derived from the

right anterior cerebral artery through the anterior communicating vessel). This blue-injected left middle-cerebral field extends medially to involve the dorso-lateral tip of the head of the caudate nucleus—the rest of the head is only sparsely injected with both suspensions. The whole of the septum is exclusively black-injected apart from there being

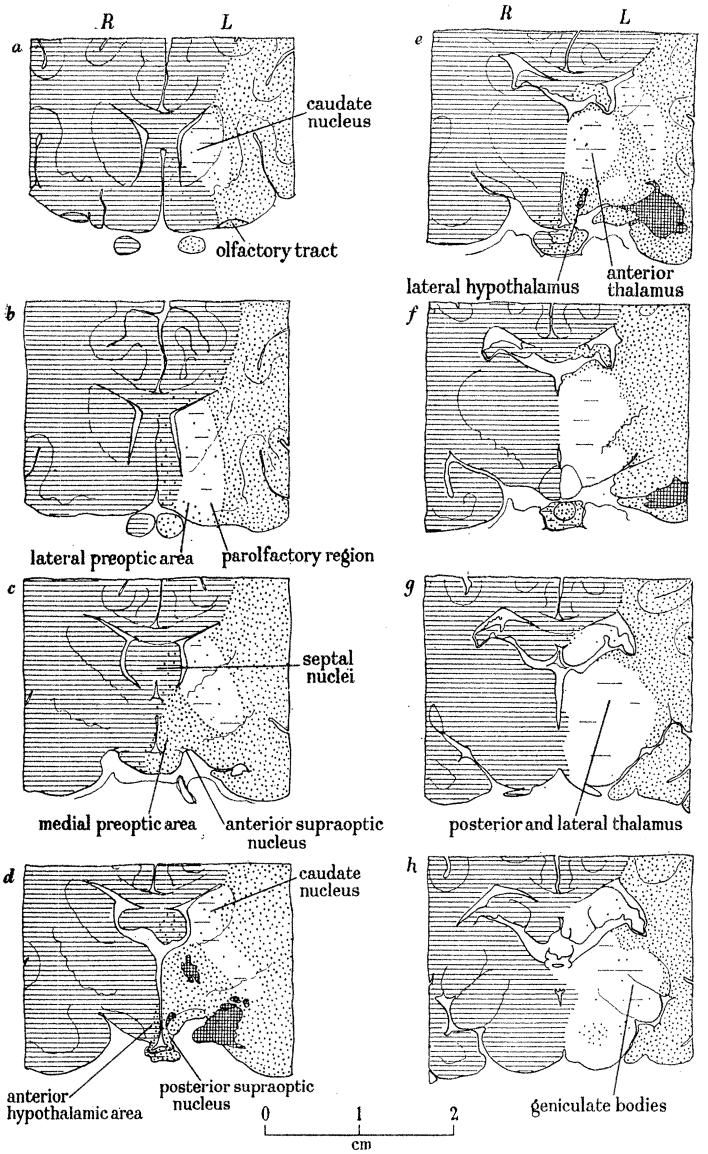

FIGURE 46. 'Girl', no. 416. Maps of selected sections to show the diencephalic distribution of the suspensions. Blue suspension infused into the left carotid—dots; black into the right—lines. The distances between the anterior surfaces of the sections *a* and *b*, *b* and *c*, etc., are 5, 3, 1·25, 3, 2·45, 3 and 2 mm respectively. The cross-hatched areas represent cystic lesion.

a trace of blue contamination in its left half. The preoptic areas on the right side carry predominantly the black suspension and on the left side they carry predominantly the blue.

Turning now to the distribution of bloods in the thalamus and hypothalamus—the distribution is illustrated in figure 46—we find that on the left side the whole of the thalamus is, with a small exception, practically devoid of suspension, i.e. it is supplied exclusively, through the left posterior cerebral artery and the thalamic branch of the left posterior

communicating artery (behind the ligature), with blood of vertebral origin. The small exception is a thin shell lying on the antero-dorsal surface of the thalamus (in the region of the antero-dorsal nucleus and the stria medullaris) and spreading to involve posteriorly the dorso-lateral part of the pulvinar, and ventrally the site of the narrow nucleus reticularis. This shell is well injected with the blue suspension and marks the medial limit of the region supplied by the left middle cerebral artery. The whole of the right thalamus, on the other hand, is well and exclusively injected with the black suspension. The left hypothalamus carries exclusively the blue suspension anteriorly, but just behind the level of the ligature on the posterior communicating artery it becomes practially devoid of suspension. Thus in the tuberal or infundibular middle region the following nuclei and areas can be excluded from the left carotid field: the posterior extent of the ventro-medial hypothalamic nucleus, the ventral extent of the perifornical area where the fornix approaches the mamillary body, the posterior extent of the dorsal hypothalamic area and the entire posterior hypothalamic area. In the caudal or mamillary region all the nuclei and areas are excluded. The whole of the right hypothalamus, on the other hand, is well injected with the black suspension, and exclusively so, except for a narrow strip on the ventro-medial aspect of the anterior hypothalamic area and for the most medial part of the posterior supraoptic nucleus where there is intrusion of a little blue from the opposite side.

Much of the left piriform lobe is damaged and cystic. Moreover, there is a small cyst in the left thalamus and hypothalamus. The cyst shows little neuroglial reaction around it, and its structure is apparently the same as that of the 'fluid-filled cyst surrounded by a minimal amount of astrocytic and connective tissue hypertrophy' which Evans & McEachern (1938) have described in the monkey's brain 3 to 9 months after occlusion of the middle cerebral artery. The cyst begins (figures 46, 47) in the dorso-medial region of the left internal capsule at the level of the anterior commissure and, enlarging as it is traced posteriorly, comes to replace most of the anterior portion of the left reticular and ventral thalamic nuclei. Its maximum vertical and transverse dimensions are 3·0 and 2·0 mm respectively. Its main body disappears 2·35 mm behind the level at which the cyst first appears in the sections, but its foot continues backwards for a further 1·0 mm as a horizontal tongue (2·5 by 0·6 mm) in the subthalamic and dorsal hypothalamic regions. Here it involves the dorsal part of the left lateral hypothalamic area and sends a finger ventrally towards the dorsal surface of the diverging optic tract to reach the brain base just lateral to the ventro-medial hypothalamic nucleus. The total antero-posterior extent of the cyst is 4·35 mm. Another and smaller cyst begins on the dorso-medial aspect of the left optic tract just as this tract is separating from the chiasma, expands on the surface of the tract to dimensions of $1{\cdot}50 \times 0{\cdot}38$ mm, and then tapers around the medial tip of the optic tract to disappear at the brain base after a total antero-posterior course of 3 mm.

Accompanying, or as a result of these cystic developments, there is destruction or degeneration of certain nuclei in the left thalamus and hypothalamus (figure 47). The nuclei of the anterior pole of the thalamus—the antero-dorsal and antero-medial nuclei and their commissural elements—show severe cell loss (figure 47, III, IV), as do also the medial nuclei throughout most of their extent—the nuclei medialis dorsalis, parataenialis, paracentralis, centralis lateralis and submedius (figure 47, V). The nuclei of the midline are

similarly affected—centralis medialis, rhomboidalis and reuniens—with the exception of the paraventricular nucleus which shows a cell population little diminished from normal. The medial part of the ventral nuclear complex, and the reticular nucleus, show severe cell loss at anterior levels and mild cell loss in the posterior thalamus (figure 47). The

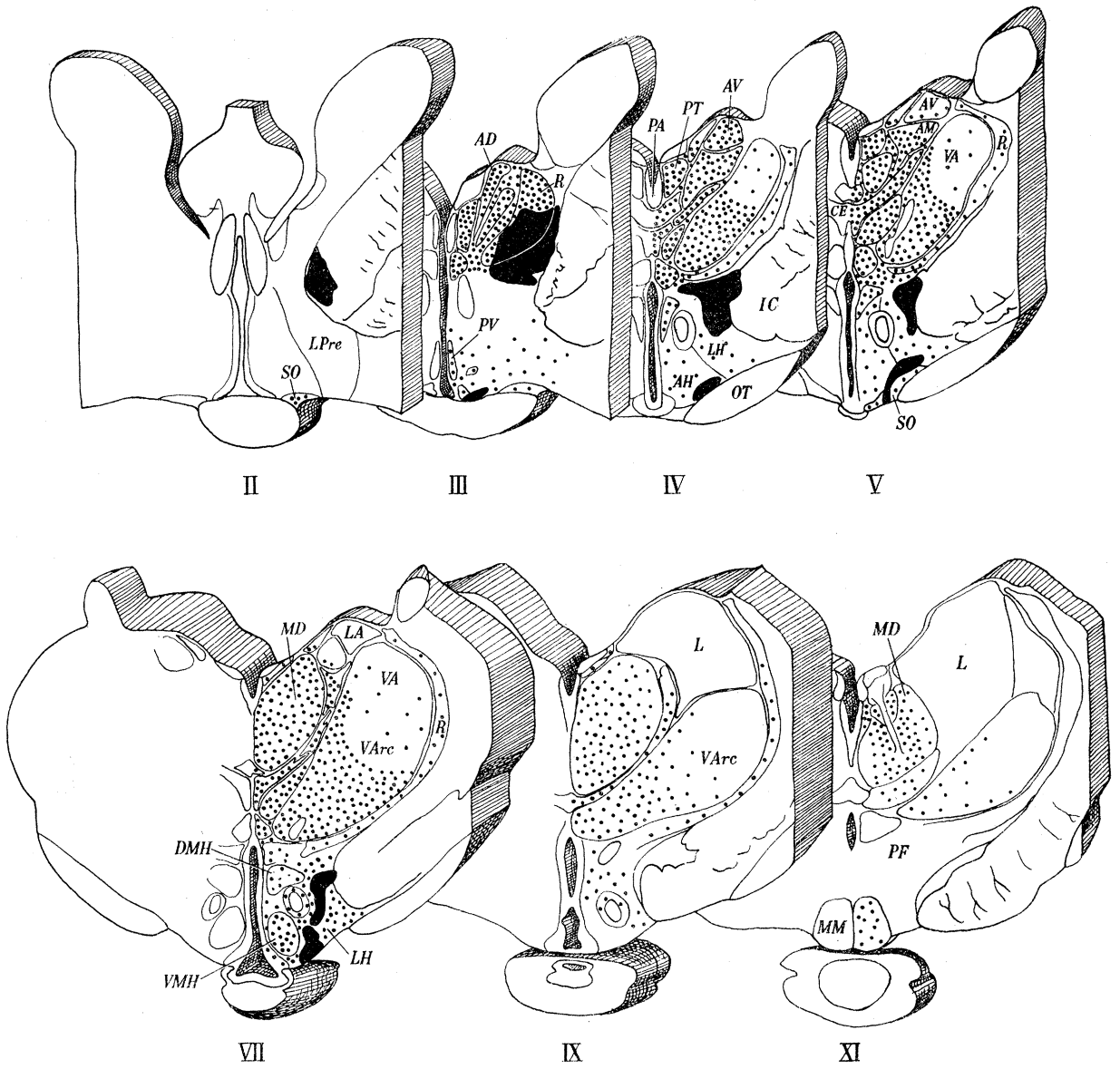

FIGURE 47. 'Girl', no. 416. Nuclei of the diencephalon which have suffered degenerative lesion. The sections are selected from those shown in figure 27 and carry the same numerals. Cystic lesion shown black; nuclei showing cell loss indicated by black dots. Abbreviations as in figure 27.

degeneration therefore has involved the medial segment of the thalamus (with the exception of the paraventricular nucleus) as far back as a level just anterior to the habenula nucleus. In the hypothalamus the medial mamillary nucleus of the left side has degenerated, and the larger cyst described above has involved the dorsal part of the lateral hypothalamic area. This cystic destruction has been accompanied by severe cell loss from the

middle tuberal region. Furthermore, *the smaller cyst has destroyed a large part of the posterior division of the left supraoptic nucleus* and is possibly responsible for the considerable cell loss that has occurred in the left paraventricular hypothalamic nucleus. There is some cell loss, too, in the anterior division of the left supraoptic nucleus: the position of the cyst is such that it would have interrupted descending fibres from this nucleus. Nevertheless, the posterior lobe shows no obvious evidence of shrinkage or other deformity.

As with some of the other animals, it was felt that it would be helpful to have a measure of the supraoptic nuclear volume occupied exclusively by each of the suspensions. This was obtained by the technique previously described, the distribution of the suspensions being first mapped on the projection drawings, and the nuclear outlines later projected on to the same drawings after the originally unstained sections had been stained with toluidin blue and remounted. The whole anterior division of the right supraoptic nucleus is well injected, and exclusively so, with the black suspension; and this applies, too, to more than half of the posterior division. On the left side the whole of the anterior division and the remnant of the posterior division are well injected, and exclusively so, with the blue suspension. The numerical expression of these facts is given in table 2 (p. 280). It is remarkable that so big a reduction in the volume of the supraoptic nucleus on the left side should have been associated with the retention of osmotic responses to infusions into the left carotid which were not clearly different from those given before operation; and this becomes all the more striking when one takes into account the fact that the density of cells in the residual volume (0·76 cu.mm) of nuclear material is abnormally low.

Discussion. The results with this animal have afforded valuable evidence of an excluding character on the location of the receptors. The whole central mass of the thalamus is excluded, since this region is, on the left side, devoid of the blue suspension that was used to trace the distribution of the left carotid blood, and yet responses had been obtained from infusions on this side, and no blue suspension was present in the corresponding region of the right thalamus. Furthermore, the posterior part of the hypothalamus is excluded through similar reasoning. Moreover, much of the anterior part of the left thalamus, as detailed above, shows degenerative changes or has been directly involved by the cyst. The results suggest, then, that the receptors are in the anterior part of the hypothalamus. Now if they lie within the supraoptic nuclei, while the retention and possible increase of the response to right intracarotid infusions after operation (figure 45, cf. *a* and *c*) may find explanation in terms of increased involvement of the osmoreceptive field with smaller osmotic-pressure increment owing to the increased carotid blood flow, such considerations of a balance between extent of field and osmotic-pressure increment give rise to difficulties when invoked in explanation of the retention of response (figure 45, cf. *b* and *d*) to infusions into the left carotid. For as we have seen, there was, after operation, a very marked decrease in the volume of nuclear material supplied exclusively by left carotid blood; and although it is reasonable to infer that the osmotic stimulus from a given intracarotid infusion was greater after than before operation, it is difficult to believe that this would have effectively counterbalanced the markedly reduced and cell-impoverished field (left supraoptic and paraventricular nuclei) on which, by hypothesis, the stimulus was now operating, unless a compensatory increase in sensitivity of the reduced field had occurred. On the other hand, 0·91 cu.mm of the right posterior division, i.e. 44% of it (table 2),

was reached by both carotid bloods, and one is ignorant of the degree to which, before operation, vertebral blood was contributing to the total supply to the nuclei. Nevertheless, the evidence from this animal alone is against the view that independently functioning receptors of unvarying sensitivity are present in the supraoptic nuclei. We therefore decided at this stage to collect such detailed information as was available up till then concerning the areas and nuclei in the thalamus and hypothalamus that could be excluded from being the receptor site by evidence from blood distribution and from destructive or degenerative lesion.

(10) *The parts of the diencephalon excluded as sites for the receptors, by collected evidence from animals in which responses were retained after operation; and inference therefrom of the region in which the receptors lie*

In the recapitulation of the evidence for the localization of the osmoreceptors presented on p. 277 the inference is drawn that the receptors lie in the region comprised by the anterior hypothalamus and the preoptic area, and, possibly, the parolfactory region. It has now become possible, with the further evidence that has accrued from the experiments on two other animals ('Jink' and 'Girl'), to substantiate this assertion and to define the region with anatomical precision. The evidence will be summarized, and it will now be noted that it has been collected in such a manner as to allow a secure statement of the regions that can be *excluded* from being the anatomical seat of the receptors. The reasons for adopting this approach have been discussed before (p. 244).

The parts of the diencephalon not directly responsible for the osmotically determined release of antidiuretic hormone have been ascertained by mapping the regions which, on both sides, were not receiving blood from one carotid when release of hormone had been obtained from infusions of hypertonic solutions into the same carotid artery. This evidence has been supplemented by mapping the regions which, in some of the animals, were found to have suffered destructive or severe degenerative lesions as a result of operation, without suppression of response to intracarotid infusions on the side of the lesion and without the blood of that carotid crossing to reach the corresponding contralateral diencephalic structures. The conclusions are presented diagrammatically in figure 48 (facing p. 251), where the regions of the diencephalon so excluded are indicated in heavy black stipple.

The entire thalamus (with one qualification, and this will be mentioned later) has been excluded from being the site of the receptors; moreover, with the exception of one or two of its smaller nuclei, it can be excluded independently by the evidence from both blood distribution and degenerative cell loss. In 'Girl' almost the entire thalamus is devoid of suspension from the carotid of the operated side, from which responses were still being obtained. The pulvinar and posterior extent of the reticular nucleus are excepted in that they carry some suspension, but these regions can with certainty be excluded since they are uninjected or only sparsely injected in four other animals (experiments with 'Paris', 'Toby', 'Whitethroat', and 'Regan'). The antero-dorsal nucleus and most-anterior parts of the midline commissural nuclei, the most anterior extent of the parataenial nucleus and adjacent paraventricular nucleus, and the anterior extent of the reticular nucleus also carry some suspension in 'Girl'; they are the only regions of the thalamus which cannot be excluded by the evidence from blood distribution alone. However, the operation in 'Girl'

had caused cystic lesions to appear with consequent degenerative changes in the thalamus, and these same nuclei, with the exception of the paraventricular nucleus, have suffered such severe cell loss (see figure 47) that they could have played no part in the persisting osmotic responses.

These observations on 'Girl' are wholly supported by the experiments with 'Whitethroat', in which animal just as extensive a part of the thalamus appears to be uninjected from the responsive side (but see p. 259 for certain reservations about the interpretation of the histological appearances in this animal), and in part by the experiments with six other animals ('Molly', 'Paris', 'Toby', 'Regan', 'Root' and 'Jink') in which varying extents of the posterior, lateral, ventral, and medial groups of thalamic nuclei were not receiving blood from the same carotid as had earlier yielded responses to the infusion of hypertonic solutions.

Mention should here be made of one region of the telencephalon that at the earlier recapitulation of localizing evidence (p. 277) could not definitely be excluded, namely, the parolfactory region. The fact that this region lies so near to the intradural trifurcation of the carotid artery has resulted in its nearly always receiving carotid blood from a responsive side. The experiments with 'Molly', in which the asymmetry of carotid distribution in the anterior cerebral arteries resulted in the parolfactory regions of both sides being dominantly supplied by one carotid, had afforded some evidence that these regions were not involved in the responses. More conclusive evidence, however, is now forthcoming from the experiments with 'Girl'. Here the ligation of the anterior cerebral artery has excluded carotid blood of that side from the greater part of the parolfactory region (see figure 46). It is reasonable to conclude that the region plays no direct role in the osmotic response, but since more complete evidence is desirable it is indicated in only light-black stipple in figure 48.

The experiments with 'Brindle', in which the left hemisphere had been removed, provide alternative evidence for the exclusion of almost the entire thalamus on the grounds of degenerative cell loss from the nuclei of the responsive side. The regions of the thalamus of this animal in which some cells persist are described on p. 251; they comprise the anterior and posterior divisions of the paraventricular nucleus, the parataenial nucleus, the commissural nuclei of both anterior and ventral groups and the paracentral nucleus. Of these nuclei only the paraventricular nucleus appears to retain its full complement of cells, as indeed it does in all the experimental animals at present under consideration; the rest are but sparsely populated. The participation of these latter nuclei in the osmotic response seems unlikely, but convincing evidence for their exclusion is forthcoming from other animals. In 'Girl', as already mentioned, the parataenial nucleus and the commissural nuclei of the anterior group have suffered severe cell loss, whilst in both 'Girl' and 'Jink' the paracentral nucleus and ventral commissural nuclei are amongst those that show degenerative changes. It may be added that in both these animals the degenerative lesion in the thalamus involved many more nuclei than the specific group under discussion, and thus general confirmation is given to the results found in 'Brindle'.

On these grounds, then, the entire thalamus has, with one qualification, been excluded from being the site of the receptors (figure 48). This qualification concerns the thalamic paraventricular nucleus. This nucleus could not be excluded on the grounds of degenerative

cell loss, it being intact in all cases where responses were present, but it is improbable that it is the seat of osmoreceptive elements, since in 'Girl' only a fraction of its extent was receiving carotid blood from the responsive side. It is a structure about which more information would be desirable, and so in the diagram (figure 48) it has been treated like the parolfactory region and has been indicated in lighter black stippling.

On the same grounds that the thalamic nuclei have been excluded so some parts of the ventral diencephalon can be eliminated from being the site of the receptors. However, of these hypothalamic regions, only the mamillary complex will be considered now because the evidence for its exclusion is unequivocal whereas that for adjacent nuclei is not. The major evidence for excluding the mamillary complex comes from observations on blood distribution. In three animals, 'Paris', 'Whitethroat' and 'Girl', the complex, together with the posterior hypothalamic area antero-dorsal to it, are devoid of the suspension infused into the carotid from which osmotic responses were previously being obtained; in a fourth animal, 'Root', the complex is but sparsely injected with suspension. Minor evidence is forthcoming from the observation that in both 'Girl' and 'Jink' the medial mamillary nucleus had suffered degenerative cell loss on the responsive side, and suspension from that side had not reached the contralateral mamillary body.

The conclusion, then, to be derived from the evidence so far collated is that the osmoreceptors lie somewhere in the region comprised by the anterior part of the hypothalamus and the preoptic areas. The region consists of the following nuclei and areas: the medial and lateral preoptic areas, the suprachiasmatic nucleus, the nucleus supraopticus diffusus, the anterior hypothalamic area, the supraoptic nucleus, the paraventricular nucleus, the dorsal and lateral hypothalamic areas, the dorsomedial and ventromedial nuclei and the periventricular system. In every animal these structures were again examined to confirm that none could be excluded by the criteria formulated above; all had received 'marked' blood (though some more completely than others) in each instance where osmotic responses had been present. The region is demarcated by the red stipple in figure 48. Its volume (unilateral) is of the order of 120 cu.mm.

It would be possible, using the criteria we have adopted, to bring the dividing line between receptive and non-receptive regions more mediad as well as a little farther forward in the tuber cinereum (figure 48). Since, however, an attempt to narrow the site of the receptors within the gross field in which we have shown them to lie also involves speculative deductions of post-operative changes in carotid blood flow, and can therefore give evidence of a suggestive character only, we shall defer this attempt to the final discussion.

The evidence from the animals whose responses we have severally considered earlier in this paper, and especially the composite evidence acquired in the last section, points unequivocally, we submit, to the anterior hypothalamus as the region in which osmotic-pressure changes in the arterial blood are causally associated with the secretion of the antidiuretic hormone. We were therefore encouraged to attempt to restrict the blood of one internal carotid artery to this region only. Such restriction can, as we have seen, be attained in theory by tying on one side the anterior cerebral, middle cerebral and posterior communicating arteries just beyond the origins of the tiny vessels which, together with those arising from the internal carotid immediately proximal to its trifurcation, convey

internal carotid blood to this region of the diencephalon. We shall now describe our attempts to do this, and the results obtained.

(11) *Restriction of the blood of one carotid artery to the anterior hypothalamic region by unilateral ligation of the anterior cerebral, middle cerebral and posterior communicating arteries, and its effects on the osmotic release of antidiuretic hormone*

(*a*) *Preliminary observations*

That this procedure would be calculated to have the required effect has, as we have already reported (p. 231), been demonstrated on a cadaver in which, after the three vessels had been tied, coloured gelatin masses were infused into the carotid vascular beds. Before, however, attempting to tie the vessels in the living animal in the form of a surgical procedure, we thought it expedient to tie them in a bitch under chloralose anaesthesia, and thereafter to determine in the usual way the distribution of suspensions infused into the carotid arteries. For this purpose an animal with two carotid loops was used ('Daphne', no. 364).

The left occipital artery was first tied; and after the dura had been exposed (as in the operations described before) the brain was shrunk by the slow intravenous injection of 11 ml 30 % NaCl. The dura was then incised, and a clear view was obtained of the region of the left internal carotid artery. The artery's three primary branches were tied about 3 mm from their origins. Incidentally, before the ligatures were applied one could see the posterior communicating vessel enlarge when the left common carotid was occluded. The ligature on the anterior cerebral artery was just beyond its internal ophthalmic branch, and that on the middle cerebral just beyond its choroidal branch. No haemorrhage was encountered in this manoeuvre, except that at one stage during the freeing of the posterior communicating vessel there was a small and temporary suffusion of blood over the anterior part of the pars distalis. The dural flaps and the temporal muscle were loosely replaced, and the skin edges sutured together. In order to obtain a record of the urine flow, the fundus of the bladder was excised and a self-retaining catheter passed into the residual stump *per urethram*. The animal was now turned so as to lie on its right side in a position similar to that adopted during intracarotid infusions, the upper jaw being loosely supported in a holder. The urine flow was low and showed rhythmic variations between 0·2 and 0·9 ml./min during the subsequent 3 h, except for a marked and transitory rise when on two occasions 5 % dextrose in distilled water was given intravenously. There was no diuresis response to 400 ml. water when on two occasions this was given by stomach tube. The black and the blue suspension were then infused into the right and the left carotid respectively, the animal was killed at the eighth second after the suspensions had reached the infusion needles, and the brain was fixed, embedded and sectioned in the usual way.

Examination of the sections showed that on the left side the caudate nucleus, the internal capsule ventral and lateral to it, and the lentiform nucleus formed an exclusively blue-injected band stretching out to the ventro-lateral surface of the brain. All other regions of the cerebral hemispheres, with the exception of a small patch in the left olfactory tract which was blue, were black-injected; and the suspension, except in the anterior region, was much sparser on the left side than on the right. The thalamus, except for a small zone

in the most anterior and dorsal region on the left side, which was blue-injected, carried exclusively black suspension, this being sparser on the left than on the right side. Similarly the anterior cerebellar areas of both sides were black-injected, the left side more sparsely so than the right. The posterior cerebellar areas, the medulla and the spinal cord were free from suspension. Clearly the right carotid blood had crossed the front and the back of the circle of Willis, and in the latter course had become mixed with unmarked vertebral blood.

Both optic nerves carried black suspension only, with the exception that in the more anterior sections the left nerve was blue-injected. The chiasma, however, in its major and posterior extent carried blue suspension predominantly, on the left side exclusively, and the pars distalis carried both suspensions; the intercarotid anastomosis had evidently escaped from damage during the tying of the vessels. Further survey of the hypothalamic region showed that on the right side this region carried exclusively black suspension. On the left side a small area carrying only blue suspension appeared at the edge of the third ventricle and immediately dorsal to the posterior part of the chiasma; as one traced the sections in a backward direction this blue-injected area at first expanded dorsally and laterally and then contracted so that it disappeared just anterior to the mamillary nuclei. Elsewhere the left hypothalamus was sparsely injected with the black suspension. The blue zone was similar in position and extent to the left half of the cone of hypothalamic tissue which we recognized earlier (figure 11; and figure 15*A*, plate 12) as being part of the carotid field. Most of the left paraventricular nucleus came within this blue half-cone. The anterior division of the left supraoptic nucleus was sparsely black-injected, and only in its extreme medial tip did it carry any blue suspension. The entire extent of the posterior division carried blue suspension only, but it was very sparse. It is not clear why the anterior division carried the black rather than the blue suspension; possibly the tiny vessels supplying it were ruptured when the ligature on the anterior cerebral artery was being applied, and the capillary bed of the medial preoptic area, which was carrying black suspension on both sides, thereupon extended to include the nucleus. That there had been damage to both divisions of the left nucleus was evident from the fact that they contained an inordinate amount of blood pigment, suggesting a degree of stasis in the capillary bed that may have accounted for the sparseness of the suspensions in this nucleus. The point, however, which we feel should be emphasized in the results of this experiment is that, unlike the finding in 'Doris', the whole of the posterior division of the left nucleus was receiving blood of left carotid origin only; the ligature on the posterior communicating artery was preventing any possible contamination with blood from the posterior part of the circle of Willis. We hoped, therefore, that if the cerebral operation was successfully performed under survival conditions, any damage to the minute vascular supply to the nuclei would be of a temporary nature, and that the anterior division of the left nucleus would recover its original supply from the left anterior and middle cerebral arteries. In that event the antero-ventral hypothalamic region on the left side, including both divisions of the supraoptic nucleus, would be receiving left carotid blood; and the opportunity would thereby be presented of obtaining independent evidence for or against our previous conclusion that the osmoreceptors lie in this region of the brain. It was, then, with a feeling of optimism that we embarked on investigations with this end in view.

(*b*) *Experiments with 'Juno', no.* 439

This animal was prepared in the usual way by perineotomy and the formation of two carotid loops; and the responses to intracarotid infusions, before the cerebral vessels were tied, will first be described.

Responses before ligation of the left anterior cerebral, middle cerebral and posterior communicating arteries. Over a period of 5 weeks four infusions were made into the right and five into the left carotid. With right-sided infusions no release of antidiuretic hormone could be detected from 1·20, 1·28 and 2·05M-NaCl, when each was given at the rate of 1·05 ml./min for 15 min. Figure 49*a* shows the absence of such response when 1·20M (graph B) and 2·05M (graph C) were so given. Left-sided infusions, however, even when their strength was as low as 0·86M, gave well-marked inhibitions of urine flow. They are illustrated in figure 49*b*. Here are shown the responses to 0·86M (graph B) and 1·37M (graph C), each given for a period of 15 min, and to 2·05M (graph D) given for a period of 10 min.

The anterior cerebral, middle cerebral and posterior communicating arteries were then tied on the left side by the technique previously described. The anterior cerebral was first tied beyond its internal ophthalmic branch, then the middle cerebral about 2 mm from its origin, and finally the posterior communicating 3 to 4 mm posterior to its junction with the internal carotid. During these manipulations there was a certain amount of damage to the cortex of the piriform lobe by the posterior limb of the retractor. Recovery from the operation was complete and uneventful, and the animal was returned to its standard diet of meat, biscuits and milk a fortnight after operation. During this period 'Juno' always circled to the left when walking, and although later she would progress in a straight line she showed, when turning, a preference for wheeling to the left. The effects of intracarotid infusions of hypertonic solutions of sodium chloride were then tested over a period of 6 weeks, and these effects will now be described.

*Responses after operation.** Five infusions were made into the right carotid and five into the left. They are illustrated in figure 49*c* and *d*. On the right side, infusions which, before operation, had given no response (figure 49*a*) now caused well-marked inhibitions of urine flow. The response to 0·86M-NaCl is given in figure 49*c*; a larger response was seen to 1·33M given for the same period (15 min) and a still larger to 2·05M infused for 7 min only. (Such appearance of response to infusions into one carotid when that to infusions into the other had been suppressed by ipsilateral operative interference was, it will be recollected, encountered in 'Root', in which animal the operative interference involved intradural ligation of the right internal carotid artery.) When, however, in 'Juno', infusions were made into the left carotid no response whatsoever could be elicited (figure 49*d*) from 0·86M (graph B) and 1·50M (graph C), each given for a period of 15 min, or from 2·05M (graph D) given for a period of 10 min. These negative results were a chastening experience, and invited consideration of possible events which, at or after the operation, would make the results compatible with the retention of our previous conclusion that the osmoreceptors are in the anterior hypothalamic region. There were seemingly three possibilities. First,

* During an experiment 6 weeks after operation it was noticed that pressure on the pads of the right forefoot elicited a rhythmic (frequency about 5/s) plantar flexion of the foot which continued while the pressure was being maintained. This clonus was not seen in any of the other feet when pressure there was similarly applied.

at the operation the tiny vessels supplying this region might have been so damaged that they become thrombosed, the region thereupon receiving its blood supply by a movement into it of the capillary bed of the right internal carotid or the left posterior cerebral artery. The answer to this possibility would have to await post-mortem examination. Secondly, a cyst might have developed in the left anterior hypothalamic region and caused destruction, direct or degenerative, of the osmoreceptive site. The answer to this possibility, too, would have to await post-mortem examination. Thirdly, the linear velocity of blood in the left internal carotid and anastomotic arteries might, as a result of tying the three vessels, have become so reduced that there was inordinate delay before the advancing front of raised osmotic activity reached the hypothalamus, and that when it did arrive, so much sodium chloride had been infused as to obtrude upon the action of the released hormone. This seemed a most unlikely explanation because even in those experiments in which the left internal carotid had been tied intradurally, the coloured suspension infused into its common carotid trunk had reached the intercarotid anastomosis within 6 or 7 s. Nevertheless, we decided to test the possibility by determining the responses to short-period intracarotid injections of hypertonic solutions of sodium chloride. The results are shown in figure 49*e* and *f*. When a rapid and short infusion of 0·51 M (0·45 ml./s for 20 s) was made into the right carotid a well-marked inhibition of urine flow was seen (figure 49*e*), whereas when the same infusion was made into the left carotid no inhibition whatsoever resulted (figure 49*f*). Now in order to minimize the risk of the picture of distribution of the two carotid bloods being, when we came to determine it, complicated by a contamination of the vertebral blood with blood of left carotid origin (through the left occipito-vertebral anastomosis), we tied the left occipital artery, and then tested the responses to right and left intracarotid infusions to make sure that they had not been significantly changed as a result of this procedure. No significant change was seen. An infusion of 0·86 M-NaCl into the right carotid at the rate of 1·05 ml./min for 15 min gave a response very like that to the same infusion before the left occipital had been tied (figure 49*c*); and infusions of 0·86 and 1·71 M into the left carotid at the same rate and for the same period gave no inhibitory response (cf. figure 49*d*). On this animal we also made at this stage, and by the technique described earlier (p. 235), three experiments involving simultaneous infusions into each carotid, into the left carotid and saphenous vein and into the right carotid and saphenous vein. The results of these will be given in discussion at the end of this paper.

Tracing the cerebral distribution of the carotid blood. Six days after the last of the above observations had been made the animal was killed by the usual technique during the intracarotid infusion of coloured suspensions (figure 9). In this instance we used as the black suspension Messrs Acheson Colloids' colloidal graphite (solid content about 7 % in water). The blue suspension (Monastral fast blue) was infused into the right carotid, the black into the left, and the animal was killed by the intracardiac injection of chloroform at the ninth second after the suspensions had reached the carotid needles. The brain was prepared for histology by embedding it in celloidin in the usual way and serial frontal sections were made. These revealed the following features.

The three vessels are completely occluded by the ligatures. That on the posterior communicating artery lies just anterior to the thalamic branch of this vessel; this branch is carrying vertebral blood to the middle and anterior thalamus. The ligature on the middle

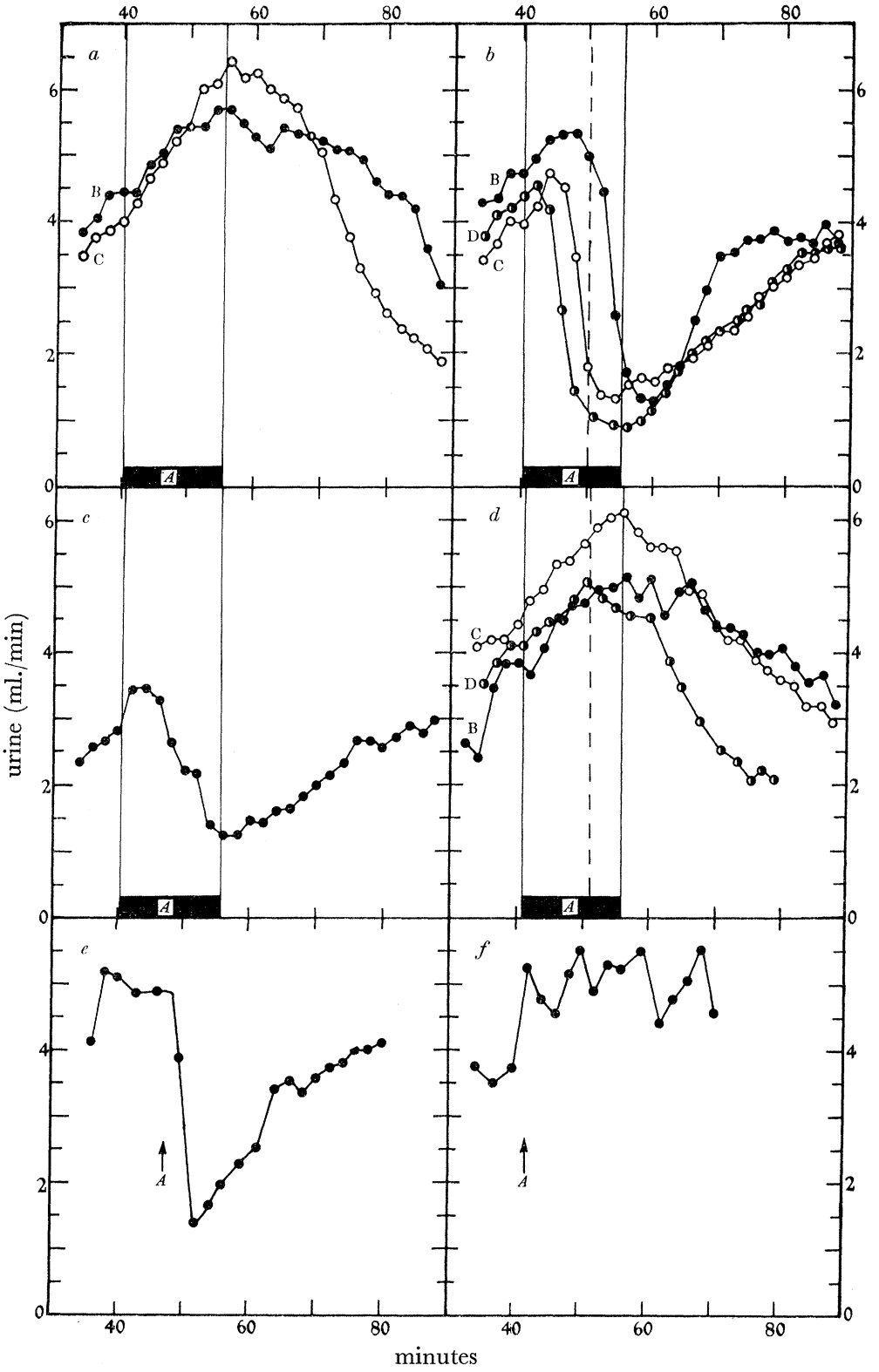

FIGURE 49. 'Juno', no. 439. Responses to infusions of hypertonic solutions of sodium chloride during established water-diuresis. The infusions during the ten or fifteen minute-periods shown by the rectangles *A* were made at 1·05 ml./min., *a* and *b* before, *c*, *d*, *e* and *f* after ligation of the left anterior cerebral, middle cerebral and posterior communicating arteries. *a*, infusions into right common carotid: 1·20M (graph B), 2·05M (graph C). *b*, infusions into left common carotid: 0·86M (graph B), 1·37M (graph C), each given for a period of 15 min, and 2·05M (graph D) given for a period of 10 min. *c*, infusion into right common carotid, 0·86M. *d*, infusions into left common carotid: 0·86M (graph B), 1·50M (graph C), each given for a period of 15 min., and 2·05M (graph D) given for a period of 10 min. *e*, infusion into right common carotid at the arrow *A* of 0·51M at the rate of 0·45 ml./s for 20 s. *f*, the same infusion as in *e*, but given into the left common carotid. Abscissae: time after the test dose of water (*a* and *b*, 450 ml.; *c*, *e* and *f*, 400 ml.; *d*, 400 ml., graph B, and 450 ml., graphs C and D).

cerebral artery lies just proximal to its large choroidal branch, and that on the anterior cerebral just distal to the internal ophthalmic artery.

There is a cyst in the left dorsal hypothalamus (figures 50 and 51) immediately dorso-medial to the ligature knot on the posterior communicating vessel. The cyst extends for 3·2 mm in an antero-posterior direction from the coronal level of the posterior edge of the optic chiasma to a plane just posterior to the median eminence. The dorsal limit is at the

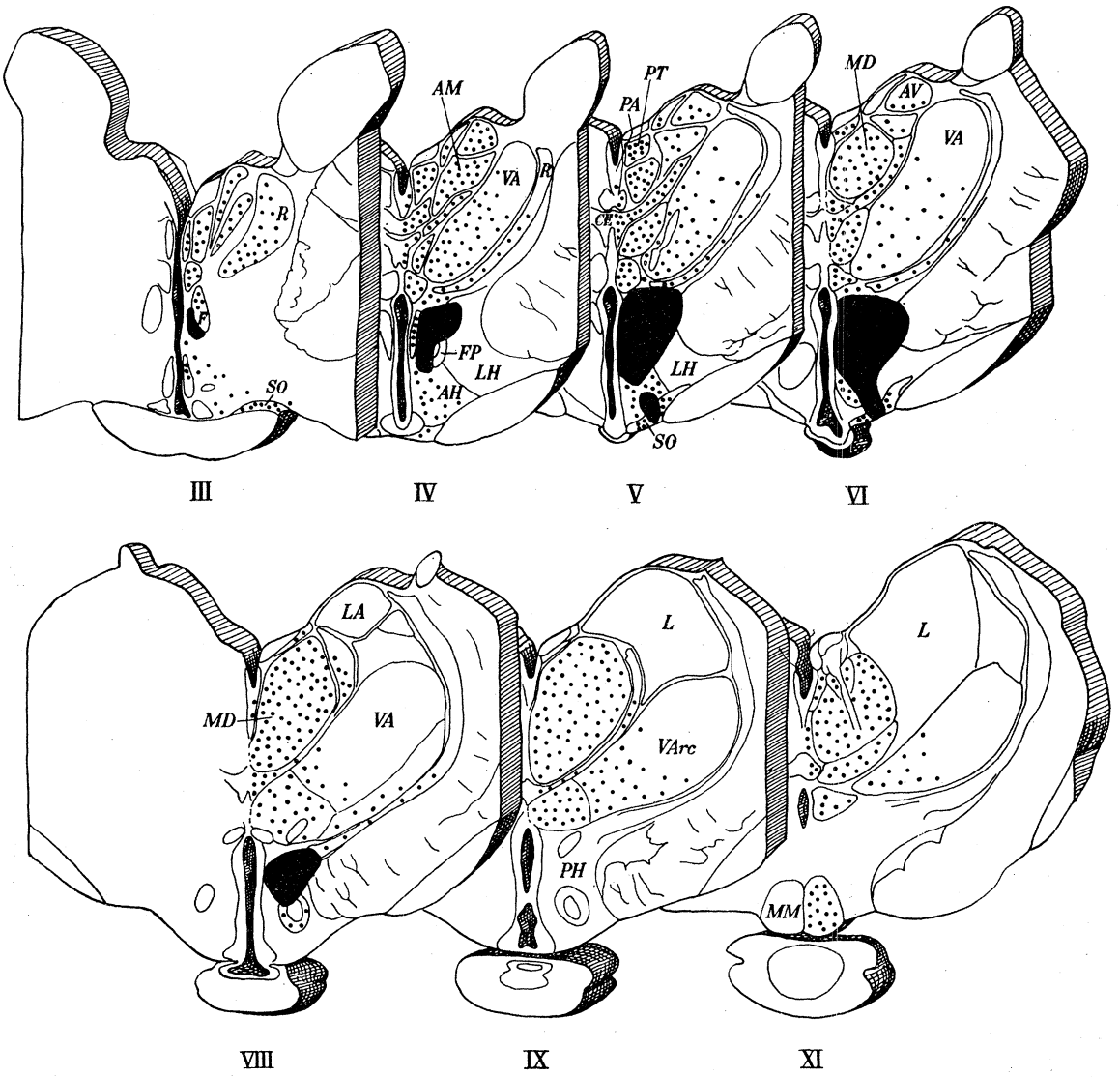

FIGURE 50. 'Juno', no. 439. Nuclei of the diencephalon which have suffered degenerative lesion. The sections are selected from those shown in figure 27 and carry the same numerals. Cystic lesion shown in black; nuclei showing cell loss indicated by dots. Abbreviations as in figure 27.

level of the sulcus limitans, and there is no extension into the thalamus. A ventral tongue passes down to the floor of the tuber cinereum through the posterior division of the supra-optic nucleus (figure 50, V and VI). The greatest dorso-ventral extent of the cyst, excluding this tongue, is 1·9 mm. The nuclei and areas involved in the cyst are the dorsal part of the anterior hypothalamic area, the fornix and the perifornical area, the postero-lateral quadrant of the paraventricular nucleus, the dorso-medial nucleus and adjacent

dorsal hypothalamic area. Also involved are the lateral hypothalamic area, fibre bundles of the internal capsule lateral to it, and the middle part of the posterior division of the supraoptic nucleus together with part of the ventro-medial nucleus dorsal to it.

In addition to the nuclei and areas directly involved in the cyst certain other left hypothalamic regions exhibit cell loss. In the paraventricular nucleus this loss is severe, cells surviving only in the most antero-ventral extent. The anterior division of the supraoptic nucleus is similarly almost devoid of cells, although a conspicuous group of accessory supraoptic cells lying midway between this division and the paraventricular nucleus persists. The posterior division of the nucleus is, as already stated, cleft by the ventral tongue of the cyst, and this has left severely reduced sections of the nucleus, in which the cells are very sparse, adjacent to the median eminence and to the optic tract. The posterior lobe shows no obvious shrinkage or other abnormality. Other regions of the middle hypothalamus show a compacting of the cells consequent upon fibre loss. The posterior hypo thalamic area and the medial mamillary nucleus both show cell loss, and the mamillothalamic tract is reduced in size. The cyst lies so close to the third ventricle that the separating wall of tissue is reduced in places to a thickness of 180μ; periventricular cells persist in this thin partition, but much of the periventricular system of fibres descending from the midline nuclei of the thalamus to the hypothalamus must have been destroyed.

The left thalamus, too, shows cell loss in many of its nuclei (figure 50). At anterior levels all thalamic nuclei show such loss. The intralaminar and medial nuclei are almost completely devoid of cells; but the anterior group of nuclei, the ventral nucleus and the reticular nucleus have persisting cells, the density of which increases at more posterior levels. The paraventricular nucleus persists but is reduced in extent. At midthalamic levels the parataenial nucleus is still thinly populated and the dorsomedial nucleus is pale and with few cells. The midline nuclei, intralaminar nuclei and the nucleus submedius are not in evidence, but the paraventricular nucleus is comparable in extent and cell density with its fellow of the right side. The more laterally situated of these nuclei (e.g. centralis lateralis) recover a normal appearance more posteriorly, but the medial and midline nuclei remain sparse in cells to the posterior limit of the thalamus. The work of Powell & Cowan (1954; Cowan & Powell 1955) would suggest that the degenerative lesions just described in the thalamus are owing to the interruption by the cyst of fibres of the inferior thalamic radiation and to damage in the piriform lobe of the cortex (figure 51).

With respect to the general distribution of the suspensions, this is for the most part as would have been expected from the position of the ligatures. The right carotid blood (blue suspension) is passing posteriorly in the posterior communicating artery to mix at the back of the circle with vertebral blood which itself contains some blue (but no black) suspension that has been carried into it through the right occipito-vertebral anastomosis. The right carotid blood passes also across the front of the circle to supply the left anterior cerebral field, and in doing so picks up a little black suspension that is reaching it through the internal ethmoidal arteries; both olfactory bulbs are well injected with the black suspension. The left middle-cerebral field, curiously enough, is well and predominantly injected with the black suspension, and this may in part have been carried there through anastomoses from the hypothalamic vessels, since no other obvious anastomoses with origins proximal to the ligatures could be found.

The distribution in the diencephalon is illustrated in figure 51. Blue suspension is distributed throughout the thalamus of both sides, being sparser on the left than on the right owing to the vertebral contribution to the flow in the left posterior communicating vessel behind the site of its ligature. In the hypothalamus the black suspension from the left carotid is distributed throughout the optic chiasma and the adjacent portion of the optic nerves and tracts of both sides. The whole of the left anterior hypothalamus carries black suspension, but, as we have seen, it is in part cystic and denuded of cells. However, the left carotid blood has reached a number of contralateral anterior hypothalamic structures, the ones exclusively injected with the black suspension being the periventricular system and medial part of the suprachiasmatic nucleus, the greater part of the nucleus supraopticus diffusus and the median eminence, the medial part of the anterior hypothalamic area and the antero-medial part of the posterior division of the supraoptic nucleus. The amount of black in the right anterior divison of the supraoptic nucleus is negligible, whilst only the antero-ventral tip of the paraventricular nucleus is invaded by the black suspension. Blue suspension is present throughout the right hypothalamus in the region lateral, dorsal and posterior to the black-injected zone. Thus the anterior division of the supraoptic nucleus, the greater part of the paraventricular nucleus and the lateral part of the posterior division of the supraoptic nucleus have received blood of exclusively right carotid origin.

As in other animals the volume of supraoptic nuclear material and the proportions of it supplied exclusively by right and left carotid blood were computed. The figures are given in table 2. The proportions of total supraoptic nuclear material occupied exclusively by the black and the blue suspensions were approximately the same, but about 55 % of that supplied by left carotid blood was so denuded of cells that it could have been taking little if any share in neurohypophysial function.

Discussion. The fact that the left anterior hypothalamus is for the most part cystic and denuded of cells would adequately account for the disappearance, after operation, of the antidiuretic response to left carotid infusions; as although the left carotid blood has encroached a little into the right anterior hypothalamus, only about 0·9 cu.mm of supraoptic nuclear material with normal cytological appearance is supplied exclusively with blood of left carotid origin. The amount supplied exclusively by blood of right carotid origin, however, is about 2 cu.mm. This distribution would account for the appearance, after operation, of responses to right-sided infusions if we assume that before operation (when no such responses were obtainable) the cerebral distribution of left preponderated strongly over that of right carotid blood. We have already noticed that in 'Rita' (p. 254 and table 2), the other animal in which no responses could be elicited from infusions into the one carotid, there was such dominance of the cerebral field supplied by the contralateral carotid. On the other hand, the results from this animal, taken in conjunction with those from the previous one ('Girl'), favour the view that the supraoptic nucleus is not a particular area in the anterior hypothalamic region with the independent property of functionally responding to osmotic pressure changes in the arterial blood. The amount of histologically normal supraoptic nuclear material supplied by left carotid blood was small and about the same (0·9 cu.mm) in each animal, yet responses to left-carotid infusions were retained in 'Girl' and abolished in 'Juno'; and this discordance is intensified by the

fact that the blood supply to this small volume of normal nuclear material is, in 'Juno', from the left carotid exclusively, and, in 'Girl', from both left and right carotids. Now the most obvious difference between the post-mortem diencephalic findings in these two animals is the position of the cyst (cf. figures 47, 50). While in 'Girl' the cyst (the larger one)

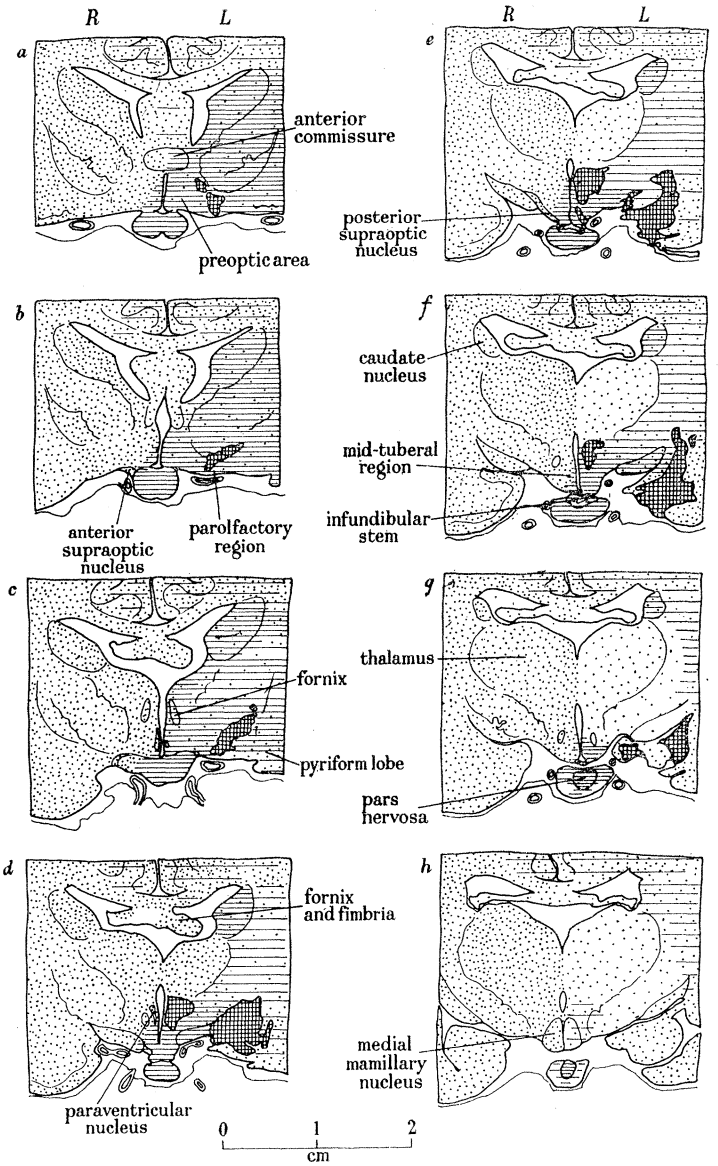

FIGURE 51. 'Juno', no. 439. Maps of selected sections to show the diencephalic distribution of the suspensions. Blue suspension infused into the right carotid—dots, black into the left—lines. The distance between the anterior surfaces of sections *a* and *b*, *b* and *c*, etc., is 1 mm, except between *g* and *h* where it is 2 mm. The cross-hatched areas represent cystic lesion.

involves the anterior part of the ventro-lateral regions of the thalamus and only invades the hypothalamus in its most dorsal and lateral regions, in 'Juno' the cyst is entirely in the left anterior hypothalamus, and, furthermore, extends so near to the wall of the third ventricle that many periventricular thalamo-hypothalamic pathways must have been divided. In the hope of obtaining more information on the possible role of the thalamus

in modifying the responsiveness of the anterior hypothalamus to osmotic pressure changes, we undertook another investigation similar to that on 'Juno', and the procedures and results will now be given.

(*c*) *Experiments with 'Linda', no.* 385

This animal was prepared for investigation by perineotomy and the formation of two carotid loops. The left occipital artery was tied at the same operation as that at which the right carotid loop was made.

Responses before ligation of the left anterior cerebral, middle cerebral and posterior communicating arteries. Over a period of 3 weeks eight tests were made of the effects of intracarotid and intravenous infusion of hypertonic solutions of sodium chloride. The results are illustrated in figure 52*a* and *b*. Each infusion was at the rate of 1·05 ml./min and for a period of 10 min. In figure 52*a* are given two responses to infusions into the right carotid, the one (graph B) being the response to 1·37M-NaCl, the other (graph C) the response to 1·54M-NaCl. In figure 52*b* are given two responses to infusions into the left carotid, the one (graph B) being the response to 1·20M-NaCl, the other (graph C) the response to 1·37 M-NaCl. The graph D shows the absence of any inhibition when an infusion of 1·37M-NaCl is given intravenously. Having measured the responses to intracarotid infusions we proceeded to tie the three main branches of the left internal carotid.

When exposure of the region had been completed the posterior communicating artery was tied (see p. 258) as it crossed the lateral face of the pars distalis and some 4 to 5 mm posterior to its origin from the carotid. The anterior tag of the knot was unfortunately unduly long and it projected on to the lateral aspect of the median eminence, but the risk involved in shortening it appeared at the time too grave to take. The anterior cerebral was then tied as it passed on to the dorsal surface of the optic nerve and immediately beyond its internal ophthalmic branch. After this had been done, the middle cerebral was tied 3 mm from its origin. There was no haemorrhage nor macroscopic damage to the brain during these manipulations. The operation was completed in 7 h, and recovery was uninterrupted (see figure 53, plate 12). Experiments on the osmotic release of anti-diuretic hormone were resumed a fortnight after the operation, and continued over a period of 4½ months. These experiments will now be described.

Responses after operation. In figure 52*c* are given two responses to infusions into the right carotid. Each infusion was at the rate of 1·05 ml./min and for a period of 10 min. Graphs B and C give the responses to 1·71M-NaCl. Fifteen weeks elapsed between the last two experiments, and the results demonstrate that the responses to infusions into the right carotid were not changing appeciably with time. Comparison of these responses with those obtained before operation (figure 52*a*) will show, as may have been expected, that the response after operation is a little smaller than that before operation. In this instance it will be seen that the response to 1·54M-NaCl before operation (figure 52*a*, graph C) equates closely with those to 1·71M-NaCl after operation (figure 52*c*, graphs B and C). When, however, infusions were made into the left carotid no response whatsoever could be elicited (figure 52*d*). Here we see that there was no response to 1·37M-NaCl (graph B; cf. the effect of the same infusion before operation, graph C, figure 52*b*), and that when the molarity was raised to 1·71 no certain difference was detectable between the

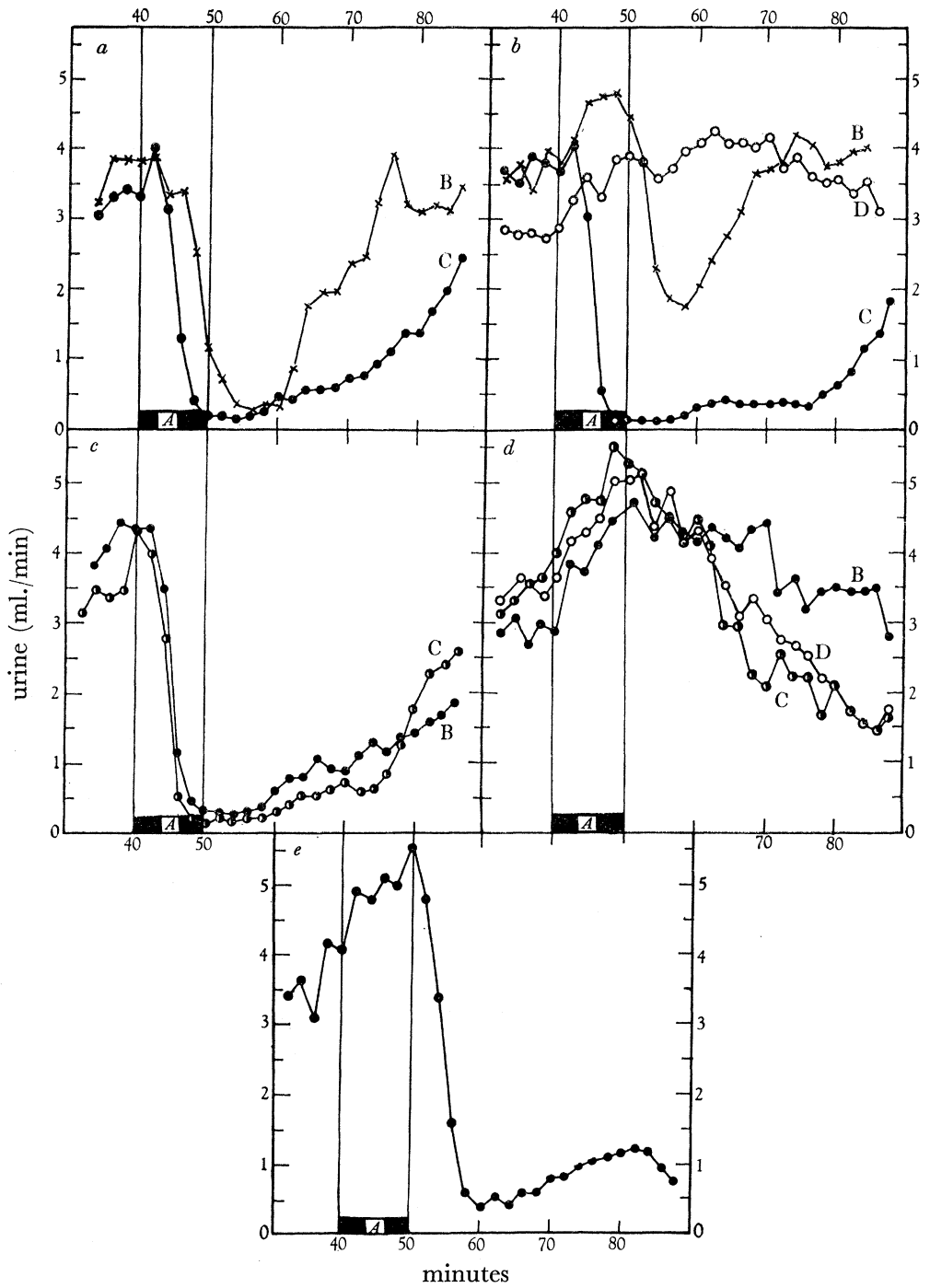

FIGURE 52. 'Linda', no. 385. Responses to infusions of hypertonic solutions of NaCl during established water-diuresis. The infusions were made at 1·05 ml./min during the 10 min periods shown by the rectangles *A*. *a* and *b* before, *c*, *d* and *e* after ligation of the left anterior cerebral, middle cerebral and posterior communicating arteries. *a*, infusions into the right carotid: 1·37M, graph B; 1·54M, graph C. *b*, infusions into the left carotid: 1·20M, graph B; 1·37M, graph C. Graph D shows the effect of an intravenous infusion, 1·37M. *c*, infusions into the right carotid: 1·71M, graphs B and C. There was an interval of 15 weeks between these two experiments. *d*, infusions into the left carotid: 1·37M, graph B; 1·71M, graph C. Graph D shows the effect of an intravenous infusion 1·71M. *e*, infusion into the left carotid, 2·57M. Abscissae: time after the test dose (350 ml.) of water.

effects of the intracarotid (graph C) and those of the intravenous infusion (graph D). Four further tests were made with this solution—one intravenous infusion, three left intracarotid infusions—with negative results. It was necessary with these strong (1·71 M) solutions to make the intravenous infusions into the left external jugular vein, as the blood flow through the malleolar vein was not large enough to prevent sensory disturbances from originating from the intima. When the strength of the intracarotid infusion was raised even to 2·57 M (figure 52*e*) no certain response was obtained; when at 53 min, the urine flow was beginning to fall the water load was 197 ml., which is very close to the isotonic volume of the 1·575 g of sodium chloride which had by then been infused, viz. 185 ml. As in 'Juno' (p. 299) the effects of short and rapid intracarotid infusions were also tested. When 10 ml. of 0·513 M-NaCl were injected into the right carotid at 0·5 ml./s the response shown in figure 54*a* was obtained; there was a faint whine from the twelfth to the fourteenth second of the injection and a

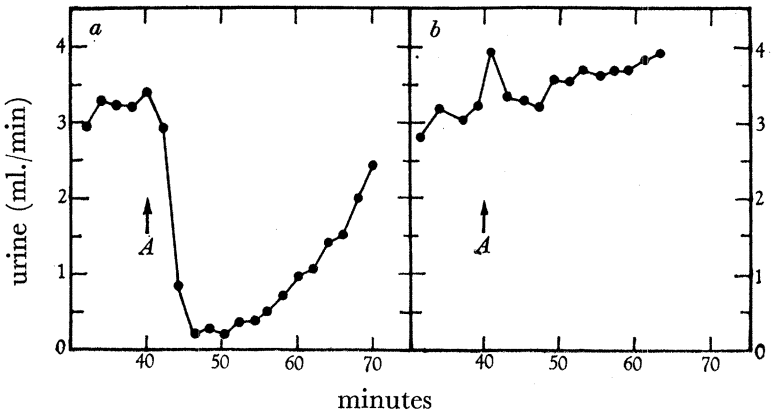

FIGURE 54. 'Linda', no. 385. The effects, during established water-diuresis, of an injection at the arrow *A* of 10 ml. of 0·513 M-NaCl at 0·5 ml./s into *a* the right carotid, and *b* the left carotid. The injections were made during the twenty-first week after the left anterior cerebral, middle cerebral and posterior communicating arteries had been tied. Abscissae: time after the test dose (350 ml.) of water.

little lip-smacking just after its end, but the animal was seemingly quite undisturbed by the procedure. When the same injection was made into the left carotid there was again a faint whine from the twelfth to the fourteenth second of the injection and a little lip-smacking just after its end, but no inhibition whatsoever in the rate of urine flow occurred (figure 54*b*). We may with reason infer from these results that the left carotid blood is reaching some part of the brain associated with conscious perception, but not—as is the case with the right carotid blood—involving the osmoreceptive region; or, alternatively, that if this region is being reached by the left carotid blood, either the region has become cystic or subsensitive to an otherwise effective osmotic stimulus, or the concentration of this blood in the total supply to the region is insufficient to elicit a detectable release of antidiuretic hormone. We then decided to determine the cerebral distribution of the right and left carotid bloods in this animal by the method previously described.

Tracing the cerebral distribution of the carotid blood. The animal received the same preliminary treatment and lay on the table in the constant-temperature room in the same position (figure 9, plate 10) as in experiments in which intracarotid and intravenous

infusions had previously been given. The apparatus for the infusion of the coloured suspensions was arranged so that the blue suspension entered the left and the black the right carotid. The infusion rate was 0·2 ml./s into each carotid, and the animal was suddenly killed at the sixth second after the suspensions had reached the infusion needles. There was no technical hitch during the procedure and there were no manifestations of the animal being in any way disturbed while the suspensions were being infused. Death occurred at the thirty-sixth minute after the test dose of water, and by then a volume of urine had been secreted which was within the range of the volumes secreted in the same period in the earlier experiments. At death the left half of the tongue was blue, with a sharp medial partition line. The head was removed, placed in a jar of fixative in the refrigerator, and later dissected by stages. There was no trace of any infection at the site of the operation 5 months earlier; perfect bony union of the zygoma had occurred, and over the opening in the skull there had formed a thin fibrous sheet to which fibres of the temporal and lateral pterygoid muscles were attached, and to the deep surface of which the brain was not adherent. The whole brain was later embedded in celloidin and sectioned on the plan already described. Examination of the sections showed, as was expected, that there had been marked narrowing of the lumina and retraction of the walls of the left internal carotid and of the anterior and middle cerebral arteries and the posterior communicating artery as far as the three ligatures, and that proximal to the ligatures these vessels were filled with the blue suspension; beyond the ligatures the vessels were of normal size and were filled with the black suspension. The left internal ophthalmic artery contained blue suspension only (the anterior cerebral ligature was placed beyond its origin), and the intercarotid anastomosis was carrying blue suspension from left to right through its whole extent, only its right mouth being filled with the black suspension. Maps of selected sections to show the diencephalic distribution of the suspensions are given in figure 55.

Anterior to the chiasma the only regions that were not exclusively black-injected were the anterior part of the left olfactory bulb which carried both suspensions, and the optic nerves which were predominantly blue-injected. The chiasma carried blue suspension almost exclusively, and as it expanded into the optic tracts a blue-injected area appeared on the left side of the base of the third ventricle and rapidly expanded dorsally as far as the antero-medial region of the thalamus, and laterally to involve the whole of the anterior hypothalamic region. As the sections were traced further posteriorly the blue-injected area contracted and disappeared just behind the level of the posterior median eminence. All the rest of the brain, and the cervical cord, were well injected, and apparently uniformly so, with the black suspension; and it is evident that the right occipito-vertebral anastomosis was making a weighty contribution to the cerebral supply. The operation, then, had been successful in the sense that the left carotid blood had been restricted to the left anterior hypothalamic and ventral thalamic regions.

A complication, however, had occurred in the anterior thalamus in the form of a cyst which had replaced most of the normal anterior thalamic structures on the left side. Its size and position are shown in figures 55 and 56; and in figure 57, plate 13. It stretched for a distance of 6 mm antero-posteriorly from the anterior end of the thalamus to a coronal level just anterior to the mamillary bodies. At its greatest width its lateral border reached the medial edge of the internal capsule. At anterior levels (figure 56, IV) the cyst has

replaced all the left thalamic nuclei with the exception of denuded portions of the nuclei anterodorsalis, anteromedialis, reticularis and ventralis pars anterior. At more posterior levels it is the nuclei medial to, and included in, the internal medullary lamina that are directly involved (figure 56, VII). The cyst ends as a tongue in the internal medullary lamina (figure 56, IX), but the posterior medial and midline nuclei are characterized by

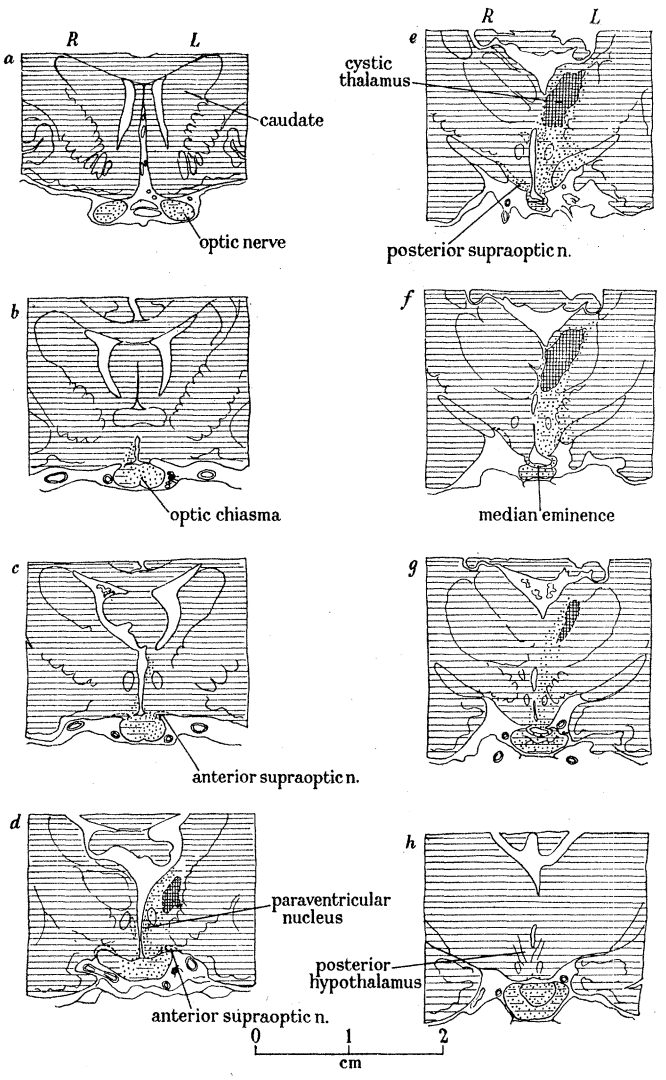

FIGURE 55. 'Linda', no. 385. Maps of selected sections to show the (cerebral) distribution of the suspensions. Blue suspension infused into the left carotid—dots; black into the right—lines. The distances between the anterior surfaces of sections *a* and *b*, *b* and *c*, etc., are 4, 1·3, 1, 2, 1, 1·3 and 2 mm respectively. The cross-hatched areas represent cystic lesion.

almost complete cell loss. The structure of the cyst was similar to that of the one in 'Girl' (p. 290). This and the nuclear degeneration associated with the cyst are illustrated in the photomicrographs of figure 57, plate 13. The only parts of the left thalamus that appeared normal histologically were part of the antero-dorsal and antero-ventral nuclei, part of the ventral nucleus (principally pars arcuata) and of the reticular nucleus, the lateral nuclei, posterior nuclei and pulvinar and the habenular nuclei. The stria habenularis remained

intact on the dorsal surface of the cystic area. It is to be noticed that the degeneration has extended right up to the midline and that the most striking difference between the thalamic picture in 'Brindle' (p. 251)—in which animal well-marked responses were being obtained from left intracarotid infusions—and that in 'Linda' is that in the latter animal the

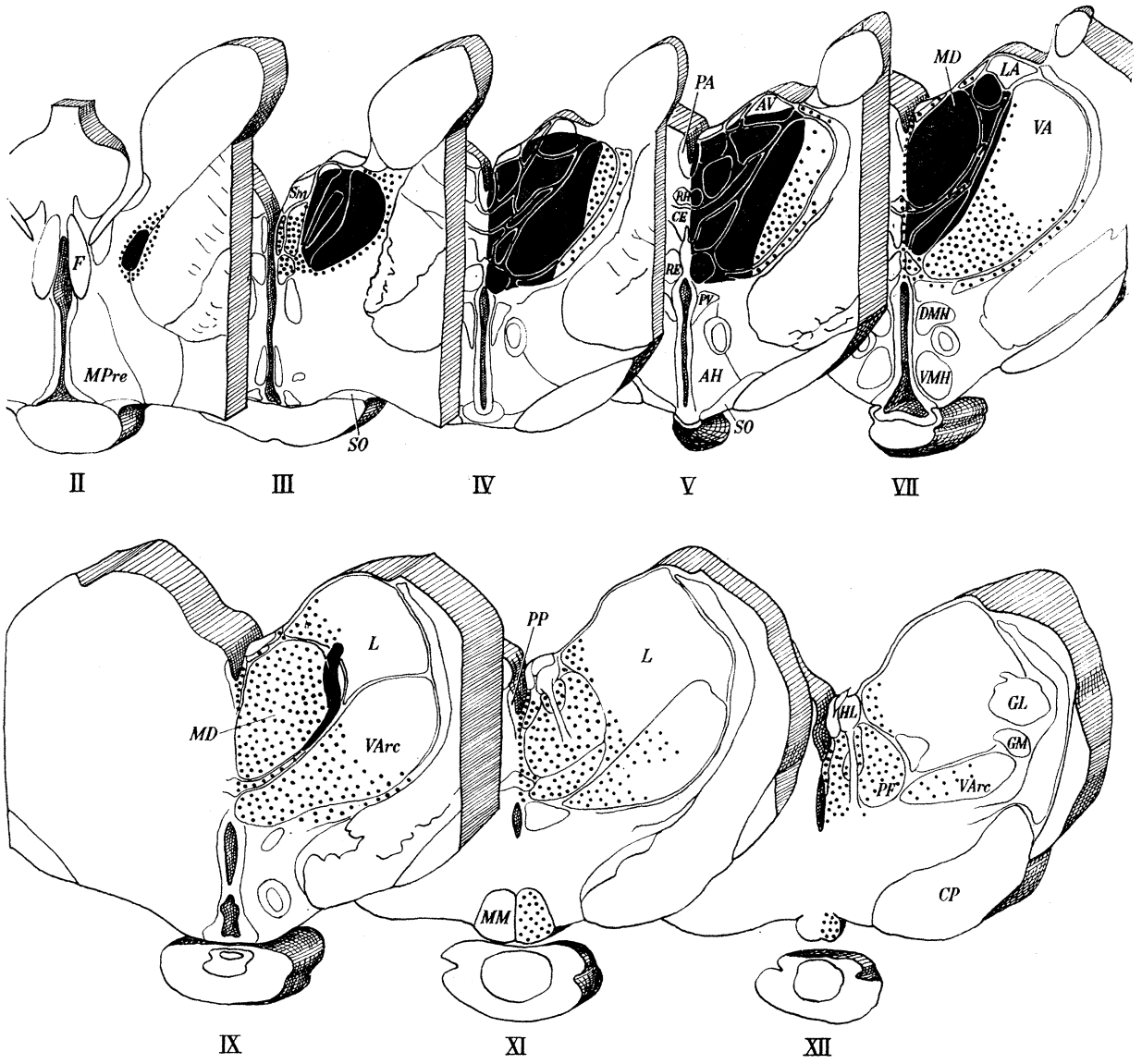

FIGURE 56. 'Linda', no. 385. Nuclei of the diencephalon which have suffered degenerative lesion. The sections are selected from those shown in figure 27 and carry the same numerals. Cystic lesion shown black; nuclei showing cell loss indicated by black dots. Abbreviations as in figure 27.

thalamic paraventricular nucleus has degenerated, together with a large part of the periventricular system of fibres, many of which must have been passing to the hypothalamus. In spite of this extensive destruction of the left anterior thalamic nuclei in 'Linda', the only change noticed in her behaviour was that during about the last month of life she was a little more reactive in the sense that she would not tolerate other dogs' playful attention; she would head them away, but was never vicious.

In the hypothalamus the left medial mamillary nucleus has completely degenerated owing to direct destruction of the mamillothalamic tract by the cyst; and the only other abnormality noticed in this region—this was seen only in one section—was compaction of cells in the anterior part of the left ventro-medial nucleus, this possibly indicating some fibre loss.

Attention was then given to the distribution of the suspensions in the hypothalamic paraventricular and supraoptic nuclei. The right paraventricular nucleus was exclusively black-injected, the left predominantly or exclusively blue-injected. The distribution in the supraoptic nuclei was determined by the method already described, that is to say, projection drawings of the serial sections were made at a magnification of 30 × and the distribution of the suspensions in the nuclear regions carefully recorded on them. The unstained sections over the extent of the posterior divisions, as well as those of the anterior divisions,

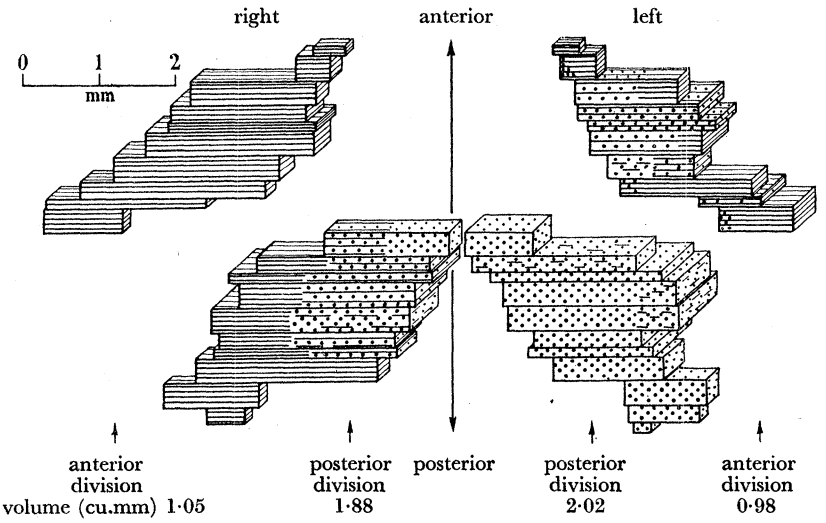

FIGURE 58. 'Linda', no. 385. Plan and isometric projection of the series of blocks of nuclear material comprising the supraoptic nuclei, and the distribution of the suspensions within them. Black suspension—thin parallel lines. Blue suspension—dots.

with which difficulty was experienced in defining the borders, were then remounted after they had been stained with toluidin blue, the nuclei outlined on the original drawings, and all the nuclear areas cut out and weighed. The plan and isometric projection of the series of blocks of nuclear material—each block having a volume equal to that of the nuclear material in the corresponding section—and the distribution of the suspensions within them are given in figure 58. On the right side the whole of the anterior division and the lateral and posterior parts of the posterior division were exclusively black-injected; these regions therefore, were receiving blood of raised osmotic pressure when infusions were being made into the right carotid artery. It is not possible to say whether the black suspension in the 'vertebral field' of the posterior division was being carried there by the internal carotid, by the right occipito-vertebral anastomosis, or from both of these sources; though from the marked posterior extension of the carotid field in previous animals in which the contralateral carotid supply had been restricted, it would seem highly probable that the source of the black suspension was the internal carotid alone. The antero-medial parts of the posterior division carried

blue suspension contaminated over most of its extent with black suspension; the extreme antero-medial part was exclusively blue-injected. Evidently the presence of blue suspension in the antero-medial part of the posterior division was owing to left-sided pressure preponderance in the intercarotid anastomosis (an observation which we mentioned earlier) and therewith in the remaining members of that group of vessels which we have named the arteries of the glandular hypophysis, median eminence and posterior supraoptic nucleus; the pars distalis and median eminence were, indeed, injected predominantly with the blue suspension. On the left side the intermediate zone of the anterior division carried both suspensions, but the extreme antero-medial tip and the postero-lateral part carried exclusively the black suspension. This exclusive injection of the extremities of the anterior division with the black suspension is presumably owing to the ligatures on the anterior and middle cerebral arteries not having been placed quite distal enough to include within the field of the left internal carotid the branches supplying these parts of the nucleus. *The whole of the posterior division, however, was well injected with the blue suspension, and almost exclusively so*, the ligature on the left posterior communicating vessel having ensured that the postero-lateral part of the division was shielded from its normal vertebral source of supply.

Discussion. On the right side the whole of the anterior division of the supraoptic nucleus and 44 % of the posterior division (figure 58) were well and exclusively injected with the black suspension, and were therefore receiving, after operation, blood of right carotid origin. This blood was supplying in addition about 34 % of the anterior division of the left nucleus, this proportion of the division being exclusively black-injected. The total volume of nuclear material so injected was 2·22 cu.mm, 0·71 cu.mm less than the total volume of the right supraoptic nucleus (table 2). These findings are clearly compatible with the hypothesis that the receptors are in, or in the region of the nucleus; and on this hypothesis the diminution observed after operation in the response to infusions into the right carotid may have been caused not only by an increase in carotid flow but also by a decrease in the proportion of osmoreceptive field which this carotid then supplied. On the left side, however, the responses to intracarotid infusions were, as we have seen, completely and permanently suppressed by the ligation of the anterior and middle cerebral arteries and the posterior communicating vessel, in spite of the fact that the whole of the posterior division of the nucleus on that side was well supplied with blood of left carotid origin. Furthermore, the volume of the nuclear material and the histological appearance of the cells were normal, and there had been no injury to the supraoptico-hypophysial tract; the anterior tag of the ligature on the posterior communicating vessel was lying on the lateral surface of the pars distalis, and there was no sign of degenerative shrinkage of the pars nervosa. Of the total nuclear volume as much as 35 % was well and almost exclusively supplied with blood of left carotid origin (see table 2). It seems to us highly improbable that, were normally responsive receptors present in, or in the immediate neighbourhood of the nucleus, no release of antidiuretic hormone should have been detected in 'Linda' in response to the large increases in osmotic pressure which were produced in the left carotid blood during the infusion experiments. Yet no release could be elicited. But in this animal a cyst was replacing most of the anterior thalamus on the left side and was, as we have seen, associated with extensive degeneration of the anterior

and medial thalamic regions. It is difficult not to associate this lesion with the absence of osmotic release; and in view of the strong evidence previously presented that the receptors are in the anterior hypothalamus one is led to infer from the findings in 'Linda' that the responsiveness of the receptors is dependent on the integrity of certain centripetal hypothalamic pathways; we have already noted that in 'Linda', and in contrast with the findings in the animals in which responses were retained after operation, the thalamic paraventricular nucleus had degenerated.

The position, then, at which we have now arrived is that the receptors are in the anterior hypothalamus but only function when their connexions with the antero-medial thalamus are preserved. For in both 'Linda' and 'Juno' responses to left-sided intracarotid infusions vanished after operation; and in the former animal the whole of the left antero-medial thalamus was destroyed (figure 56), while in the latter the hypothalamic cyst was so close to the wall of the third ventricle (figure 50) that thalamo-hypothalamic pathways in the periventricular system would inevitably have been directly involved. In 'Girl' and 'Jink', on the other hand, responses were retained after operation. In the former animal the cyst (figure 47) was much more laterally placed than in 'Linda' and 'Juno', and although most of the antero-medial thalamus showed severe cell loss the thalamic paraventricular nucleus was preserved. This nucleus was preserved, too, in 'Jink' (figure 44); here there was much less degenerative involvement of the antero-medial thalamus than in 'Girl'. Lastly, in 'Brindle', in whom responses were retained after left hemispherectomy, all the dorsal thalamic nuclei with the exception of the paraventricular nucleus and cell groups in some other medial nuclei had degenerated (figure 28).

With all the animals listed in table 2 in which tests of antidiuretic responses to intracarotid infusions were made, the results, with the sole exception of those obtained with 'Girl', are consistent with, and in many instances strongly supportive of the hypothesis that the receptors are in, or in the near region of the supraoptic nuclei but functionally dependent upon their linkage with the antero-medial thalamus; the particular structure in the antero-medial thalamus that would appear to be suspect in this connexion is the paraventricular (thalamic) nucleus.

We have now finished the description of our investigation so far as it has yet been carried; so in the general discussion which follows we shall, first, aline our evidence on the gross site of the receptors; secondly, consider any evidence that is forthcoming for their narrower localization within this site; and, thirdly, indicate the sort of experiments that could best be done to test the more conjectural elements in the hypothesis at which we finally arrive.

III. DISCUSSION

There is no need to repeat the evidence by which the posterior lobe of the pituitary, the rhombencephalon, the mesencephalon and the telencephalon (with the exception of the preoptic areas) have been excluded from being the receptors' site; this has been done on the sure criterion of retention of osmotic response from intracarotid infusion of hypertonic solution and either absence of this carotid blood from the region under review, or structural or functional absence of the region as a result of operative removal or post-operative lesion of destructive or degenerative character. The same criterion applied to the diencephalon

has excluded the whole of the thalamus (with the exception of its paraventricular nucleus) and the posterior part of the hypothalamus, i.e. the posterior hypothalamic area and the mamillary complex. These results have been presented in figure 48 (facing p. 251), and there the non-excluded part of the hypothalamus, i.e. the region in which, by inference, the receptors lie, has also been demarcated (red stipple). This region, then, received in each instance blood from that carotid from infusions into which osmotic responses had been obtained. And this was so with the last two animals, 'Juno' and 'Linda'; at the time the evidence summarized in figure 48 was collected, experiments on these animals had not been made.

Before attempting to see whether our experimental results contain any evidence favouring a narrower localization than that shown in figure 48, it will be convenient to consider the status of the thalamic paraventricular nucleus as a possible primary site of the receptors. This nucleus has remained undamaged in all instances where osmotic responses were present; and in 'Linda', as we have seen, its destruction on the left side was associated with the disappearance of responses to intracarotid infusions on that side, although the ipsilateral anterior hypothalamus was intact and was being well supplied by ipsilateral carotid blood. We have, therefore, re-examined our material with the object of gaining evidence for or against this nucleus being primarily involved in osmoreception. Evidence of such nature comes from six animals. In 'Root' (p. 265) responses to infusions into the left carotid were much increased when the right internal carotid had been tied. The extreme anterior end only of the left nucleus carried left carotid blood (blue suspension, see figure 36), and even here the injection was not so intense as in the antero-ventral hypothalamus. The rest of the nucleus was uninjected or sparsely and patchily injected and had apparently received its blood supply as a mixture, in the posterior cerebral artery, of left carotid and unmarked vertebral blood. It is difficult to believe that, before operation, when a minimal response only could be elicited from infusions into the left carotid, the supply of left carotid blood to the nucleus was even less than after operation. The facts, then, are against the primary involvement of this nucleus in the osmotic response. As mentioned earlier, we made in this animal a few double-infusion experiments, i.e. simultaneous infusion into each carotid or into one carotid and a systemic vein. The idea prompting these experiments was that if the thalamic paraventricular nucleus was 'integrated' with the antero-ventral hypothalamus in an osmoreceptive function, and some of the fibres from the nucleus decussated in the periventricular system to reach contralateral hypothalamic structures, an infusion into each carotid might elicit a larger response than, in this animal, that obtained from infusion into the left carotid and a systemic vein. Actually no such effect was seen. When 0·86M-NaCl was infused into each carotid simultaneously for 15 min, each infusion being at the rate of 1·05 ml./min, the response was indistinguishable from that to the same infusions given simultaneously into the left carotid artery and the malleolar vein. Infusions at that time into the right carotid were, as we have seen, ineffective. We, of course, did not then know the diencephalic distributions of the two carotid bloods; and later it was found (figure 36) that the right carotid was supplying a small fraction only of the anterior hypothalamic region—in terms of supraoptic nuclear material some 5 % of the total (table 2)—and that none of this blood was reaching the paraventricular nuclei. The results of these double-infusion experiments,

therefore, give no evidence on the question of such integration of function between the thalamic paraventricular nucleus and the anterior hypothalamus as we had envisaged. The evidence from the responses to left-carotid infusions and from the diencephalic distribution of left-carotid blood is, however, as we have seen, distinctly against this nucleus playing a primary role in osmoreception. Supporting evidence in the same sense comes from the experiments on 'Whitethroat' (p. 261). Here, after the left internal carotid had been tied, responses to infusions into the right carotid were still present, but no right-carotid blood was reaching the region of either the right or the left thalamic paraventricular nucleus. Again, in 'Rita' (p. 255) no responses were obtainable from infusions into the left carotid although the left thalamic paraventricular nucleus was richly supplied throughout almost its whole extent with blood of left-carotid origin; and, conversely, in 'Paris' (p. 241), although suspension from the left carotid reached the nucleus, the injection was very sparse and patchy and was contaminated with suspension from the right carotid; yet responses to left-carotid infusions had been obtained. Again, in 'Jink' (p. 285), the left nucleus was very sparsely and patchily injected with the blue suspension (left-carotid blood), the blood supply of the nucleus being predominantly of vertebral origin; yet responses to infusions into the left carotid were greater than before the middle cerebral and posterior communicating vessels had been tied. Moreover, the right nucleus was much better injected with the black suspension than was the left with the blue, and yet no responses were obtainable to right-sided infusions. Lastly, in 'Girl' (p. 289), ligation of the left anterior cerebral and posterior communicating arteries produced no certain change in the response to infusions into either left or right common carotid trunk. The responses to infusions on either side were well marked (figure 45); and while on the right side the thalamic paraventricular nucleus was well supplied throughout with blood of right-carotid origin, on the left side the extreme anterior end only of the nucleus received blood of left-carotid origin (figure 46). The collected evidence from these six animals is, then, strongly against this nucleus being the site at which the antidiuretic response to a rise in the osmotic pressure of the arterial blood is initiated, and *per contra* reinforces the conclusion previously reached that the receptors are in the anterior hypothalamic region. If we accept this evidence, as we feel we must, how are we to explain the complete disappearance of responses to left-sided infusions in 'Linda' (figure 52) when the left anterior cerebral, middle cerebral and posterior communicating arteries had been tied? The left anterior hypothalamus was being well supplied by left-carotid blood and showed no histological abnormality, but the left thalamic paraventricular nucleus had been completely destroyed by the cyst (figure 56). In 'Brindle' (p. 251), on the other hand, well-marked responses to left-sided infusions were seen after left hemispherectomy (figure 25), and the paraventricular nucleus was intact, although the extent and degree of degeneration of the remaining thalamic structures was at least as great as that encountered in 'Linda' (cf. figures 28 and 56). Moreover, 'Linda' is the only one of our animals in which operative procedures have resulted in the destruction of this nucleus. By the present evidence, then, we are led to infer that the receptors are in the anterior hypothalamus but their responsiveness to a rise in the osmotic pressure of their environment is dependent upon the functional integrity of and their linkage with the thalamic paraventricular nucleus. Such a hypothesis seems, perhaps, not inordinately improbable when we recollect that a connexion

between the thalamus and the neurohypophysis is evinced by the release of antidiuretic hormone in response to emotional stress or afferent nerve stimulation (Klisiecki, Pickford, Rothschild & Verney 1933; Theobald 1934; Theobald & Verney 1935; Rydin & Verney 1938; O'Connor & Verney 1942), that such release can be inhibited by an immediately preceding intravenous injection of quite small amounts of adrenaline (O'Connor & Verney 1945) or tyramine (Verney 1947), and that the block so produced is probably a direct effect of these compounds on synaptic transmission in the effector pathways (Verney 1947). At all events, the hypothesis enunciated above is one that is susceptible to the test of experiment; the ways and means will be discussed later.

The precise anatomical connexions of the thalamic paraventricular nucleus are unknown. It, in contradistinction to the remaining midline nuclei, appears to be spared from retrograde degeneration after experimental lesions in the corpus striatum and adjacent cortex of the medial surface of the rabbit's hemisphere (Cowan & Powell 1955). Walker (1936) has described slight degeneration in the posterior part of this nucleus in the monkey after lesions anterior and posterior to the optic chiasma, the posterior lesion involving the supraoptic nucleus and the floor of the third ventricle rostral to the mamillary bodies; and in some of the experiments of Powell & Cowan (1954) on the rat, degeneration in this nucleus was associated with damage to the preoptic areas and the anterior and lateral hypothalamic areas. On the other hand, Cowan & Powell (1955) found that in a rabbit in which a well-localized lesion was made in the medial preoptic area (destroying its dorsal third) and the anterior hypothalamus, no degeneration in any of the thalamic nuclei supervened. However, Rioch (1931) has demonstrated, with Weigert-prepared material in the dog, a fibre connexion (in the periventricular system) between the midline nuclei of the thalamus and the medial preoptic and hypothalamic areas. We, too, in Weil-stained preparations, have seen fine medullated fibres streaming down from the thalamic paraventricular nucleus into the thalamic commissural network, and thence into the dorsal part of the medial preoptic and anterior hypothalamic areas. Moreover, Clark & Boggon (1933) have shown, in the cat, that after lesions limited practically to the midline nuclei, degeneration is restricted to commissural fibres and periventricular fibres; in no case could degenerated periventricular fibres be traced into the substance of the hypothalamus. Thus it would appear that the fibre connexions between the midline nuclei and the hypothalamus are established by short relays of neurones. Indeed, according to Ingram (1940) the periventricular system is characterized by the presence of small cells arranged in vertical rows. Possibly, then, the small and inconsistent retrograde effects on the thalamic paraventricular nucleus of preoptic and hypothalamic lesions are explicable in terms of relays on the course of the intervening tracts.

In connexion with the hypothesis that we have adopted, the results obtained in 'Juno' (p. 298) and in 'Girl' (p. 287) merit further discussion than has already been given to them. In 'Juno' ligation of the left anterior cerebral, middle cerebral and posterior communicating arteries led, as in 'Linda', to the complete suppression of responses to left-sided intracarotid infusions. But in 'Juno' the left thalamic paraventricular nucleus suffered only a little diminution in cell density, and this was confined to the anterior third of the nucleus. Had, therefore, the hypothalamic conditions been as in 'Linda' one would have expected, from hypothesis, that responses would have been retained. There was, however,

in 'Juno' an antero-dorsal hypothalamic cyst (figure 50) that extended so close to the third ventricle that any fibre connexions between the thalamic paraventricular nucleus and the functioning remainder of the anterior hypothalamus would have been destroyed. Such destruction, as we have seen, need not necessarily have caused retrograde degeneration in the nucleus. Much, however, of the hypothalamic osmoreceptive region (figure 48) was destroyed in 'Juno' (figure 50), so this destruction alone may have been the prime cause of the disappearance of responses to left intracarotid infusions. Nevertheless, if we postulate that within the osmoreceptive region the supraoptic nucleus itself is predominantly functional in this regard, then the results in 'Girl' (p. 287) suggest that the disappearance of response in 'Juno' was owing to structural interruption of thalamic paraventriculo-hypothalamic pathways rather than to the cell impoverishment of the supraoptic nucleus itself. For in 'Girl' (p. 292) the amount of histologically normal supraoptic material supplied by left carotid blood was about the same as in 'Juno', and yet in the former animal responses to left intracarotid infusions were retained. As already stated (p. 299), the effects of simultaneous bilateral intracarotid infusions and simultaneous intracarotid and intravenous infusions were determined in 'Juno'. Each infusion was at the rate of 1·05 ml./min and for a period of 15 min. The antidiuretic response to 0·86M-NaCl infused into each carotid was indistinguishable from that to the same solution infused into the right carotid and the malleolar vein. A raised osmotic pressure in the environment of the right thalamic paraventricular nucleus, therefore (see figure 51), did not sensitize the residuum of supraoptic nuclear material reached by left carotid blood to such degree as to cause a detectable increase in response over and above that given to simultaneous infusions into the right carotid and malleolar vein. The later disclosure of the left hypothalamic cyst, however, makes the interpretation of these results equivocal. It is a pity that similar experiments were not made on 'Linda', as if an increase in osmotic pressure in the environment of the thalamic paraventricular nucleus does indeed increase the sensitivity of the hypothalamic receptors, and if at the same time some of the connecting pathways decussate in the intrathalamic or suprachiasmatic regions, then simultaneous infusions into the two carotids might have been expected to give a larger antidiuretic response than a combination of infusions into the right carotid and the malleolar vein. In the absence of further facts it would be unprofitable to pursue discussion of the possible role, and its mode, of the thalamic paraventricular nucleus in the osmotic release of antidiuretic hormone.

Let us now turn to the osmoreceptive region itself with a view to seeing whether there is any evidence to suggest that a particular area within this region has a higher claim than any other to being the receptive site. The region, shown by the red stipple in figure 48, comprises the following areas and nuclei: the lateral and medial preoptic areas, the suprachiasmatic nucleus, the nucleus supraopticus diffusus, the anterior hypothalamic area, the supraoptic nucleus, the paraventricular nucleus, the dorsal hypothalamic area, the lateral hypothalamic area, the periventricular system, the ventro-medial nucleus and the dorsomedial nucleus. In assessing the claims of each of these structures we shall take into account considerations other than the gross one through which the outer limit of the receptive region was defined, viz. the invariable presence within it of blood of that carotid from infusions into which antidiuretic responses were elicited. These other considerations comprise, first, the relation of a post-operative change in degree of response with the inferred

post-operative change in carotid volume flow and in carotid hypothalamic field, and secondly, the fact of a nucleus or area being partly involved in a post-operative lesion or being fed in part by blood from a source other than that of the carotid which supplied the effective osmotic stimulus.

With respect to the first consideration, there are experiments in which the response to a given intracarotid infusion has been greater after operation than before. Now in some instances, as after ligation of the occipital, middle cerebral and posterior communicating arteries in 'Jink' (p. 284), it is reasonable to infer that the volume flow of blood in the ipsilateral common carotid diminished: the accompanying increase in ipsilateral responses could then have been owing to the production of a greater osmotic increment by a given infusion. In other instances, on the contrary, as after intradural ligation of an internal carotid in 'Root' (p. 265), it is reasonably certain that an increased carotid flow (in this case on the side contralateral to that of the operation) accompanied the recorded augmentation in response. Here it is suggestive that there had been an extension of the field of distribution of this carotid into osmoreceptive regions previously supplied from other sources. Such circumstance could offer valuable information about the narrower localization of the receptors. For any crossing of carotid blood to contralateral hypothalamic nuclei or areas would strongly implicate these structures, while any posterior extension into ipsilateral structures that may be supposed normally to have received some vertebral blood would strengthen their candidature as the site of the receptors. These contingencies have been taken into account in the construction of table 3, where a summary of observations on all relevant experimental material is presented in simplified form. We would emphasize that the data refer only to conditions under which antidiuretic responses to intracarotid infusions were being observed.

With respect to the second consideration, it is not justified to conclude that such regions could have played no part in the primary receptive process, since it is not known what proportion of the total number of osmoreceptive elements need be engaged in order to initiate the responses; nor can the possibility be ruled out that, were part of the receptive field damaged, there might be some recruitment or facilitation of the remaining elements. However, the nuclei and areas that fall into these categories have been specifically noted in table 3.

The only area of the preoptic and most anterior hypothalamic regions for which the sum of evidence favours its exclusion as the receptive site is the lateral preoptic area, and that with the same reservations that were applied to the medial parolfactory region. Indeed, the area is immediately adjacent to the parolfactory region, and evidence for its exclusion comes from the same animals ('Molly' and 'Girl') as that for the latter. It is, however, a region about which confirmatory evidence is desirable.

The medial preoptic area has received marked blood in every instance where responses were present. In two animals ('Molly' and 'Toby') asymmetry of distribution of the suspensions indicates that the most medial part of this area was at least receiving mixed carotid blood, but this evidence is too slight to give definite support to its exclusion. The medial preoptic area, then, must be considered as a region in which the receptors possibly lie. Continuous posteriorly with the medial preoptic area is the anterior hypothalamic area. This area lies at the centre of the receptive region and has been richly supplied with

TABLE 3. SUMMARY OF EVIDENCE FOR OR AGAINST THE PARTICIPATION OF NUCLEI AND AREAS OF THE ANTERIOR HYPOTHALAMUS IN THE OSMOTIC RESPONSES

+, evidence consistent with the view that the nucleus or area is directly involved in reception; −, evidence suggesting that the region is not directly involved in reception

	side on which responses were retained	response after compared with before operation	probable change in blood flow in common carotid after operation	medial preoptic area	lateral preoptic area	supra-chiasmatic nucleus	nucleus supra-opticus diffusus	anterior hypo-thalamic area	supra-optic nucleus	para-ventricular nucleus	dorsal hypo-thalamic area	lateral hypo-thalamic area	peri-ventricular system	ventro-medial nucleus	dorso-medial nucleus
'Molly', p. 228	right	—	—	+ (−)(4)	+ (−)(4)	+	+	+	+	+	+	+	+	+	+
	left	—	—	+	+	+	+	+	+	+	+	+	+	+	+
'Paris', p. 241	right	diminished	uncertain	+	+	+	+	+	+	+	+	+	+	+	+
	left	diminished	uncertain	+	+	+	+	+	+	+	− (4)	− (4)	+	+	− (4)
'Toby', p. 242	right	not diminished: probably increased	increased	+ (1)	+	+	+ (1)	+	+ (1)	+	+	+	+	+	+
	left	increased	uncertain	+ (−)(4)	+	+	+ (−)(4)	+	+ + (2) (−)(4)	+ + (2)	+ + (2)	+ + (2)	+ + (2)	+ + (2)	+ + (2)
'Brindle', p. 247	left	increased (after tying occipital)	reduced (after tying occipital)	+	+	+	+	+	+	+	+	+	+	+	+
'Rita', p. 253	right	—	—	+	+	+	+	+	+	+	+	+	+	+	+
'Regan', p. 264	right	diminished (despite some crossing to left side)	increased	+	+	+	+	+	+	+	+	+	+	+	+
'Root', p. 265	left	increased	increased	+ + (1)	+	+	+ + (1)	+ + (1)	+ + (1, 2)	+ + (1, 2)	+ + (2)	+ + (2)	+ + (1, 2)	+ + (1)	+ + (2)
'Doris', p. 271	right	diminished	increased	+	+	+	+	+	+	+	+	+	+	+	+
'Jink', p. 284	left	increased	reduced	+ (+) (1)	+	+	+ (+) (1)	+ (+) (1)	+ (+) (1)	+	+	+	+ (+) (1)	+ (1) (−)(3)	− (3)
'Girl', p. 288	right	unchanged	increased	+	+	+	+	+	+	+	+	+	+ (−)(4)	+	+
	left	unchanged	reduced	+	−	+	+	+ (−)(3)	+ (1) − (3)	− − (3)	− (3)	− (3)	+	− (3, 4)	+ + (2)
'Linda', p. 305	right	slightly diminished	increased	+	+	+	+	+	+	+	+	+	+	+	+
'Juno', p. 298	right	increased (appeared)	increased	+ (−)(4)	+	(−)(4)	+ − (4)	+ (−)(4)	+ (2) (−)(4)	+ (+) (2)	+ + (2)	+ + (2)	+ (−)(4)	+	+ + (2)

The data concern the distribution of carotid blood in the hypothalamus where responses to intracarotid infusions of hypertonic solutions were present, and thus relate to the structures of the ipsilateral side of the hypothalamus only, except where note (1) applies.

(1) Suspension has crossed to reach the same structure of the contralateral side.
(2) Presumed posterior extension of carotid supply into the nucleus or area on the same side.
(3) Cystic damage or cell loss.
(4) Part of the region not injected with suspension.

Compare with Table 2 for information on operations and responses. Symbols in brackets imply that the indications were of a minor character only.

marked blood in every instance where responses were present. In one animal, 'Girl', the area has suffered some cell loss owing to a nearby cystic lesion, but this may be considered compensated by the fact that a narrow medial band of the contralateral area has received blood from the same carotid. The status of the area as a possible receptive region is thus not compromised.

The cells of the medial preoptic area and the anterior hypothalamic area are very similar in type, and the suprachiasmatic nucleus, which occurs at the border between the two areas, appears histologically to be merely a condensation of these cells. Similarly, the nucleus supraopticus diffusus may be part of the same complex, and is distinguished by forming the bed nucleus of the supraoptic decussations. As would be expected from their anatomical position, therefore, and as reference to table 3 will show, there is little evidence to exclude these nuclei from the receptive region.

The dorsal hypothalamic area and lateral hypothalamic area may be linked in discussion since they have suffered a parallel fate in all the experiments. In all but two of the animals they are well injected throughout the greater part of their extent, with some sparseness of the colours tending to appear at most posterior limits only. In one animal, however ('Paris'), quite extensive sparse patches appear in these areas on one side, the areas having been partly vascularized by vessels carrying unmarked vertebral blood. In 'Girl' the posterior limits of the areas are similarly devoid of suspension, and the injected portions are heavily damaged by cystic lesion. Of the total antero-posterior extent of the lateral area which is injected (5 mm), 2 mm are directly destroyed or are devoid of cells; similarly, of the 3 mm antero-posterior extent of the injected dorsal area 1·5 mm are cystic or devoid of cells. Moreover, carotid blood of this (left) side has not reached the lateral or dorsal hypothalamic area of the contralateral side. The likelihood of these areas being involved in reception would thus, at first sight, appear to be small. However, they cannot be rejected without reservation, since it will be recalled that the interpretation of the results in 'Girl' is beset with difficulties (see pp. 292 and 303). In particular, in this animal, some damage or cell loss had occurred to most of the left anterior hypothalamic nuclei, yet responses were fully retained to infusions into the left carotid. It is possible that compensatory factors of the kind suggested earlier in this section, coupled with the probability that the blood flow in this left carotid was reduced after the operation, had contributed towards the retention of responses; so far as the lateral and dorsal areas are concerned, such factors would enhance the importance of the fact that the anterior extents of the areas were intact. However, on the sum of evidence on the status of these areas it would seem improbable that either of them is the receptive site.

With considerations on this particular animal ('Girl') still in mind, it will be convenient to turn to the dorsomedial nucleus next. In 'Girl' this nucleus was conspicuous on the left side in being the one least damaged by the cystic lesion. It showed cell condensation but no obvious cell loss. Moreover, it was well injected with the colour suspension of the left carotid, and it may be supposed that there had been a post-operative extension of the normal carotid field into the posterior part of the nucleus. Evidence from this animal, then, is consistent with an osmoreceptive role of the dorso-medial nucleus. This fact is recorded in table 3, but reference to the appropriate column of the table will show that two other animals offer evidence of an entirely contrary nature. In 'Paris' the left nucleus is only

patchily supplied with left carotid blood, but responses were present to left-sided infusions. In 'Jink' the greater part of this nucleus is discretely destroyed by a small cyst in the left hypothalamus (see figure 44), yet responses to left-sided infusions were not only retained but were greater than before the operation. On sum it seems necessary to relegate the dorso-medial nucleus to the same status as the dorsal and lateral hypothalamic areas.

The ventro-medial nucleus has been well and, as a rule, completely injected in all cases where responses have been present, and the only specific evidence that would tend to disqualify it as a site of receptors comes from experiments on 'Girl'. Here the nucleus had suffered some cell loss and was not completely injected, but these deficiencies were seemingly not sufficiently marked to impugn the status of the nucleus as a potential site for the receptors.

The periventricular system need be given no special discussion, since although it has often been involved in an asymmetry of distribution of the carotid bloods, or has been in part uninjected, the system is so extensive that a substantial part of it has never failed to be injected with suspension from that carotid from infusions into which antidiuretic responses had earlier been obtained (see table 3).

There remain to be considered the two cell groups most intimately connected with the neurohypophysis—the supraoptic and paraventricular nuclei. These two nuclei cannot be differentiated by the histological appearance or cytochemical reactions of their cells, nor has any clearly confirmed distinction been made between them in the functions they subserve in controlling the neurohypophysis. The nuclei should therefore possibly be considered as two parts of a single functional unit, and whether or no this view is taken will have an important bearing on their role as prospective osmoreceptive centres. If the paraventricular nucleus acts as a receptive centre subsidiary to the supraoptic nucleus, then its inclusion in, or exclusion from, a given carotid distribution would presumably affect the magnitude of the responses observed. This interpretation would fit well with some of the results we have obtained. On the other hand, there is evidence from one series of experiments that makes it appear almost certain that the paraventricular nucleus is not the exclusive site of the receptive elements. This evidence comes from 'Girl', where the cell population of the paraventricular nucleus was found to be so drastically reduced on the left side that it is not possible to believe that the few remaining cells could have mediated the persisting responses (table 3).

The remaining structure in the osmoreceptive region, viz. the supraoptic nucleus, is the one to which, throughout this work, specific attention has been given with respect to its status as the site of the osmoreceptors. Quantitative data concerning this nucleus have been given in table 2. In most instances the evidence, as we have seen, is not merely consistent with but strongly supportive of the view that the receptors are in or in the close region of this nucleus, the result in two animals only being such as to demand some modification of the simple hypothesis that independently functioning receptors of fixed sensitivity are placed in the nucleus. On the one hand, the results with 'Linda' have led us to suggest that the osmoreceptive function of the nucleus is dependent upon its connexion with the thalamic paraventricular nucleus; and, on the other hand, those with 'Girl' seem to require the postulation of recruitment or facilitation in the reduced field of supraoptic cytons when some of them have been destroyed. No such quantitative data

as those given in table 2 have been obtained for the other structures in the osmoreceptive region, and in the absence of this information the claims of some of them—the medial preoptic area, the suprachiasmatic nucleus, the nucleus supraopticus diffusus, the anterior hypothalamic area, the periventricular system and the ventro-medial nucleus (table 3)—must for the present be regarded as not inferior to those of the supraoptic nucleus itself. If, for structural reasons, we regard the medial preoptic area, the anterior hypothalamic area and the periventricular system as weak candidates for an osmoreceptive role, we are left with the following structures as possible sites for the receptors: the suprachiasmatic nucleus, the nucleus supraopticus diffusus, the ventromedial nucleus and the supraoptic nucleus. According to Rasmussen (1940), after hypophysectomy or hypophysial stalk section there are no well-established retrograde changes in any region of the hypothalamus other than the supraoptic and paraventricular nuclei. This, however, does not exempt the remaining nuclei in the list above from a possible osmoreceptive role, since their axons may well relay in the supraoptic nucleus or end in the median eminence itself. Indeed, it may be that the receptors are not confined to a particular hypothalamic nucleus, small increments in osmotic pressure activating receptors of low threshold at one site, and increasing increments recruiting receptors of increasing threshold at other sites. It follows from the preceding discussion that while the supraoptic vesicles mentioned at the beginning of this paper lie within the osmoreceptive zone there is no evidence that they themselves mediate the osmotic release of the antidiuretic hormone.

We suggest, therefore—now that the gross osmoreceptive site is of manageable dimensions, viz. about 100 cu.mm—that it would be profitable to make further studies on the lines adopted in the present investigation with a view to seeing whether the osmotic release of antidiuretic hormone is quantitatively affected by punctate destruction in the anterior hypothalamus of one or other of those nuclei (other than the supraoptic nucleus) which we have failed to exclude from having an osmoreceptive role. A possible surgical approach in such an investigation is, as we have convinced ourselves, the diasphenoid route to the anterior chiasmatic region. But before this is done, it would seem best to attempt to clarify the functional status of the thalamic paraventricular nucleus. The anterior and major part of this nucleus lies superficially in the wall of the third ventricle and could, we believe, be reached without undue difficulty by removing the cortex and striatum at anterior thalamic levels and elevating the lateral edge of, or removing, the fornix and fimbria. It would, indeed, be of great interest, in view of the results discussed before, to see whether destruction of this nucleus led to a disappearance or impairment of the osmotic release of antidiuretic hormone. Should this prove to be so our anatomical and physiological studies of blood distribution to the thalamus offer the opportunity of devising means whereby an osmotic stimulus might be carried to the thalamus and not to the anterior hypothalamus. As we have seen, the main routes of blood supply to the thalamus are branches of the posterior cerebral and the thalamic branch of the posterior communicating vessel. That is to say, the source of supply is almost exclusively the vertebral arteries. If, therefore, the posterior communicating vessels were tied just anterior to their thalamic branches after a vertebral artery had been made accessible for direct infusion, the effect, on the release of antidiuretic hormone, of an osmotic stimulus to the thalamus, and, in particular, the paraventricular nucleus, could be determined. The formation of a vertebral artery loop

was once attempted ('Brandy', no. 336), and although the result was a failure we are convinced that the technique could become a successful surgical procedure. In brief, the technique involved the removal of the posterior part of the right sternohyoid muscle, the detachment of the scalenus anterior muscle from its insertion into the first rib, removal of the transverse process of the seventh cervical and the full opening up of the vertebral canal in the sixth cervical vertebra. The vertebral artery, freed from its origin to its disappearance into the fifth vertebra, was then enclosed in a strip of skin and the formation of the loop completed. Unfortunately, a patch of gangrene appeared on the loop 4 days later and this necessitated its excision. The mistake in the technique was the completion of the loop at the same operation as that at which the vertebral artery was freed and the skin brought down to the surface of the vessel. Postponement of the actual formation of the loop until some weeks after the first stage had been completed would, we think, lead to a successful technical issue, and therewith the opportunity of making the kinds of investigation we have outlined above. These, as well as tests of the *quantitative* effects, on the osmotic release of antidiuretic hormone, of punctate destruction of each of the structures in the osmoreceptive region, are, however, for the future.

We are much indebted to Dr C. M. Scott and Imperial Chemical (Pharmaceuticals) Ltd for the gift of the two dispersions used in most of our experiments, to Messrs Acheson Colloids Ltd (Product Development Department) for the gift of dispersions of colloidal graphite, and to the B.B. Chemical Company Ltd for the gift of neoprene latex and certain colouring agents. We also gratefully acknowledge the kindness of Dr F. Howarth in performing a hemispherectomy on one of our animals. Professor E. C. Amoroso kindly arranged for the photography and colour photography of some of our specimens in his Department; to him and to Mr A. Goffin we express our best thanks for their valuable help. Other colour photomicrographs were made by Dr E. Weston Hurst (Imperial Chemical (Pharmaceuticals) Ltd); to him we are much indebted. It is also a pleasure to acknowledge the help given us by Dr Powell and Dr Cowan in connexion with the anatomy and histopathology of the thalamus.

Mr A. Hogwood has given technical assistance throughout, and his efficient help is gratefully acknowledged.

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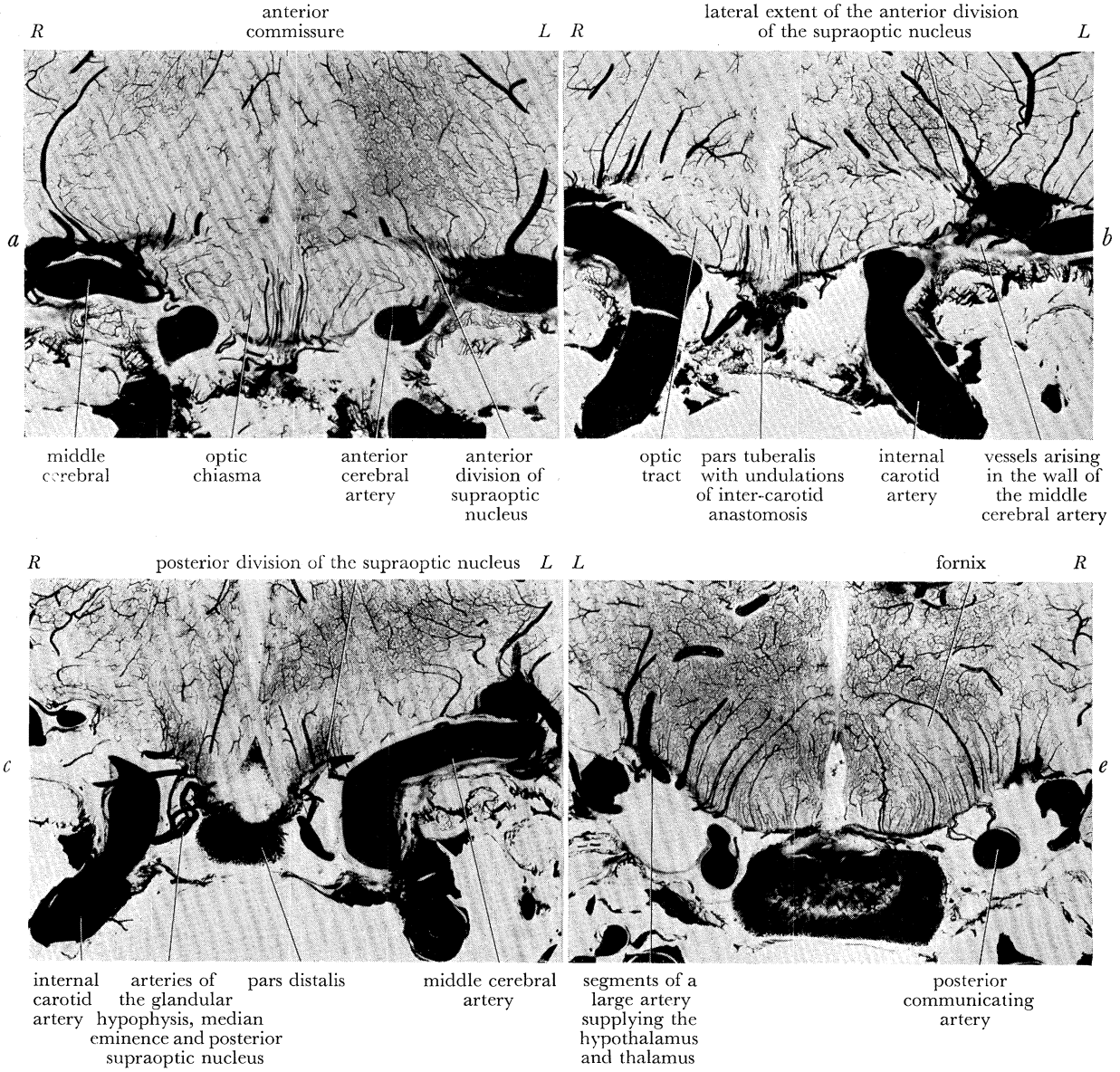


FIGURE 3. Photomicrographs of five sections selected from a frontal series through the diencephalon of dog D 5. One section intervenes between *b* and *c*, and three sections between *d* and *e*. The blood vessels were injected with Indian-ink plasma mass. Sections *a*, *b* and *c* are seen from the anterior, *d* and *e* from the posterior surface. Each is 500μ thick. (Magn. *a*, *b*, *c*, *e*, $\times 6$; *d*, $\times 4{\cdot}5$.)

(*Facing p.* 324)

FIGURE 4. Photomicrograph of frontal section through the hypothalamus and adjacent structures of the sella turcica of dog D 3 *a*. Basisphenoid bone decalcified *in situ*. 200 μ thick; van Gieson stain. To show the origin of the intercarotid anastomosis. (Magn. × 6.)

FIGURE 5. Photograph of neoprene cast of the circle of Willis and its branches in the dog. Left lateral aspect to show especially the blood supply to the thalamus. The right side of the cast has been removed. (Magn. × 1·5.)

FIGURE 7. Photograph of neoprene cast to show the posterior-lobe artery originating on one side from the anastomotic artery and on the other from the junction of the anastomotic with the internal carotid artery. (Natural size.)

FIGURE 9. The technique of infusion of coloured suspensions into the carotid blood streams. The animal is 'Linda' (p. 305). The arrangements here illustrated are only representative of the actual procedure: the needles are lying subcutaneously in the carotid loops. *a*, constant-speed motor, reduction gearing and micrometer screw. *b*, brass plate placed across the plungers of the two 10 ml. syringes *c* which contain the coloured suspensions. To the brass plate are fixed the rods *d*, which are free to move in the guides *e*. *f*, three-way taps. *g*, 20 ml. syringes containing 0·85 % NaCl. *h*, rubber or polythene tubes leading to the infusion needles *j*. Close to each needle is interpolated a short length of glass tubing. *k*, metronome. *l*, extension tube leading from the catheter to the delivery tube *m* which opens over a series of graduated tubes (*n*) held in a brass disk (*o*) which can be rotated by hand. The black spot on the thorax marks the apex beat. In an actual experiment this area and the tissues in the underlying interspace are infiltrated with 2 % procaine HCl.

FIGURE 13. Monochrome of colour photomicrograph to show the appearance of the black suspension in the anterior division of the right supraoptic nucleus, SO ('Doris', no. 379). (Magn. × 50.)

FIGURE 14. Monochrome of colour photomicrograph to show the appearance of the blue suspension in the anterior division of the right supraoptic nucleus, SO ('Toby', no. 395). (Magn. × 50.)

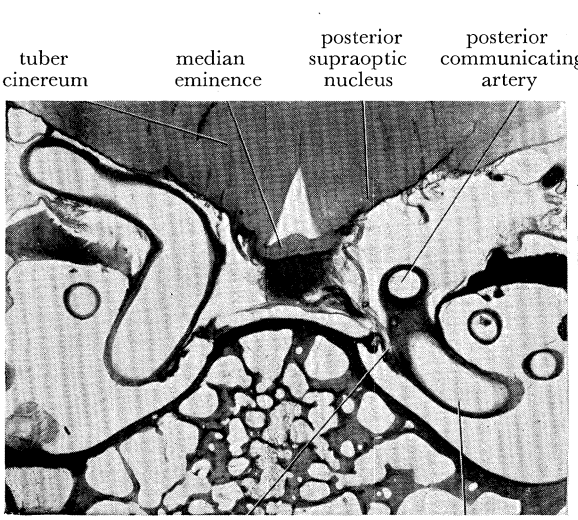


FIGURE 4

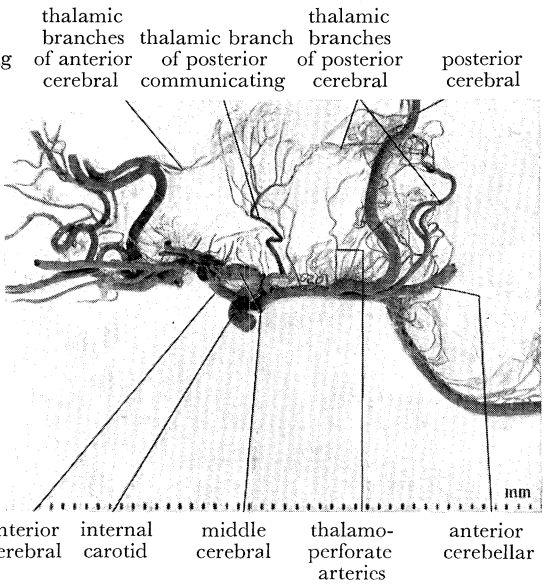


FIGURE 5

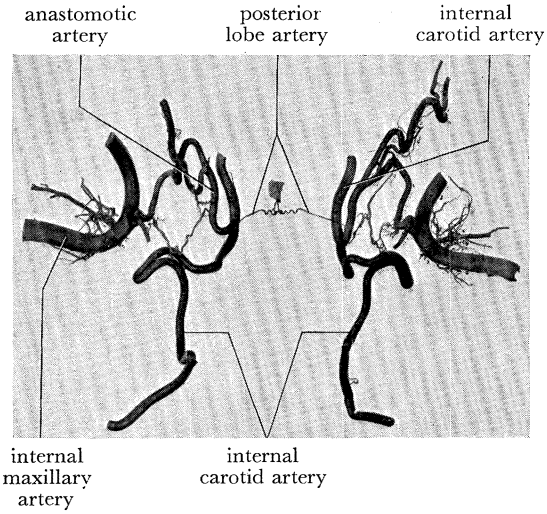


FIGURE 7

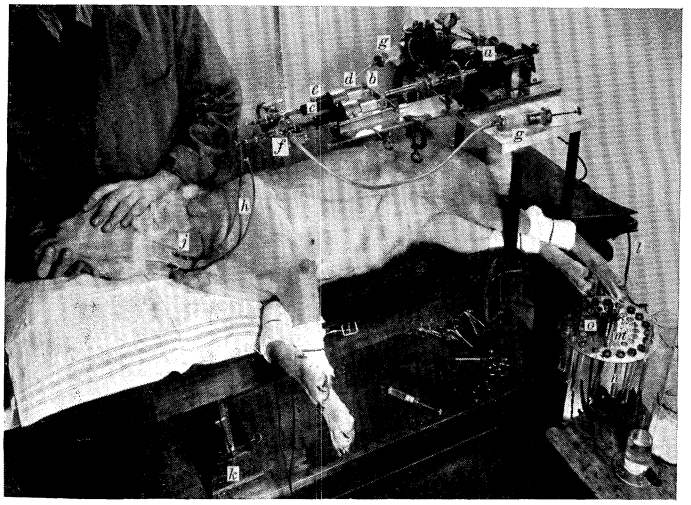


FIGURE 9

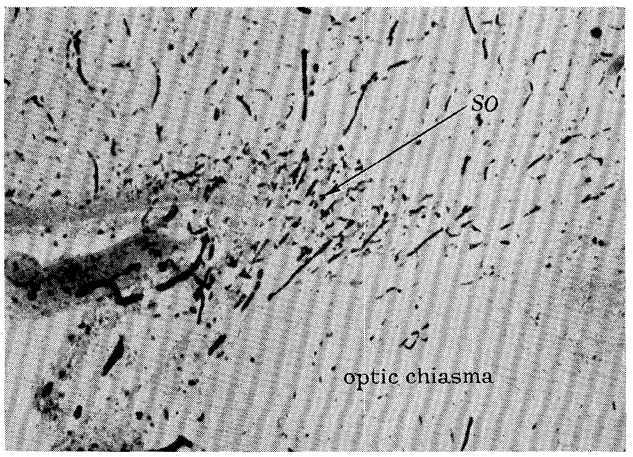


FIGURE 13

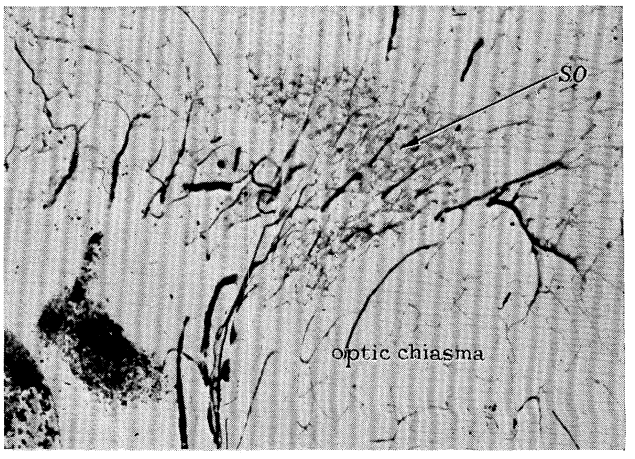


FIGURE 14

FIGURE 6. Reconstruction of the course of the thalamic branch of the posterior communicating artery on the left side of dog D 5. The blood vessels were injected with Indian-ink plasma mass. The reconstruction was made by the superposition of portions of photomicrographs of serial frontal sections. A posterior branch of the artery and its distribution to the lateral and medial thalamic nuclei is shown separately reconstructed to the right of the figure, its point of connexion with the parent vessel being indicated by the interrupted lines. (Magn. $\times$ 9.)

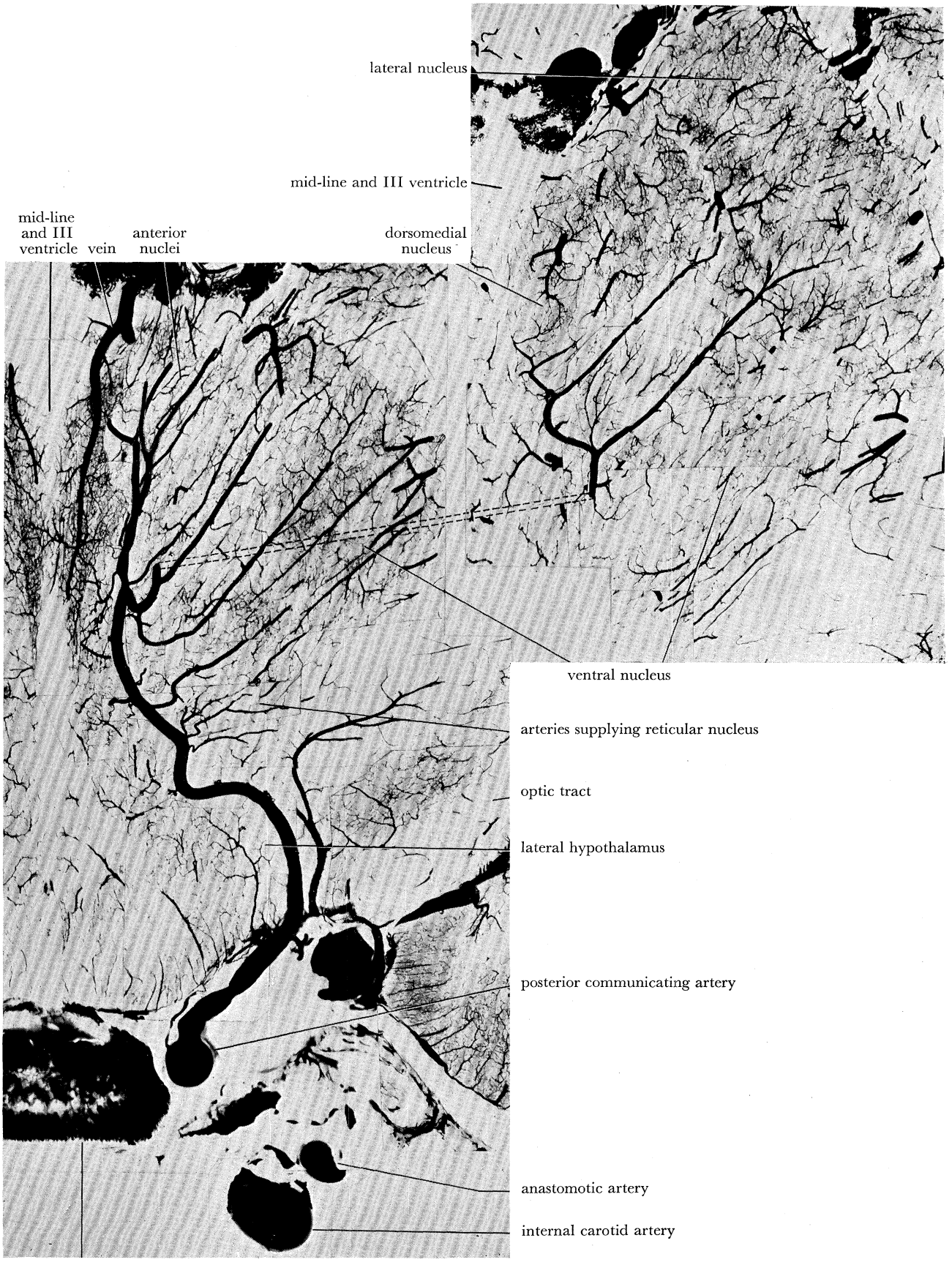

lateral nucleus
mid-line and III ventricle
mid-line and III ventricle
vein
anterior nuclei
dorsomedial nucleus
ventral nucleus
arteries supplying reticular nucleus
optic tract
lateral hypothalamus
posterior communicating artery
anastomotic artery
internal carotid artery
hypophysis

FIGURE 6

FIGURE 15. *A*: Monochrome of colour photomicrograph to show the appearance and distribution of suspensions in the posterior divisions of the supraoptic nuclei. Dog 377. Black suspension (*Bk*) infused into the right carotid and blue (*Bl*) into the right vertebral. *B* is a photomicrograph of the adjacent section stained with toluidin blue to show the extent of the nuclei (*P*). Note in *A* that the postero-lateral parts of the nuclei have received vertebral blood only (blue suspension), and the medial parts carotid blood only (black suspension in the right nucleus, no suspension in the left nucleus). Note also the 'cone' of tissue in the ventro-medial hypothalamus supplied by carotid blood, and the division of the blood supplies to median eminence and glandular hypophysis between the two carotid sources. (Magn. × 8.)

FIGURE 20. 'Molly', no. 341. Monochrome of colour photomicrograph to illustrate the fact that in this animal the posterior lobe was supplied solely by the right carotid artery. Black suspension had been infused into the right, and blue into the left carotid. The posterior lobe carries exclusively the black suspension, as does also the right half of the pars distalis. The left half of the pars distalis carries predominantly the blue suspension, shown enclosed by white dashes. (Magn. × 10.)

FIGURE 31*b*. Monochrome of colour photograph of the intradural exposure of the left internal carotid artery (*ic*) and its trifurcation. To the right the pars distalis (*h*) can be seen with the posterior communicating artery (*pc*) running lateral and dorsal to it; at the top, receding beneath the retractor is the middle cerebral artery (*mc*); to the left the anterior cerebral artery (*ac*) with its internal ophthalmic branch (*io*) lies adjacent to the optic nerve (*on*). *tl*, temporal lobe.

FIGURE 32. Photograph of special instruments used in the surgical procedure of tying the anterior cerebral, middle cerebral and posterior communicating arteries. For description see text, p. 255, and figure 31*a*, p. 257.

FIGURE 53. Photograph of 'Linda', no. 385, 21 weeks after ligation of the left anterior cerebral, middle cerebral and posterior communicating arteries. Immediately after recovery from the operation there was some weakness of the left circumocular muscles—some of the temporal and zygomatic branches of the facial nerve had inevitably been divided at operation—and in blinking the nictitating membrane was drawn across the eye. Later the elevators of the upper lip were brought into action in blinking, and the nictitating-membrane response then disappeared. These phenomena were also seen with the other animals in which the same operative approach to the pituitary region had been made.

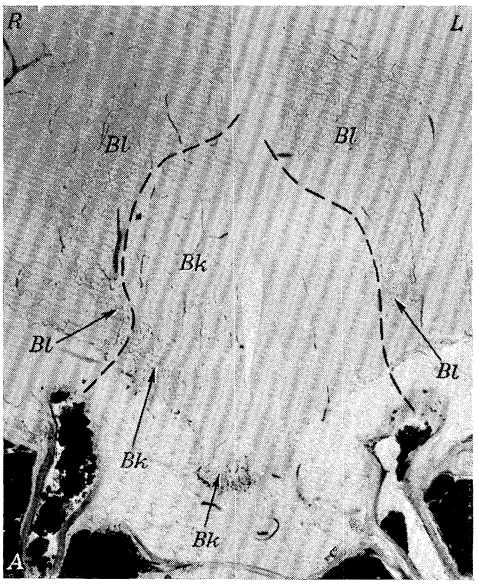


FIGURE 15*A*

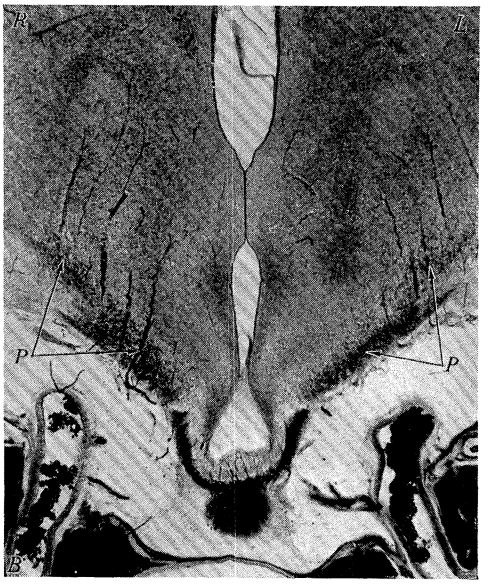


FIGURE 15*B*

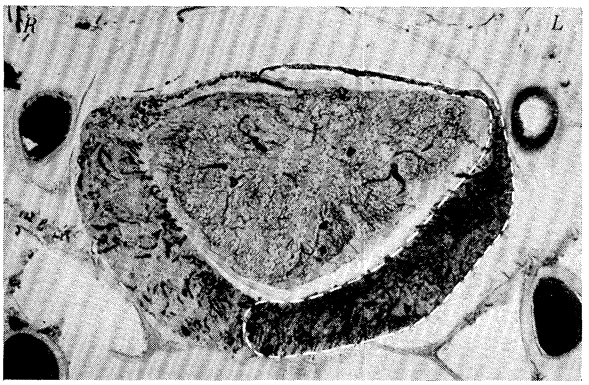


FIGURE 20

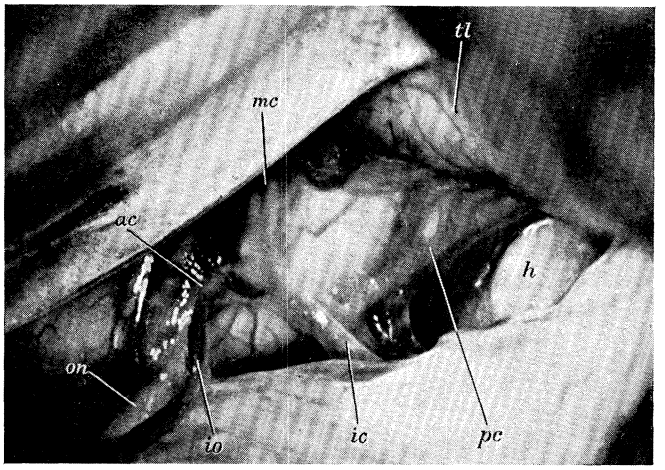


FIGURE 31*b*

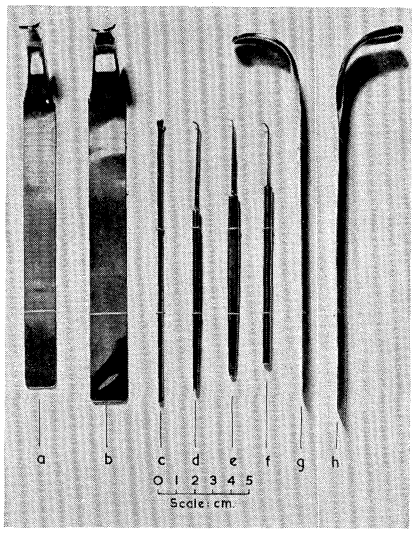


FIGURE 32

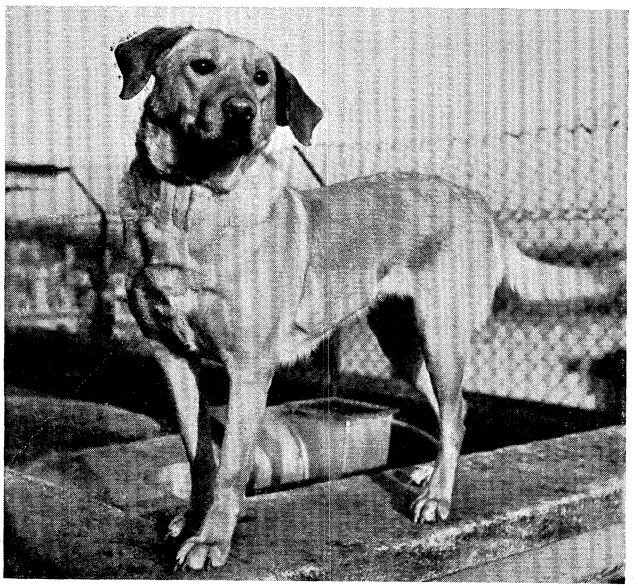


FIGURE 53

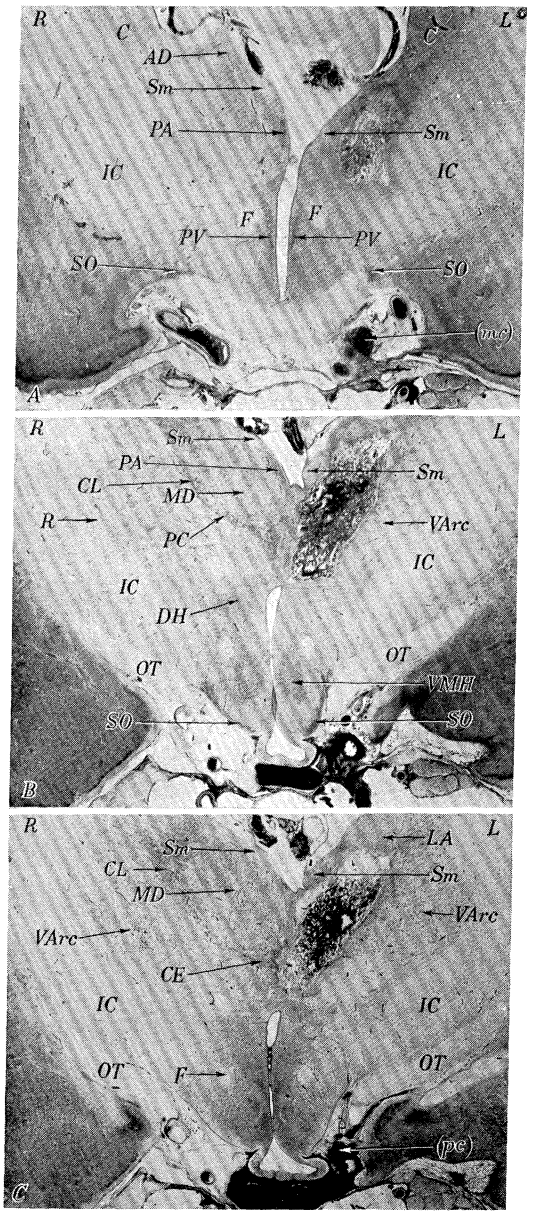


FIGURE 57. 'Linda', no. 385. Monochromes of colour photomicrographs of sections (125μ thick) stained with toluidin blue to show the cyst in the left anterior thalamus, its position and structure, and the nuclear degeneration associated with it. The anterior face of the sections is presenting. *A*, through the optic chiasma; *B* and *C*, through the median eminence. *B* is 2 mm posterior to *A*, and *C* is 1 mm posterior to *B*. Note that both anterior and posterior divisions of the supra-optic nucleus are well preserved on the left side: the appearance and density of the cells in the nuclei of the two sides are indistinguishable. Part of the knot, (*mc*), of the ligature on the left middle cerebral artery can be seen (stained intensely) just ventral to the optic tract in *A*; and in *C*, at (*pc*), is seen the occluded left posterior communicating artery with its ligature tag. For key to lettering see legend to figure 27. (Magn. $\times 4$.)

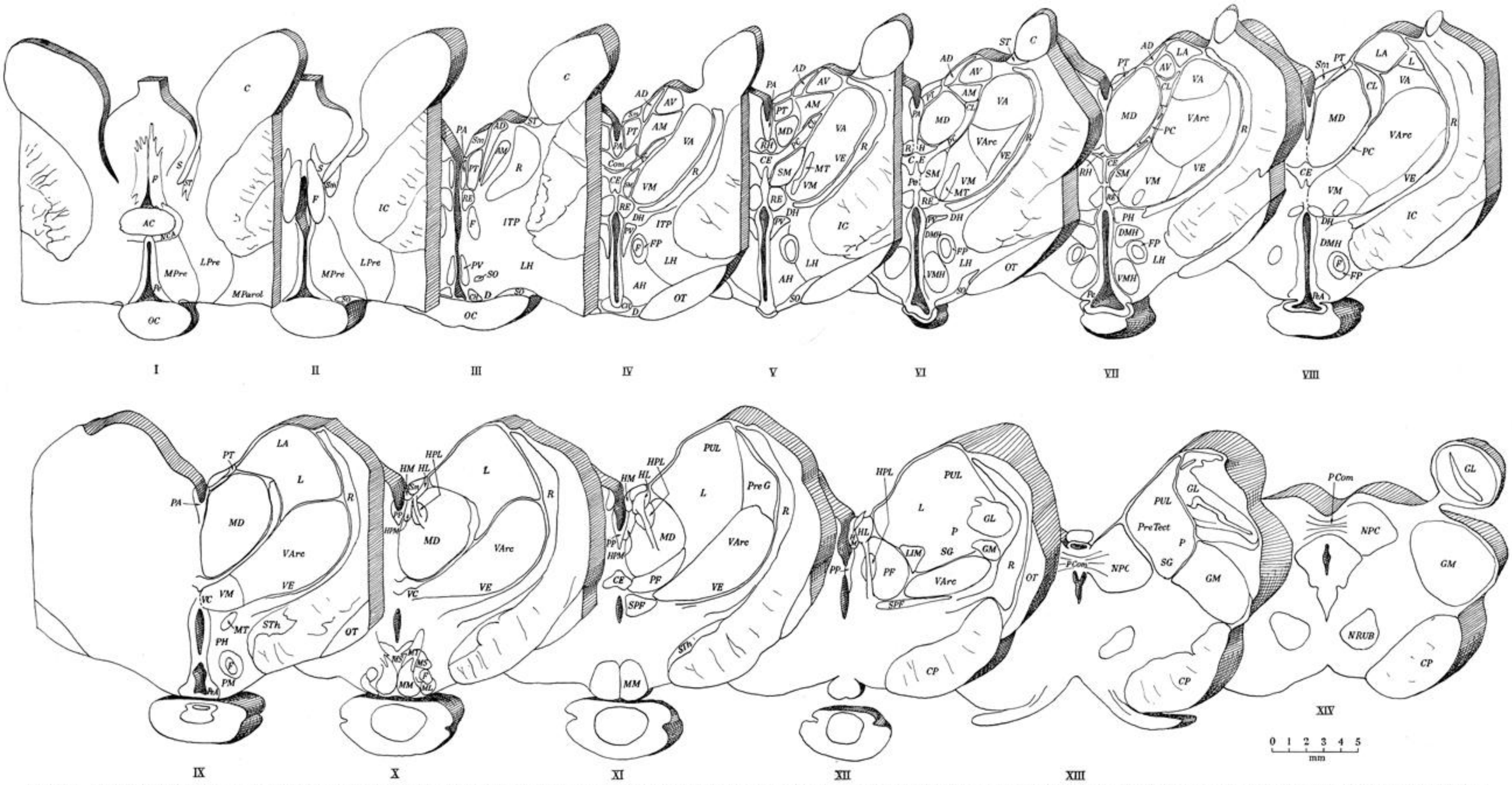


FIGURE 27. Serial frontal sections of the diencephalon to show the topography of the nuclei and areas in the thalamus and hypothalamus of the dog. The heads of two animals under chloralose anaesthesia were perfused through the carotids with 0·9 % NaCl at room temperature, and when the effluent from the external jugular veins was fairly clear the perfusing fluid was changed to the formalin-acetic acid-ethanol fixative. Blocks were cut from the brains so as to include the whole diencephalon, and serial sections were made of the celloidin-embedded material. The sections shown form a complete series, and the projected thickness of each is isometric in the sense that the length of the inclined lines of shading represents the actual depth of the section. For further description see text. The key to nomenclature is given opposite.

(Facing p. 250)

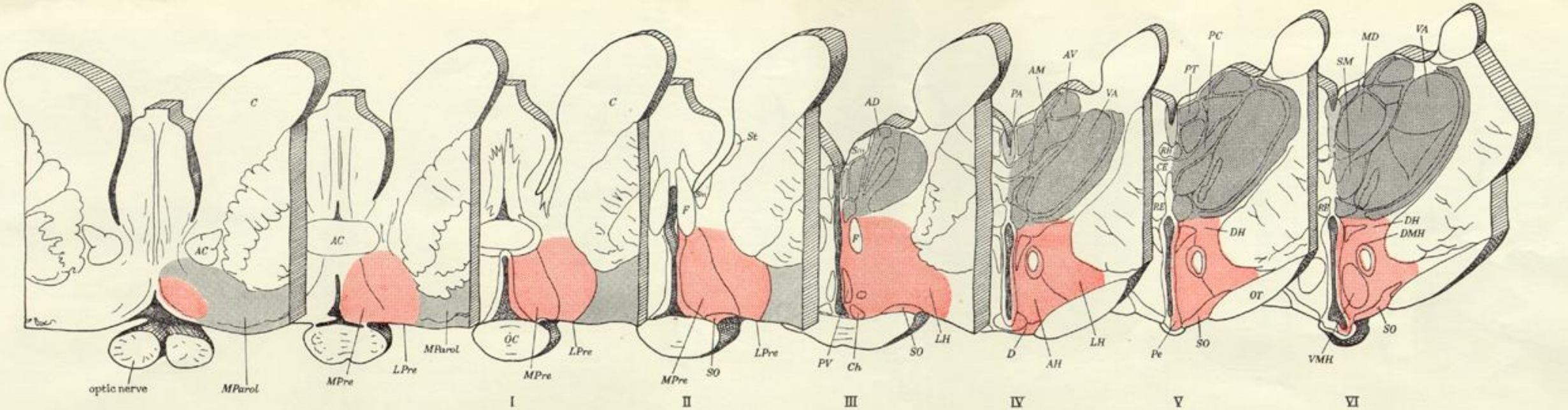
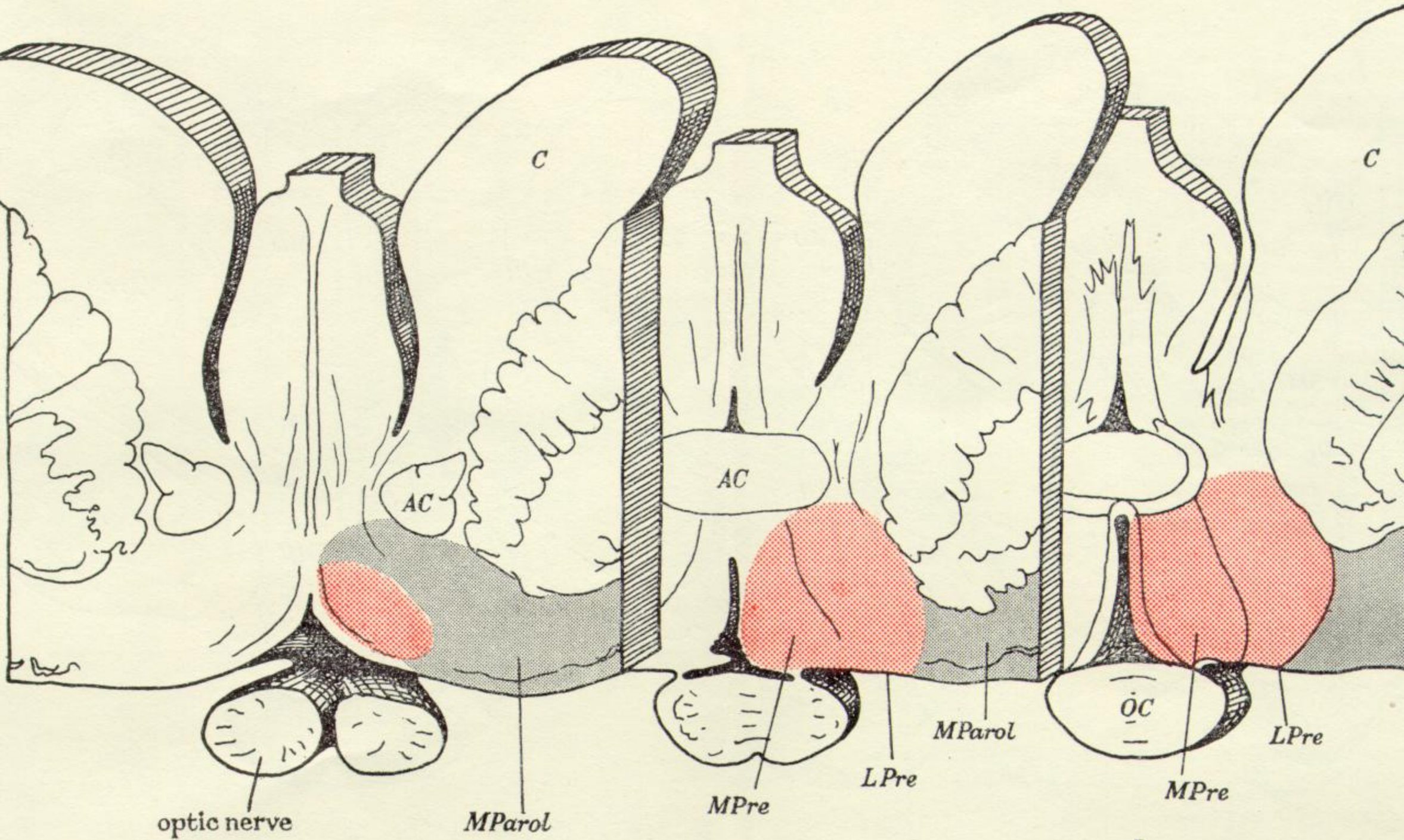


FIGURE 48. Diagrammatic representation of the parts of the diencephalon excluded as sites for the osmoreceptors by collected evidence from animals in which responses were retained after operation, and showing the region in which, by inference, the receptors lie. The heavy black stipple indicates nuclei and areas excluded by evidence from blood distribution or degenerative cell loss or both. Light black stipple indicates regions not completely excluded by evidence from blood distribution, and for which confirmatory evidence is desirable. Red stipple indicates the region in which the receptors lie and which has always received carotid blood where responses have been present. The figure should be studied in conjunction with figure 27 in which the nomenclature of all the nuclei is given; corresponding sections carry the same numeral.



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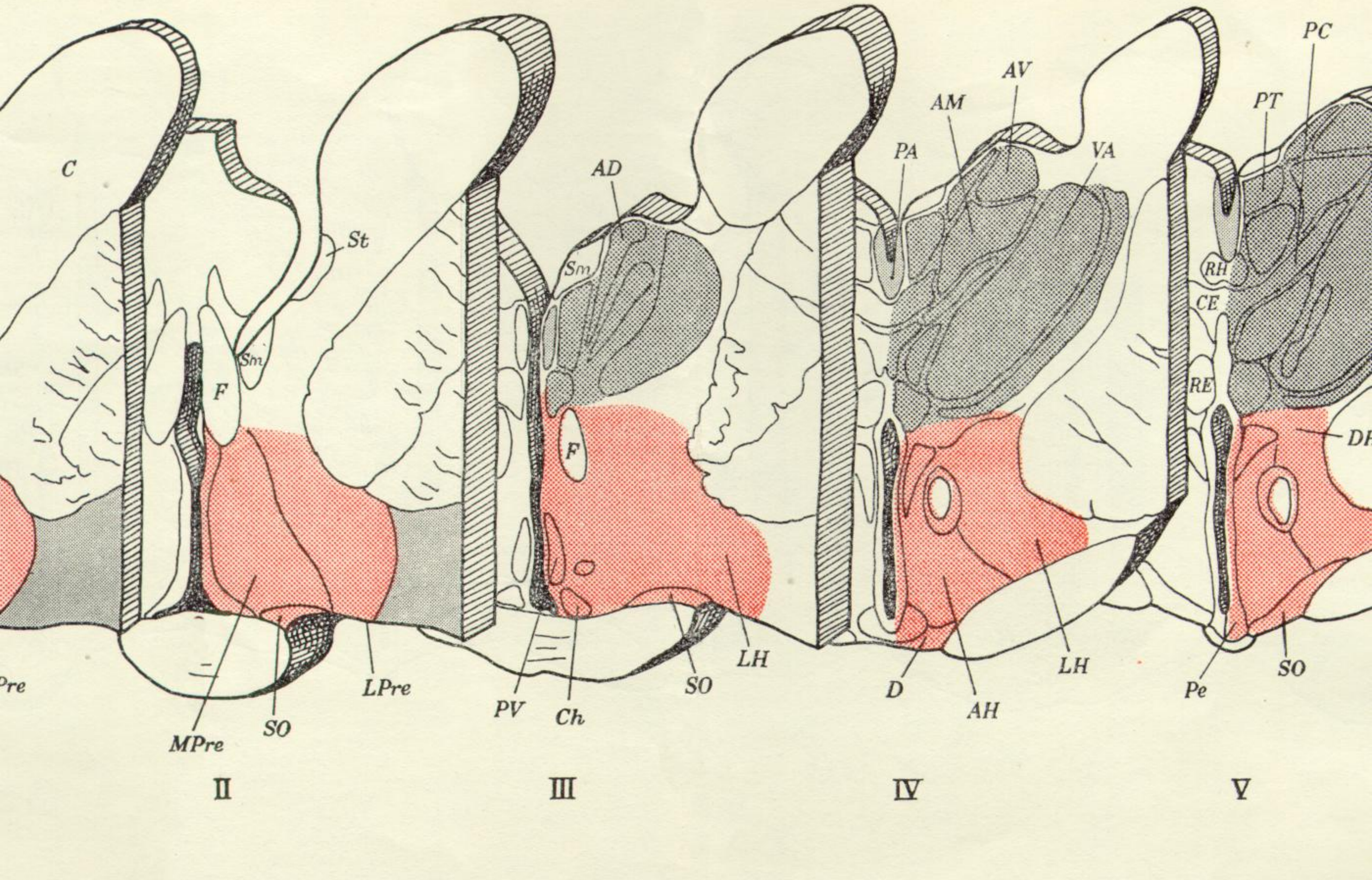

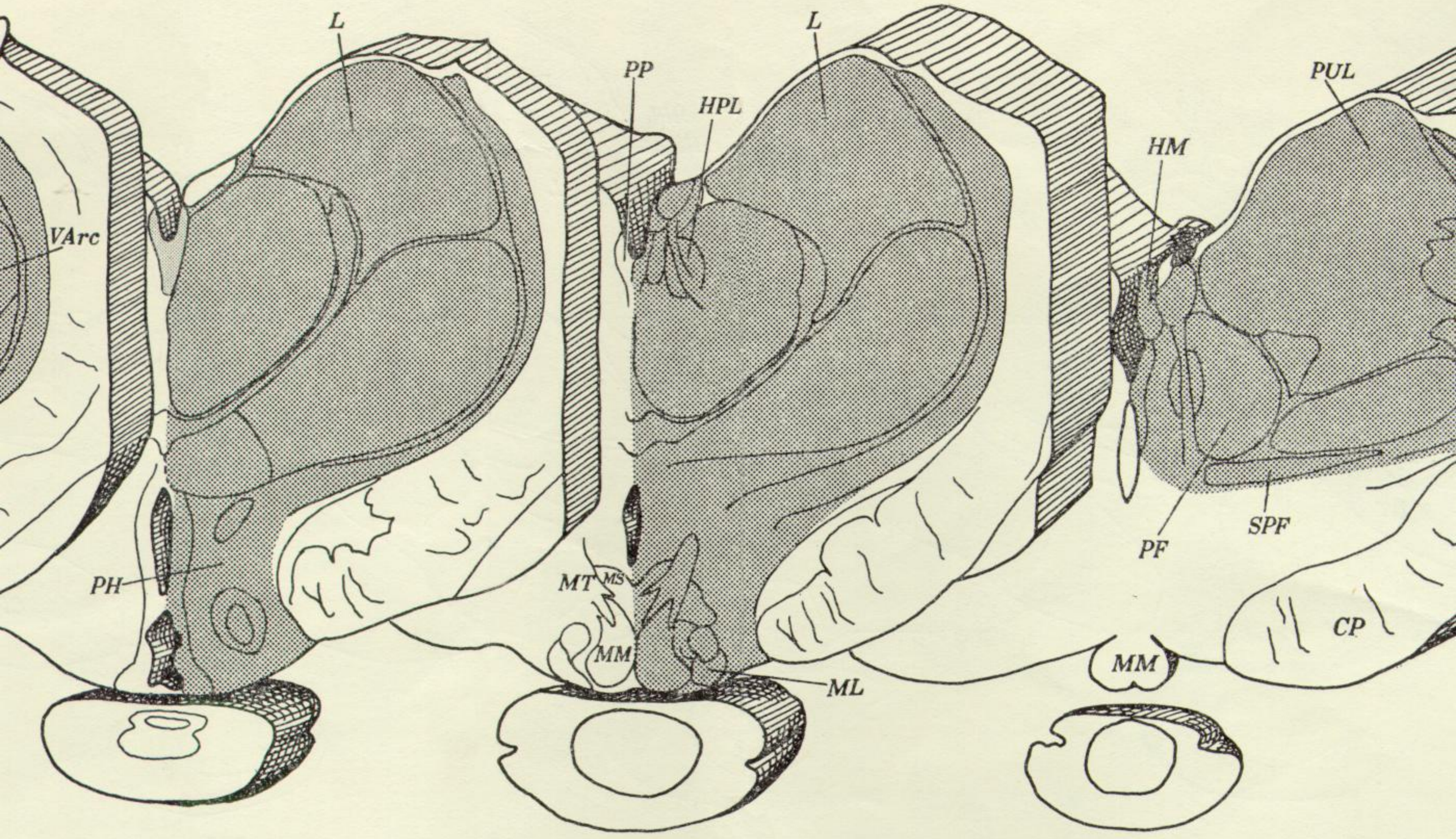

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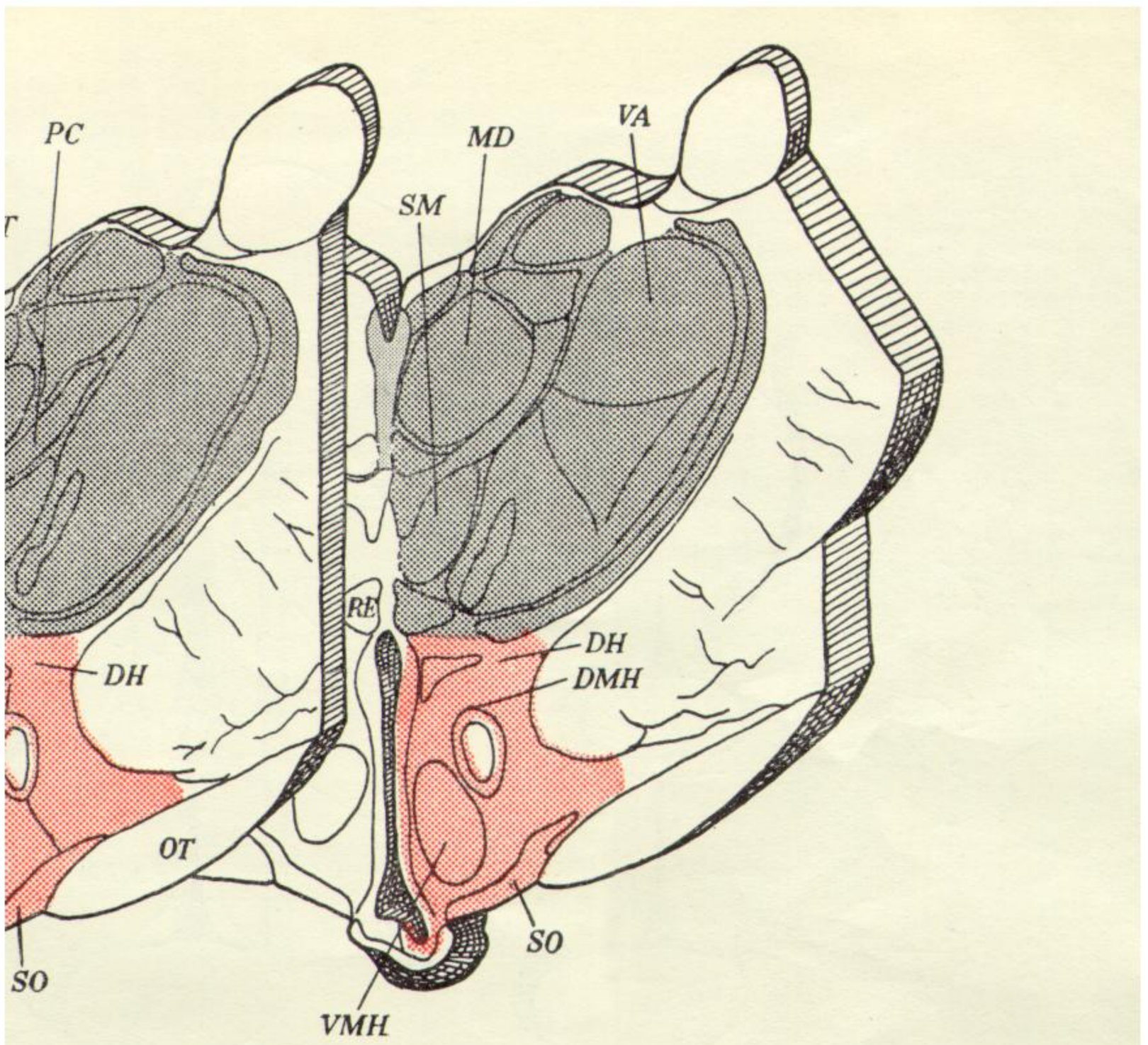

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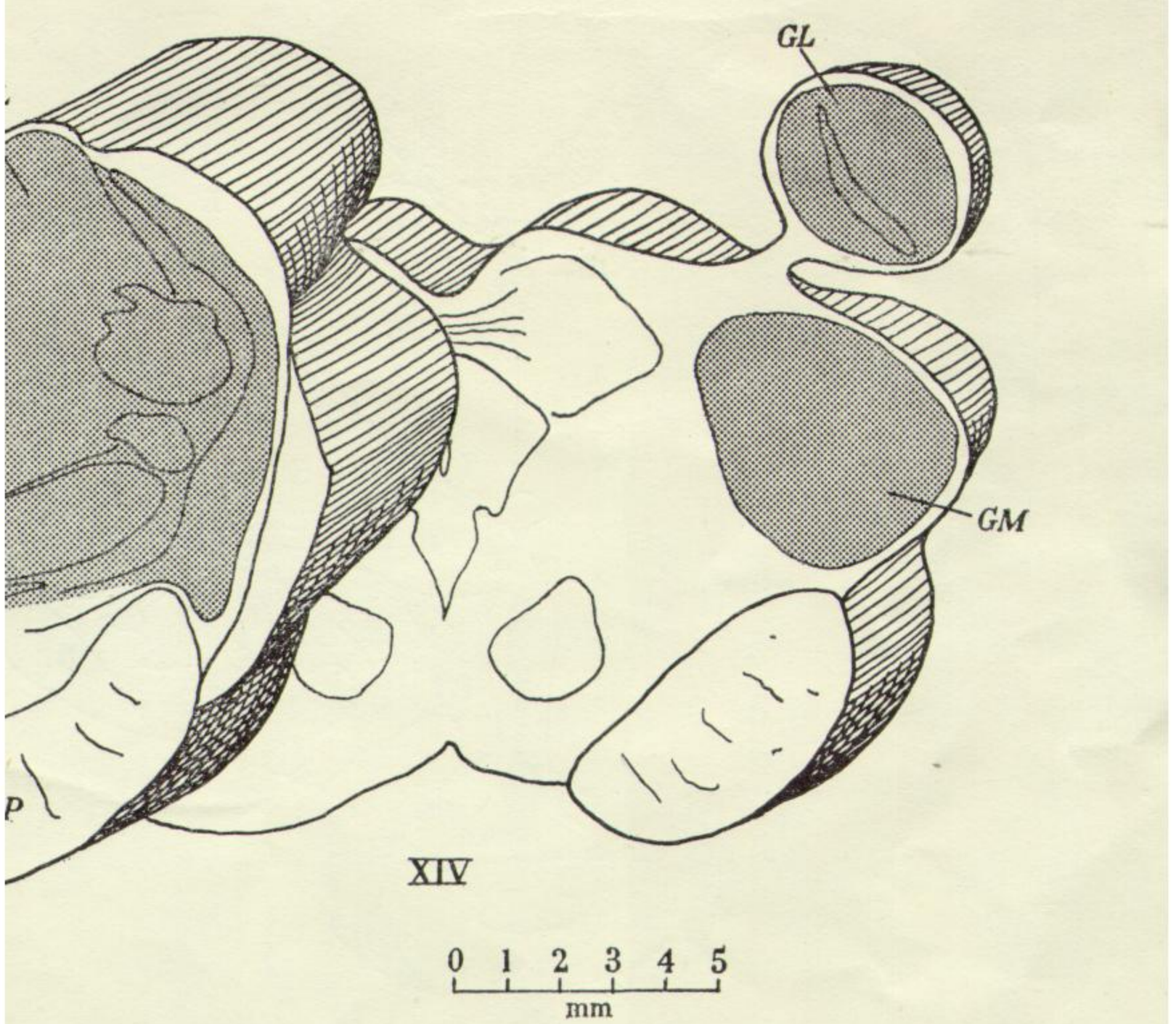

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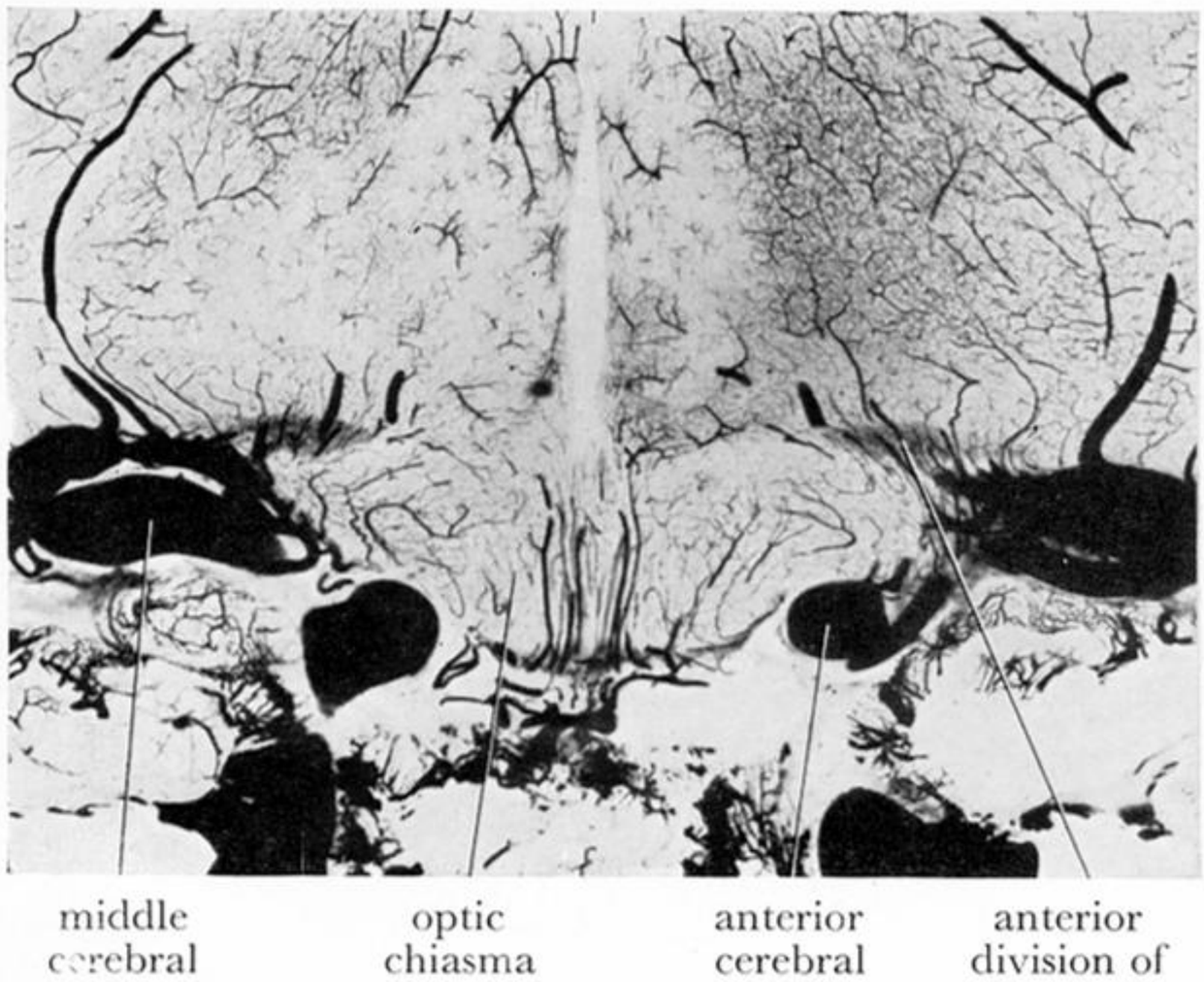

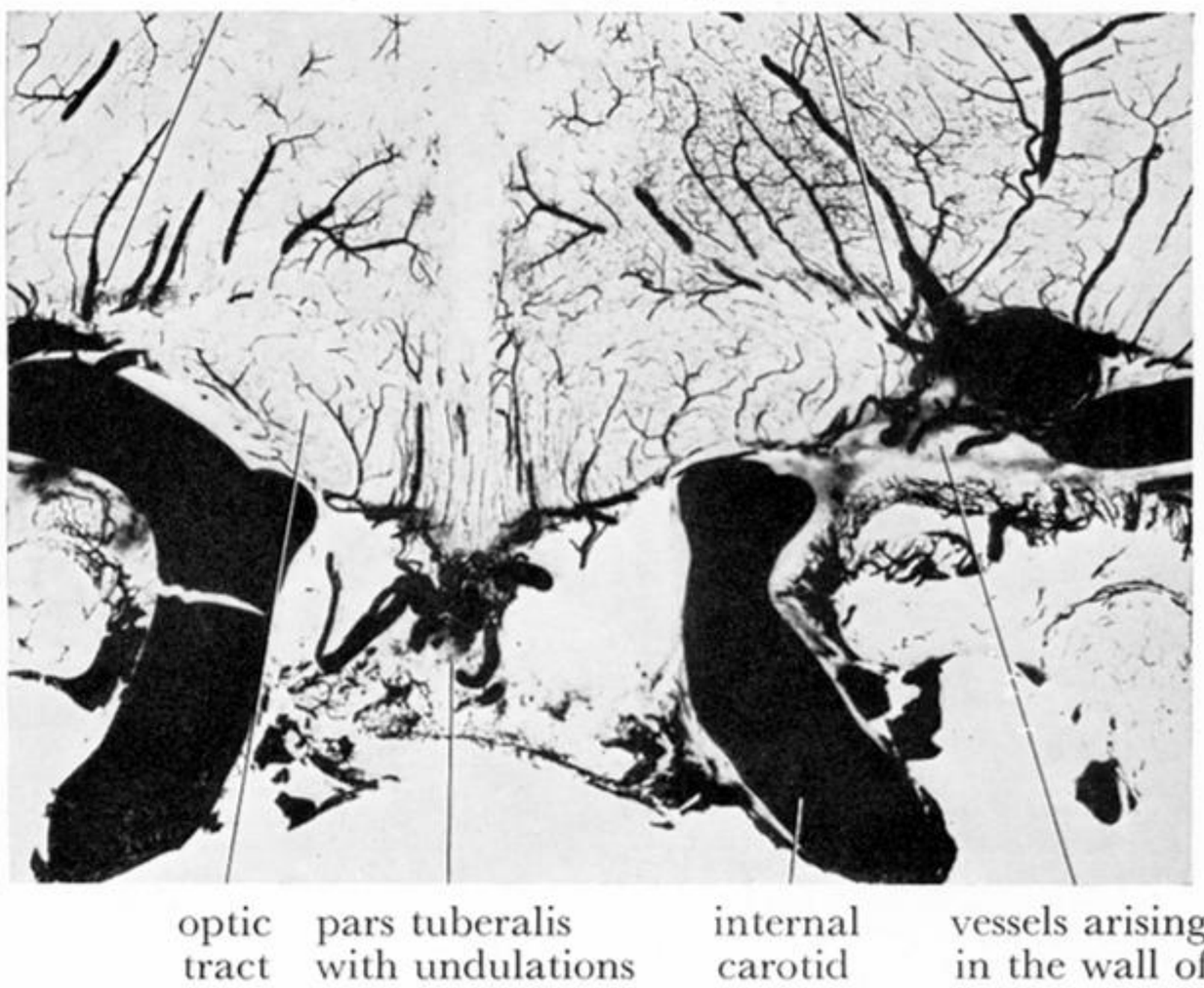

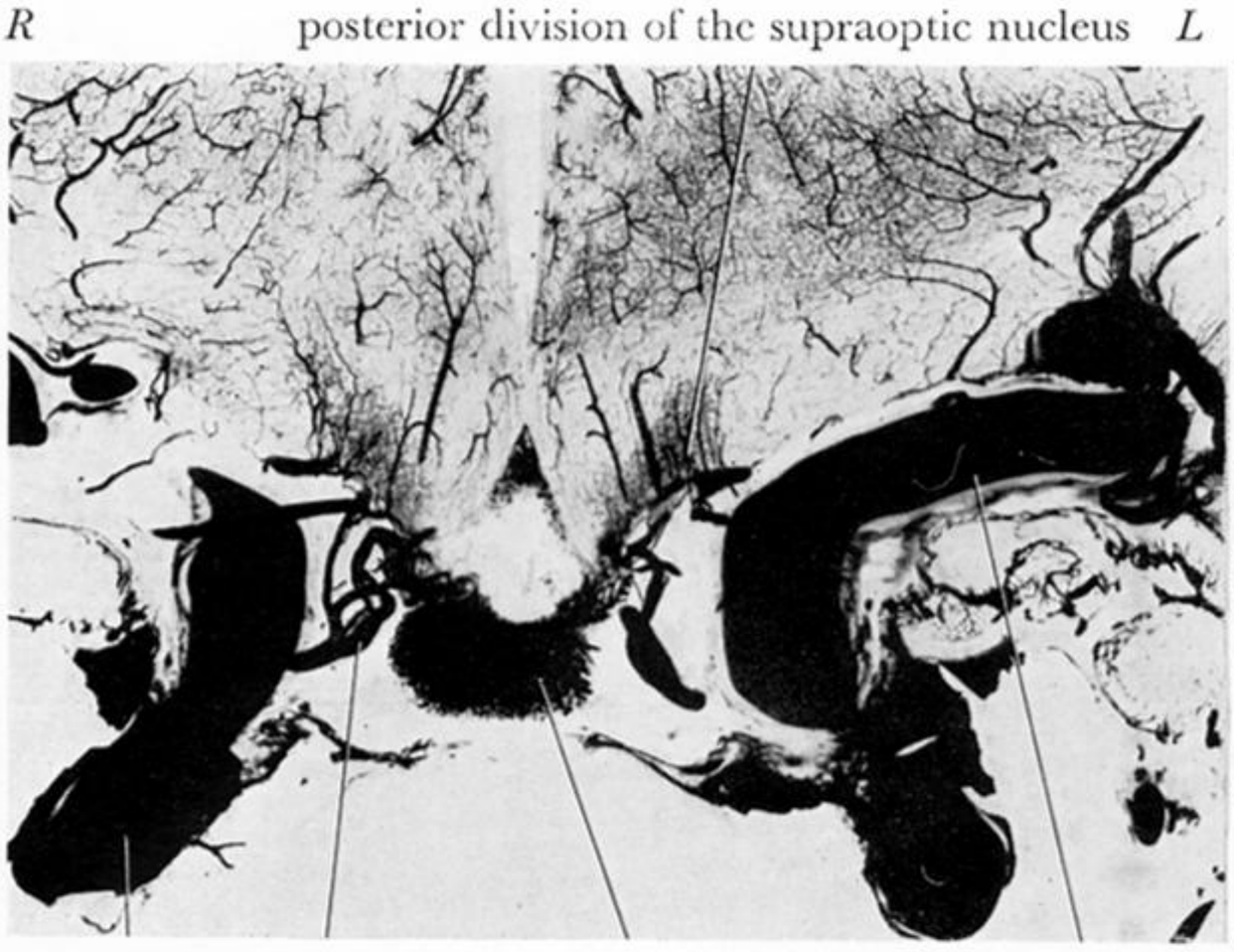

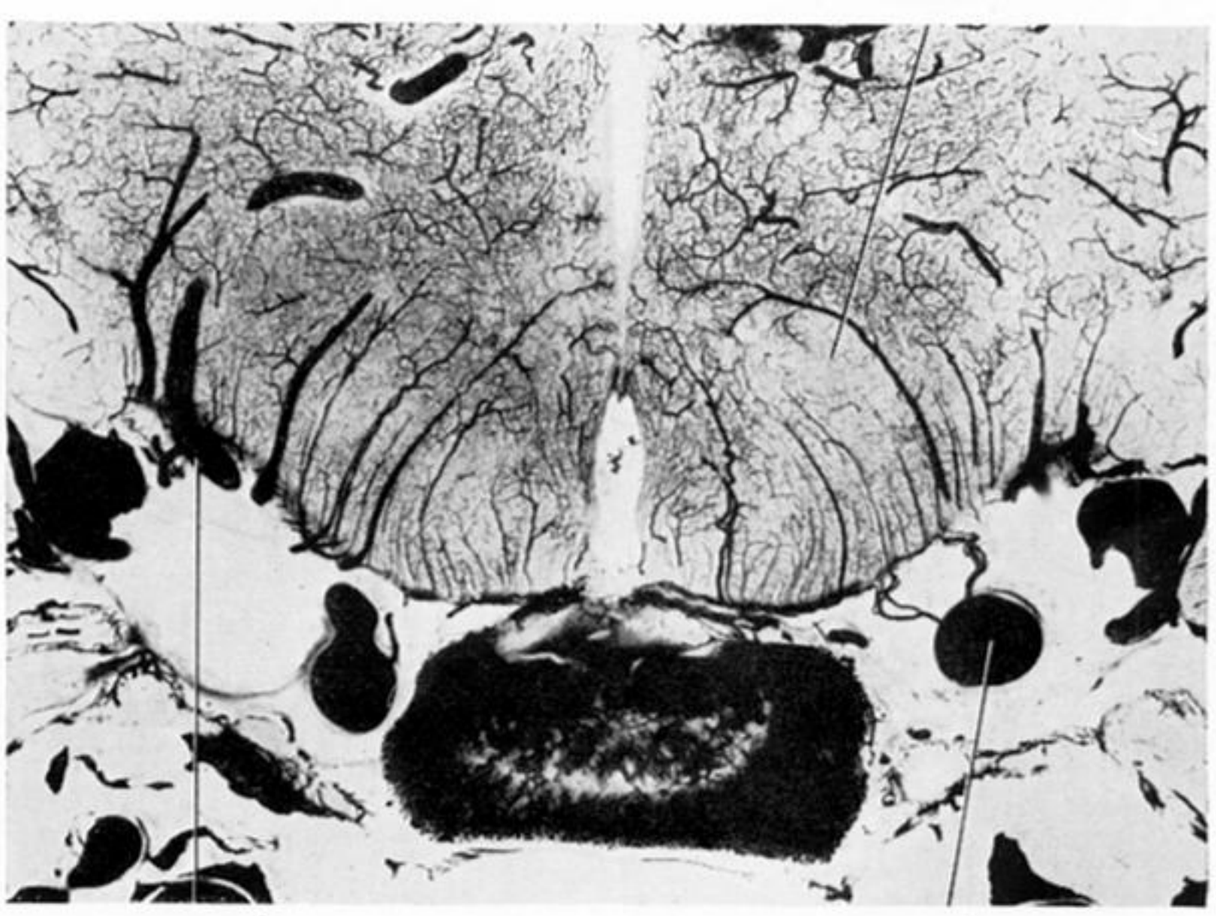

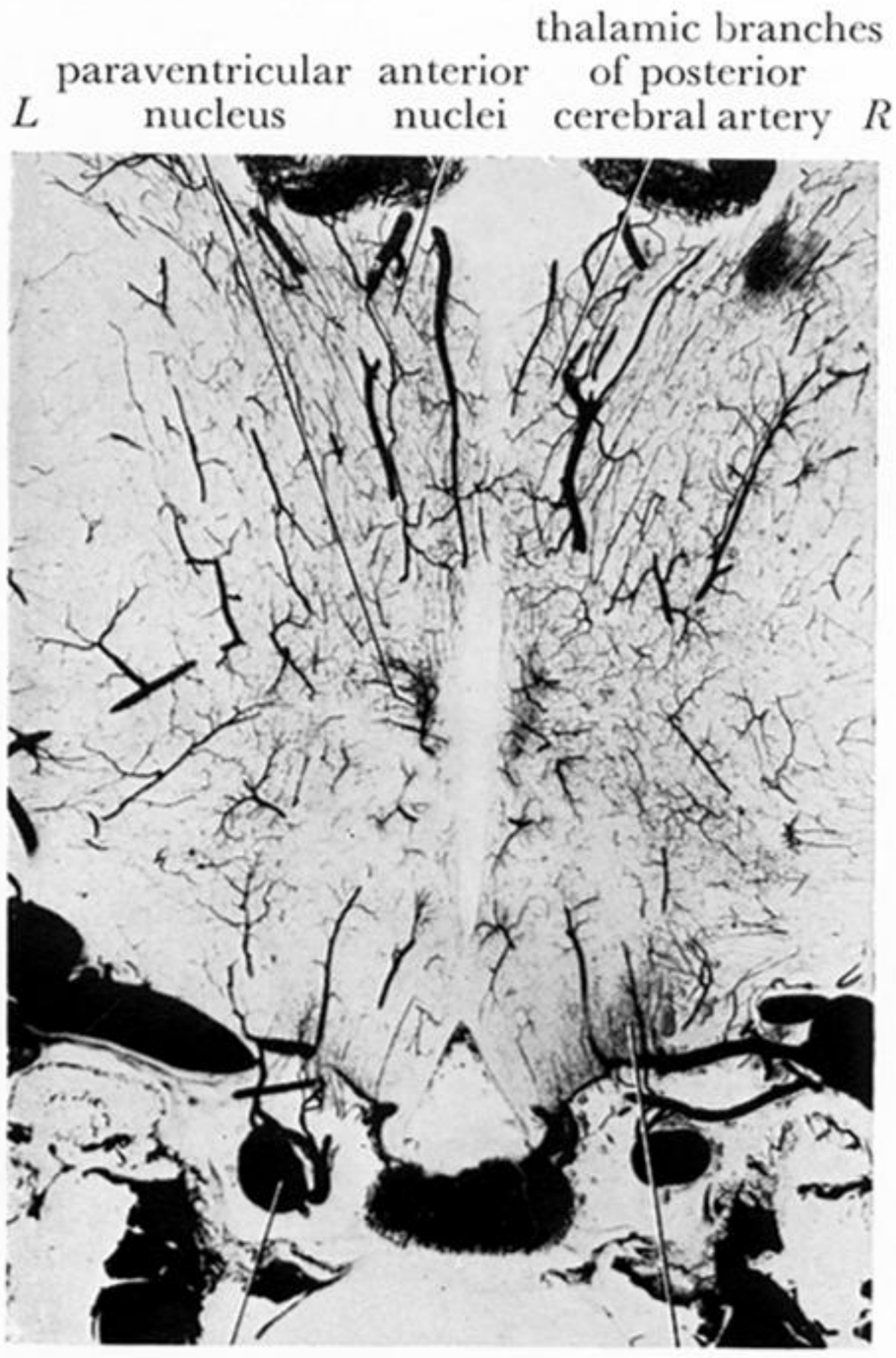

FIGURE 3. Photomicrographs of five sections selected from a frontal series through the diencephalon of dog D 5. One section intervenes between *b* and *c*, and three sections between *d* and *e*. The blood vessels were injected with Indian-ink plasma mass. Sections *a*, *b* and *c* are seen from the anterior, *d* and *e* from the posterior surface. Each is 500 μ thick. (Magn. *a*, *b*, *c*, *e*, $\times 6$; *d*, $\times 4{\cdot}5$.)

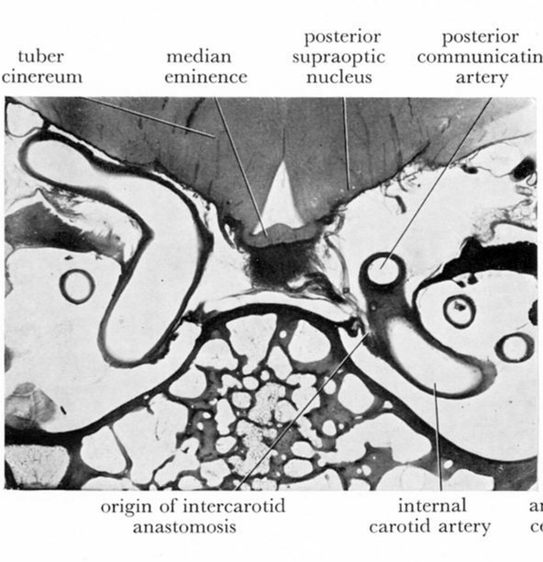

FIGURE 4

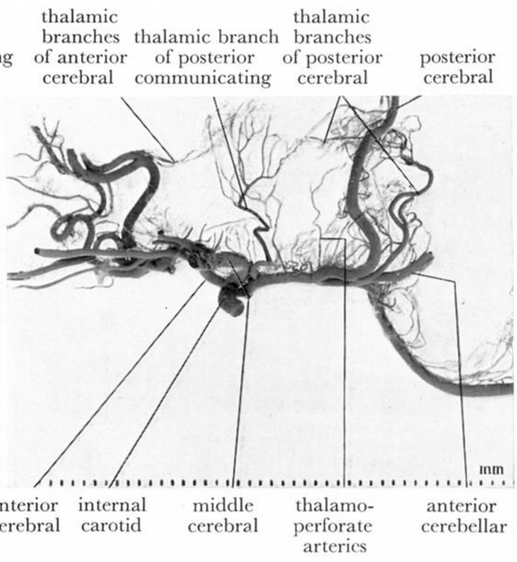

FIGURE 5

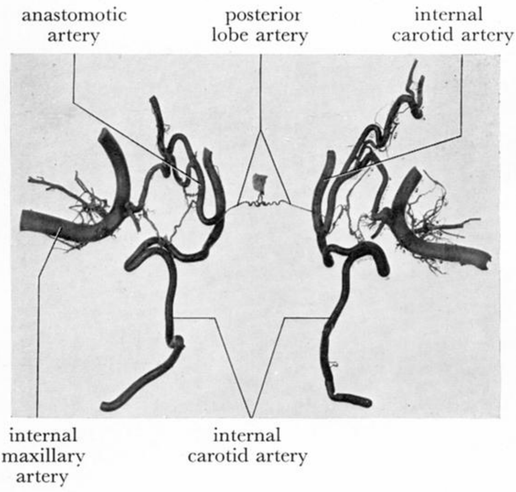

FIGURE 7

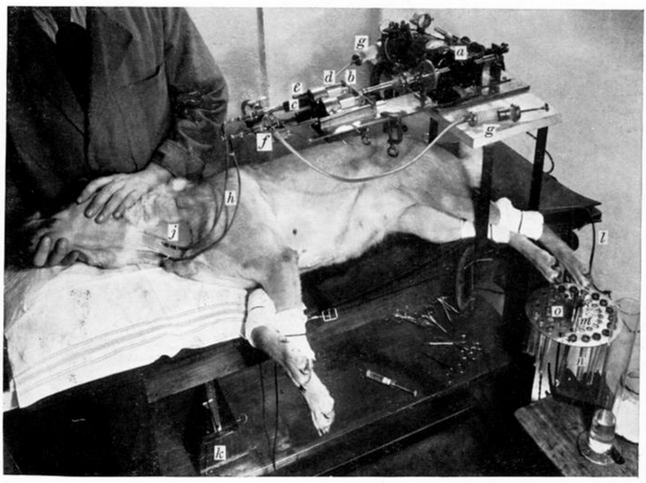

FIGURE 9

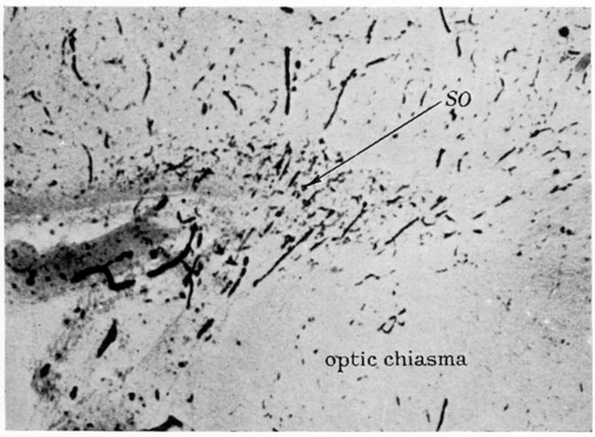

FIGURE 13

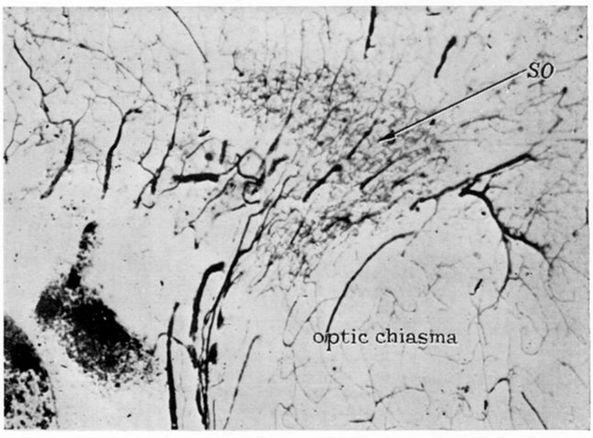

FIGURE 14

FIGURE 4. Photomicrograph of frontal section through the hypothalamus and adjacent structures of the sella turcica of dog D 3 *a*. Basisphenoid bone decalcified *in situ*. 200 μ thick; van Gieson stain. To show the origin of the intercarotid anastomosis. (Magn. × 6.)

FIGURE 5. Photograph of neoprene cast of the circle of Willis and its branches in the dog. Left lateral aspect to show especially the blood supply to the thalamus. The right side of the cast has been removed. (Magn. × 1·5.)

FIGURE 7. Photograph of neoprene cast to show the posterior-lobe artery originating on one side from the anastomotic artery and on the other from the junction of the anastomotic with the internal carotid artery. (Natural size.)

FIGURE 9. The technique of infusion of coloured suspensions into the carotid blood streams. The animal is 'Linda' (p. 305). The arrangements here illustrated are only representative of the actual procedure: the needles are lying subcutaneously in the carotid loops. *a*, constant-speed motor, reduction gearing and micrometer screw. *b*, brass plate placed across the plungers of the two 10 ml. syringes *c* which contain the coloured suspensions. To the brass plate are fixed the rods *d*, which are free to move in the guides *e*. *f*, three-way taps. *g*, 20 ml. syringes containing 0·85 % NaCl. *h*, rubber or polythene tubes leading to the infusion needles *j*. Close to each needle is interpolated a short length of glass tubing. *k*, metronome. *l*, extension tube leading from the catheter to the delivery tube *m* which opens over a series of graduated tubes (*n*) held in a brass disk (*o*) which can be rotated by hand. The black spot on the thorax marks the apex beat. In an actual experiment this area and the tissues in the underlying interspace are infiltrated with 2 % procaine HCl.

FIGURE 13. Monochrome of colour photomicrograph to show the appearance of the black suspension in the anterior division of the right supraoptic nucleus, SO ('Doris', no. 379). (Magn. × 50.)

FIGURE 14. Monochrome of colour photomicrograph to show the appearance of the blue suspension in the anterior division of the right supraoptic nucleus, SO ('Toby', no. 395). (Magn. × 50.)

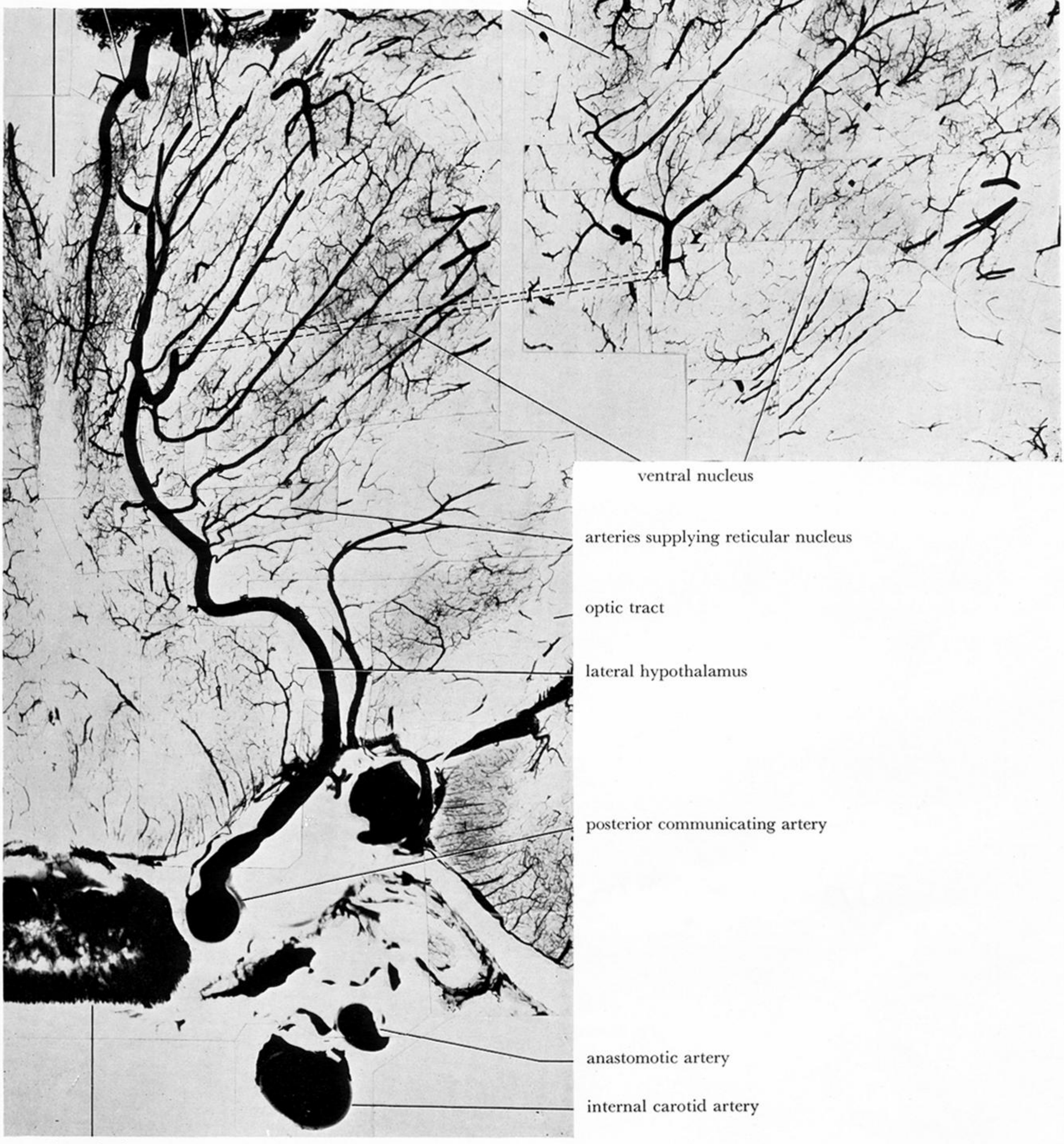

FIGURE 6

FIGURE 6. Reconstruction of the course of the thalamic branch of the posterior communicating artery on the left side of dog D 5. The blood vessels were injected with Indian-ink plasma mass. The reconstruction was made by the superposition of portions of photomicrographs of serial frontal sections. A posterior branch of the artery and its distribution to the lateral and medial thalamic nuclei is shown separately reconstructed to the right of the figure, its point of connexion with the parent vessel being indicated by the interrupted lines. (Magn. × 9.)

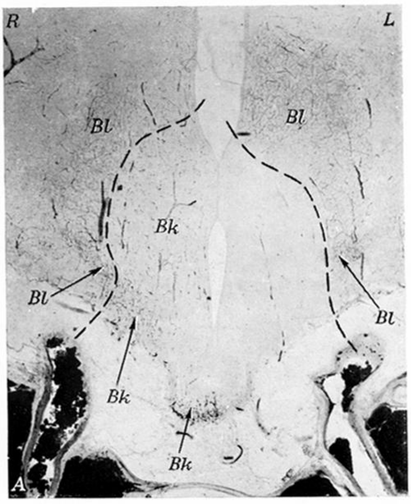

FIGURE 15*A*

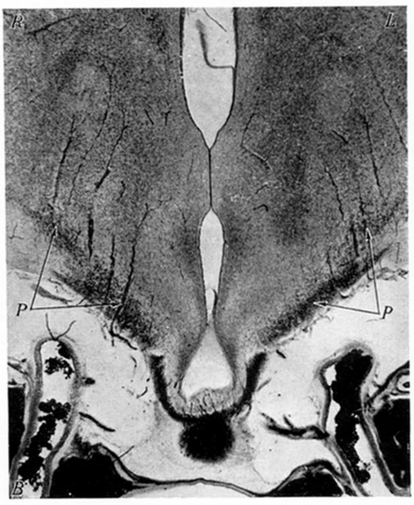

FIGURE 15*B*

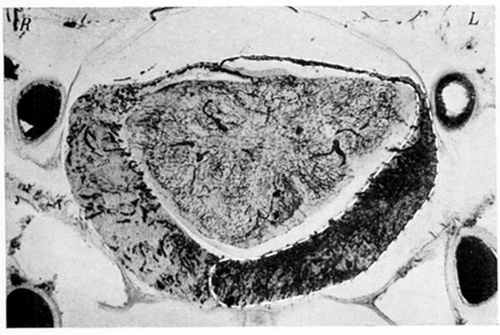

FIGURE 20

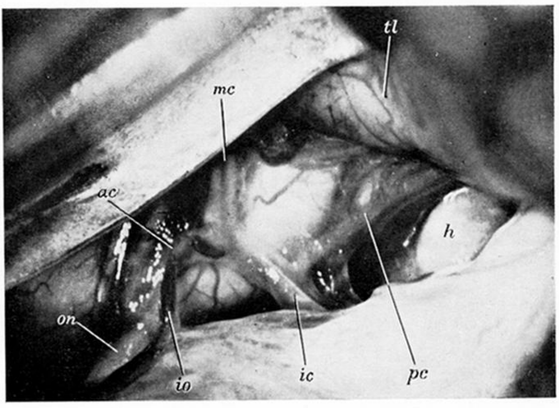

FIGURE 31*b*

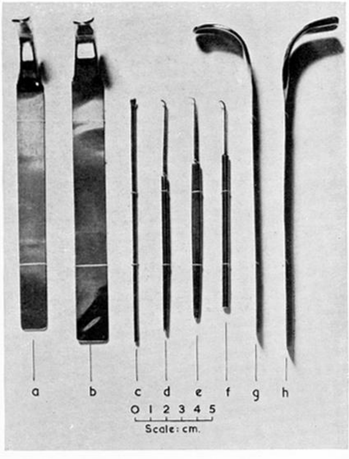

FIGURE 32

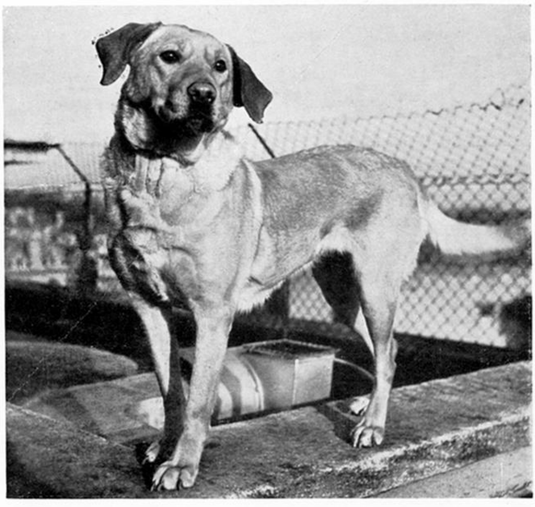

FIGURE 53

FIGURE 15. *A*: Monochrome of colour photomicrograph to show the appearance and distribution of suspensions in the posterior divisions of the supraoptic nuclei. Dog 377. Black suspension (*Bk*) infused into the right carotid and blue (*Bl*) into the right vertebral. *B* is a photomicrograph of the adjacent section stained with toluidin blue to show the extent of the nuclei (*P*). Note in *A* that the postero-lateral parts of the nuclei have received vertebral blood only (blue suspension), and the medial parts carotid blood only (black suspension in the right nucleus, no suspension in the left nucleus). Note also the 'cone' of tissue in the ventro-medial hypothalamus supplied by carotid blood, and the division of the blood supplies to median eminence and glandular hypophysis between the two carotid sources. (Magn. × 8.)

FIGURE 20. 'Molly', no. 341. Monochrome of colour photomicrograph to illustrate the fact that in this animal the posterior lobe was supplied solely by the right carotid artery. Black suspension had been infused into the right, and blue into the left carotid. The posterior lobe carries exclusively the black suspension, as does also the right half of the pars distalis. The left half of the pars distalis carries predominantly the blue suspension, shown enclosed by white dashes. (Magn. × 10.)

FIGURE 31*b*. Monochrome of colour photograph of the intradural exposure of the left internal carotid artery (*ic*) and its trifurcation. To the right the pars distalis (*h*) can be seen with the posterior communicating artery (*pc*) running lateral and dorsal to it; at the top, receding beneath the retractor is the middle cerebral artery (*mc*); to the left the anterior cerebral artery (*ac*) with its internal ophthalmic branch (*io*) lies adjacent to the optic nerve (*on*). *tl*, temporal lobe.

FIGURE 32. Photograph of special instruments used in the surgical procedure of tying the anterior cerebral, middle cerebral and posterior communicating arteries. For description see text, p. 255, and figure 31*a*, p. 257.

FIGURE 53. Photograph of 'Linda', no. 385, 21 weeks after ligation of the left anterior cerebral, middle cerebral and posterior communicating arteries. Immediately after recovery from the operation there was some weakness of the left circumocular muscles—some of the temporal and zygomatic branches of the facial nerve had inevitably been divided at operation—and in blinking the nictitating membrane was drawn across the eye. Later the elevators of the upper lip were brought into action in blinking, and the nictitating-membrane response then disappeared. These phenomena were also seen with the other animals in which the same operative approach to the pituitary region had been made.

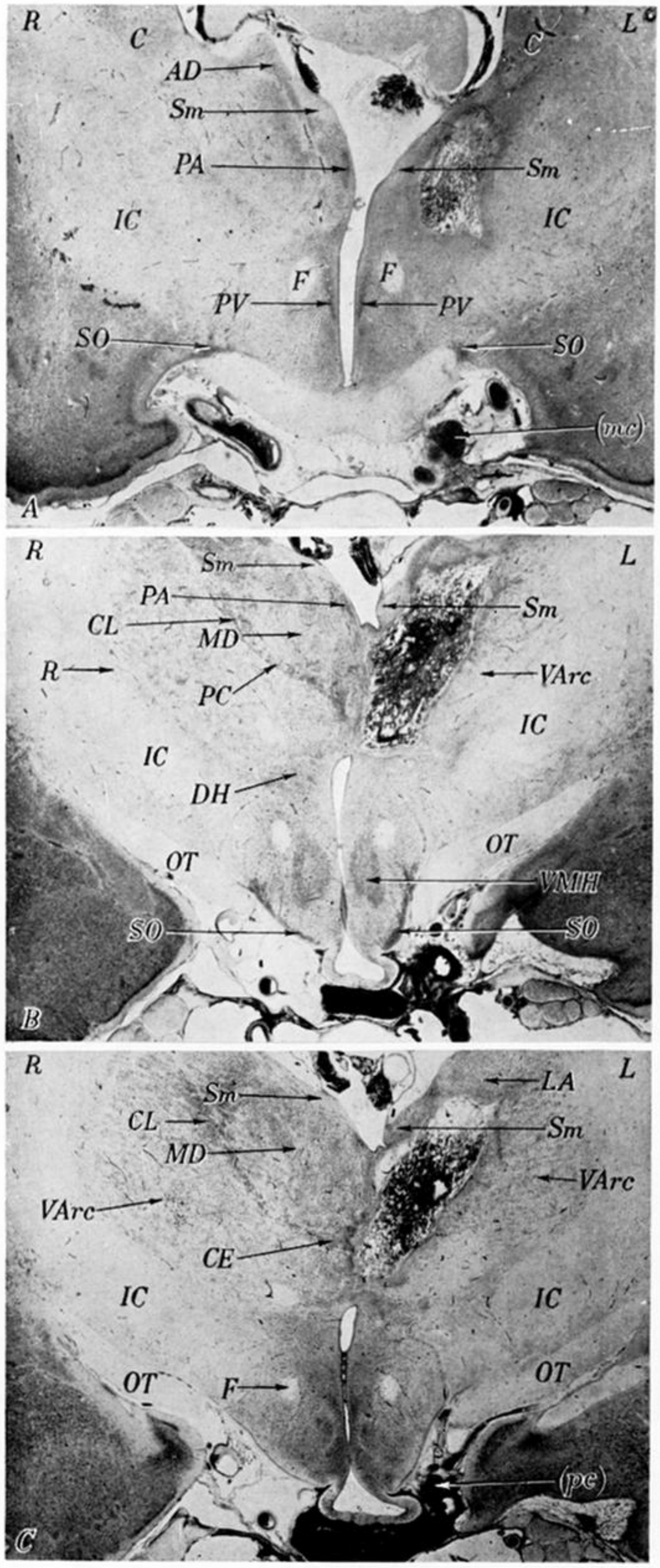

FIGURE 57. 'Linda', no. 385. Monochromes of colour photomicrographs of sections (125μ thick) stained with toluidin blue to show the cyst in the left anterior thalamus, its position and structure, and the nuclear degeneration associated with it. The anterior face of the sections is presenting. *A*, through the optic chiasma; *B* and *C*, through the median eminence. *B* is 2 mm posterior to *A*, and *C* is 1 mm posterior to *B*. Note that both anterior and posterior divisions of the supra-optic nucleus are well preserved on the left side: the appearance and density of the cells in the nuclei of the two sides are indistinguishable. Part of the knot, (*mc*), of the ligature on the left middle cerebral artery can be seen (stained intensely) just ventral to the optic tract in *A*; and in *C*, at (*pc*), is seen the occluded left posterior communicating artery with its ligature tag. For key to lettering see legend to figure 27. (Magn. $\times 4$.)