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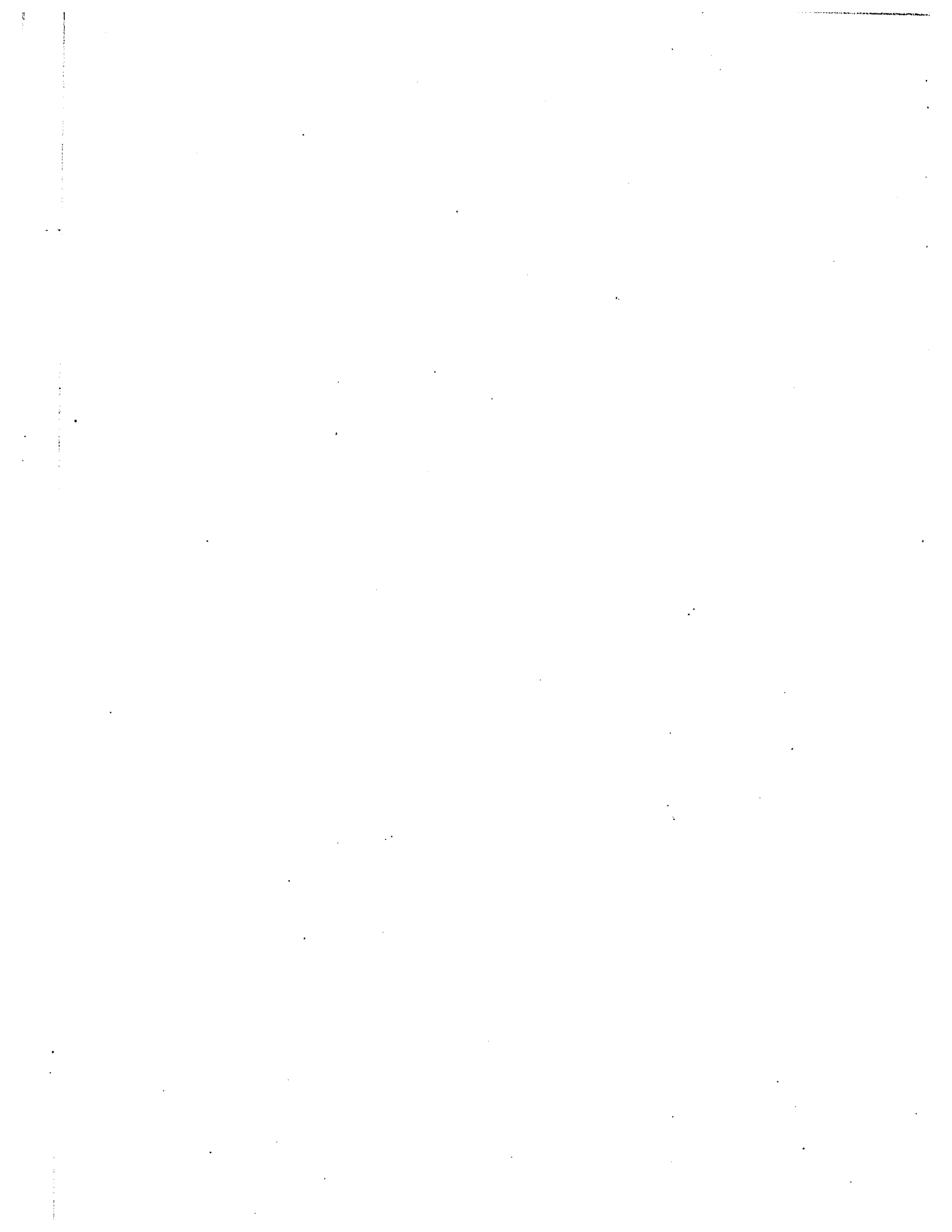
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HS

EXPERIMENTAL STUDIES ON NEUROSECRETION

BY

JOSEPH F. WETHINGTON

A THESIS

Submitted to the Faculty of the Graduate School of the University of Ottawa in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Department of Anatomy



OTTAWA, JUNE, 1957



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## CHAPTER I

### LITERATURE REVIEW AND FORMULATION OF THE PROBLEM

Introduction.— The hypothalamus is phylogenetically a very ancient portion of the diencephalon, originating mainly from the alar material of the earlier prosencephalon (Kingsbury, 1922). It has become increasingly clear in the past few years that the hypothalamus exerts control over a whole series of vegetative functions, e.g. increase in blood pressure, erection of hairs, respiration etc. This control by the hypothalamus is mainly carried out by descending pathways from the hypothalamus to the brain stem and the spinal cord, from where the impulses are relayed through the peripheral vegetative nervous system. However, one of the special pathways through which the hypothalamus exerts a vegetative influence is by means of the hypothalamic-hypophyseal tract to the neural lobe of the hypophysis. In the following chapters we shall attempt to show how certain hypothalamic nuclei are related to this vegetative function.

Normal Structure of the Hypothalamus. As shown in Fig. 1 the hypothalamus in cats extends from the level of the optic chiasm rostrally to the mammillary bodies caudally. The internal capsule and the subthalamus form the lateral boundaries of the hypothalamus while the third ventricle divides this area of the diencephalon into

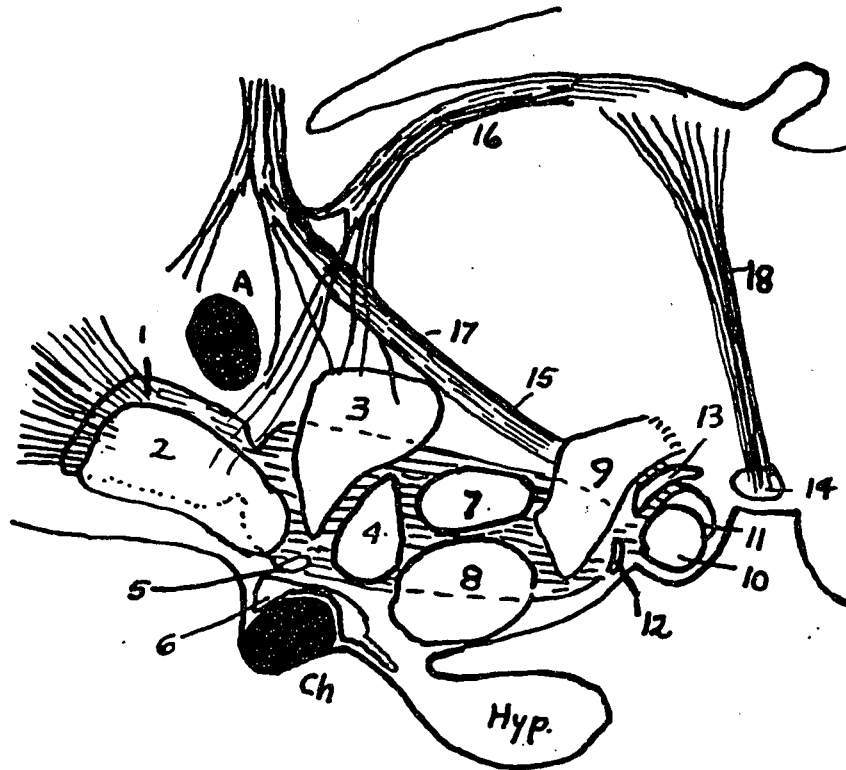


Fig. 1. Diagram showing relative positions in a sagittal plane of hypothalamic nuclei in average mammalian brain, and their relation to fornix, stria habenularis, and fasciculus retroflexus. A, anterior commissure; Ch, optic chiasma; Hyp, hypophysis; 1, lateral preoptic nucleus (permeated by the medial forebrain bundle); 2, medial preoptic nucleus; 3, paraventricular nucleus; 4, anterior hypothalamic area; 5, supra-chiasmatic nucleus; 6, supra-optic nucleus; 7, dorsomedial hypothalamic nucleus; 8, ventromedial hypothalamic nucleus; 9, posterior hypothalamic nucleus; 10, medial mammillary nucleus; 11, lateral mammillary nucleus; 12, pre-mammillary nucleus; 13, supra-mammillary nucleus; 14, interpeduncular nucleus (a mesencephalic element in which the fasciculus retroflexus terminates); 15, lateral hypothalamic nucleus, (permeated by the medial forebrain bundle); 16, stria habenularis; 17, fornix; 18, fasciculus retroflexus of Meynert (habenulopeduncular tract). (From Le Gros Clark et al., *The Hypothalamus*, London, Oliver and Boyd, 1938).

lateral halves. According to La Gros Clark (1938) the hypothalamus is subdivided into four areas.

1. The preoptic area. This area extends above and in front of the optic recess and reaches from the level of the optic chiasm to the lamina terminalis, and dorsally to the anterior commissure.
2. The supraoptic area. This area extends from the optic chiasm rostrally, to the anterior border of the infundibulum, caudally.
3. The infundibular area. This area is located behind the caudal extent of the supraoptic region extending to the mammillary region, caudally. It is in immediate relation to the infundibulum of the hypophysis.
4. The mammillary area. This area is located in the caudal part of the hypothalamus, characterized by the presence of two well circumscribed bodies and the area adjacent to the caudal region of the floor of the third ventricle.

The Hypothalamic Nuclei 1. The preoptic area. According to Clark (1938), and Aver (1951), this region is part of the telencephalon as it develops from the telencephalon median between the outpocketing telencephalic vesicles and the diencephalon. These authors include this area in the hypothalamus because functionally it is closely related to the hypothalamus.

The lateral group of preoptic nuclei is a direct extension forward of the lateral hypothalamic nucleus. It is composed of medium sized cells scattered diffusely in the course of the median forebrain bundle to which it contributes some fibers. This bundle

occupies an extreme lateral position in all four hypothalamic areas.

The medial preoptic nucleus lies near the ependymal lining of the third ventricle and is composed of small cells. La Gros Clark has shown that the medial preoptic nucleus has short fiber connections with the parolfactory area and the nucleus of the diagonal band in front and the hypothalamic nuclei behind.

2. The supraoptic area. This area extends from the optic chiasm to the rostral end of the infundibulum. The area consists of four nuclear groups, the supraoptic, paraventricular, supra-chiasmatic and the anterior hypothalamic nuclei.

Heynert in 1872 was the first author to describe the supra-optic nuclei, referring to them as the "basal optic ganglion". This basal optic ganglion was first called the supraoptic nucleus by Lenkosssek in 1887. Further description of this hypothalamic nuclear group was made by Malon (1910), Kappers, Huber and Crosby (1936).

A frontal section of the hypothalamus shows that the supra-optic and the paraventricular nuclei are well defined in vertebrates. Topographically the two nuclei differ greatly since the paraventricular group lies close to the third ventricle and reaches up just above the optic chiasm to the level of the ventral medullary lamina of the thalamus. The supraoptic nucleus forms a sharply circumscribed cell group which is adjacent to the optic chiasm and to the beginning of the optic tract on each side. The rostral extremity of the nucleus is seen on the lateral aspect of the optic chiasm, while its caudal extremity lies close against the medial aspect of the optic tract.

Cytologically, the supraoptic and paraventricular nuclei are similar. The cells are large and closely grouped among the fibers. Vacuolations and a peripheral distribution of the Nissl substance with multinucleated cells is observed in both groups. It was this observation which led Gaupp and Scharrer (1933), Roussy and Mosinger (1934), to believe that these cells had a secretory function. Scharrer and Gaupp suggested that the supraoptic nuclei have endocellular capillaries penetrating the cell bodies of the nuclei. Finley (1938, 1939), and Florentin (1934), criticized the opinion of Scharrer and Gaupp on the basis that a secretory activity of a nerve cell is incompatible with the structure and function of a neuron. In 1940, Finley suggested that the cells of the supraoptic and paraventricular nuclei are chemoreceptors, and Verney (1947), ascribed osmoreceptor functions to the cells of the supraoptic area.

According to the work of Palay and Wissig (1953), a second granular body, about the size of a mitochondrion, is located within the cytoplasm. Palay and Wissig further described a striated appearance of the Nissl substance in fresh supraoptic nuclei. This striated appearance is due to a system of lamellae or canaliculi present in the supraoptic nuclei and identified in electron-micrographs of tissue culture cells of liver and pancreas by Porter (1952). The reticulum is particularly prominent in the basophil regions of the cells and consists of strands of canaliculi arranged in parallel bundles. Palay and Wissig do not consider the reticulum, located in the Nissl substance of the supraoptic nuclei as an artefact, because the remainder of the cell in which it is found appears to be

in perfect condition. After two hours of observation, the reticulum disintegrates into a pale granular mass, before the nucleus has begun to lose its translucence. This same structure called the "endoplasmic reticulum" by Porter, varies in diameter from 0.2 microns to 0.07 microns with a dense membrane 0.6 microns in thickness. The endoplasmic reticulum has not been seen in nerve cells from parts of the central nervous system other than the hypothalamus. Further description of the cellular structure of these so-called magno-cellular nuclei will be presented on page 30.

The nucleus suprachiasmaticus is a compact group of small granular cells lying above the middle of the optic chiasm on each side of the midline. Anteriorly, it is continuous with the medial preoptic nucleus.

The anterior hypothalamic nuclei are practically absent in man; they are small cell groups located immediately behind the level of the nucleus suprachiasmaticus at the anterior border of the tuber cinereum and lying between the periventricular layer of cells medially and the medial forebrain bundle laterally. This group of nuclei receives fibers from the medial forebrain bundle and is also in relation with the terminal fibers of the stria terminalis.

3. The infundibular area. In this area the nucleus hypothalamicus ventromedialis and dorsomedialis are located. These cells are an oval eminence of gray matter in and above the tuber cinereum lying between the optic chiasm and the mammillary bodies.

The nucleus hypothalamicus ventromedialis is a dense mass of small rounded cells lying close to the third ventricle medial of the

fornix, caudal to the supraoptic and suprachiasmatic cells and ventral to the dorsomedial hypothalamic nuclei.

The nucleus hypothalamicus dorsomedialis consists of round cells located immediately dorsal to the nucleus hypothalamicus. This group of nuclei has also anatomical connections with the medial fore-brain bundle. (Le Gros Clark, 1938).

4. The mammillary area. Topographically, this area of the hypothalamus is well differentiated and consists of a homogeneous spherical mass of cells circumscribed by a capsule of white fibers. This mass of small cells is composed of a lateral and medial mammillary group. Two well defined tracts, the fornix and the fasciculus mamillothalamicus, are associated with the mammillary group of nuclei.

In lower mammals a nucleus supramammillaris is formed as a small group of cells covering dorsally the medial mammillary nucleus. Behind, the supramammillary nucleus appears to become continuous with the interpeduncular nucleus, which may be considered as a caudal continuation of the median hypothalamic nuclei. (Auer, 1951).

Between the tuber cinereum and the mammillary body of the hypothalamus is a collection of large and small cells, the nucleus hypothalamicus posterior which occupies the area between the ventro-medial and dorso-medial hypothalamic nuclei, rostrally and the mamillothalamic tract, caudally. Medially, this collection of cells lies adjacent to the ependyma of the lateral wall of the third ventricle, laterally by the fornix and dorsally it continues with the gray matter of the midbrain tegumentum.

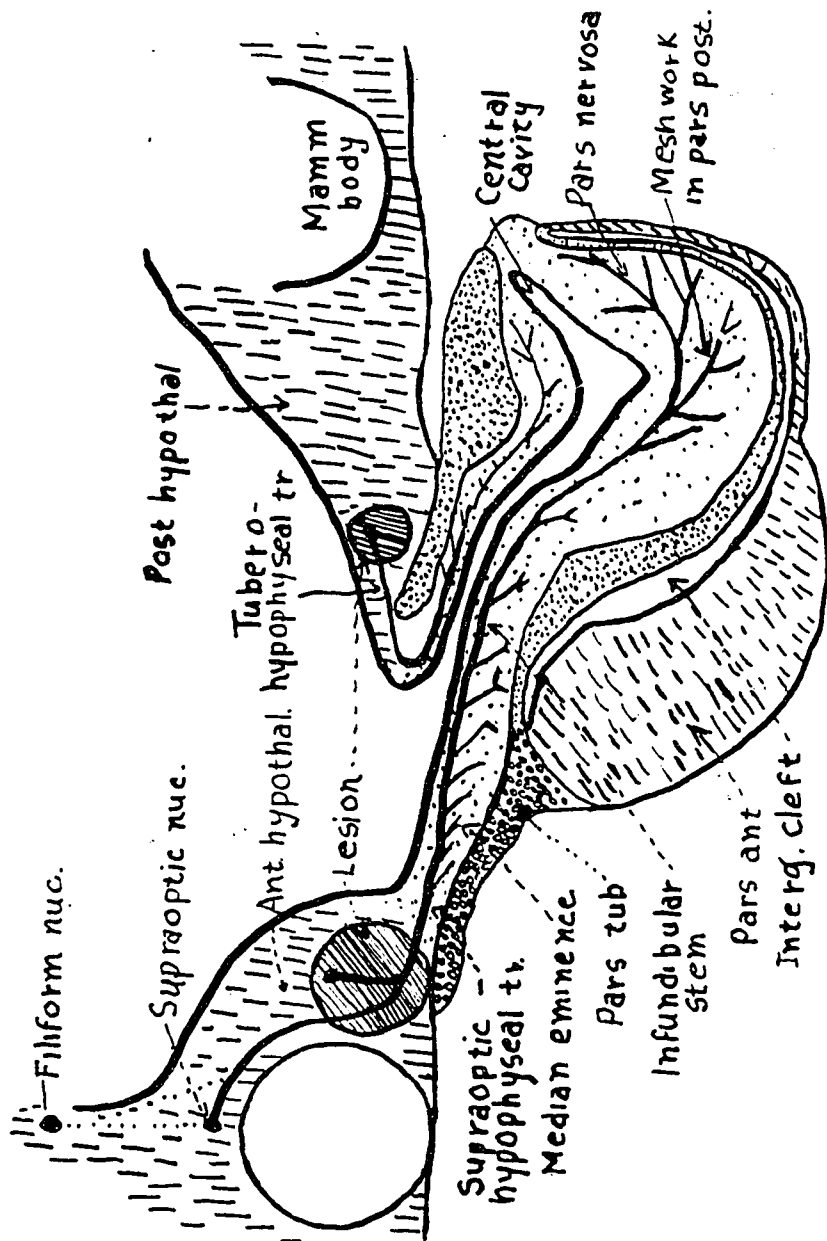


FIG. 2

The Anatomy of the Hypophysis The hypophysis of the cat and other mammals consists of two main divisions, the adenohypophysis and neurohypophysis. Each of these two divisions is subdivided into the following anatomical regions:

<u>Divisions</u>	<u>Subdivisions</u>
Adenohypophysis	Pars distalis Pars tuberalis Pars intermedia
Neurohypophysis	Pars nervosa Infundibular stem Median eminence

A diagram of the hypothalamic-hypophysal system is represented in Figure 2.

Caudal to the optic chiasm in the region of the infundibular area, the hypothalamus forms a large swelling which contains an extension of the third ventricle. Tilney (1936), labelled this swelling the median eminence. This structure is attached to the base of the hypothalamus and continues caudally with the infundibular stem. The infundibular stem is a long structure which extends obliquely backwards and terminates in the pars nervosa. Its ventral wall is continuous with the median eminence and its dorsal hypothalamus. As will be shown later, all three parts of the neurohypophysis have the same histological structure. In cats the third ventricle extends into the neural lobe of the hypophysis.

The adenohypophysis consists of three parts which are morphologically and functionally distinct. The pars tuberalis covers the median eminence rostrally; caudally it surrounds the first part

of the infundibular stem, while dorsally it is in contact with the posterior hypothalamus. The pars intermedia is a very thin sheet of well differentiated cells that closely invests the pars nervosa, without apparent structural continuity between the pars intermedia and pars nervosa. The pars distalis is separated from the pars intermedia by an interglandular cleft. In this work, the term hypophyseal stalk or neurohypophysis will include the median eminence, the infundibular stem and the pars nervosa.

Histology of the Neurohypophysis The pars nervosa in cats is surrounded by a capsule composed of collagenous connective tissue fibers. Penetrating the capsule are blood vessels, which are surrounded by fibers that enter into the interior of the gland. Within the substance of the neurohypophysis, as revealed by cresyl-violet and silver stains, are the following structures:

1. Unmyelinated nerve fibers which are collected in dense bundles in the median eminence. Near the periphery of the gland, many ramifications of the axons of the hypothalamic-hypophyseal tracts spread out in a fan-like fashion (see page 8).
2. Blood vessels. Hematoxylin and eosin sections of the pars nervosa reveal a rich capillary plexus within the interior of the gland. Wislocki and King (1936), in their investigations of the blood supply of the pars nervosa, found that branches from the inferior hypophyseal arteries enter the capsule of the gland, take an irregular course into the interior of the pars nervosa, where they break up into a close meshed plexus of ra-

ther wide capillaries. The blood vascular pattern of this region will be discussed in more detail later (see page 13).

3. Ependymal cells. These cells line the projection of the third ventricle into the neurohypophysis, including the median eminence, the infundibular stem and the central cavity of the pars nervosa.
4. Reticular fibers. According to the work of Bucy (1932), Bielschowsky's stain demonstrates a dense network of reticular fibers which form the supporting framework for the neurohypophysis.
5. Mast cells. Koim (1910), and Gray (1935), have described mast cells which are located along the walls of the capillary plexus. The cells are large with a definite nucleus. The cytoplasm contains coarse red granules and processes stretch out along the capillary network.
6. Pituicytes. According to Romais (1940), there are four specialized glial cells in the neurohypophysis; reticulo-pituicytes, micropituicytes, fibropituicytes and adenopituicytes. These glial cells are classified as such, largely on the basis of a unipolar, bipolar or multipolar processes. The processes may reach 900 microns in length, although they are only two to three microns in thickness. These processes are much longer than processes of glial cells which are seen in other parts of the nervous system. Very frequently these processes divide dichotomously, but more often they give off numerous shorter branches. The nucleus of the pituicyte is very irregular in shape and

the cytoplasm may contain numerous lipoid granules. In man, the granules may be pigmented and will reduce silver directly, or blacken with the methods of Bielschowsky and Hortege.

The Hypothalamic-Hypophyseal Tracts In Fig. 2, it can be seen that the median eminence becomes continuous with the anterior wall of the infundibular stem, while the posterior hypothalamus becomes continuous with the dorsal wall of the infundibular stem.

Fisher, Ingram, Ranson, and Magoun (1936), in using silver staining techniques, found that there are a large number of unmyelinated nerve fibers in both the dorsal and ventral wall of the infundibulum. Rasmussen (1940), has estimated this number to be 100,000. Traced distally, these fibers are seen to course throughout the length of the infundibular stem and to spread out and ramify in the pars nervosa. Proximally, these fibers were reported to have their cell bodies in two areas of the hypothalamus, the supraoptic and the mammillary regions (see page 43).

Fisher, Ingram, Ranson, and Magoun have shown that most of the fibers, coursing through the ventral wall of the infundibular stem, arise from the cells of the supraoptic nuclei. This group of fibers has been named the supraoptic hypophyseal tract. From its cellular origin these fibers emerge close to the dorsal and caudal surfaces of the optic chiasm, and continue along the inner side of the rostral end of the optic tract, turn medially to enter the median eminence, through which they pass into the ventral wall of the infundibular stem.

These investigators also described a bundle of unmyelinated nerve fibers which course through the dorsal wall of the infundibular stem. These fibers appear to arise from neurons in the posterior hypothalamus. This bundle of fibers has been designated as the tubero-hypophyseal tract. The tract is not as heavy as that in the ventral wall of the infundibular stem and its final axonic branches have not been demonstrated clearly in the pars nervosa.

The third fiber bundle, arising from the paraventricular nuclei, seems to contribute only a few fibers to the hypothalamic-hypophyseal system. Rasmussen, Fisher, Ingram, and Hanson believe that many of these fibers run ventro-laterally to end in relation to the supraoptic nuclei.

Neurosecretion The earliest active investigation concerning the function of the pars nervosa of the hypophysis was a demonstration by Oliver and Schafer (1895), that the blood pressure of an animal increases after injection of an extract from the hypophysis. It was later shown by Howell in 1898 that this effect was due to a substance from the pars nervosa as opposed to a substance from the pars distalis. In 1901, Magnus, Schafer and Dale showed that extracts from the pars nervosa also have an antidiuretic and oxytocic action.

Several reviews of the current understanding of the pars nervosa, including O'Connor's (1947), show that, although much progress has been made in the physiology, chemistry and pharmacology of hypophyseal hormones, much uncertainty remains regarding

the identification of the cells responsible for their synthesis. The concept that certain hypothalamic nuclei may exhibit glandular activity was first advanced by Speidel in 1922. Scharrer in 1928 independently proposed a similar theory based on cytological investigations of the hypothalamus of Phloxin.<sup>?</sup> Collin and Stukinsky in 1949 observed the same phenomena and the consensus of all these workers is that products of most glandular cells first become visible as granules which grow, undergo various kinds of transformation, and eventually they are discharged.

The histological structure of the pars nervosa did not encourage the earlier workers to believe that the active principle which could be extracted from it was elaborated within it. Herring (1908), whose name is now applied to the hyaline bodies often seen in the hypophyseal stalk, held that the posterior lobe hormone was produced by the cells of the pars intermedia, and that it passed back into the pars nervosa and up the hypophyseal stalk. His interpretation was accepted by many other workers, and particularly by Harvey Cushing (1933). Cushing's belief was that the posterior lobe hormones not only entered the blood stream, but that after making their way up the hypophyseal stalk, they passed through the ependymal lining of the third ventricle and so entered the cerebrospinal fluid. Garsh in 1939, stated that the pituicytes of the infundibulum secreted the posterior lobe hormones. Scharrer and Scharrer in 1937, maintained that the supraoptic and paraventricular nuclei are the responsible units that secrete these hormones. None of these theories gained credence, because of technical difficulties, until 1949, when Bargman showed that with the Gomori (1939), chromalum-hematoxylin technique, a stainable material could be readily

demonstrated, in mammals as well as in many other vertebrates, in the neurohypophyseal system. This so-called neurosecretory material is characteristically aggregated in cytoplasm of the supraoptic, the paraventricular cells and the axons of these neurons. Since Bargman's original observation, two principle hypothesis have been introduced with respect to the site of origin of neurosecretion.

The first hypothesis was proposed by Scharrer (1936), Palay (1945), Scharrer and Scharrer (1945), Bargman (1949), Ortman (1951), Hill and Zetler (1951), who have suggested that the nerve cells of the hypothalamic-hypophyseal system secrete the material, then transport the material along their axons to the axon terminal in the pars nervosa. This hypothesis was further substantiated by Hill in 1951, who demonstrated the accumulation of Gomori positive neurosecretion above the severed hypophyseal stalk and observed the depletion of the material distal to the ligature. Mosier and David (1954), in their studies of the chick embryo have found neurosecretory substance appearing in the nuclei of the supraoptic region as early as ninety-seven hours of incubation and it was present in the axon after five days of incubation. The substance was found in the infundibulum by the sixth day and increased in concentration in the infundibulum throughout embryonic life.

The second hypothesis was proposed by Palay (1953), and Bodian (1951). According to these workers the entire hypothalamic-hypophyseal system may play an active part in neurosecretion. Palay has since discarded this theory but Bodian believe that the rich

axon terminals in the pars nervosa of the opticum and their close association with neurosecretory substance tends to support this hypothesis.

The chemical nature of the neurosecretory material has been equally difficult to determine as has been the site of synthesis of this complex material. However, it is known that stainable material is identified with the oxytocic, vasopressor and antidiuretic hormones of the pars nervosa as has been shown by Hild and Zetler in 1951. Besides the investigations of Hild and Zetler, the chemical nature of neurosecretion has been actively investigated by Barnnett (1954), and Sloper (1955), and particularly by the latter. Barnnett has demonstrated, that the infundibulum of the neurohypophysis of normal rats and other animals contains disulfide positive material, that this material can be depleted from the neurohypophysis after dehydration of rats and moreover that it will reaccumulate after rehydration. Sloper and Adams found, after a very extensive investigation of the histochemistry of neurosecretion, published in 1955, little concentration of lipid or carbohydrate throughout the hypothalamic-hypophysial system. Their histochemical findings indicated that neurosecretion is not a glycolipo-protein, but a protein rich in cystine. Sloper suggested that this material may represent cystine-rich ostepeptides which show marked oxytocic, vasopressor and antidiuretic activity.

It has been shown by Gilman and Goodman (1937), that the need for water conservation which occurs in physiological water deprivation is a stimulus for the release of the antidiuretic hormone.

Ortmann (1951), and Hill and Zetler (1952), have demonstrated in rats, dehydrated by water deprivation, that the amount of antidiuretic hormone in the pars nervosa is reduced. This reduction of hormone content is manifested by the disappearance of Gomori positive material in the pars nervosa. It has likewise been shown by Bodian (1951), that subcutaneous injections of 3-hydroxy-2 phenylcinchoninic acid into the opusculum for four days will produce a decrease in the amount of stainable neurosecretory material in the neurohypophysis of this animal. Bodian suggested that the action of the antidiuretic change occurs by means of release of neurosecretory substance from the neurohypophysis. It has been demonstrated recently by Levangne and Scharrer (1953), that a 2.5% saline solution as drinking fluid for a period of thirteen days in adult rats will produce a complete depletion of neurosecretory material in the pars nervosa. If after reaching this stage, the animals are again given drinking water, the neurosecretory material reappears in the pars nervosa in the perivascular area, which Vasquez Lopez (1942), described as areas where the fibers of the hypothalamo-hypophysial tracts terminate. These physiological methods of decreasing the content of neurosecretory material in the pars nervosa and the infundibular stalk is therefore directly related to the severity of dehydration either by water deprivation or hypertonic saline and also to the quantitative amount of antidiuretic drug injected.

In the investigations concerning the site of secretion of the hormones of the pars nervosa, the blood vascular pattern of the

hypothalamic-hypophyseal system has been mentioned as an anatomical link between the hypothalamus and the hypophysis. Pope and Fielding (1938) were the first to suggest a hypophyseal-portal circulation. They believed that the blood flowed from the hypophysis to the hypothalamus. Wislocki and King (1936) and Wislocki (1936) suggested that the blood flow is from the median eminence to the pars distalis. Since Wislocki and King's work, there has been much uncertainty as to the direction of flow in this system of vessels and also about the possibility of the portal system acting as a vascular shunt from the hypothalamus to the anterior and posterior hypophysis. The original observations of Pope and Fielding, that the flow of blood is from the hypophysis to the hypothalamus has been discarded after the findings of Harris and Green (1949), and Barnett and Groop (1951). These workers have demonstrated by India Ink injections and in situ preparations in rats that the flow is from the median eminence, extends into the hypophyseal stalk and collects to form the large portal trunks which may be observed to fan out into the sinusoids of the pars distalis. Landsmeer (1947), reported that the arterial blood supply to the pars distalis is derived from branches of the posterior communicating artery, while the pars nervosa is supplied by pontine branches from the basilar artery and small branches from posterior communicating artery. The venous drainage, according to Landsmeer, is from the veins originating in the tuber cinereum extending into the pars distalis and thence flowing into a vein of the pars nervosa.

These investigations concerning the blood supply of the hypothalamic-hypophyseal system were influenced by the disbelief in the existence of any nervous connection exerting control over the hypophysis. It was therefore necessary to consider a hypophyseal portal shunt which in some way permitted the hypothalamus to exercise control over the anterior lobe of the hypophysis via the vascular system. The hypothesis, which has been advanced concerning this control was introduced by Harris (1948). He believed that some unknown "chemotransmitter" is liberated from the hypothalamus as a result of nervous stimulation, and then would subsequently enter into the portal vessels. The "chemotransmitter" then would pass down the longitudinal channels of the hypophyseal stalk into the sinusoids of the pars distalis where it would exert its influence on the secretory cells of the pars distalis.

In view of the work of Zucherman (1954), who separated the pars distalis from the hypothalamus in female ferrets and found no histological change in the pars distalis, most observers have disposed of the view that the hypophyseal-portal veins are essential for the activation of the pars distalis. In any case, since the pars distalis has so many varied functions, it would seem highly unlikely that there would be a separate "chemotransmitter" for each of these functions.

In view of the divergent opinions concerning the significance of the hypothalamus in relation to the neurohypophysis and the pars distalis, it was thought advisable to formulate the following problems as the object of investigation for this work:

1. An analysis of the anatomical areas of the hypothalamus, which may be responsible for the secretion of the hormones or their forerunners of the pars nervosa in cats.
2. An investigation of the effects on neurosecretion following a destructive lesion in different areas of the hypothalamus.
3. A study of some physiological conditions affecting the site of origin of the posterior lobe hormone.

## CHAPTER II

### MATERIALS AND METHODS

Twenty-three adult cats, and twenty albino rats were used for this study.

Lesions, with the method of electrocoagulation, were made in the hypothalamus of cats with the Horsley-Clarke stereotaxic instrument. With this instrument, one can insert a fine electrode from any direction through the brain. Accurately placed electrolytic lesions can be so inflected at any desired point. This apparatus itself consists of a rigid frame which can be fixed to the animal's head by means of clamps on the orbits and bars in the external auditory meatus. The bars are graduated in millimeters so arranged that the needle carrier can be brought to a point over the brain and the needle inserted to a predetermined depth. In order to use the instrument, it is necessary to determine the location of the point to be reached in terms of rectilinear coordinates. These coordinates were determined with the aid of Jasper and Ajmono-Marsan's "Stereotaxic Atlas of the Diencephalon of the Cat". Coordinates were determined in order to make lesions in the dorsal, anterior and lateral hypothalamic areas. The position of the coordinates was accurately calculated with reference to a fixed zero point. In order to do this three planes must be established:

First, the basal plane, which extends through the lower edge of the orbit and the center of the external auditory meatus; secondly, the zero horizontal plane which is parallel to the latter plane but above it, approximately one third of the distance from the interaural line to the vertex; the zero point lies in the interior of the brain at the intersection of the horizontal plane with the midsagittal plane and with the third plane, which lies at right angles to two former planes: this third plane passes also through the interaural line and is known as the zero frontal plane. It is therefore possible to insert the tips of the needle electrode in any desired position with reference to this zero point. For example, a lesion in the lateral hypothalamus would be  $\frac{1}{2}$  cm. to the right or left, 12mm. rostral and 3mm. in depth from the zero line.

For the purpose of making a lesion, a unipolar electrode was used. The electrode was made of 22 gauge wire with the thickness of less than one millimeter and was so rigid that it penetrated the dura without distortion. A spherical lesion was made by means of an electro-surgical-cautery unit, applying a direct current of 7 amperes to the inserted electrode for approximately 30 seconds.

All operations were performed under sterile conditions. The animal was anesthetized by intra-peritoneal injection of sodium pentothal, calculated on the basis of body weight, and placed in the stereotaxic instrument. The hair was removed from the calvarium with a razor, soap and water. The vertex and surrounding

operative area was sterilized with iodine and the skin opened by means of a sagittal incision. The temporalis and frontalis muscles were retracted by means of a hemostat. Bilateral foramina were made in the parietal bone by use of a small trephine. The needle electrode was inserted to the desired depth, and the area cauterized.

At the termination of the operation, the incision was closed with metal clips, and iodine applied to the operative area. The animals were placed in a warm cage and post-operative care administered until the animals were sacrificed.

In the period before exsanguination, which varied from one to seven days, some of the animals were given preventive therapy with antibiotics. They were fed orally by means of an eye dropper. The animals were sacrificed after having been anesthetized with sodium pentothal. A midline incision was then made from the manubrium sternum to the xiphoid process, the anterior rib cage was excised a twenty gauge needle was inserted into left ventricle. The superior vena cava was removed at the same time. Ten animal were then perfused with 100 cc. of isotonic saline solution followed by 100 cc. of 10% formalin-sublimate (formalin 1 part, saturated Hg Cl<sub>2</sub> 3 parts, H<sub>2</sub> O-3 to 6 parts). The brain was removed and placed in 10% formalin-sublimate for 6 weeks. This slices of the brain were embedded in paraffin. Twelve micron<sup>sections</sup> were made and stained with the following Gomori-alum-chrome-hematoxylin phosine technique.

1. Treat every tenth paraffin section with Bouin's solution

containing 3 to 4 gms. of Chrome alum per 100 cc, at 37 degrees for 12 to 24 hours.

2. Oxidize with the following mixture for 1 to 2 minutes: 2.5% solution of  $K_2CrO_7$  and 5% solution of  $H_2SO_4$ , 1 part each; distilled water 6 to 8 parts. Rinse.
3. Bleach with 1 to 3% solution of sodium bisulfite or oxalic acid, and wash with tap water.
4. Stain in the following solution for about 20 minutes or until the beta cells stand out dark blue against a much paler background:

Equal volumes of 1% aqueous hematoxylin and 2% chrome alum solutions were mixed. To each 100 cc. of this mixture, 2 cc. of a 5%  $K_2Cr_2O_7$  and 1 cc. of 5%  $H_2SO_4$  are added. The mixture is ripe after 48 hours and will keep in the ice box for many months. It should have a deep opaque, somewhat purplish blue shade and be filtered before using.

5. Differentiate in 0.5% acid-alcohol for about  $\frac{1}{2}$  minute, and wash with tap water for 2 to 3 minutes.
6. Counterstain with 0.5% aqueous solution of phloxin for 3 minutes and rinse.
7. Immerse in a 5% solution of phosphotungstic acid for 1 to 2 minutes.
8. Sections are then washed under the tap for 5 minutes. They should regain their red shade.
9. Differentiate in 85 to 95% alcohol until alpha cells stand out deep red.

10. Transfer to 95% and absolute alcohols, clear in xylene and mount in balsam.

Twenty rats were used for special studies of the hypothalamic nuclei and the pars nervosa of the hypophysis. Ten rats were placed on a water deprivation regime for a period of 6 days. The animals were weighed each day of the experiment and one animal was sacrificed every day commencing on the first day of the experiment. The brain was placed in 10% formalin-sublimate for a period of 2 weeks. Serial sections, 6 microns in thickness, were made of the hypothalamus and stained separately with thionin and Gomori's chrome-alum-hematoxylin stain. Observations of the hypothalamic nuclei of both stained and unstained sections were carried out with a Zeiss Phase Microscope using a bright field oil immersion objective.

Ten albino rats were placed on a 1% saline drinking solution for a period of 6 days. Daily weights were recorded and one animal was sacrificed each day of the experiment. The brain and the detached hypophysis were placed in a 10% formalin-sublimate solution for a period of 2 weeks. Staining procedure and observations were carried out in the same manner as in the former dehydration study.

## CHAPTER III

### OBSERVATIONS

Microscopic Appearance of the Neurohypophysis of a Normal Animal. Frontal sections of the hypothalamic supraoptic area of a normal cat stained for neurosecretory material reveal evidence of blue-black granules within the cytoplasm of the supraoptic and paraventricular nuclei. The concentration of this so-called Gomori positive substance is equally distributed throughout the cells, with the exception of the area adjacent to the cell nucleus which does not contain the same concentration of neurosecretory granules. Small granules of neurosecretory material are observed in the axons of those cells which are localized along the ventral surface of the hypothalamus, especially in the axons of the supraoptic nuclei. In the infundibular stalk the neurosecretory material, as represented in Figure 3, is distributed along the fiber tracts as small and large granules. In the infundibular stalk large Gomori positive structures are found; these structures are commonly referred to in the literature as Herring bodies (Herring, 1908). These structures differ in size but not in the concentration of neurosecretory material. In Figures 3 and 4, the pars nervosa of the normal control animal, a homogeneous mass of material is seen, which is heavily concentrated in the peripheral areas of the gland

and masks completely, with the exception of the blood vessels, the terminal axons of the hypothalamic-hypophyseal tracts, pituicytes and other structures normally observed with Bielschowsky's or hematoxylin-eosin stains. The central area of the gland adjacent to the central cavity does not contain as much neurosecretory material as the peripheral area. In the central area one may observe Herring bodies with the same structural appearance and size as those observed in the infundibular stalk. There is no microscopic evidence to indicate that neurosecretory material is located in either the pars intermedia or pars distalis.

Phase Contrast Studies of Neurosecretory Material in the Supraoptic Nuclei of Rats The presence of neurosecretory material as revealed with Gomori's chrome-alum-hematoxylin stain is consistent with the conclusion of Smith (1951), that these granules are minute, viscous, coacervate particles, and may therefore be considered to reflect the physiological state of the secretory process of the nerve cells.

In order to study these granules in greater detail, the phase contrast microscope was employed. This type of instrument represents a method of observation which makes the finest structures visible by substantially increasing the contrast by optical means. Furthermore, the phase contrast microscope provides an image which is equal in sharpness and clearness to those taken in the bright field. It reduces likewise the necessity to employ stained preparations with the concomitant danger of altering and damaging the

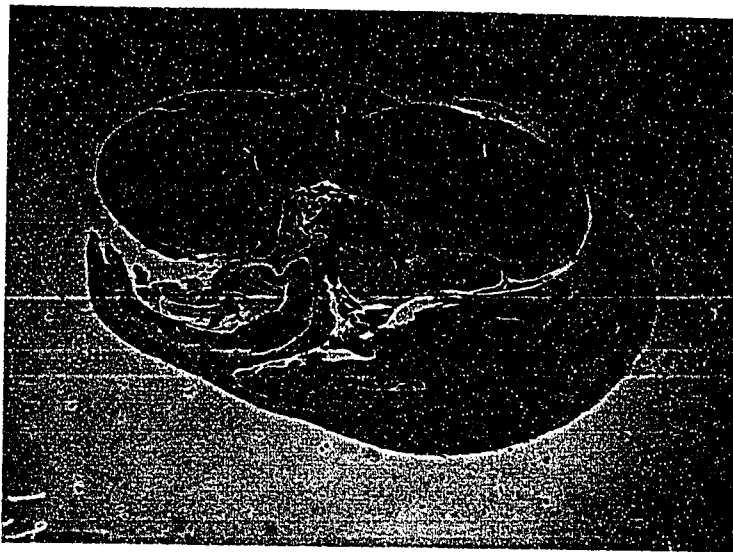


Fig. 3. Pars nervosa, pars distalis, pars intermedia, pars tuberalis, median eminence and infundibular stem of normal control animal. Neurosecretory material is observed in the median eminence, infundibular stem and pars nervosa. The pars intermedia encloses the pars nervosa. Neurosecretory material is equally distributed in the glandular substance. X5



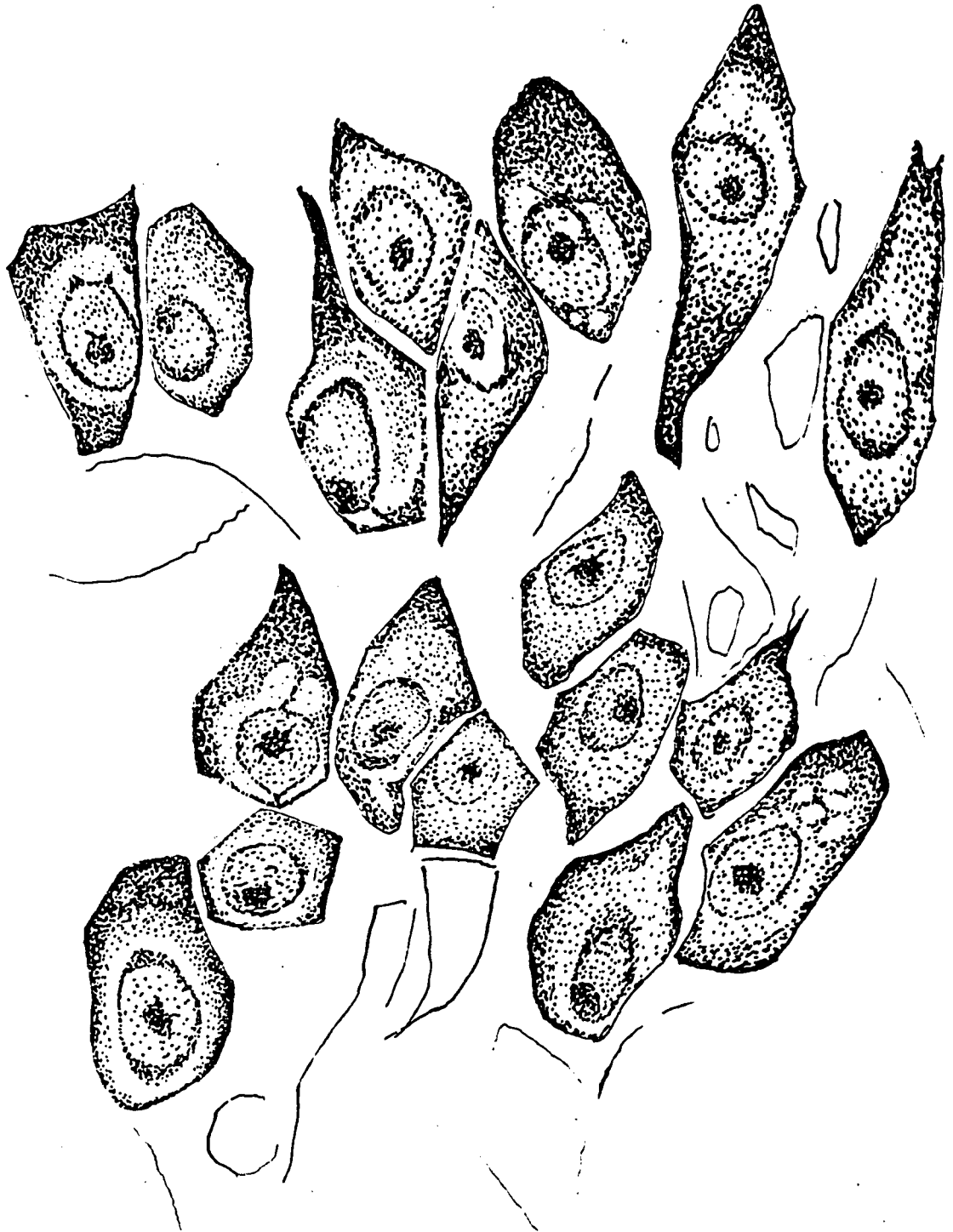
Fig. 4. Pars nervosa and pars intermedia of a normal control animal stained with Gomori's chrome-alum-hematoxylin-phloxine stain, and demonstrating evidence of neurosecretory material in the peripheral area of the gland. Light areas within the gland are blood vessels. Note the heavy concentration of material adjacent to the pars intermedia. XIII6

structures to be examined.

Phase Contrast Appearance of Normal Supraoptic Nuclei

The supraoptic nuclei are easily distinguished because of their large size and the granularity of the cytoplasm. The granular bodies are dispersed throughout the cytoplasm, especially near the cell membrane of the neuron as shown in Figures 5 and 6. The granular bodies are the secretory elements because they do not appear in the nuclei of the cerebral or cerebellar cortex and they resemble the granules found in the neurohypophysis of many other vertebrate animals including rats and cats used in this study. The granules appear to be round and approximately of equal size. The nucleus of the cells appears optically homogeneous, except for a small tangled mass of material within the nucleus which is identified as the nucleolus. Occasionally, cells are seen which are multinucleated and which contain vacuoles. In the axons of supraoptic cells, the granules vary in size and seem to be much smaller in the distal portion of the axon. The paraventricular nuclei consist of smaller cells which do not contain granules of comparable size as those observed in the supraoptic nuclei.

Rat 1a



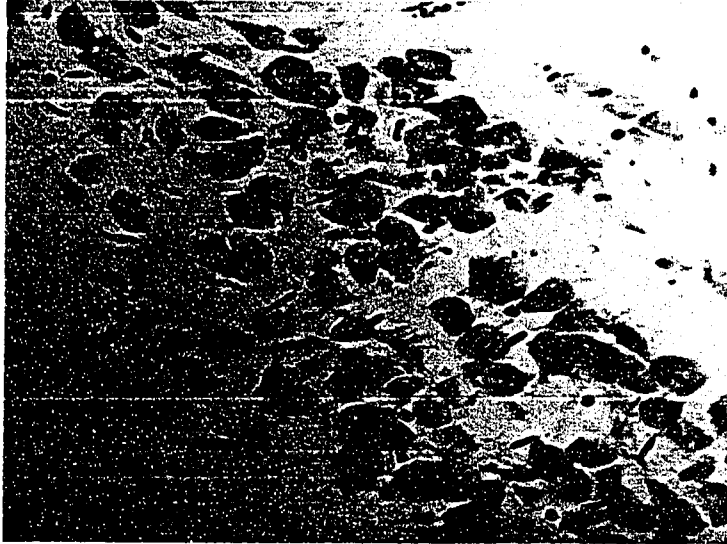


Fig. 6. Supraoptic nucleus of a normal rat stained with Gomori's chrome-alum-hematoxylin-phloxine method. The neurosecretory granules are localized in the peripheral area near the cell membrane. The cytoplasm near the nucleus of the nerve cells does not contain a heavy concentration of material. Note the nucleoli within the nucleus of the cell body. X 4,000

Thionin stains of serial sections of the rat hypothalamus demonstrate granular Nissl bodies near the cell membrane. The nuclei of these cells have different shapes and are usually located near the central area of the cell. Adjacent to the nuclei is a clear area which is believed to represent the location of the Golgi apparatus. Except for the shape, no significant structural difference was observed between the supraoptic nuclei of rats and adult cats.

## EXPERIMENTAL OBSERVATIONS

Bilateral Lesions of the Supraoptic Nuclei Eight adult cats were used for the cauterization of the supraoptic region. The following description explain in detail the postoperative findings in two of these animals.

The lesion in animal 1-A, Figures 7 and 8, (Table 1), extended from the supraoptic area rostrally, to the infundibular area caudally. The anterior extent of the lesion destroyed bilaterally, a portion of the supraoptic nuclei, the ansa lenticularis, lateral hypothalamus, the fornix and touched the optic chiasm.

Microscopic examination of the neurohypophysis of this animal reveals a major decrease in the amount of stainable neurosecretory material in the pars nervosa. This decrease in granulation is illustrated in Figure 9. The reduction in granulation has occurred largely in the areas adjacent to the central cavity of the pars nervosa, and to a lesser extent in the peripheral areas. There is a corresponding decrease of neurosecretory material in the infundibular stalk and the supraoptic nuclei adjacent to the lesion. In the hypothalamic area of necrosis the supraoptic cells have undergone karyolysis with disappearance of neurosecretory granules in the cytoplasm. The remaining cells of the supraoptic nuclei which are not directly involved in the lesion appear normal in size and the cytoplasm



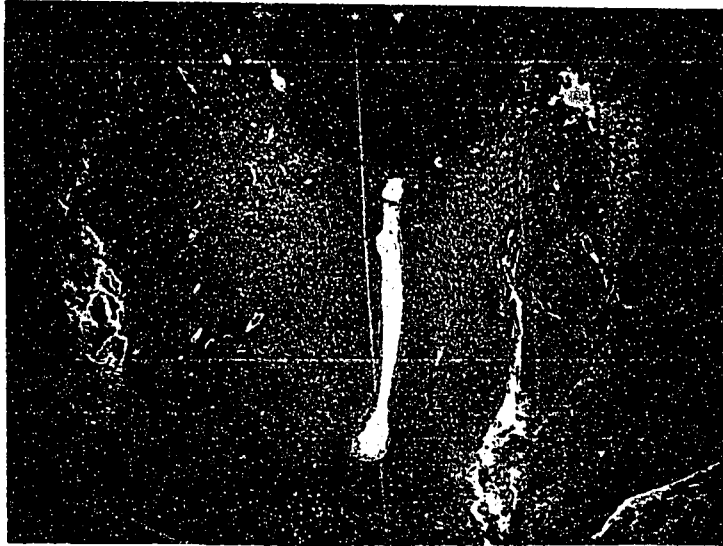


Fig. 8. Bilateral lesions in the supraoptic area of animal 1-A. Undestroyed stained supraoptic cells are noted bilaterally above the optic tracts. The lesion has destroyed a major portion of these cells, especially in the right supraoptic area. The paraventricular cells are observed in the midline localized on both sides of the third ventricle. X12

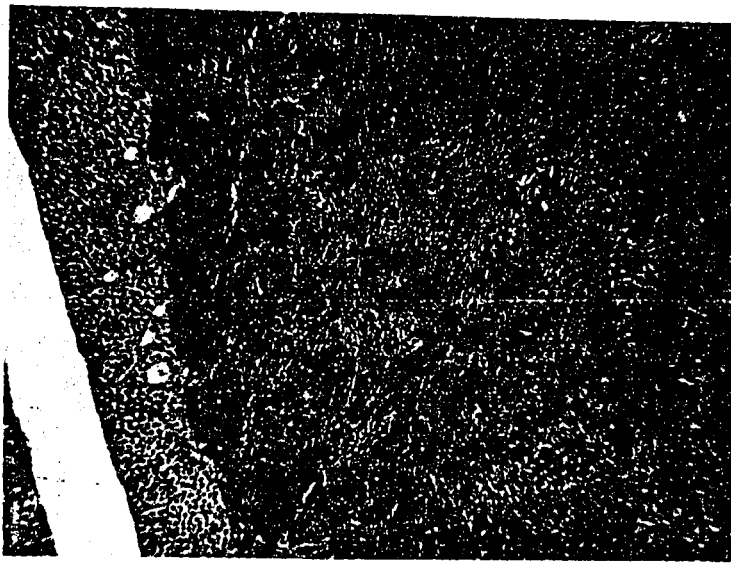


Fig. 9. Pars nervosa of animal 1-A. Note the reduction in neurosecretory material in the central and the peripheral areas of the gland. Herring bodies are observed in the glandular substance near the central cavity of the third ventricle. X58

of these cells contain a full complement of Gomori positive material. Herring bodies are seen in the pars nervosa and in the distal portion of the infundibular stalk. These structures are located in the peripheral areas of the gland but are better observed when there is a depletion of neurosecretory material near the terminal axons of the hypothalamic-hypophysial tracts. Bodian (1951), stated that Herring bodies are storage structures for neurosecretory material. It is observed that there is a reduction in the amount of neurosecretory material in these structures when compared with the control section, thus confirming the findings of Bodian who studied these structures in the opossum. In order to further identify the possible chemical nature of Herring bodies, serial sections of the neurohypophysis of three different animals were made and stained for glycogen by the Schiff method. Microscopic examination of the neurohypophysis of these animals, and especially the pars nervosa revealed no evidence that the Herring bodies are Schiff positive.

Animal 2-A in this series demonstrated a bilateral lesion extending from the supraoptic area to the infundibular area caudally. Figures 10 and 11 represent frontal sections somewhat primarily in the antero-lateral hypothalamus. Some of the supraoptic cells along the optic chiasm have been destroyed completely, while a portion of the right supraoptic group is still intact. There is no involvement of the paraventricular group of nuclei, nor of the structures which are adjacent to the lateral wall of the third

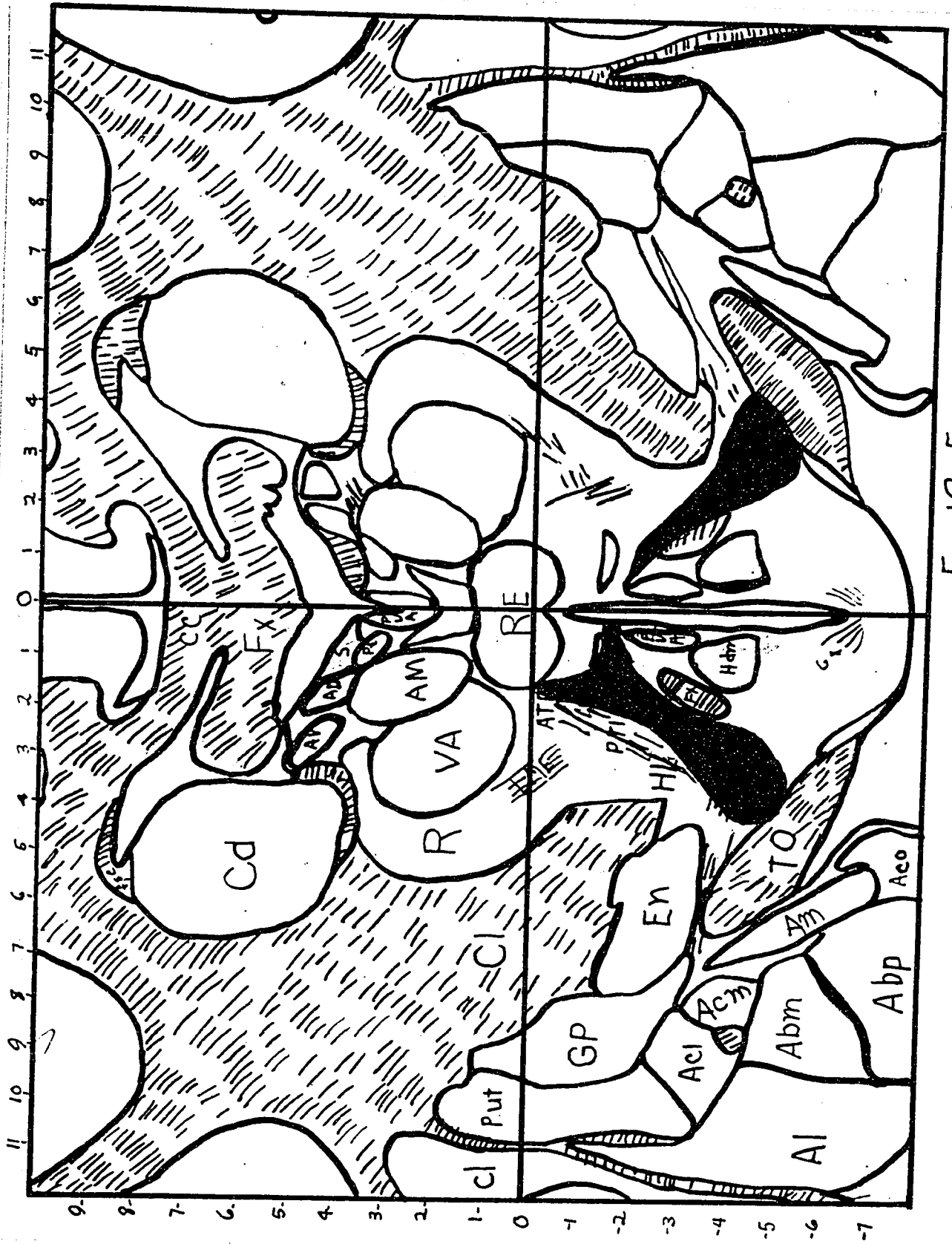
ventricle.

Histological examination of the hypothalamic-hypophyseal system of this animal as shown in Figure 12 of the pars nervosa and the infundibular stalk, reveals a significant reduction in the amount of neurosecretory material.

The axons of the supraoptic nuclei observed near the floor of the third ventricle show small beads of granular Gomori positive material. The amount of neurosecretory material in these axons is much less than those observed in a normal animal. There is a similar reduction of neurosecretory material in the supraoptic nuclei of the cells involved in the cauterized specimens. The infundibular stalk reveals a marked reduction of neurosecretory material as well as in the Herring bodies in this region. Serial sections of the pars nervosa reveal an equal reduction of neurosecretory material throughout the peripheral and central portions of the gland. The gland is extremely vascular and one can observe pituicytes and fiber tracts which are not seen in the control animal due to the heavy concentration of neurosecretory material.

The parenchymal cells of the neurohypophysis and the pars intermedia show no evidence of any significant change in histological structure, not do the blood sinuses of the pars distalis reveal neurosecretory material within the lumen.

Bilateral Lesions of the Paraventricular Nuclei Frontal sections of the supraoptic area of the hypothalamus stained with Gomori's chrome alum-hematoxylin stain, show a small group of paraventricular nuclei near the third ventricle positive for neuro-



Fr. 12.5

FIG. 10

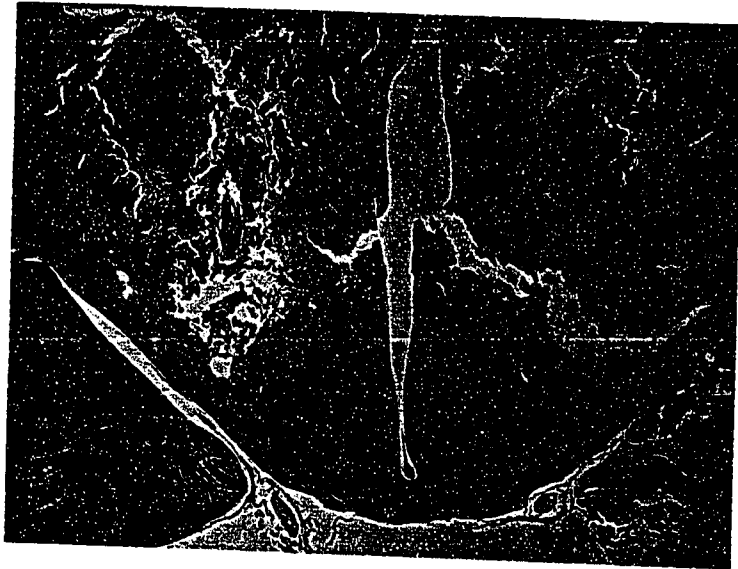


Fig. 11. A bilateral lesion in the supraoptic area of animal 2-A. The paraventricular nuclei are not destroyed and are located above the fornix near the third ventricle. The section is caudal to the optic chiasm and a few supraoptic cells which have not been destroyed are localized near the floor of the third ventricle. X5



Fig. 12. Pars nervosa of animal 2-A. Note the reduction in neurosecretory material in the pars nervosa. Except for localized areas the periphery of the gland is well depleted of material. The central cavity is not observed in this section. A corresponding decrease in Gomori positive substance is observed in the infundibular stem leading to the glandular portion of the neurohypophysis. X15

secretory material. As mentioned earlier in this work (page 13), it is questionable whether or not all the axons of these cells extend to the pars nervosa. It has been suggested by Nicolesco in 1929, that axons of the paraventricular nuclei terminate around the cell bodies of the supraoptic nuclei. Rasmussen (1940), has found that after stalk resection, the paraventricular nuclei of cats show a slight atrophy. It is estimated by Rasmussen that twenty per-cent of the cells disappear in monkeys and possibly a greater atrophy of the paraventricular nuclei in man after stalk resection.

Since these cells from all normal cytological appearances secrete Gomori positive material, a bilateral lesion was made in this area to destroy these nuclei without involvement of the supraoptic group. The lesion in animal 1-B extended from the supraoptic area to the infundibular area caudally, destroying the paraventricular cells, the fornix and the anterior commissure. The extent of necrosis was well circumscribed in that the supraoptic nuclei were isolated from the main area of the lesion.

Examination of serial sections of the supraoptic area revealed no evidence that the axons of the paraventricular nuclei contain neurosecretory material. Granular structures were only evident in the perikarya of these cells.

The pars nervosa, as observed in Figure 14, demonstrates a decrease in neurosecretory material in the peripheral and central areas of the gland. The infundibular stalk likewise contained a decreased concentration of positive material. There is no cytological evidence to indicate a change in the amount of cy-



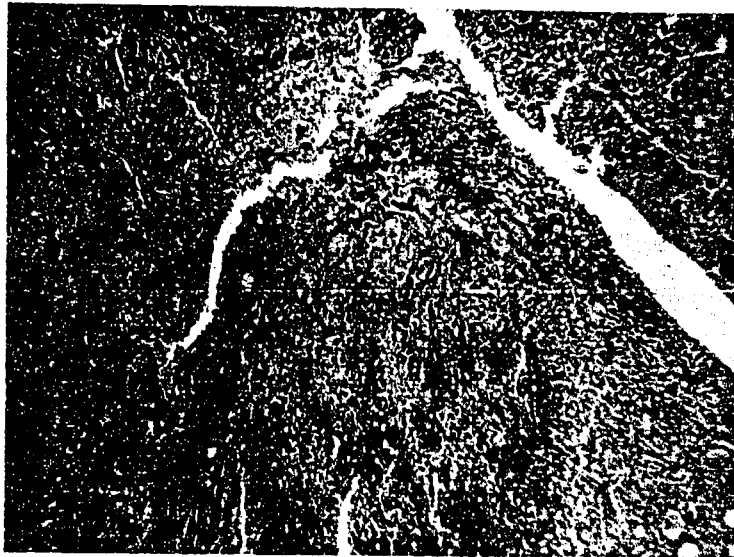


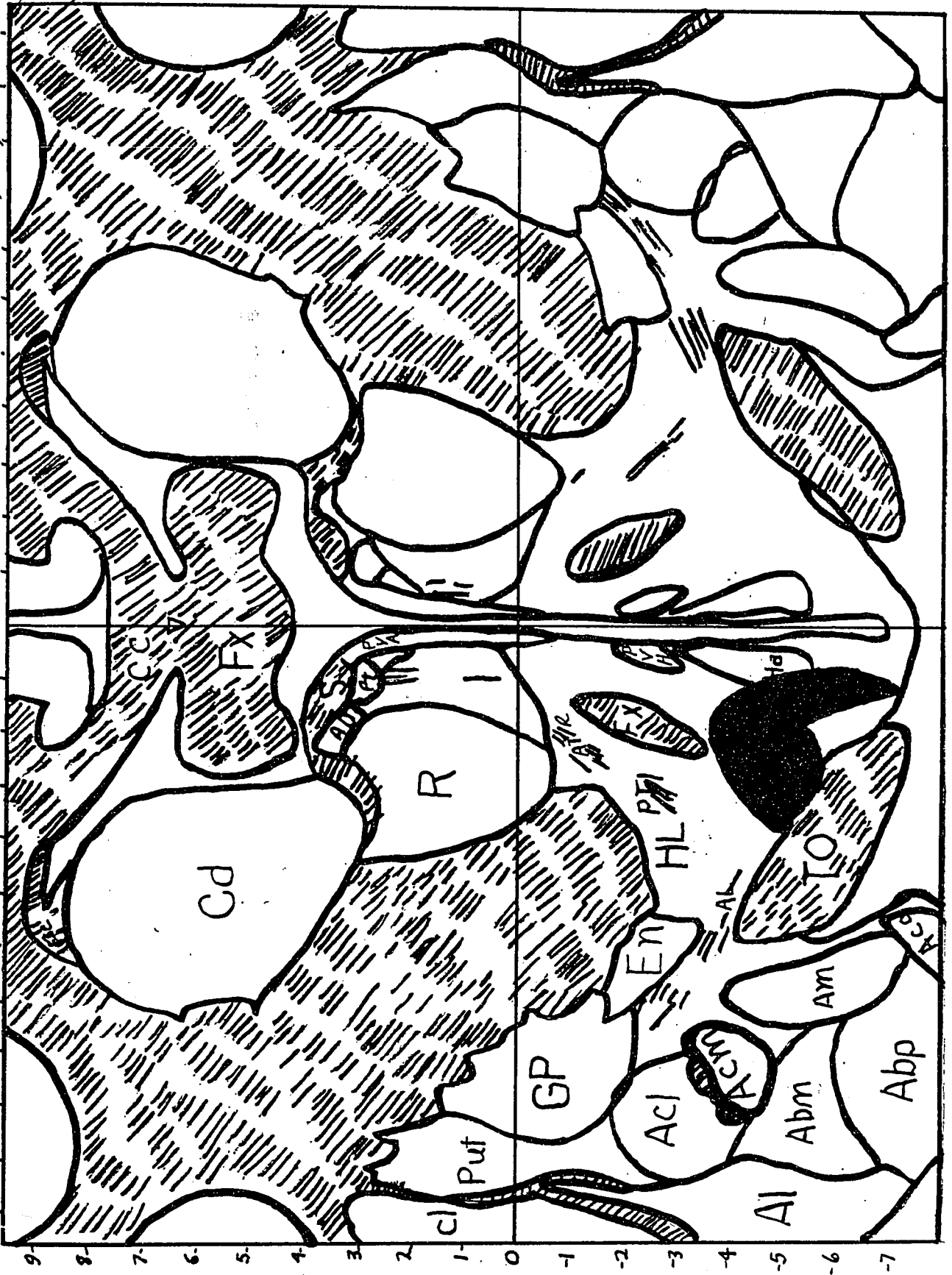
Fig. 14. Para nervosa of animal 1-3. There is a decrease in neurosecretion in the peripheral area of the gland. Note the mobilization of neurosecretory material around the blood vessels and the Herring bodies in the area of major depletion of Gomori positive material. X58

toplasmic neurosecretory granules in the supraoptic nuclei of their axons.

Unilateral lesions of the supraoptic Area These lesions were made in four animals in order to determine whether an electrolytic unilateral lesion of the supraoptic nuclei will produce a quantitative reduction in neurosecretory material in the neurohypophysis. (Table 3).

Figures 15 and 16 represent one animal in this operative series in which the lesion was made in the supraoptic area of the hypothalamus. The area of necrosis extended rostrally from the supraoptic area to the infundibulum caudally. The left group of supraoptic cells were destroyed, as well as the lateral hypothalamus and a portion of the fornix. The paraventricular nuclei are intact bilaterally and there is no histological evidence to indicate that the paraventricular and the supraoptic nuclei not involved in the lesion have an increased or decreased content of neurosecretory material within the cytoplasm.

As shown in Figure 17, of the pars nervosa of animal 1-C, there is a reduction in the concentration of neurosecretory material in the central and peripheral areas of the gland. The equal reduction of neurosecretory material makes the Herring bodies more visible throughout the gland. The concentration of neurosecretory material in the pars nervosa and the infundibular stalk does not show the same amount of reduction of neurosecretory material as an animal with a bilateral lesion of the supraoptic



Fr. 13.0

Fig. 15



Fig. 16. Unilateral lesion in animal 1-C. The lesion extends from the supraoptic area, rostrally, to the infundibular area, caudally. The left supraoptic group of cells were destroyed completely. The right supraoptic nuclei are localized above the optic tract. X12



Fig. 17. Pars nervosa of animal 1-C. There is a decrease in neurosecretory material throughout the peripheral and central areas of the gland. The large, dark stained bodies adjacent to the central cavity, are Herring bodies. X15

nuclei.

The three remaining animals used in this experimental series had discrete lesions in the supraoptic area. Histological examination of the neurohypophysis of these animals revealed evidence of a reduction in neurosecretory material. (Table 3), comparable to the reduction observed in animal 1-C.

Bilateral Lesions of the Mammillary Area Fisher, Ingram, Ranson and Magoun (1938), describe a tuberohypophyscal tract extending from the posterior hypothalamic area to the dorsal region of the pars nervosa. According to Rasmussen (1940), this tract may arise from the posterior end of the supraoptic nuclei. Smith (1951), has reported in his findings that the mammillary nuclei are Gomori positive. A further search of the literature revealed no evidence of the posterior hypothalamic nuclei contributing to the neurosecretion in the neurohypophysis.

In order to ascertain whether the axons of the dorsal hypothalamic neurons have neurosecretory connections with the neural lobe of the hypophysis, bilateral lesions were made in the mammillary area of four adult cats. (Table 4).

In animal 1-D (Figures 18 and 19), the lesion extended from the infundibular area to the mammillary bodies caudally. The principal structures cauterized include the median forebrain bundle, fornix, mammillo-thalamic tracts and the mammillary bodies.

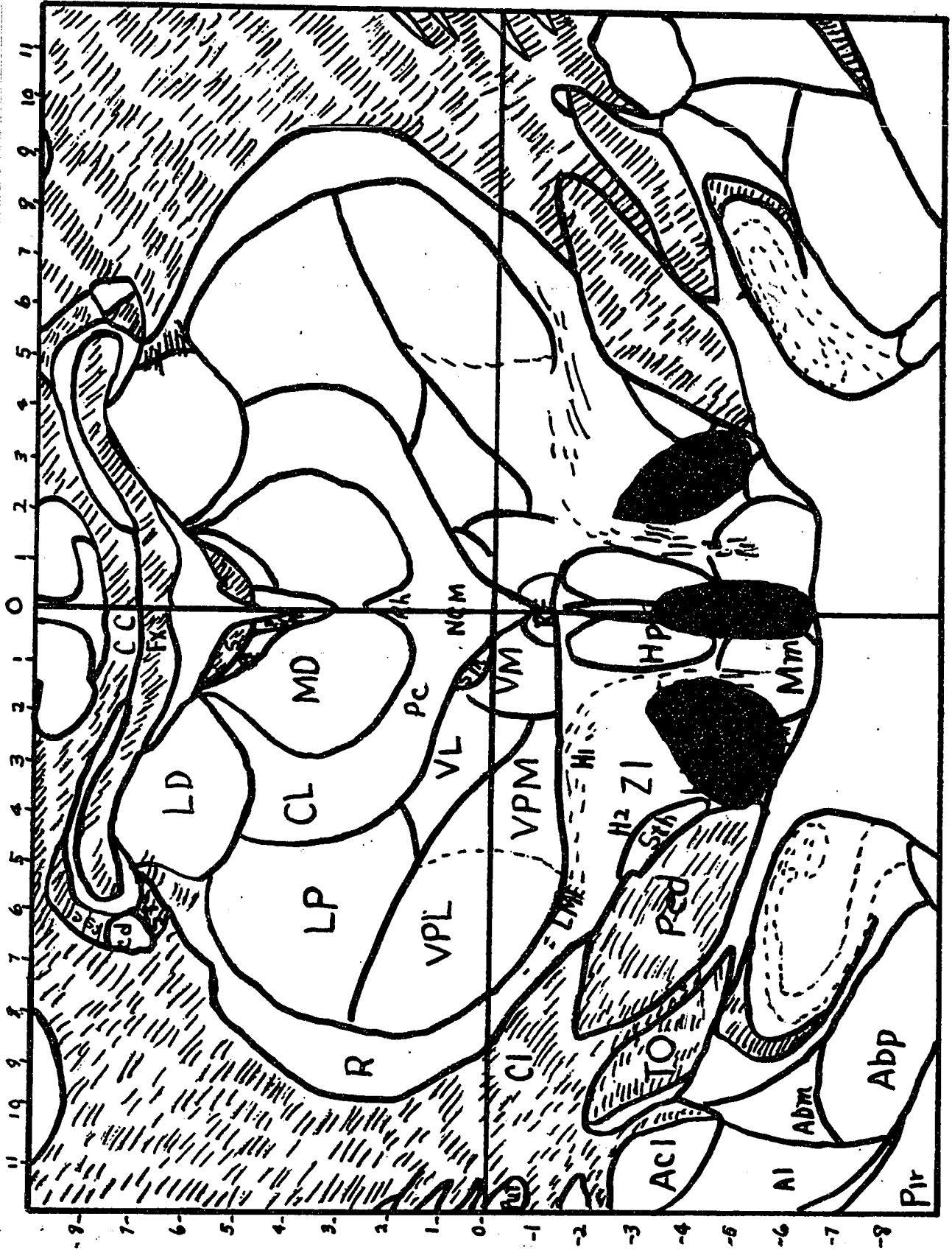
Figure 20 represents the pars nervosa of this animal. It is observed, when comparing the concentration of neurosecretory material in the pars nervosa of this animal with the control animal, that a slight reduction in neurosecretory material has occurred in the experimental animal.

When compared with the control sections, there is no change in the concentration of neurosecretory material in the infundibular stalk, not in the supraoptic nuclei. The periphery of the gland demonstrates a heavy concentration of material equally distributed throughout the area.

The results obtained in animal 5-A reveal no positive evidence that the mammillary nuclei or other nuclei of the mammillary area provides neurosecretory material to the hypothalamic-hypophysial system. After histological examination of the cells that are located in this region, no neurosecretory granules were observed within the cytoplasm of the mammillary nuclei.

Lesions were also performed in the same region in three additional animals. The post operative findings indicated no significant reduction of neurosecretory material in the infundibular stalk or the pars nervosa and all observations were similar to those described for animal 1-D.

Bilateral Lesions of the Lateral Hypothalamus Anatomically, these lesions were made bilaterally either in the supraoptic, infundibular or mammillary areas lateral to the for-



Fr. 9.5

FIG. 1B

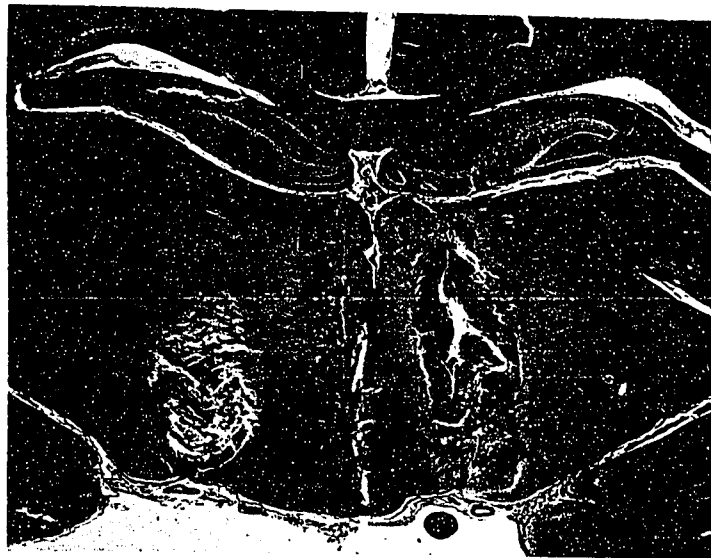


Fig. 19. Bilateral lesion of the mammillary and lateral hypothalamic areas in animal 1-9. The right lesion involves a portion of the medial and lateral mammillary group of nuclei. The left lesion has destroyed only a portion of the left lateral mammillary group. X5

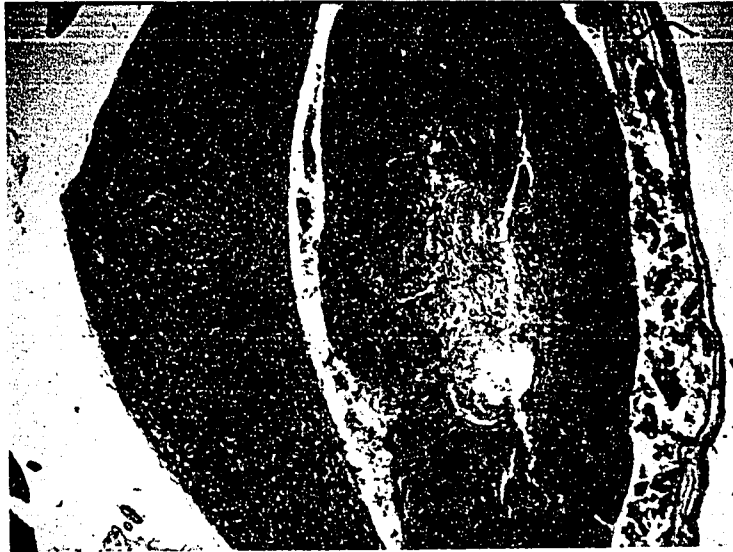


Fig. 20. Pars nervosa, pars distalis and pars intermedia of animal 1-D. A small decrease in neurosecretory material is observed in the pars nervosa, especially in the area adjacent to the central cavity. X15

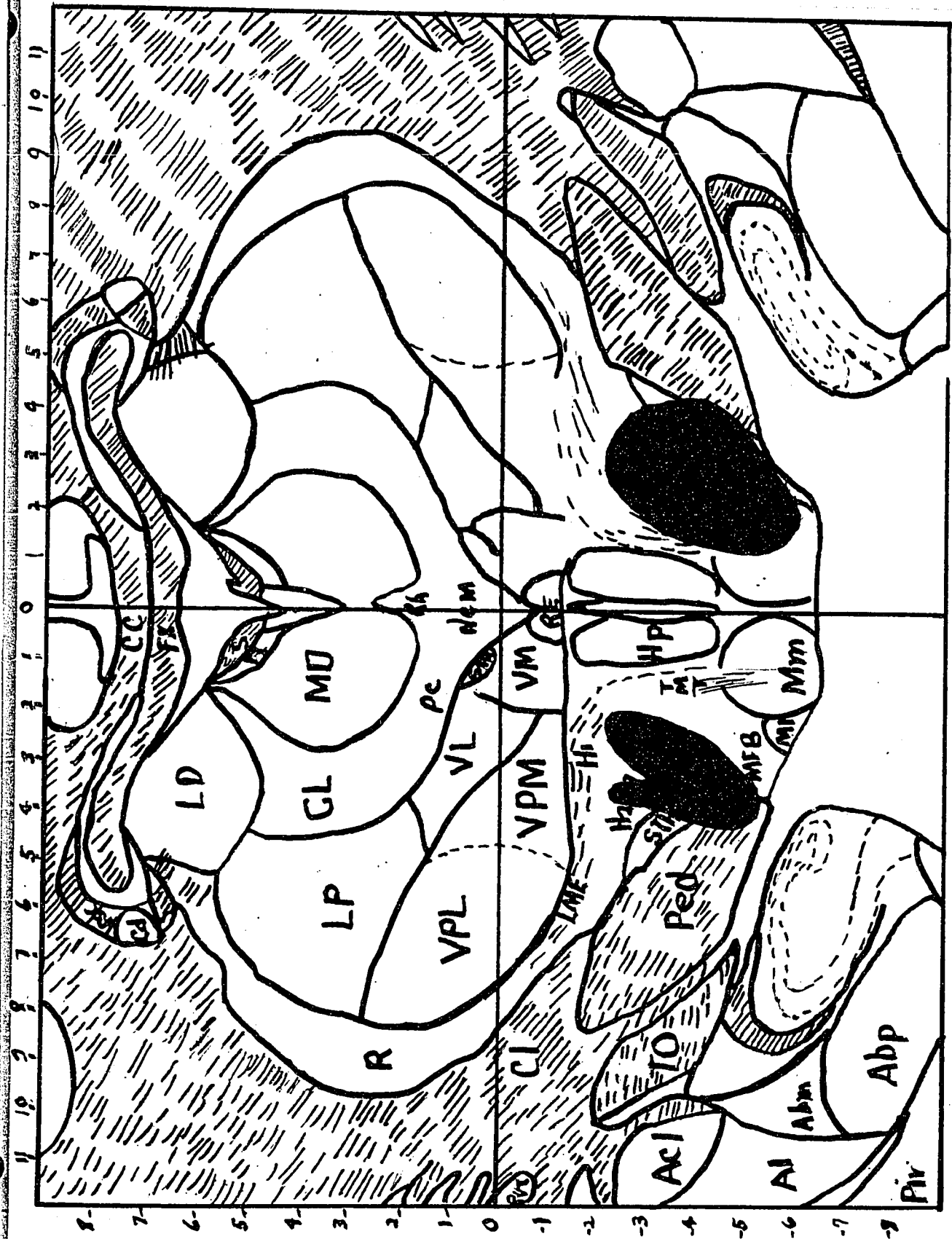
nix and medial to the internal capsule.

In animal 1-E (Figures 21 and 22, Table 5), the lesion extended rostrally from the infundibular to the mammillary area caudally. The principal structures characterized by this bilateral lesion included the median forebrain bundle, the mamillo-thalamic tracts and a portion of the right lateral mammillary nuclei. The remaining mammillary nuclei and the nuclei of the supraoptic area were not involved in this lesion.

The concentration of neurosecretory material in the neurohypophysis of this animal (as shown in Figure 23), was slightly reduced in the glandular area adjacent to the central cavity. The supraoptic nuclei presented no evidence of reduction in the amount of Gomori positive material in the cytoplasm of these cells.

In order to compare the effects of a lateral hypothalamic lesion not involving the destruction of the supraoptic nuclei, with the effects produced by a lesion in the lateral hypothalamus destroying the supraoptic nuclei unilaterally, lesions were made in the following areas of animal 2-E: A left lesion extending from the infundibular area rostrally, to the caudal extent of the left mammillary area, destroyed the left lateral mammillary nuclei, the median forebrain bundle and the mamillo-thalamic tract. In the right hypothalamus of this same animal a small, well circumscribed lesion was made in the supraoptic area destroying the right group of supraoptic nuclei.

Figure 27, illustrating the pars nervosa of this animal



Fr. 9.5

Fig. 21



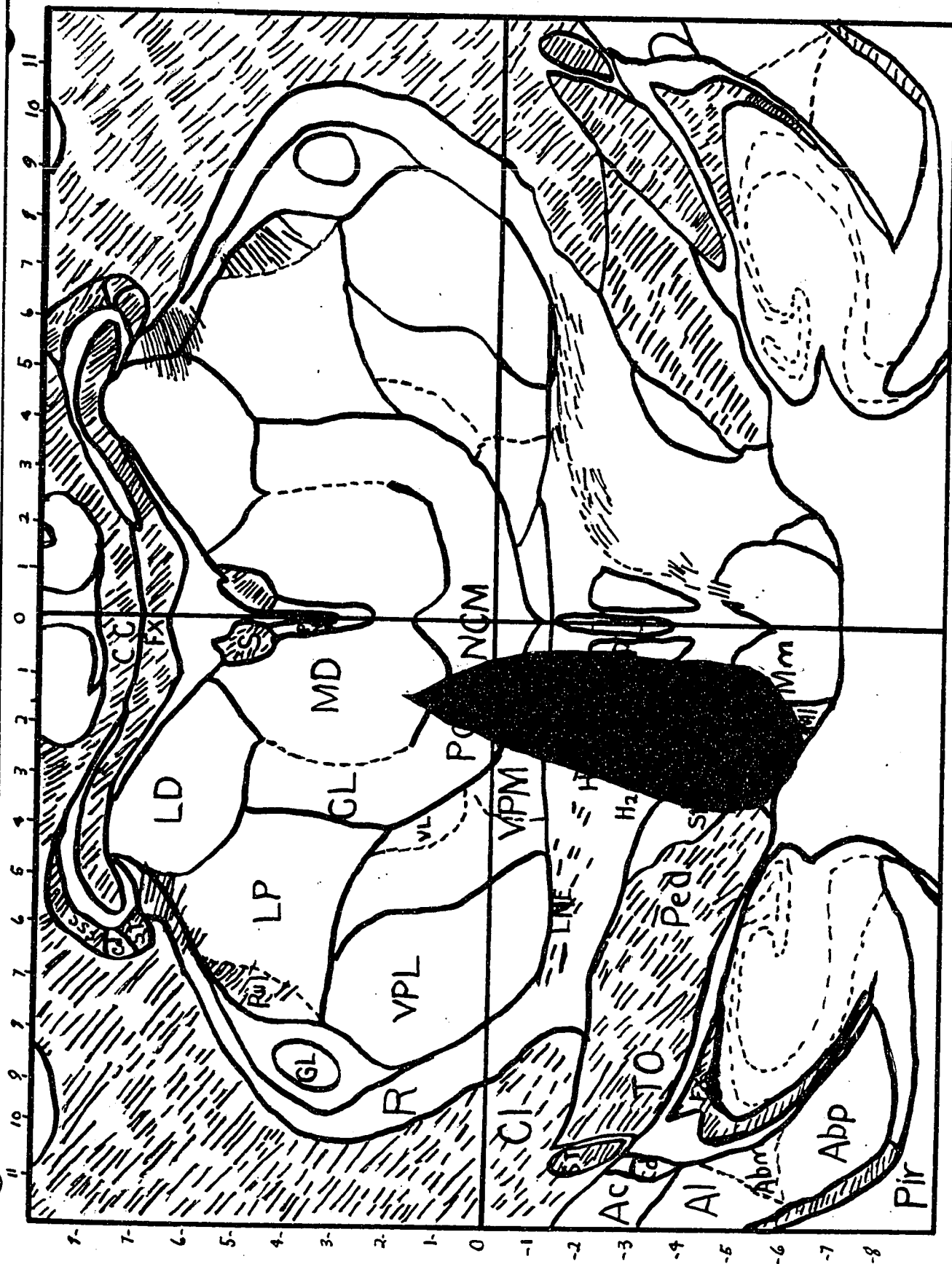
Fig. 22. Bilateral lesion of the lateral hypothalamus. The supraoptic, the paraventricular and the maxillary nuclei were not destroyed by this lesion. X5



Fig. 23. Pars nervosa and pars intermedia of animal 1-E.  
Herring bodies are noted near the central cavity and there is  
a small decrease in neurosecretory material in the central area  
of the gland. Note the extreme vascularity of the gland.

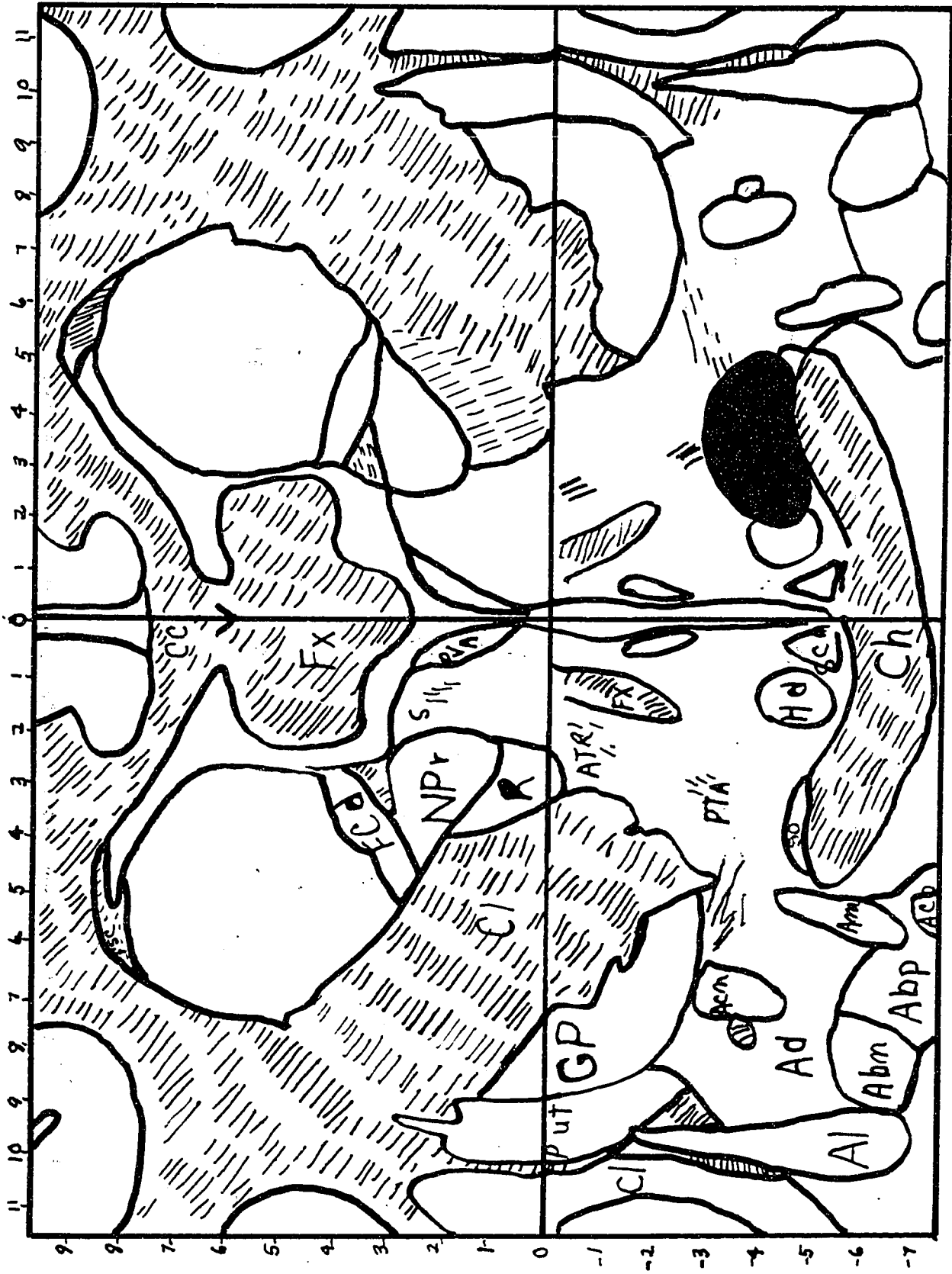
X58

demonstrated a major reduction in the concentration of neurosecretory material. The Gomori positive material is concentrated in small isolated accumulations while the remaining glandular areas are free of neurosecretory material. The infundibular stalk of this animal likewise demonstrates a marked reduction of neurosecretion. The results obtained with these latter two animals and the post operative findings after electrolytic lesions of the supraoptic and mammillary nuclei indicate that the supraoptic nuclei are largely responsible for the secretion of the Gomori positive material of the pars nervosa.



Fr. 9.0

Fig. 24



Fr. 13.5

FIG. 25

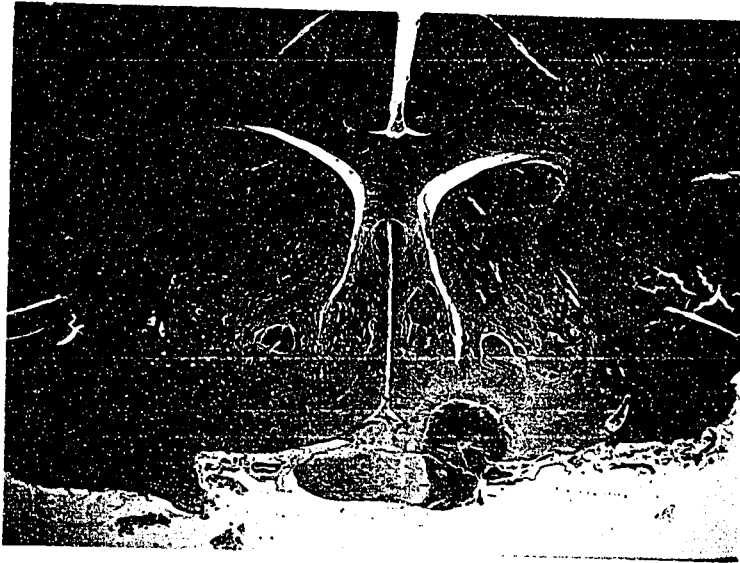


Fig. 26. Unilateral lesion of the right supraoptic area in animal 2-E. This lesion has destroyed a major portion of the right supraoptic nuclei. The left lesion extends from the infundibular area, caudally, to the mammillary area as shown in Fig. 23. There is no involvement of the left supraoptic nucleus, as the main area of necrosis is in the lateral hypothalamus. X5

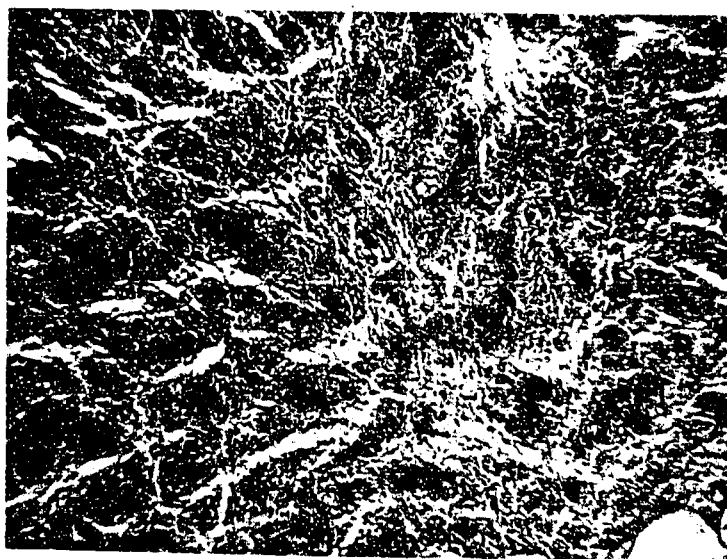


Fig. 27. Pars nervosa of animal 2-5. Note the reduction in neurosecretory material in the peripheral area of the gland. The decrease is due to the lesion of the supraoptic nucleus in this animal. XII 6

TABLE ONE

EXPERIMENTAL DATA

OF

BILATERAL LESIONS OF THE

SUPRAOPTIC NUCLEI

Cat 1-A

- Horsley-Clarke Sterotaxic Coordinates:

Frontal 13.5 - 11.5

Lateral 3.0

Horizontal 2.5 - 5.5

- Post Operative Period: Two days.

- Post Operative Findings: Animal notable to consume liquids. Weak extension and flexion in extremities. Left pupillary reflex abolished.

- Perfusion: No.

- Localization of Lesion: Supraoptic area bilaterally. No involvement of paraventricular, but major destruction of supraoptic nuclei.

- Concentration of Neurosecretory material in the Infundibular stalk: Marked reduction.

- Concentration of Neurosecretory material in the Pars Nervosa: Marked reduction.

Cat 2-A

- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 13.0 - 11.0
  - Lateral 4.5
  - Horizontal -5 - 6
- Post Operative Period: Three days.
- Post Operative Findings: Weak extension and flexion reflexes; reaction of pupil to light normal.
- Perfusion: 50 cc of Saline, 100 cc of 10% Formalin - Sublimate
- Localization of Lesion: Supraoptic area bilaterally. Major area of necrosis rostrally to the tuber cinereum. Paraventricular nuclei intact. Major destruction of supraoptic nuclei.
- Concentration of Neurosecretory material in the Infundibular stalk: Very marked reduction.
- Concentration of Neurosecretory material in the Pars Nervosa: Marked reduction.
- Remarks: No evidence of involvement of adenohypophysis. Parenchymal cells appear normal.

Cat 3-A

- Horsley-Clarke Stereotaxic Coordinates:

Frontal 13.5 - 11.0

Lateral 3.0

Horizontal -2.5 - 6.0

- Post Operative Period: Ten days.

- Post Operative Findings: Left pupillary light reflex abolished. Diminished flexion and extension reflexes.

- Perfusion: No.

- Localization of Lesion: Right supraoptic area with destruction of supraoptic nuclei and optic chiasm. No involvement of left supraoptic nuclei or paraventricular nuclei.

- Concentration of Neurosecretory material in the Infundibular Stalk: Slight decrease.

- Concentration of Neurosecretory material in the Pars Nervosa: Slight decrease.

Cat 4-A

- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 13.0 - 10.0
  - Lateral 4.0
  - Horizontal -4.0 - 6.5
- Post Operative Period: One day.
- Post Operative Findings: Pupillary reflex normal.  
Very weak extension and flexion reflexes in the extremities. Supportative therapy given.
- Perfusion: No.
- Localization of Lesion: Rostrally in the supra-optic area there is necrosis of the right supra-optic nuclei only. Further caudally in this same area there is bilateral destruction of the supra-optic and paraventricular nuclei. There is no apparent involvement of mammillary bodies.
- Concentration of Neurosecretory material in the Infundibular stalk: In the distal portion of the stalk there is a marked reduction in neurosecretory material and a decrease in staining reaction of the Herring bodies.
- Concentration of Neurosecretory material in the Pars Nervosa: There is a major decrease of material in the peripheral area of the gland. Gomori positive material is localized around the blood capillaries.

Cat 5-A

- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 12.5 - 10.0
  - Lateral 3.5
  - Horizontal -2.5 - 5.0
- Post Operative Period: Fourteen days.
- Post Operative Findings: Two days after the operation the animal was able to walk, but with impaired movement of the right front extremity. Respiratory rate and pupillary reflexes normal. Second Operation performed twelve days after the first operation. The post operative condition two days after the second operation was very poor. Extremity reflexes diminished, but pupillary reflex normal.
- Perfusion: 100 cc of saline and 100 cc of 10% formalin-sublimate.
- Localization of Lesion: There is a well circumscribed bilateral lesion in the supraoptic area of the hypothalamus. This lesion does not involve destruction of the supraoptic nor the paraventricular nuclei. Caudally, the bilateral lesion extends to the infundibular area in the lateral hypothalamus. There is destruction on the left side of the fornix and median forebrain bundle, and a portion of the internal capsule.
- Concentration of Neurosecretory material in the Infundibular Stalk: No decrease.
- Concentration of Neurosecretory material in the Pars Nervosa: No decrease.

Cat 6-A

- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 13.5 - 11.0
  - Lateral -3.0
  - Horizontal -2.0 - 6.0
- Post Operative Period: Four days.
- Post Operative Findings: Animal very lethargic with rigidity of the muscles of extension in hind extremity. There is diminished flexion reflexes in all extremities, and no left pupillary reflex.
- Perfusion: 100 cc of saline, 100 cc of 10% formalin-sublimate
- Localization of Lesion: Rostrally, a well circumscribed lesion involves a unilateral destruction of the right supraoptic nuclei and the optic chiasm in the supraoptic area. The paraventricular and the left supraoptic nuclei are intact. Near the anterior portion of the infundibular area a bilateral lesion is observed in the hypothalamus, involving destruction of the nucleus periventricularis and the fornix.
- Concentration of Neurosecretory material in the Infundibular Stalk: Slight decrease.
- Concentration of Neurosecretory material in the Pars Nervosa: There is no evidence of decrease in neurosecretory material in the periphery, but a slight decrease in the area near the central cavity of the gland.

Cat 7-A

- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 13.5 to 12.5
  - Lateral 4.5
  - Horizontal -1 to -5
- Post Operative Period: Six days.
- Post Operative Findings: Animal lethargic and not able to use extremities for the first three days after the operation. Four days after the operation the animal was able to walk and there was dilatation of the right pupil.
- Perfusion: 100 cc of 10% formalin-sublimate.
- Localization of Lesion: The lesion is located primarily in the rostral extent of the supraoptic area. There is unilateral destruction of the right paraventricular and supraoptic nuclei. A lesion is noted in the left supraoptic area but the paraventricular and the supraoptic nuclei are not involved.
- Concentration of Neurosecretory material in the Infundibular Stalk: No evidence of any significant reduction.
- Concentration of Neurosecretory material in the Pars Nervosa: There is a slight reduction in neurosecretion.

Cat 8-A

- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 13.0 to 12.0
  - Lateral 3.5
  - Horizontal -4.0 to -6.0
- Post Operative Period: Two days
- Post Operative Findings: Diminished extension and flexion reflexes in extremities. Animal lethargic but able to consume liquids. Pupillary reflex normal in both eyes.
- Perfusion: 100 cc of saline, 100 cc of 10% formalin-sublimate.
- Localization of Lesion: There is a bilateral, well circumscribed lesion located in the supraoptic area involving destruction of a major portion of the supraoptic nuclei. The paraventricular nuclei, and the fornix are intact. This lesion does not extend much further than the caudal portion of the supraoptic area.
- Concentration of Neurosecretion in the Infundibular Stalk: Marked reduction.
- Concentration of Neurosecretion in the Pars Nervosa: Marked reduction. Neurosecretory material that is present appears to be mobilized around blood capillaries.

TABLE TWO

EXPERIMENTAL DATA

OF

BILATERAL LESION OF THE

PARAVENTRICULAR NUCLEI

Cat 1-B

- Horsley-Clanke Stereotaxic Coordinates:
  - Frontal 13.0 to 11.5
  - Lateral 1.0
  - Horizontal -3.5
- Post Operative Period: Four days.
- Post Operative Findings: Animal lethargic but able to consume liquids. Pupillary, extensor and flexor reflexes of extremities normal.
- Perfusion: No perfusion.
- Localization of Lesion: Supraoptic area bilaterally with no involvement of the supraoptic nuclei.
- Concentration of Neurosecretion in the Infundibular Stalk: Moderate decrease.
- Concentration of Neurosecretion in the Pars Nervosa: Moderate reduction.

TABLE THREE

EXPERIMENTAL DATA

OF

UNILATERAL LESIONS OF THE

SUPRAOPTIC NUCLEI

Cat 1-C

- Horsley-Clarke Stereotaxic Coordinates:

Frontal 13.0 to 11.0

Lateral 3.5

Horizontal -4.0 to -6.5

- Post Operative Period: Three days

- Post Operative Findings: The extremities were not paralyzed. The right pupillary reflex was diminished. Animal very lethargic and not able to take food without the employment of a eye dropper.

- Perfusion: 100 cc of saline and 100 cc of 10% formalin-sublimate.

- Localization of Lesion: The lesion extended unilaterally in the left hypothalamus from the supraoptic area rostrally, to the infundibular area caudally.

- Concentration of Neurosecretory material in the Infundibular Stalk: Slight decrease.

- Concentration of Neurosecretory material in the Pars Nervosa: A decrease in neurosecretory material is more evident in the pars nervosa than in the infundibular stalk.

Cat 2-C

- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 13.5 to 14.5
  - Lateral 4.4
  - Horizontal -2.0 to -4.5
- Post Operative Period: Six days.
- Post Operative Findings: Animal very lethargic. Two days after the operation there was slight movement of the extremities and constriction of the pupil to light. Five days post operatively, the animal was able to walk, but would not consume food except liquids.
- Perfusion: 100 cc of saline, 100 cc of 10% formalin-sublimate.
- Localization of Lesion: Rostrally, the lesion extended into the anterior region of the supraoptic area. The fornix and paraventricular nuclei were destroyed bilaterally, and unilaterally the supraoptic nuclei on the right side. Dorsally, the lesion extended into the infundibular area with necrosis bilaterally of the lateral hypothalamus and chiefly the nucleus ventralis medialis.
- Concentration of Neurosecretory material in the Infundibular Stalk: Moderate reduction.
- Concentration of Neurosecretory material in the Pars Nervosa: Moderate decrease with a concentration of neurosecretory material around the blood capillaries.

Cat 3-C

- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 12.5 to 11.5
  - Lateral 4.5
  - Horizontal -1 to -4.5
- Post Operative Period: Three days.
- Post Operative Findings: Pupillary reflex normal. Flexion and extension reflexes of extremities present, but very weak.
- Perfusion: 50 cc of saline, 100 cc of 10% formalin-sublimate.
- Localization of Lesion: Area of necrosis involved the dorsal region of the supraoptic and the anterior region of the infundibular area. The lesion is well circumscribed and involves only the destruction of the left supraoptic nuclei.
- Concentration of Neurosecretory material in the Infundibular Stalk: No secretion available for microscopic study.
- Concentration of Neurosecretory material in the Pars Nervosa: Slight decrease of neurosecretory material when compared with control sections.
- Remarks: Histological appearance of pars distalis was normal. No neurosecretory substance observed in areas other than the pars nervosa.

Cat 4-C

- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 13.0 to 11.5
  - Lateral 3.5
  - Horizontal -3.5 to -5.0
- Post Operative Period: Two days.
- Post Operative Findings: Diminished reflexes.  
Pupillary reflex normal. Animal lethargic and not able to consume food except liquids.
- Perfusion: 100 cc of saline, 100 cc of 10% formalin-sublimate.
- Localization of Lesion: There is a unilateral lesion in the left supraoptic area which has destroyed a portion of the fornix, median forebrain bundle and the left supraoptic nuclei.
- Concentration of Neurosecretory material in the Infundibular Stalk: Slight decrease.
- Concentration of Neurosecretory material in the Pars Nervosa: Slight decrease.

TABLE FOUR

EXPERIMENTAL DATA

OF

BILATERAL LESIONS OF THE

MAMMILLARY NUCLEI

Cat 1-D

- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 9.5 to 11.0
  - Lateral 2.0
  - Horizontal -3.5 to -5.5
- Post Operative Period: Three days.
- Post Operative Findings: Flexion and extension reflexes normal. Animal lethargic but able to consume liquids.
- Perfusion: 100 cc of saline, 100 cc of 10% formalin-sublimate.
- Localization of Lesion: Rostrally from the infundibular area to the mammillary area caudally. Destruction of the median forebrain bundle and the mammillothalamic tract bilaterally. Partial necrosis of the lateral and medial mammillary nuclei.
- Concentration of Neurosecretory material in the Infundibular Stalk: No major reduction.
- Concentration of Neurosecretory material in the Pars Nervosa: No major reduction.

Cat 2-D

- Horsley-Clarke Stereotaxic Coordinates:

Frontal 10.0 to 9.0

Lateral 2.0

Horizontal -2.0 to -6.0

- Post Operative Period: Two days.

- Post Operative Findings: Similar to Cat 1-D.

- Perfusion: 100 cc of saline, 100 cc of 10% formalin-sublimate.

- Localization of Lesion: The lesion primarily involved bilaterally, the mammillary area with destruction of the medial and lateral mammillary nuclei, medial forebrain bundle and the mammillo-thalamic tracts.

- Concentration of Neurosecretory material in the Infundibular Stall: No decrease.

- Concentration of Neurosecretory material in the Pars Nervosa: No reduction.

Cat 3-D

- Horsley-Clarke Stereotaxic Coordinates:

Frontal 9.0 to 11.5

Lateral 3.0

Horizontal -2.0 to -6.0

- Post Operative Period: One day.

- Post Operative Findings: Animal able to consume liquids. Flexion and extension reflexes of extremities normal.

- Perfusion: 50 cc of saline, 100 cc of 10% formalin-sublimate.

- Localization of Lesion: Mammillary area bilaterally. Right medial and lateral mammillary nuclei destroyed, left medial mammillary cells intact. Lesion also has destroyed the median forebrain bundle and fornix.

- Concentration of Neurosecretory material in the Infundibular Stalk: No reduction.

- Concentration of Neurosecretory material in the Pars Nervosa: Slight reduction.

TABLE FIVE

EXPERIMENTAL DATA

OF

LESIONS OF THE

LATERAL HYPOTHALAMUS

Cat 1-E

- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 9.0 to 11.5
  - Lateral 3.5
  - Horizontal -2.5 to 5.5
- Post Operative Period: Six days.
- Post Operative Findings: Animal presented no evidence of diminished pupillary or extremity reflexes.
- Perfusion: 90 cc of saline, 100 cc of 10% formalin-sublimate.
- Localization of Lesion: Lesion in the lateral hypothalamus extending through the mammillary and infundibular areas bilaterally.
- Concentration of Neurosecretory in the Infundibular Stalk: Slight reduction.
- Concentration of Neurosecretory in the Pars Nervosa: Slight reduction in the pars nervosa. Slight reduction in the central area of the gland.

Cat 2-E

- Horsley-Clarke Stereotaxic Coordinates:

Frontal 8.5 to 13.5

Lateral, left 2.5; right 4.5

Horizontal -2.0 to -5.5

- Post Operative Period: Two days.

- Post Operative Findings: The reflexes of extension and flexion in the right hind extremity diminished. Left extremity reflexes appear normal; pupillary reflex normal.

- Perfusion: No perfusion.

- Localization of Lesion: In the left hypothalamus the lesion is located in the infundibulum and mammillary area. In the right hypothalamus the lesion involves only the supraoptic area.

- Concentration of Neurosecretory in the Infundibular Stalk: Marked reduction.

- Concentration of Neurosecretory in the Pars Nervosa: Marked reduction. Some areas completely depleted of material.

## DEHYDRATION EXPERIMENTS

Experimental Physiological Conditions Which Affect the Secretion Granules of Supraoptic Neurons. As has been mentioned earlier, (page 15) it has been demonstrated by Ortmann, Hild, Leveque and Scharrer, that various types of stimuli are capable of causing depletion of neurosecretory substance in the neurohypophysis. The type of stimuli varied from severe dehydration either by a salt load, depriving the animals of drinking fluid, or by the injection of desoxycorticosterone with a hypertonic saline drinking fluid. The depletion of neurosecretory material was demonstrated by these workers at the perivascular nerve endings of the supraoptic-hypophysial tract in the pars nervosa.

The experimental dehydration methods carried out in the present work are different from the methods employed by Ortmann, Hild, Leveque and Scharrer. First of all, the period of dehydration either by salt load or water deprivation was approximately a week less than the period of dehydration used by Leveque and Scharrer. Secondly, the concentration of salt in the drinking fluid in the present experimental procedure was 1% whereas these workers employed a 2.5% solution.

With the exception of Ortmann's work, these studies were primarily concerned with effects of dehydration on the neurosecretory substance in the neurohypophysis, especially in the pars nervosa. For example, Hild studied the reaction of neurosecretion before and

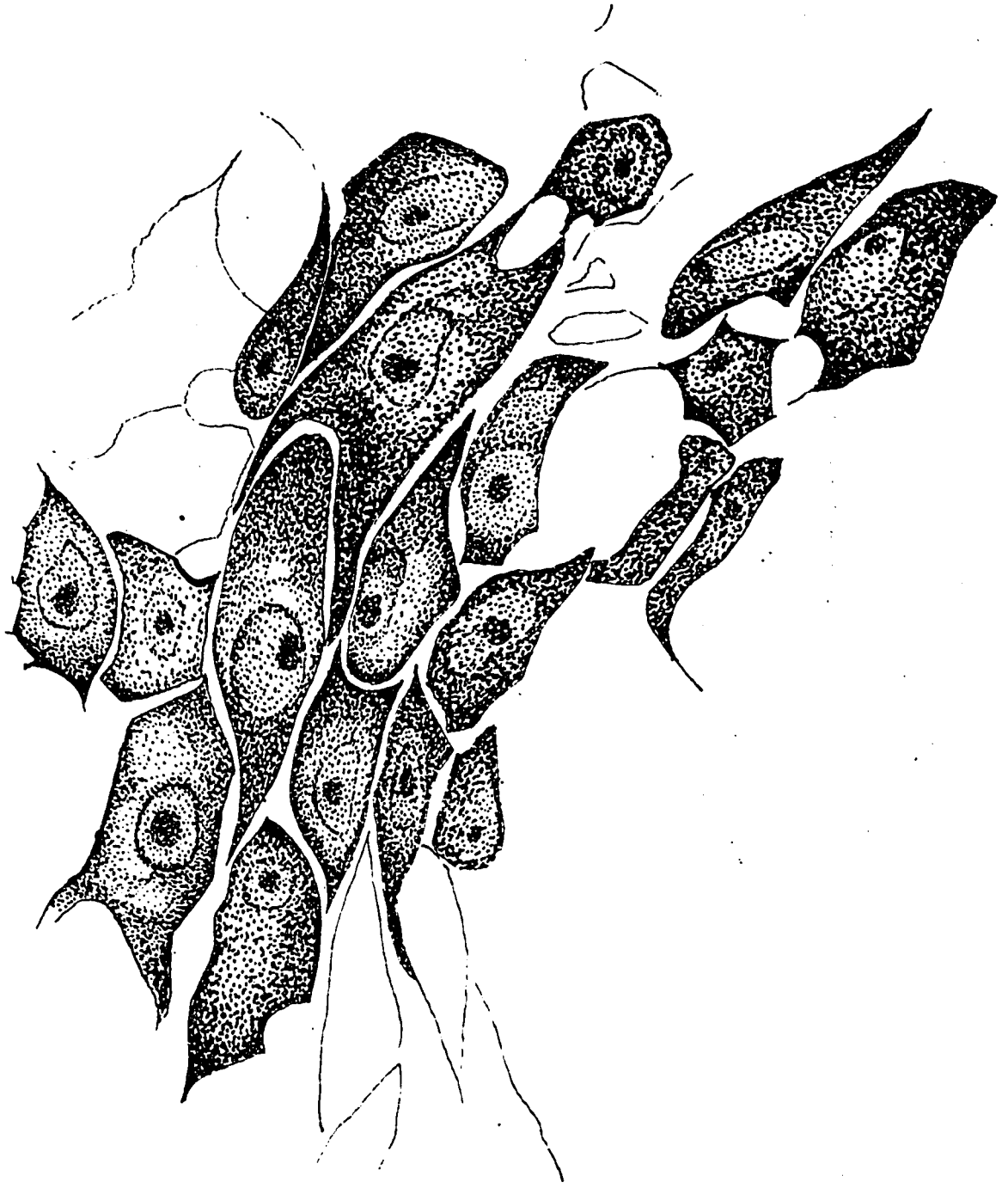
after ligation of the infundibular stalk in animals subjected to salt and water dehydration, while Loveque and Scharrer using the same type of dehydration methods focused their attention on the functional activity of the pituocytes in the pars nervosa.

In the following experimental dehydration methods, the cellular changes accompanying neurosecretion in the supraoptic nuclei have been the primary object of study. In view of the reported decrease of Gomori positive material of the neurohypophysis by dehydration, it may be assumed that dehydration for several days before cauterization of the supraoptic nuclei will further enhance the decrease of neurosecretory material in the pars nervosa and infundibular stalk. In order to investigate this problem, the following experiments were carried out:

Water Deprivation. Ten young adult rats were placed on a water deprivation diet for a period of six days. One rat was sacrificed each day of the diet. The fixation and staining procedures was the same as in the earlier experiments.

Figure 6 is a schematic drawing of the supraoptic nuclei of a normal rat. The large size of the cells and the distribution of neurosecretory granules is noteworthy. Figure 23 is likewise a schematic drawing of the supraoptic nuclei of an animal dehydrated by water deprivation for a period of six days. There appears to be an increase in granulation in the cytoplasm of the cells. The granulation is more concentrated in the periphery of the cells and also in the area adjacent to the nucleus. The increase of cytoplasmic

Kat 5



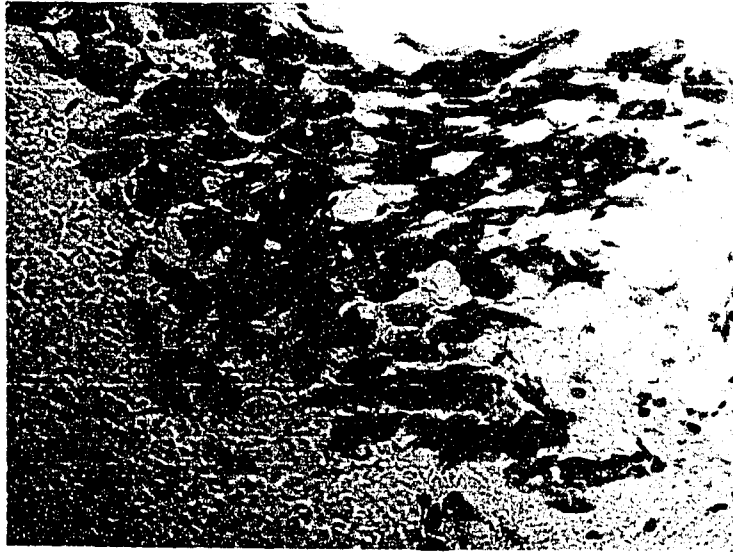


Fig. 29. Supraoptic nucleus of a rat dehydrated by water deprivation for a period of six days. Note the heavy concentration of Gomori positive material in the cytoplasm of the neurons. The nucleus in the cell bodies of the supraoptic nucleus is observed in only a few cells. X400

material is demonstrated by an increased cellular staining affinity with the Gomori and Thionin stains. Further observation shows that when one compares Figure 29 with Figure 6, there is a much heavier concentration of granular material in the latter cell group.

The concentration of neurosecretory material in the infundibular stalk and in the pars nervosa of the animal sacrificed on the last (sixth) day of the experiment was sharply decreased. This decrease in neurosecretory material began on the second day of the diet and continued until the termination of the experiment. In the pars nervosa the depletion of neurosecretory substance is observed in the periphery of the gland with an increased concentration around the blood vessels.

Salt Diuresis. Ten young rats were placed on a one per-cent hypertonic saline solution as drinking fluid for six days. Figure 31 is a schematic drawing of the supraoptic nuclei of an animal sacrificed on the last day of the experiment. In Figure 30, cell bodies shows a marked depletion of granulation in the cytoplasm when compared with the normal control animal. The depletion of neurosecretory substance is very noticeable adjacent to the nucleus of the cell and along the axons. The reduction of granulation was noted from the second day of the diet until the termination of the experiment. The neurohypophysis of the animal sacrificed on the last day of the diet demonstrated a marked reduction in neurosecretory material primarily in the peripheral area of the pars nervosa.

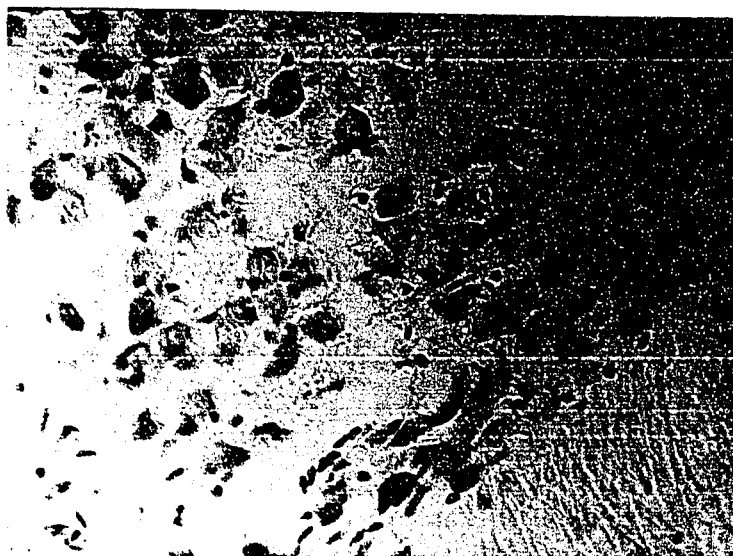


Fig. 30. Supraoptic Nucleus of a rat allowed to drink a one per-cent hypertonic saline solution for a period of six days. There is a major depletion of neurosecretory granules in the cytoplasm of the cell bodies. The nucleus is observed occupying a major portion of the cell. Some nucleoli are visible. X400.

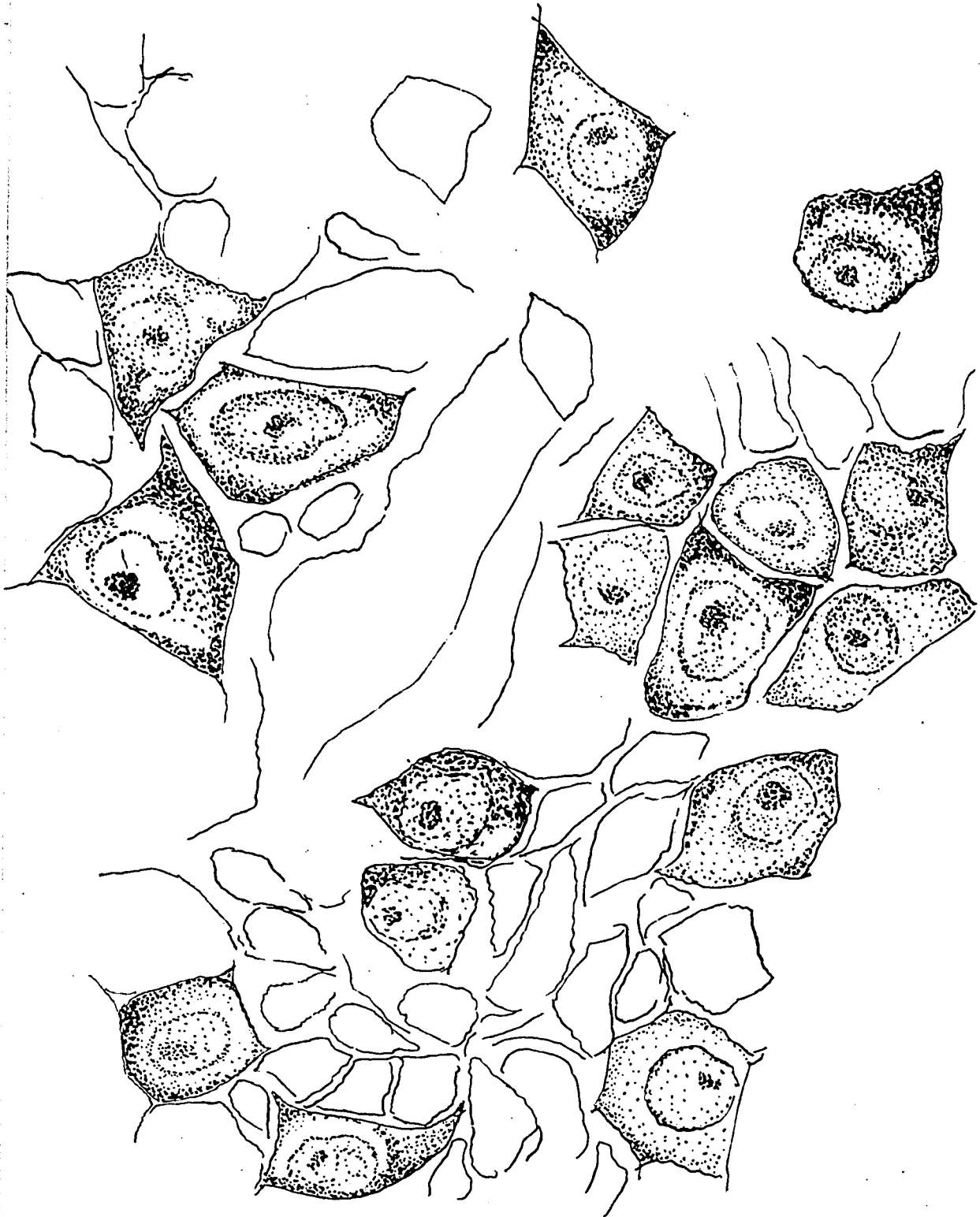


Fig. 31

EXPERIMENTAL LESIONS OF THE SUPRAOPTIC NUCLEI  
IN CATS PREVIOUSLY SUBJECTED TO DEHYDRATION

In view of earlier observations in which bilateral lesions of the supraoptic neurons failed to produce a total depletion of neurosecretory material in the hypothalamic-hypophyseal system, it was decided that dehydration of the animals before the operation, followed by a destructive lesion of the secretory nuclei might bring about a total depletion of neurosecretory material in the neurohypophysis.

Since it has been shown by Levoque, Scharrer, Ortman and Hild, and by the experiments performed in this study that dehydration will decrease the amount of neurosecretory material in the neurohypophysis, four adult cats were dehydrated for a period of five days prior to the operation. Bilateral lesions were made in two hypothalamic areas, supraoptic and mammillary.

Bilateral Lesions of the Supraoptic Area in two Cats  
Previously Subjected to Water Deprivation and Salt Diuresis.

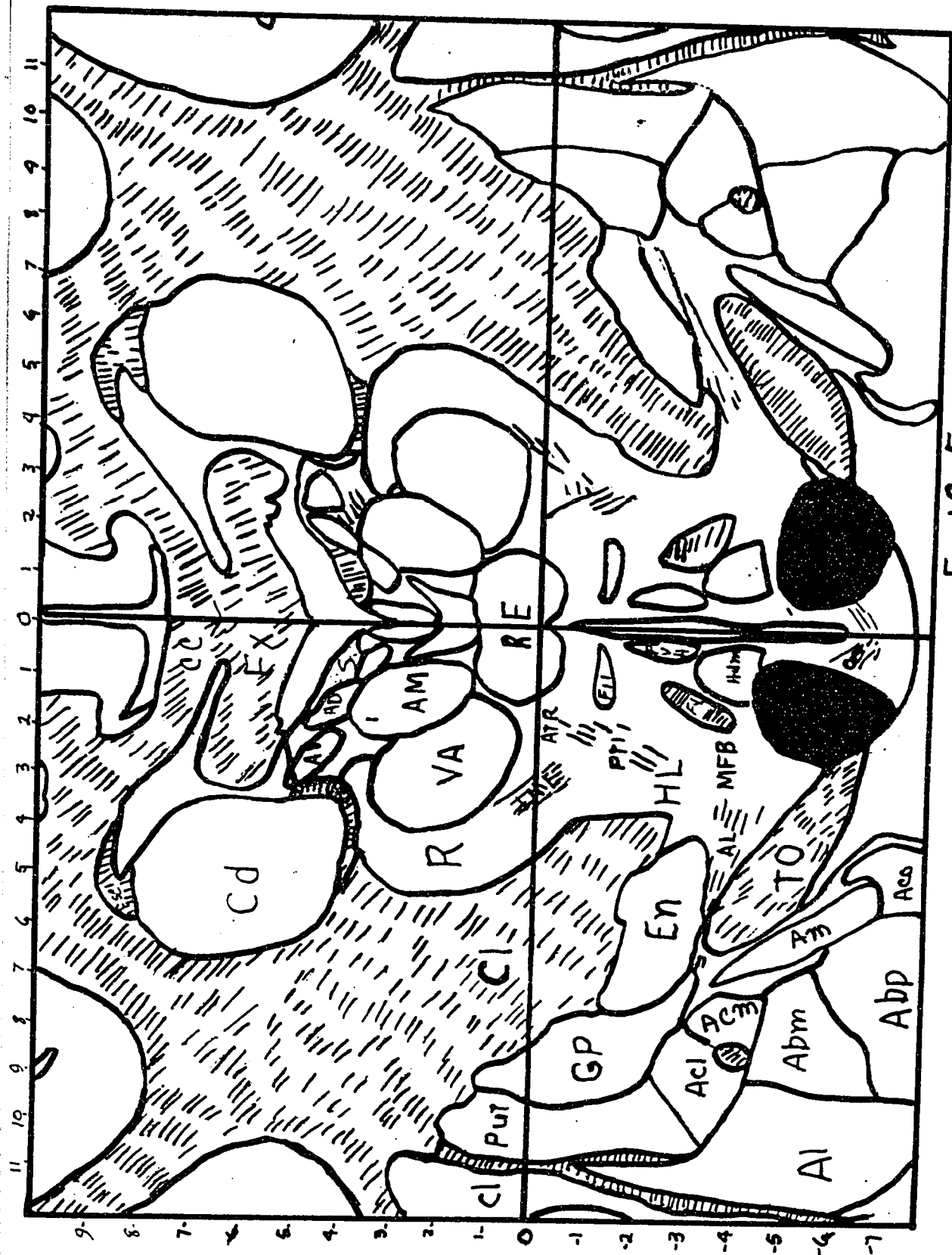
Control Animals. Two adult cats were dehydrated for a period of five days by the following methods:

One animal was subjected to water deprivation for a period of five days, sacrificed, and serial sections of the hypothalamus and neurohypophysis stained for neurosecretory substance.

The second animal received a 1% hypertonic saline solution for five days, sacrificed, and serial sections of the hypothalamus stained for neurosecretory material.

Microscopically the first animal dehydrated by water deprivation demonstrated an increase of neurosecretory granules in the supraoptic nuclei. The material appeared to be homogeneous and very few nuclei of the cells were observed due to intense accumulation of neurosecretory substance. The neurohypophysis reveals a reduction of Gomori positive material in the infundibular stalk, pars nervosa and in the Herring bodies. The supraoptic nuclei of the second animal shows a marked reduction of granulation in the cytoplasm of the cell body. The nuclei and nucleoli are easily observed in almost all the cells, while the neurosecretory material is localized adjacent to the cell membrane. The neurohypophysis of this latter animal likewise demonstrates a reduction in Gomori positive material. Thus it can be seen that the results obtained by dehydration methods in these two control cats, are similar and comparable to the findings obtained in dehydrated adult rats.

Experimental Animals. The animals used in this operation were without fluids for a period of five days before the operation. The lesion involves the destruction bilaterally, of the paraventricular, the supraoptic nuclei, lateral hypothalamus and fornix. The rostral-caudal extent of the lesion is of such width as to sever completely the nerve pathways of the hypothalamic-hypophyseal sys--



Fr. 12.5

FIG. 33

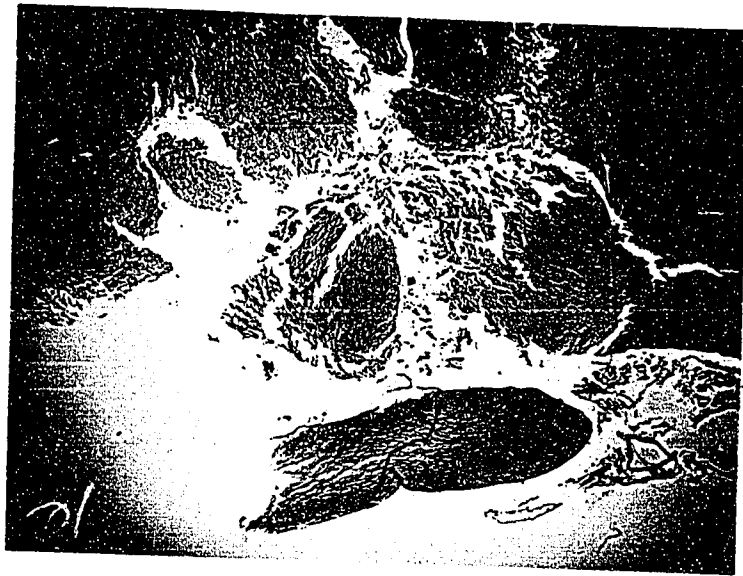


Fig. 34. Bilateral lesion of the supraoptic area of animal 1-F. The supraoptic and paraventricular nuclei have been destroyed completely. X12.

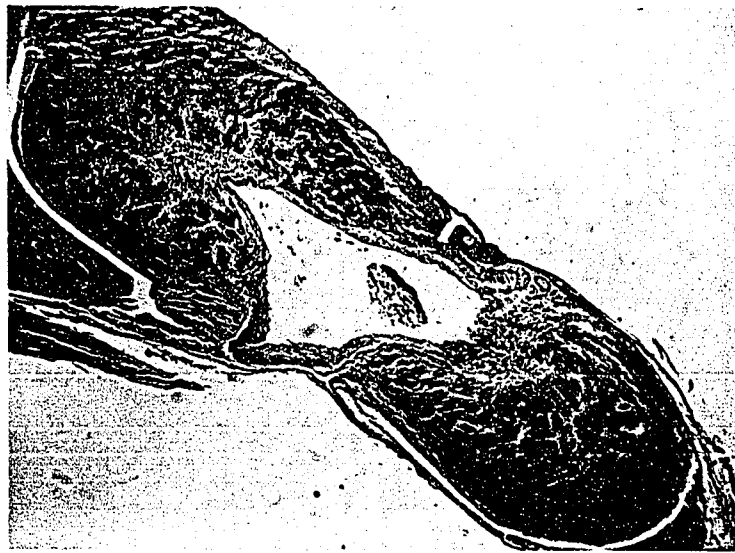
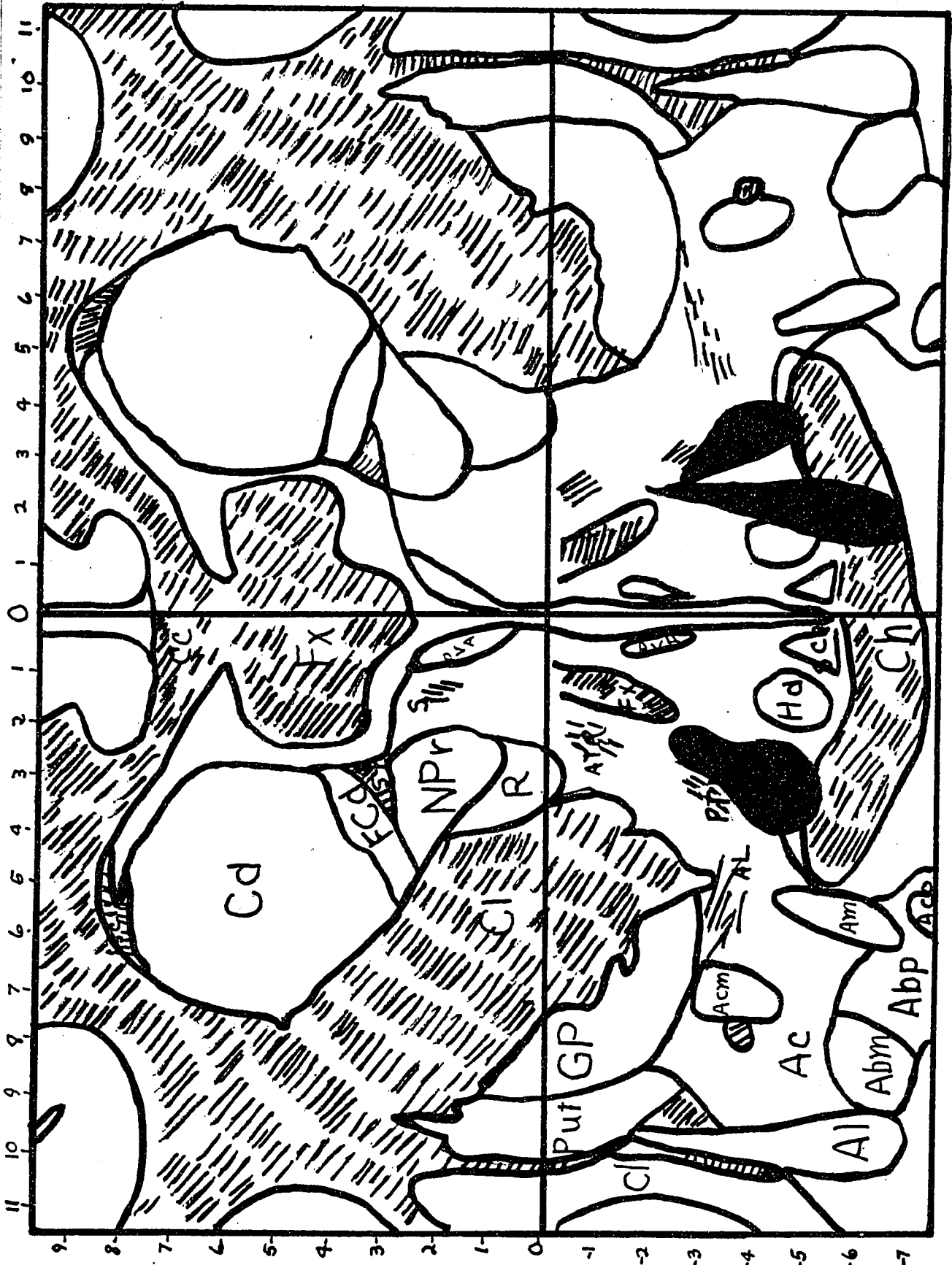


Fig. 35. Pars nervosa of animal 1-F. Note the large decrease in neurosecretion in the central and peripheral areas of the gland. There is a mobilization of the remaining Gomori positive material adjacent to the blood vessels. X15.



Fig. 36. Pars Nervosa and infundibular stalk of a control animal dehydrated by water deprivation for five days. There is a major decrease of neurosecretory substance in the pars nervosa especially in the central area of the gland. There is a moderate concentration of neurosecretory material in the infundibular stalk. X15.



Fr. 13.5

FIG. 37

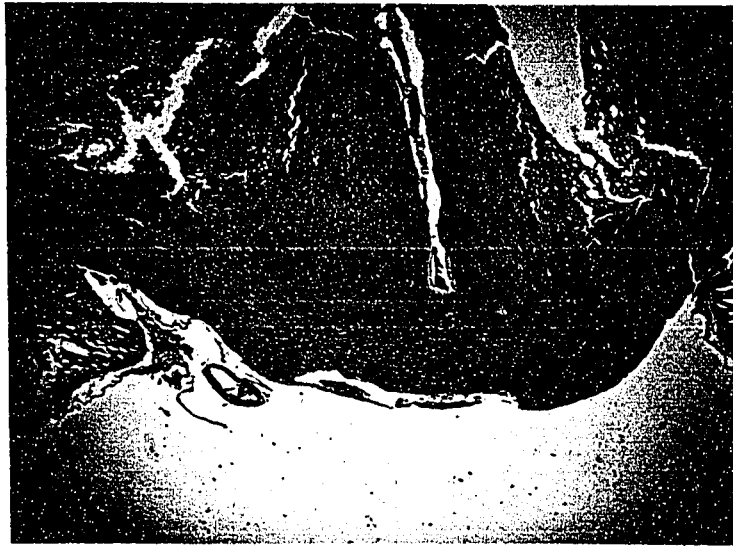


Fig. 38. Bilateral lesion of the supraoptic area of animal 2-5. A major portion of the supraoptic nuclei have been destroyed. The paraventricular nuclei are not involved.

X12.



Fig. 39. Pars nervosa of animal 2-F. There is a major decrease in neurosecretory material in the central and peripheral areas of the gland. X58.

tan, (Figure 33 and 34). As shown in Figure 35, there is a major decrease of neurosecretory granules in the pars nervosa and the infundibular stalk. This decrease is readily observed when one compares this section with the normal control section (Figure 36). It is also apparent that there is a mobilization of neurosecretory material adjacent to the blood vessels. The periphery of the gland and the area around the central cavity demonstrate a major depletion.

The second animal was given a one per-cent hypertonic saline solution diluted with milk for a period of five days prior to the operation. Figures 37 and 38 show a bilateral lesion in the supraoptic area of the hypothalamus involving destruction of the supraoptic nuclei and the lateral hypothalamic area. The paraventricular nuclei are not destroyed. This lesion extended rostrally from the supraoptic area of the hypothalamus to the infundibulum dorsally. Figure 39 illustrates the pars nervosa of this animal I-F. Again, there is a notable decrease in neurosecretion in the glandular division and also in the hypophyseal stalk. Comparison of Figure 39 and Figure 36, the control animal in this experiment, indicates that there is a major reduction in neurosecretory material in the pars nervosa and along the supraoptic-hypophyseal tracts, apparently due to the destruction of the supraoptic nuclei.

Posterior Hypothalamic Lesions in Cats Previously Sub-  
jected to Water Deprivation. Two cats were used in this series of operations. Both animals were placed on a water deprivation diet for a period of five days prior to the operation. Figure 40 represents the area involved in this bilateral posterior hypothalamic lesion. The lesion extended rostrally from the infundibular area of the hypothalamus dorsally to the mammillary area. The lesion involved the destruction of a major portion of the infundibular and mammillary nuclei without affecting the paraventricular or supraoptic nuclei.

The pars nervosa of the hypophyseal gland of this animal, as shown in Figure 41, demonstrates a decrease in neurosecretory material. The neurosecretory substance appears to be mobilized around the blood vessels of the pars nervosa. Since the decrease in neurosecretory substance in the experimental animal is comparable to the decrease in the control animal subjected to water deprivation, it appears that the depletion is due to dehydration effects and not to the lesion in the infundibular or mammillary areas. Furthermore, it has been shown in animal 1-D that a bilateral lesion in the same area in a normal animal fails to produce a major decrease in neurosecretory substance in the neurohypophysis. A second animal dehydrated by water deprivation with a bilateral posterior hypothalamic lesion involving approximately the same region and nuclei as in the preceding animal presented the same decrease in stainable neurosecretory material in the neurohypophysis due to effects of dehydration and not to cauteriza-

tion of the maxillary area.

It is apparent from the results obtained with the dehydration diets prior to cauterization of the supraoptic nuclei, that the effects of dehydration produced a further decrease in neurosecretory material in the neurohypophysis when compared with animals in which the supraoptic area nuclei were destroyed and which were kept on a normal diet.





Fig. 41. The pars nervosa of animal 1-G. Note the depletion of Gomori positive material in the glandular substance. This depletion was attributed to the water deprivation for five days prior to the destruction of the mandibular region. KSC.

TABLE SIX

EXPERIMENTAL DATA

OF

ANIMALS DEHYDRATED BY

WATER DEPRIVATION AND SALT DIURESIS

Cat 1-F

-- Water Deprivation: Five days.

-- Horsley-Clarke Sterotaxic Coordinates:

Frontal 13.0 to 10.0

Lateral 3.0

Horizontal -4.0 to -6.6

-- Post Operative Period: Two days.

-- Post Operative Findings: Animal expired two days after the operation. Flexion and extension reflexes diminished, and no pupillary reflex. Large amount of extra dural hemorrhagic exudate noted at autopsy.

-- Perfusion: No perfusion.

-- Localization of Lesion: Rostrally the lesion extended from the supraoptic area and caudally to the anterior region of the mammillary area. The structures involved in this lesion bilaterally include the supraoptic and paraventricular nuclei, the fornix, optic chiasm, median forebrain bundle and the hypothalamic-ventromedialis nucleus.

-- Concentration of Neurosecretory material in the Infundibular Stalk: There is a marked decrease of neurosecretion along the axons of the hypothalamic-hypophyseal tracts.

-- Concentration of Neurosecretory material in the Pars Nervosa: Marked decrease. Central and peripheral areas of the gland well depleted. Gomori positive material concentrated around blood capillaries.

Cat 2-F

- Drinking Solution: 1% hypertonic saline for five days.
- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 11.0 to 9.0
  - Lateral 2.5
  - Horizontal -2.0 to -6.0
- Post Operative Period: Three days.
- Post Operative Findings: Pupillary reflex normal.  
Animal lethargic but able to consume liquids.  
Extension and flexion reflexes normal.
- Localisation of Lesion: Supraoptic area. The supraoptic nuclei destroyed bilaterally. The lesion does not involve the paraventricular nuclei.
- Concentration of Neurosecretory material in the Infundibular Stalk: Marked decrease.
- Concentration of Neurosecretory material in the Pars Nervosa: Marked decrease in the peripheral and central areas of the gland.

## Cat 1-G.

- Water Deprivation: Five days.
- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 9.0 to 11.5
  - Lateral 3.0
  - Horizontal -2.5 to -6.0
- Post Operative Period: Four days.
- Post Operative Findings: Animal lethargic but able to consume liquids. Flexion and extension reflexes diminished.
- Perfusion: Perfused with 100 cc of saline and 100 cc of 10% formalin-sublimate.
- Localization of Lesions: Rostrally from the infundibular area to the mammillary area caudally. There is necrosis of the medial and lateral mammillary nuclei, mammillothalamic tracts and the median forebrain bundle.
- Concentration of Neurosecretory material in the Infundibular Stalk: Moderate reduction.
- Concentration of the Neurosecretory material in the Pars Nervosa: Marked reduction in the peripheral and central areas of the gland.

Cat 2-G

- Water Deprivation: Five days.
- Horsley-Clarke Stereotaxic Coordinates:
  - Frontal 11.0 to 9.0
  - Lateral 2.0
  - Horizontal -2.0 to 6.0
- Post Operative Period: Two days.
- Post Operative Findings: Animal very lethargic, but able to consume liquids. Pupillary, extension and flexion reflexes normal.
- Perfusion: 100 cc saline, 100 cc of 10% formalin-sublimate.
- Localization of Lesion: Bilateral lesion of the mammillary nuclei. Structures involved are principally the same as in animal 1-G.
- Concentration of Neurosecretory material in the Infundibular Stalk: Marked reduction.
- Concentration of Neurosecretory material in the Pars Nervosa: Same as in animal 1-G.

## CHAPTER IV

### DISCUSSION

The foregoing observations provide sufficient evidence that the supraoptic nuclei are the main group of hypothalamic cells that secrete a substance which may be identifiable as the neurosecretory material of the neurohypophysis. Experimental observations likewise indicate that the paraventricular nuclei secrete Gomori positive material which is contributed to the neurosecretory material in the hypothalamic-hypophyseal system. However, the axons of the paraventricular nuclei show no evidence of containing neurosecretory substance, consequently the pathway of these axons to the neurohypophysis, or to the supraoptic nuclei as suggested by Rasmussen, was not revealed by the chrome-alum-haematoxylin stain. No nerve cells other than those of the supraoptic area are chrome-haematoxylin positive. Smith (1951), has suggested that the mammillary groups are secretory in appearance and function. Close examination for a secretory function in these cells in cats did not confirm these findings.

The evidence presented here for the elaboration of a secretory material in a nerve cell is coherent with Scharrer's original observation in 1928. His conclusions have now been confirmed by many workers, especially by Hild and Stutinsky (1951), who ligated the hypophyseal stalk and found that part of the neurohypophysis distal to the ligature depleted of neurosecretory material, while the axons proximal to the ligature contained an accumulation of

### III.

Conori positive material. Furthermore, the findings of Thomson and Drager (1950), regarding the flow of neurosecretory material in the infundibular stalk substantiate also the evidence of the hypothalamic origin of the so-called Conori positive material. Thomson (1954), ligated the axons coming from a few secretory nuclei in the brain of a fly and has demonstrated the damming-back of neurosecretory material immediately proximal to the ligature. Similar findings were reported by Ortman, Hild and Zettler (1951), and were based on the fact that neurosecretory granules disappear from the hypothalamus and neurohypophysis when animals are dehydrated. These workers found that if the infundibular stalk is ligated in animals which are dehydrated and then these same animals are permitted to drink ad lib, granules of neurosecretory material reappear proximal to the ligature but not in the part below. Moreover, the granules first reappear in the cell bodies themselves as opposed to the axons. The decrease of neurosecretory substance in the neurohypophysis with the experimental dehydration procedures reported in the present study, especially with the hypertonic saline solution, tend to confirm the findings of Ortman, Hild and Zettler. It is therefore apparent that the concentration of neurosecretory material in the hypothalamus, infundibular stalk and the pars nervosa, varies with the state of hydration or dehydration of the body.

The observations reported in this work concerning the chemical nature of neurosecretory material with respect to a glycoprotein component indicated that neurosecretory substance does not con-

tain a large carbohydrate component. With the periodic acid-Schiff technique, material within the bodies of the paraventricular and supraoptic nuclei gave a faint reaction. The infundibular stalk and Herring bodies gave a negative reaction. Conversely, the control section of a newborn mouse kidney reacted strongly.

From the evidence presented in this work and particularly the findings of Sloper (1955), neurosecretory material is probably a protein which Sloper believes is rich in cystine. Whether this substance which stains by the chrome-alum-haematoxylin-phloxin method is identical with antidiuretic hormone has not been determined by the experimental findings reported here. However, it is probably associated with the antidiuretic hormone, possibly as a carrier substance and according to Scharrer and Scharrer (1954), the concentration of this neurosecretory substance in the neurohypophysis bears a direct relationship to the quantity of antidiuretic hormone present.

In view of the findings, mentioned above, the general conclusion which these studies are suggesting, is, that the pars nervosa itself is little more than a storage organ for neurosecretory material produced by the hypothalamic nuclei. However, Bodian (1951), in a careful study of the histological structure of the neurohypophysis has stated that the axon terminals in the hypothalamic-hypophyscal tracts may also be involved in secretion. Bodian believes that the "morphological complexity of the nerve terminals becomes more readily rationalized as a mechanism for serving

to increase the ratio of cell surface to cell volume, and perhaps thus favoring an enhancement of the secretory process in the pars nervosa as compared with the hypothalamic nuclei". The present experimental findings neither disprove nor confirm the findings of Bodian. In nearly all the observations concerning the location of the secretory material in the pars nervosa, it was noted that the greater concentration of material appeared in the areas of the axon terminals in the periphery of the gland. The glandular decrease in neurosecretory material occurred first in the area adjacent to the central cavity and lastly in the periphery of the pars nervosa. Since a total depletion of neurosecretory material was not obtained either by traumatic lesions or physiological methods, one cannot rule out the possibility as suggested by Bodian that the terminal axons may secrete neurosecretory material.

With respect to the findings of Bodian, the investigations of Rasmussen, Fisher, Ranson, Ingram, and especially Wild cannot be overemphasized. These workers have shown that atrophy of the pars nervosa occurs after severing the hypothalamic-hypophyseal tracts owing to the interruption of the trophic and secretomotor influence from the hypothalamus. Rasmussen (1949), has also found evidence of retrograde degeneration of the supraoptic nuclei after hypophyseal stalk lesions; the number of cells degenerating depending upon the area in which the lesion is located.

In the observations performed in the present work, the post operative survival time is an important factor which may explain the

Lack of total depletion in neurosecretory material in the neurohypophysis. The technical difficulties involved in making a circumscribed lesion in order to abolish totally the supraoptic and paraventricular cells bilaterally without severe trauma to the operative animal in this important vegetative area of the brain, was a constant problem due to excessive hemorrhage, disturbance of the sleep-waking mechanism, respiratory rate, etc. The practical impossibility to destroy all the supraoptic cells and axons of the supraoptic and paraventricular-hypophysial tracts is moreover an important second factor. The findings of Magoun, Fisher and Hanson (1959), tend to point out this fact. These workers reported that the degree of polyuria in monkeys varies with the location of a transected lesion in the neurohypophysis. In cases with partial polyuria, an average of only five to seven per-cent of the volume of the normal neurohypophysis remained proximal to the section and connected with the hypothalamus. In the cases with normal urine output, an average of twelve to sixteen per-cent of the total volume of the neurohypophysis remained connected with the hypothalamus proximal to the sections. Thus the negative results encountered in these observations may be explained either by the sizable number of supraoptic and paraventricular nuclei remaining after lesions in the supraoptic area, or the traumatic effects of the operation including hemorrhage with a subsequent renal shut-down.

The second phase of this work concerning the physiological conditions which affect the quantity of neurosecretory material in

the pars nervosa and the supraoptic nuclei raises many questions. Ortman (1950, 1951), Hild and Zettler (1953), Hild (1951), Leveque and Scharver (1953), have shown that various types of stimuli are capable of causing depletion of the neurosecretory substance in the neurohypophysis. For example, the perivascular nerve endings of the supraoptic-hypophysial tract in the neural lobe of the hypophysis of severely dehydrated animals are devoid of neurosecretory granules. Rathboller (1953), has shown that following an unpleasant stimulus such as a needle prick in rats, vasodilation and mobilization of the neurosecretory material occurs toward the lumen of the blood vessels in the infundibulum. After six minutes the area is void of neurosecretion and returns to normal again after one hour. With bioassay methods, Kovacs and Bachrach (1951), reported a reduced antidiuretic activity in the hypothalamus and pars nervosa. These workers injected intraperitoneally a 5% sodium chloride solution, and observed the reduction one hour after the first injection. It was shown in the dehydration methods performed in this work that water deprivation and salt diuresis are stimuli which will produce a mobilization of neurosecretory material towards the lumen of the blood vessels in the pars nervosa. Following the mobilization of the Gomori positive material towards the blood vessels, there is a subsequent reduction in the concentration of this neurosecretory material in the pars nervosa and the infundibular stalk. Cytologically, the supraoptic nuclei of both types of animals used in this study reveal every indication of cellular activity in either dehydrated states. It was observed that

the granulation in the cytoplasm of the supraoptic nuclei in the rats dehydrated by water deprivation for six days was increased when compared with the normal non-dehydrated animal. In the rats that were given a 1% hypertonic saline solution the granulation in the supraoptic nuclei decreased after six days of ad lib consumption. Both groups of animals showed evidence of neurosecretory depletion in the pars nervosa and in the infundibular stalk.

The explanation for these cytological findings may be as follows: One may interpret the increase in granulation during water deprivation as a hyperactivity of the cell, or as an indication of storage of neurosecretory substance. Physiologically, the renal reabsorption of water increases in this state of dehydration, consequently one may expect a higher level of antidiuretic hormone secreted by the supraoptic cells in order to increase tubular reabsorption of water in the kidney. However, hyperactivity of a cell is usually indicated histologically by a decrease in the amount of granular material in the cytoplasm. This fact may be observed in the zymogenic cells of the fundic glands of the stomach or the exocrine glands of the pancreas. In these cells, secretory activity is indicated by an increase in staining affinity before discharge of the cytoplasmic contents and a non-granular clear cytoplasm after discharge of the secretory products. In view of the findings that dehydration of rats with 1% hypertonic saline for six days produces a depletion of neurosecretory material in the supraoptic nuclei one may thus assume that the supraoptic nuclei in this dehydrated condition show evidence of a greater secretory activity than

the supraoptic nuclei of animals dehydrated by water deprivation. According to the work of Verney (1947), the release of the antidiuretic hormone is controlled by the effective osmotic pressure of the blood. In these experiments by Verney the injections of hypertonic saline solution of sodium chloride, sodium sulfate, or sucrose into the carotid artery of unanesthetized dogs markedly inhibited water diuresis. The existence of "osmoreceptors" has been implied by Verney's experiments and although their location is unknown, the extreme degree of vascularity of the supraoptic and paraventricular nuclei favors the view that changes in the effective osmotic pressure of the body fluids may be detected by osmotic receptors in the supraoptic area. It is known that the administration of water dilutes the blood and increases urine flow in proportion to the amount of water given. In contrast, water deprivation causes a rise in total solute concentration associated with a decrease urine flow and maximal concentration of urine. The urine of animals dehydrated either by restriction of water or by injection of hypertonic saline solutions possesses antidiuretic properties which do not appear in similarly treated animals with lesions of the supraoptico-hypophyseal system (Verney, 1947). Dehydration by salt administration or water deprivation also results in reduction of assayable antidiuretic hormone in the neurohypophysis. Thus the conclusion has been reached that the release of antidiuretic hormone is correlated with changes in osmotic pressure. In accordance with Verney's work, the cytoplasm of the supraoptic nuclei in both types of dehydration methods performed in this work

should theoretically contain an equal concentration of granular material.

However, one may also conclude that an animal dehydrated by water deprivation is not excreting the normal volume of urine by the renal glomeruli. Therefore, the absolute demand for antidiuretic hormone for the reabsorption of water from the kidney tubules is decreased, consequently there is an accumulation of granular material in the cytoplasm of the hypothalamic nuclei. The increase in granulation in these latter nuclei in accordance with Verney's theory is brought about by an increase of effective osmotic pressure due to water deprivation. In contrast to the above theory, the animals which received hypertonic solutions of sodium chloride the serum content of solutes was increased consequently producing a higher effective osmotic pressure. The increase of osmotic pressure stimulates the supraoptic nuclei to increase the rate for the antidiuretic factor is likewise increased. In other words, the secretion rate of hormones is greater in rats given a hypertonic saline solution which would explain the decrease in granulation in the cytoplasm of the hypothalamic nuclei. ?

↓ In conclusion, the experimental evidence presented in this work points to the nuclei of the supraoptic area as the primary source of origin of the neurosecretory material of the neurohypophysis. Further pharmacological tests must be devised in order to detect whether this material secreted by the supraoptic area nuclei is chemically identical to the hormones of the pars nervosa or possibly modified in the transport mechanism to the pars nervosa.

## CHAPTER V

### SUMMARY

1. Twenty-three adult cats and twenty rats were used in this study. The axons and nerve cells of the hypothalamic-hypophysial system were stained for neurosecretory material with the Gomori chrome-alum-haematoxylin-phloxine method.

2. A dark blue neurosecretory material was observed in the supraoptic and paraventricular nuclei of the supraoptic area of the hypothalamus. The same material was observed in the axons of these cells in the median eminence, infundibular stem and the pars nervosa of the cats and rats.

3. Histological examination of the mammillary area revealed no evidence that these cells secrete a Gomori positive material which contributes to the neurosecretory material of the neurohypophysis.

4. There is no experimental evidence to indicate that cells other than the supraoptic and paraventricular secrete a material which may be identifiable as the posterior lobe hormones.

9. 5. Bilateral lesions of the supraoptic nuclei in adult cats reduced the microscopic visible concentration of neurosecretory material in the median eminence, infundibular stem and especially the pars nervosa, one to seven days following the lesion.

6. After unilateral lesions of the supraoptic nuclei of cats, a small reduction in neurosecretory material was observed in the

pars nervosa.

7. Bilateral lesions of the posterior hypothalamic area in cats did not produce a major reduction in neurosecretory material in the neurohypophysis.

8. Bilateral lesions in the lateral hypothalamic areas of cats presented no evidence of any major decrease in the concentration of neurosecretory material in the neurohypophysis.

9. Ten adult rats, placed on a water deprivation diet for one week, showed evidence of an increase in secretion granules in the supraoptic nuclei, and a decrease of Gomori positive material in the pars nervosa.

10. Ten adult rats permitted to drink a hypertonic saline solution, showed evidence of a decrease in secretion granules in the supraoptic nuclei and a decrease of neurosecretory material in the pars nervosa.

11. Adult cats, dehydrated before bilateral lesions of the supraoptic nuclei were made, presented evidence of an increased depletion of neurosecretory material in the neurohypophysis.

12. All experimental data indicates that the supraoptic nuclei are largely responsible for the secretion of a Gomori positive material which may be identifiable as the hormones of the pars nervosa.

## RESUME

1. Nous avons usé dans ces travaux 23 chats et 20 rats adultes. Les axones ainsi que les cellules nerveuses du système hypothalamo-hypophysaire furent colorés spécifiquement pour la neurosecrétion à l'aide de la méthode de fixation au chrome-alun-hématocryline-ponce de Gomori.

2. Dans une série de rats et de chats normaux, une substance "neurosecréttoire" d'un bleu foncé fut ainsi démontrée dans les noyaux supraoptiques et paraventriculaires de la région susoptique de l'hypothalamus. La même substance fut également aperçue dans les axons de ces cellules jusque dans l'éminence cendrée, dans la tige de l'infundibulum, et dans le lobe nerveux de l'hypophyse.

3. Ce procédé ne suffit pas à indiquer que les cellules de la région mammillaire secréteraient une substance contribuant à la neurosecrétion de l'hypophyse.

4. Il n'y a également aucune preuve expérimentale que des cellules autres que celles des noyaux supraoptiques et paraventriculaires, secréteraient la substance identifiable à l'hormone du lobe postérieur.

5. D'un à sept jours après une lésion bilatérale des noyaux supraoptiques de chats adultes, la quantité de substance "neurosecréttoire", visible au microscope, fut considérablement réduite dans l'éminence cendrée, dans la tige de l'infundibulum, et tout spécialement dans le lobe nerveux.

6. Une lésion unilatérale réduisit assez peu la neurosecrétion et cela seulement dans le lobe nerveux.

7. Des lésions bilatérales dans la région postérieure de l'hypo-

thalamus des chats, n'ont pas réussi à réduire sensiblement la neurosecrétion de la neurohypophyse.

8. Des lésions effectuées de chaque côté de la région latérale de l'hypothalamus, n'eurent également que peu d'effet.

9. Dix chats adultes privés d'eau pendant une semaine semblèrent présenter une augmentation des granules dans les noyaux supraoptiques et une diminution de substance se colorant au Gomori dans le lobe nerveux.

10. Dix autres chats adultes, soumis à une diète liquide de solution saline hypertonique semblèrent exhiber une diminution de la neurosecrétion des noyaux supraoptiques et du lobe nerveux.

11. Des chats adultes déshydratés, présentèrent aucun signe d'une plus grande chute de la neurosecrétion neurohypophysaire après des lésions bilatérales des noyaux supraoptiques.

12. Toutes les données expérimentales indiquent donc que les noyaux supraoptiques sont largement responsables de la sécrétion des hormones du lobe postérieur.

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