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An Ultrastructural Analysis of Rat Posterior Pituitary with Special Reference to Hormone Release

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LOMA LINDA UNIVERSITY

Graduate School

AN ULTRASTRUCTURAL ANALYSIS OF RAT POSTERIOR PITUITARY WITH SPECIAL REFERENCE TO HORMONE RELEASE

by

William M. Hooker

A Dissertation in Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy in the Field of Anatomy

August 1969

Each person whose signature appears below certifies that he has read this dissertation and that in his opinion it is adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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INTRODUCTION

Excluding vascular elements, it can be said that the neurohypophysis arises totally from neural tissue. According to Kleeman and Cutler (1963) the mammalian neurohypophysis consists of: 1) a group of specialized hypothalamic nuclei, 2) the median eminence, 3) the neurohypophyseal tract, and 4) the pars nervosa (synonyms: neural lobe, posterior lobe of the pituitary). Nerve fibers of the neurohypophyseal tract terminate in the pars nervosa (Kleeman and Cutler, 1963).

Progress in physiological research on the neurohypophysis has far exceeded anatomical findings. For example, the presence of biologically active hormones in the neural lobe was known for more than 50 years (Oliver and Schafer, 1895) before Bargmann in 1948 was able to clearly demonstrate histologically the existence of morphological features consistent with the available physiological data. The concept of neurosecretion has developed subsequently and at present adequately fits in with the current data.

The secretory function of the supraoptic and paraventricular nuclei is now clearly understood but the exact nature of production and release of the hormones is still obscure. It is known that neurohormones are actively synthesized in the hypothalamic nuclei a.d that these hormones are conjugated in some way to carrier proteins and are packaged for transport down axons into the release area. This release area is commonly called a "neurohemal organ". These concepts have been developing over the last 20 years and through the efforts of many investigators a relatively good working knowledge of the controls and

operational factors in the hypothalamo-neurohypophyseal system have been achieved.

Neurosecretion as an endocrine process is not limited to the mammal. It is known to exist in such diverse systems as the sinus glands of crustaceans, the corpus cardiacum of insects, the annelid brain, the pericardial and postcommissure organs in crustaceans, the urophysis of fishes and in a variety of analogously constructed organs in arachnids and myriapods (Bern and Knowles, 1966).

The classical definition of neurosecretion involves the light microscopic demonstration of secretory material in neurons by standard cytological methods which distinguish glandular activity. Certain staining methods, notably including that of Gomori, were adopted by "neurosecretionists" as standard procedure for the demonstration of neurosecretory material (NSM). Unfortunately, these procedures were very nonspecific and really identified only inclusions stainable with either chrome-alum or paraldehyde fuchsin. Thus these stains may or may not indicate neurosecretory material.

The advent of the electron microscope further refined cytologic criteria that could be employed to study neurosecretory processes. The observation in neurons, for example, of electron-dense granules in the range of 1000Å to 3000Å gave rise to the hope that a dependable criteria for the identification of neurosecretory systems had been discovered. It soon became apparent that granules in this category were present in areas where there is presumably no neurosecretion (Bern and Knowles, 1966). Consequently, at the present time the definition of

such a system is somewhat more complicated than was first anticipated. The present concept of a neurosecretory system is one in which a part of the nervous system carries on hormonogenesis and the hormone is released at the site of a neurohemal organ (Bern and Knowles, 1966). This provides a location for releasing the hormone into the systemic circulation.

In the case of the vertebrate posterior pituitary, the known hormones involved are: vasopressin, synthesized mainly in the supraoptic nucleus of the hypothalamus, and oxytocin, predominantly elaborated in the paraventricular nucleus. These hormones have several functions and will be described only briefly here. Oxytocin is an octapeptide which influences the smooth muscle of the uterus and stimulates myoepithelial contraction in the breast during lactation. Vasopressin, also an octapeptide which derives its name from its vasopressor activity, is known as antidiuretic hormone because of its prominant effect on the renal tubules in preventing diuresis.

The importance of these two hormones makes the study of the system significant clinically as well as academically. For example, in diabetes insipidus there is decreased production, or total lack, of antidiuretic hormone (ADH). As a result the patient exhibits polyuria and polydipsia. An understanding of the entire system is not only important in treating this patient but also in being aware of the possible locations for the causative lesion. This is a good example of a neuroendocrine system in which the nervous system has control over the entire body by way of the vascular system. The functional ampli-

cations of such a system induced the writer to undertake a research project concerned with neuroendocrinology.

Considerable effort by many workers has been devoted to studies of the hypothalamo-neurohypophysial axis and, in particular, the methods and controls for the release of hormones under physiologic and experimental conditions.

A number of review articles are available covering the hormones of the hypothalamo-hypophysial system. A comprehensive work on hormones produced by neurosecretory cells was published in 1954 (Scharrer and Scharrer, 1954). Niels Thorn's (1958) review covers earlier research on mammalian antidiuretic hormone and the very complete work of Sawyer (1961) discussed all aspects of the neurohypophysial hormones up until that time. A complete review on the neurohypophysis by Kleeman and Cutler (1963) discussed the topic in general from 1957 to 1963. The latter perhaps is the most complete work covering the basic systemic processes in relation to their pathological and clinical correlates. The work by Acher (1966) is a treatment of the chemistry of neurohypophysial hormones. The work by Sawyer and Mills (1966) discussed the control of vasopressin secretion.

One of the best sources to date for organismic physiology of the neurohypophysis is the 1968 review by Farrell, Fabre, and Rauschkolb (1968). A review by Share (1967) is a good reference for those interested in the bioassay and physiological control of the release of vasopressin. Several other reviews may be consulted for additional discussion and references: (van Dyke, 1955; Rothballer, 1966; Gauer and

Henry, 1963; Kleeman and Cutler, 1963; Sawyer, 1966).

The cytologic components of the neurohypophysis may be divided into three categories: neurons, glial cells (the so-called pituicytes), and vascular system elements. For the historic development of the cytology of the gland, the reader is referred to Christ (1966).

The pars nervosa contains unmyelinated axons and terminals of nerve fibers whose cell bodies are located chiefly in the supraoptic and, to a lesser extent, in the paraventricular nuclei of the anterior hypothalamus. These fibers reach the neurohypophysis by way of the hypothalamo-hypophysial tracts in the infundibular stem and, after ramification in the gland their terminals expand abutting the capillary network of the gland. Bodian (1951) has described these terminal expansions as the palisade layer. Cell bodies in the hypothalamus, the axons, and the terminals all contain varying amounts of neurosecretory material which can be seen in properly stained light microscopic preparations as colloid particles inside the fibers. In electron microscopic observations these are seen as osmiophilic neurosecretion granules (NSG).

In the opossum Bodian has described three layers or zones in the neurohypophysis: the hilar zone, which is the area containing the nerve fibers and the pituicytes; the septal zone, containing blood vessels and connective tissue; and the palisade zone, which contains rows of nerve endings and pituicyte processes. This precise organization has not been demonstrated in other animals.

Nerve fibers in the neurohypophysis are similar to those found in the central nervous system generally. Their axons contain consider-

able numbers of neurotubules, mitochondria, neurofilaments as well as the NSG and occasional outpocketing of synaptic-like or "synaptoid" vesicles.

Numerous studies of the cytology and ultrastructure of the neurohypophysis are available for various species: human (Lederis, 1965); rabbits, both normal (Barer and Lederis, 1966) and drug-treated (Fujita and Hartmann, 1961); the rat in a strain with homozygous diabetes insipidus (Brattleboro strain) (Scott, 1968); the neural lobe of the rat (Bargmann and Knoop, 1957; Palay, 1957; Hartmann, 1958; Palay, 1955; Monroe, 1967; Daniel and Lederis, 1966; Monroe and Scott, 1966); the rainbow trout, Salmo irideus (Lederis, 1964); cats and dogs (Bargmann and Knoop, 1957); the common grass snake, Tropidonotus natrix (Bargmann, Knoop and Thiel, 1957); the toad, Bufo arenarum (Gerschenfeld, Tramezzani and DeRobertis, 1960); the opossum, Didelphis virginiana (Bodian, 1951; Bodian, 1963; Roth and Luce, 1964); birds (Kobayashi, 1963); the white crowned sparrow, Zonotrichia leucophrys gambelii (Bern, et. al., 1966); the desert rat (R. L. Holmes, 1968); the fetal monkey (Holmes, 1966); the hedgehog, Erinaceus europ. L (Holmes and Kiernan, 1964); the parakeet, Melopsittacus undulatus (Kobayashi, Bern, Nishioka, and Hyodo, 1961); the lizard, Klauberina riversiana (Rodriquez and LaPointe, 1969); the bullfrog, Rana catesbeiana (Oota and Kobayashi, 1963); the turtle, Clemmys japonica (Oota, 1963b); the chicken, Gallus domesticus (Duncan, 1955); the fish, Oryzias latipes (Oota, 1963a); the mouse (Oota, 1963c); and the pigeon, Columbia livia domestica (Oota and Kobayashi, 1962).

Other review articles covering the cytological aspects are available (Kleeman and Cutler, 1963; J. F. Christ, 1966).

This study was undertaken in an attempt to resolve some of the controversial problems evident in the literature.

It is known, for example, that osmotic stress causes the posterior pituitary to become depleted of its neurosecretion granules and that there is an increase in the number of synaptic vesicles in the axonal endings (Palay, 1957; Gerschenfeld, et. al., 1960; Bern, et. al., 1966; Daniel and Lederis, 1966 and others). Some have speculated that these changes are due to an increase in neurotransmitter substance at the site of release (Gerschenfeld, et. al., 1960; Kobayashi, et. al., 1965 and Koelle, 1961). Others have felt that these vesicles are the result of budding off and that they represent "ghosts" of the larger vesicles after they had lost their contents and fragmented (Holmes and Knowles, 1960; Lederis, 1964). There seemed to be some question in the literature as to whether these small vesicles were typical synaptic vesicles.

Some workers reported the appearance of swellings along the axons (Barer and Lederis, 1966) and others did not. Another factor involved in the decision to study this structure was the fact that the majority of electron microscopic descriptions of the gland were done with techniques that are now outdated. These techniques resulted in inferior and often very inadequate fixation and detail.

It must be remembered that while electron microscopy yields static pictures, they represent a stage in a dynamic, living state. With

this in mind, a detailed study and reevaluation of the ultrastructure of the posterior lobe of the pituitary seemed desirable.

In order to correlate morphological changes with functional stages of hormone release, animals were subjected to various osmotic stresses. The results obtained were considered in light of recent pharmacological, physiological, and biochemical advances.

MATERIALS AND METHODS

Approximately 60 female, 250 gram Sprague-Dawley rats were used in this study. Of this number, 26 were used as "normal" animals for controls and to find the best fixation procedures for preserving the posterior pituitary gland.

Fixation Procedure. The immersion technique so frequently used by other investigators has not resulted in consistently acceptable fixation of nervous tissue. Dripping the fixative onto the surface of living tissue allows only the superficial layers of the tissue to be studied and deeper layers are too poorly fixed to be useful. Based on the extensive work by Karlsson and Schultz (1965, 1966) and Schultz and Karlsson (1965) it was possible to employ a well-established procedure of perfusion fixation (fig. 1). A detailed discussion of this and other fixation procedures is available (Sjostrand, 1967). The technique used here involved tracheal cannulation and respiration with a gas mixture of 5% CO_2 and 95% O_2. The CO_2 facilitates vasodilation of the cerebral vessels. A discussion of this phenomenon has recently been published (Skinhoj and Paulson, 1969). An opening cut in the plastic tubing connecting the cannula to the gas cylinder allowed the animal to breathe voluntarily. Following thoracotomy the operative assistant ventilated the animal by covering the hole intermittantly with his finger. The thoracic and abdominal skin was incised along the midline and widely reflected and a slightly para-sagittal incision into the thorax was made, usually on the right side, from the xiphoid process to the right first rib. Lateral cuts were made just above the diaphragm. Two large

hemostats laterally reflected the flaps of the thoracic wall as well as occluded the severed internal thoracic artery. The flaps of the thoracic wall were then held open by means of two large hemostats. The ascending aorta was exposed by retraction of the thymus gland with two small clamps. The pericardial sac was stripped away and a loose ligature was placed around the aorta taking care not to disrupt the vasculature. The left ventricle was incised and the glass cannula for perfusion inserted into the ascending aorta and tied in place. The right atrium and/or ventricle was widely opened and perfusion started at a pressure of about 1.4 meters of water. The perfusion apparatus used was an intravenous infusion set with a squeeze-type flow regulator.

A standard fixative solution was employed for all animals used in the quantitative study. This consisted of 3% glutaraldehyde, 1% gum acacia and Sorenson's phosphate buffer (pH 7.3) adjusted to 320 mOsm. The flow rate was adjusted so the 250 ml of perfusate was completely used in 15 minutes.

Removal of Tissue. After perfusion, the brain was exposed and the optic nerves sectioned and the brain gently pulled back to gain access to the median eminence area. Great care was taken to minimize pulling up on the brain in an attempt to prevent disturbance of the infundibular stalk. In most instances, however, exposure of the pituitary area necessitated retraction of the brain with resultant breaking of the delicate infundibular stalk. No adverse effects, however, could be traced to this occurrence. The brain could then be removed leaving the pituitary exposed and accessible.

In the rat the pars nervosa is located centrally and anteriorly with a horseshoe-like ring of anterior pituitary lateral and posterior. The small pars intermedia is interposed between them. Care was taken to be as gentle as possible when excising the posterior lobe from the gland, since disruption of tissue by post-mortem mechanical manipulation is known to cause membrane breaks as well as other morphological modifications (Cammermeyer, 1960).

Since this study involved comparative values, an effort was made to sample approximately the same general area of the pars nervosa in each specimen, generally the central portion.

Preparation for Examination. The small pieces of tissue (less than 0.5 millimeter square) were placed in about 2 milliliters of 320 mOsm Sorenson phosphate buffered (pH 7.3) 1% osmium tetroxide for three hours. This post-fixation procedure was used both on the basis of its fixative reaction which is dependent on the ability of OsO_4 to act as a strong oxidizing agent (Porter and Kallman, 1953) and on the basis of the heavy metal causing a selective reaction with various parts of the tissue. This selective reaction accounts for its usefulness as an electron stain.

Following the postfixation, the tissue was dehydrated in a graded series of aqueous acetone (40, 75, 95, and 100%) and embedded in Vestopal W according to the method of Kurtz (1961). Sectioning was done on an LKB 8800 Ultrotome and the sections were mounted on copper grids with a single 1 by 2 millimeter rectangular hole, using a Formvar supporting film. Glass knives were used on the microtome and the sections

were floated onto a 5% acetone solution. While they were floating on the surface of the fluid the copper grids were brought up under them and with some manipulation they could be properly oriented on the rectangular hole.

Staining of the mounted sections was accomplished by floating the grid on one drop of lead citrate (Reynolds, 1963) for 5 minutes and uranyl acetate (Watson, 1958) for 30 minutes.

The stained, mounted sections were then placed in a vacuum evaporator and a thin coat of carbon was evaporated onto them. Examination of the specimens was carried out on a Siemens Elmiskop IA electron microscope (Siemens America Inc., 350 Fifth Ave., New York, New York, 10001) using a 200 μ condenser II aperture and a 30 μ thin metal objective aperture.

Experimental Procedures. In order to study changes of the pars nervosa during osmotic stress, two experimental approaches were employed. The first group consisting of 10 animals, was divided into 4 subgroups. In an effort to capture the dynamics of the antidiuretic response, increasing time intervals were allowed between removal of drinking water from the animals and sacrifice by perfusion with the fixative. These intervals were 1½ days, 2½ days, 4 days, and 7 days (table I).

Table I

CHRONIC DEHYDRATION GROUP

Days with No Drinking Water	Number of Animals
1½	3
2½	3
4	2
7	2

The study of the neurohypophysial response to acute hyperosmotic stress was done on Group II. Infusion of a hypertonic sodium chloride solution was employed for this purpose. The solution chosen was 10 milliliters of 6% NaCl per Kg body weight and this amount was slowly infused into the femoral vein. In order to accomplish this, a longitudinal incision was made over the area of the femoral triangle and the femoral sheath exposed. The vein was carefully dissected free and two ties were placed around it; the use of a dissecting microscope was found to be essential. A clamp was placed cephalad to the ties. The tie most caudad was tied down tightly to insure cessation of blood flow to the area beyond and a very small incision was made in the vessel wall. A small catheter tube (Intramedic Polyethylene Tubing PE 10) connected by means of a 27 gauge needle to a Sage infusion pump, model 255 W-1 (Sage Instruments, Inc., 2 Spring Street, White Plains, New York, 10601), was then inserted into the lumen of the vessel and the second tie fastened securely. The clamp was then removed allowing free communication between the catheter tube and the vessel lumen. Varying speeds of infusion were employed and for varying periods of time. The time delay before perfusion fixation was also varied in order to study the antidiuretic response to acute osmotic shock. The various time periods used are illustrated in table II.

Quantification Methods. The number of micrographs needed to adequately sample a tissue with electron microscopic techniques is unduly large when one considers the "smallness" of the sample, because of the degree of magnification and the thinness of sections used. The fact that

TABLE II

Each animal was given 10 ml. 6% Na Cl per Kg. body weight for the time period represented by the striped area and perfusion took place at the end of the entire bar.

Osmolality figures at end of each bar represent the serum osmolality at perfusion.

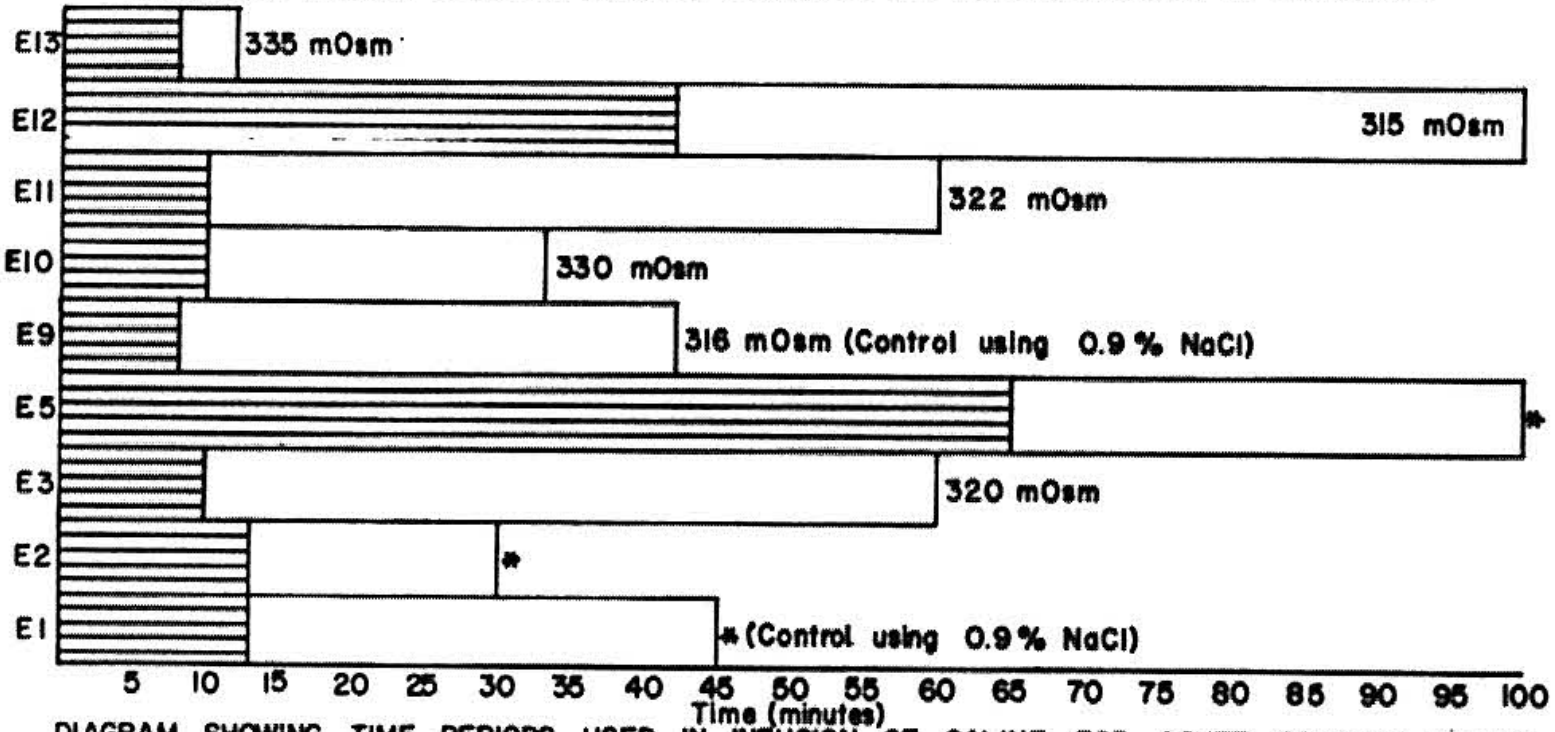


DIAGRAM SHOWING TIME PERIODS USED IN INFUSION OF SALINE FOR ACUTE OSMOTIC STRESS SERIES

*No osmolality figures available.

groups of micrographs to be compared must be matched for magnification and other variables such as individual differences from specimen to specimen related to their history and to the level of the section, must be taken into consideration. Complete quantification of all data mentioned would be beyond the scope of this project.

Some of the animals in the chronically stressed group were subjected to the following sampling methods. Measurements were made on 8 X 10 prints. A clear acetate sheet with an inscribed ½ inch grid was placed over the print to be counted. With the point-counting method the relative area of each of the components of interest (see table X) was obtained by counting the structures that were located under the intersections in the grid. This resulted in 275-300 counts per picture with the point-counting method that was used. The relative area occupied by specific components is obtained by the number of intersections found to overly those components divided by the total number of intersections counted. While complete statistical analysis of the data was not possible, the standard error was calculated for each of the measurements, using the following formula: $S^2(p) = \frac{p(1-p)}{n-1}$, where p is the fraction of the number of counts for a given component divided by the total number of counts (n), and S is the variance whose square root is the standard error (Eranko, 1955).

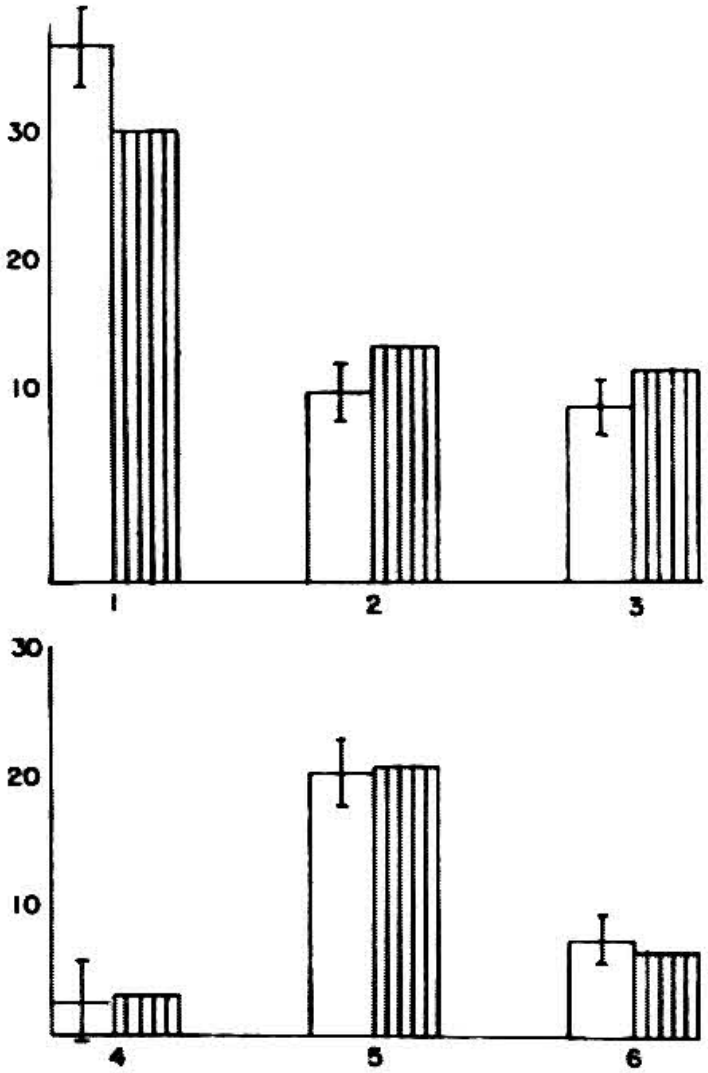
In addition, in order to check the sampling error, a measurement was made of the actual area covered by the various components. To do this as accurately as possible, the micrograph was divided into much finer subdivisions and the actual area of the components was estimated.

For this purpose the area within the ½ inch squares made by the grid previously described was divided into 4 smaller squares and the proportional area occupied by a component within these estimated on the basis of 10. This resulted in obtaining 10,220 area counts from one 8 X 10 micrograph as compared to only 275-300 point counts for the same micrograph. This facilitated a determination made of the error of the point-counting method. The results show that this error was within limits of acceptability (table III), and in most cases approximated the figures obtained for the standard error calculations.

Measurements and Magnification. The magnification for each micrograph was computed by noting the direct meter reading of the microscope for a given picture and consulting a calibration chart. The calibration chart was obtained by using a diffraction grating replica (Ernest Fullam, Inc., Catalog No. 1008). To obtain the correct final magnification, the original magnification was multiplied by the photographic enlargement factor. Sizes of granules, vesicles and membranes could then be determined by direct measurement of the enlargement and dividing by the total magnification.

The osmolality measurements were done for the acute osmotic stress series in order to provide a check on the actual osmotic state of the animal at the time of death (table II). These measurements were made with an Advanced freezing point depression Osmometer, Model 31LAS-67 (Advanced Instruments, Inc., 45 Kenneth Street, Newton Highlands, Mass., 02161).

TABLE III



COMPARISON BETWEEN POINT-COUNTING METHOD AND AREA COUNTING METHOD

☐ POINT-COUNTING

▥ AREA COUNTING

1 Small endings with no NSG
2 Extracellular space
3 NSG in dilation of axon
4 Synaptic vesicles
5 Pituicyte cytoplasm
6 Pituicyte nucleus

OBSERVATIONS

The ultrastructure of the rat neurohypophysis has been described in detail by others (Palay, 1955, 1957; Daniel and Lederis, 1966; Bargmann and Knoop, 1957; Monroe, 1967; Monroe and Scott, 1966). The use of newer procedures for fixation and embedding used in this study has made it necessary to study and describe the histology of normally hydrated rats used for controls.

The Normal Hydrated Animal

Organelles found in the neuronal processes in the posterior lobe of the pituitary are seen in figs. 6 and 7. Mitochondria are found in the axons of nerve fibers leading into the parenchyma of the gland as well as in the terminal dilations of the fibers. They are elongate or oval in shape and are typical of those found elsewhere in the central nervous system. They appear to be homogeneously intermingled with the dark neurosecretory granules and consist of an inner and outer limiting membrane with cristae. This same appearance was also noted in the experimental preparations.

Neurotubules, oriented longitudinally, are present in nerve fibers except in their terminal portions. The structure of these neurotubules is seen in cross section as well as longitudinal section (fig. 7). They correspond with the membranous system of undulating tubules observed by Palay in 1957. Palay described wide dilations in these tubules occasionally containing neurosecretion granules, but these were not seen in this material. Neurosecretory material appears as large osmiophilic granules in the nerve fibers separate from this tubular system. The

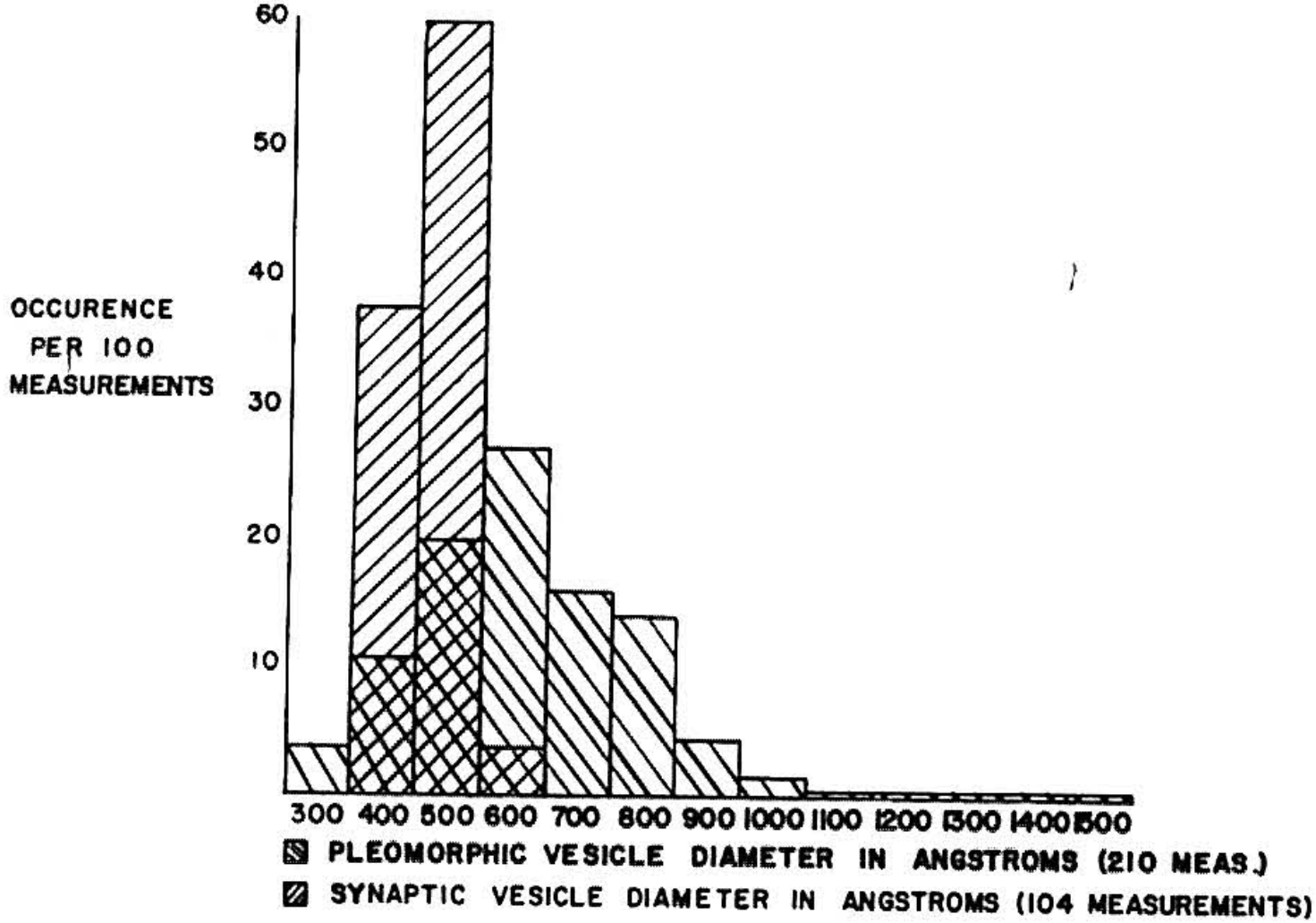
neurotubules are typical of those seen in other parts of the nervous system and appear to remain unchanged and intact in osmotically stressed animals.

The dark neurosecretion granules (NSG) are 1000 Å - 1500 Å in diameter and are seen in the nerve fibers and in their terminal dilations. The presently accepted concept is that these vesicles are manufactured in the supraoptic and paraventricular nuclei of the hypothalamus, packaged by the Golgi apparatus of the neuronal cell bodies in these areas and migrate down the axon as discrete granules, accumulating in the terminal portions of the axons. Large aggregations of these structures account for the colloidal droplets seen in light microscopic investigations, still occasionally termed Herring bodies.

There are two vesicular components in the axons of normally hydrated animals. The axon proper appears to contain only large electron dense granules. The synaptic-like vesicles, often termed synaptoid vesicles, are found in the axon endings which are frequently abutted against the outer basement membrane of the perivascular space. These vesicles, here termed synaptoid, contain no electron dense material in contrast to the larger granules. Table IV is a histogram of synaptoid vesicle diameter measurements. The average diameter was found to be 520 Å. Synaptoid vesicles are found densely packed and occasionally form a hexagonal array (fig. 9). Occasionally they are found in scattered groups among accumulations of the larger neurosecretion granules. They do not occur as isolated vesicles but as densely packed aggregations (figs. 4, 5, and 9). These vesicles resemble those found in other parts of the

TABLE IV

Histogram Comparing Size Distribution of
Synaptic and Pleomorphic Vesicle Diameters



mammalian central nervous system and in neuromuscular junctions. Their membranes measure about 50 Å in thickness.

Determinations of pleomorphic vesicle diameters are tabulated in plate IV. Notice the size distribution of the pleomorphic vesicles in comparison to the synaptic vesicles where the former vary widely from 325 Å to 1500 Å in diameter and the synaptic vesicles vary much less and range from 410 Å to 675 Å.

Large electron dense granules are a distinctive feature of the neurohypophysial nerve terminals. These granules are 1000 - 1500 Å in diameter and their limiting membrane is about 70 - 75 Å thick. They are distributed throughout the terminal among the synaptoid vesicles and the mitochondria. These large granules correspond to the neurosecretory granules of light microscopy that can be seen in sections of the neurohypophysis stained by chrome alum-haematoxylin or paraldehyde-fuschin. They occur not only in the pars nervosa but also in the hypothalamohypophysial tract and the supraoptic and paraventricular nuclei.

The endings, containing aggregations of granules, abut on the perivascular space and measure from ½ - 3 microns in diameter. Occasionally, however, much larger accumulations are seen in the neurons (fig. 18) and correspond to the Herring bodies seen in light microscopic studies. In this study one Herring body was observed which measured 21 microns in diameter.

Extracellular Space

An unusual finding in this investigation was the considerable

amount of extracellular space found in the pars nervosa. Values for this component were counted by the point counting method and the control animals varied from 14.1% to 14.8%.

The Pituicyte

The pituicytes are the only cellular structures of the posterior pituitary gland which are not neurons or connected with the vascular system. They are believed to be neuroglial in origin and are thought to be similar to the astrocytes seen in cerebral cortex (Farquhar and Hartmann, 1957; Schultz, Maynard and Pease, 1957). One can readily identify the pituicyte nuclei since they are the only nuclei located in the pars nervosa except for those in vascular elements. They are typical glial nuclei with a sparsely distributed pattern of dense chromatin material within a matrix of moderate density. The nuclei in normal animals measure from 6 to 16 microns in their longest dimension. The pituicyte cell has many ramifying processes which are intimately associated with neuronal endings and fibers (figs. 7, 11, 14, 22).

The pituicyte cytoplasm is easily distinguishable by the presence of many free ribosomes. Frequently these appear to be oriented either closely surrounding lipid droplets of the cytoplasm, or in association with rough endoplasmic reticulum (figs. 15 and 20). The cisternae of the rough endoplasmic reticulum occasionally contain a dense matrix. Mitochondria are distributed throughout the entire cytoplasm and are typical of glial cells. Microtubules are much less frequently seen than in neurons.

Smooth endoplasmic reticulum is seen occasionally but not frequently in the pituicyte cytoplasm. The most prominent example of this smooth endoplasmic reticulum is found in figure 15 where an elaborate system of tubules is easily observed.

The Golgi apparatus is very prominent and seems to be widely dispersed in the pituicyte cytoplasm. Frequently it is found in association with lipid droplets but not as intimately associated with them as are the ribosomal elements previously mentioned (figs. 15 and 28). One pituicyte seen in a montage contained 6 areas of Golgi membranes adjacent to 18 lipid droplets. The possible implications of these relationships will be discussed later.

The lipid droplets are not membrane bound and are relatively homogeneous with no apparent substructure. According to Gersh (1939) they are comprised predominantly of neutral unsaturated lipids. Diameters of these droplets measure from 600 - 1500 millimicrons with the usual size being about 1000 millimicrons. It was found in this study that the lipid droplets quite consistently show a halo effect where the periphery of the droplet exhibits greater electron density. Occasionally a large portion of the granule appears to contain this slightly darker material with small areas of lighter density. The significance of these observations is unclear. An example can be found in figs. 28 and 35. Occasional lysosomal structures are also seen in the pituicyte cytoplasm (figs. 14, 15, and 18).

Perivascular Space

Like other endocrine organs the posterior pituitary gland contains a rich vascular network. Exchange between the vasculature and parenchyma is greatly facilitated by an elaborate network of interconnecting perivascular spaces. These have been described in detail by otners (Liss, 1958; Wittkowski, 1967 and Barer, 1965).

The basement membranes are broad sheets which serve as barriers on either side of the perivascular space. The sheetlike nature of these membranes is demonstrated in fig. 13, where a tangential section fortuitously sliced the membrane longitudinally. The basement membrane has a width of about 600 Å. The inner layer of basement membrane, lying directly adjacent to the endothelial wall, is intimately associated with the endothelium and remains in close apposition to it. The outer layer is at various distances from the vessel and can be directly adjacent to the inner layer of the basement membrane. At times, however, spaces between these two layers have been observed as great as 100 μ . Although these occured in sections that stretched between adjacent vessels, it does give an idea of the extensiveness of the PVS. The usual size range is a few microns. Functional possibilities that could be correlated with this elaborate space will be discussed.

The fibroblasts are a classical component of the perivascular space. They are frequently seen and possess long, slender processes (fig. 32) reaching for considerable lengths from the perikarya. Their cytoplasm is dark with numerous ribosomes, both free and in association with rough endoplasmic reticulum (figs. 10 and 28).

Another component of the PVS is collagen fibers which are abundantly distributed throughout the PVS (figs. 4, 24, and 32). These collagen fibers demonstrate a periodicity (fig. 5) and generally tend to be about 375 - 475 Å in width. The collagen fibers are frequently seen in relation to the fibrocyte from which they are derived (fig. 32).

The two most frequently found nerve endings of the perivascular space are illustrated in fig. 8. These include endings with dark neurosecretion granules (NSG) as well as endings densely packed with tubular structures. The PVS also contains endings with synaptoid vesicles (figs. 4, 5, 6, and 9) and pleomorphic vesicles (figs. 27 and 40). The latter will be described below. Counts were made in two control animals of the relative volume of the various types of endings in the perivascular space. Table X shows that free space accounted for 69 and 61.4% of the total perivascular space for the two control animals. Those processes which contain dark NSG comprised 13.3 and 11.4% of the total PVS while the processes with no dark granules accounted for 6.8 and 12.6% of the total PVS. Thus it is apparent that there is no significant difference in the amounts of those endings with dark NSG and those without. Both occupy approximately the same amount of space in the PVS. The fibroblasts were found to occupy 10.7 and 14.5% of the total PVS for the two animals respectively. Since these counts were done at very low magnifications it was impossible to differentiate between the various types of endings that are included in the term "with no dark granules".

The degenerating Herring body seen in fig. 17 is an apparently normal constituent of the neurohypophysis. Many features indicating

degeneration can be seen such as myelin figure formations (fig. 37). These multi-lamellar bodies consist of several layers of membrane and these bodies are found in various stages of formation. Also to be found in these degenerating cell processes are remnants of neurotubules. These are present as shattered fragments of tubular material randomly scattered.

CHRONIC DEHYDRATION EXPERIMENT

In order to verify the state of the animals in this experiment, their weights were recorded during their respective water deprivation periods and these figures are given in table V.

Axons

In an animal deprived of drinking water for 2½ days there is a marked decrease in the overall number of dark neurosecretion granules (table VI). There are nerve terminals completely lacking in neurosecretion granules as well as numerous terminals with their full compliment of dark NSG. Almost invariably, however, those terminals whose dark NSG are depleted display numerous small vesicles which will here be termed pleomorphic vesicles. These vesicles are different than those encountered in the normal situation, and are different than the synaptoid vesicles normally found in the control animals. They range in size from 325 to 1500 Å and exhibit variable shapes. Their average size was 685 Å. They may be oval or spherical as are the synaptoid vesicles. They may also be irregularly shaped, appearing to be partially collapsed (fig. 27).

The degree of depletion of the NSG appears to be proportional

TABLE V

WEIGHT LOSS OF ANIMALS IN CHRONIC WATER DEPRIVATION EXPERIMENT

The following figures represent the averages of the weight in grams of 4 animals for each group.

		Days Thirsted		
Day	7 Day Thirst	4 Days	2½ Days	1½ Days*
0	about 300	315	298	303
18 hrs.	272			284
1	272			
1½		285	272	271
2	257			
2½			261	
3	246	258		
4		247		
4½	229			
5				
6	209			
7	198			

*Average of 3 animals

to the time of dehydration, with fewer present in the 4 day group and very few dark NSG in the 7 day animals. The relationships of the NSG depletions are shown in table VI where fibers containing NSG for the various groups are compared.

Table VII compares for each group the percentage of total area of the cell processes which had no dark granules in them. Since these counts were made on low power micrographs, the exact nature of each ending could not be distinguished. Consequently, some pituicyte small processes as well as neuronal processes are included.

While increased water deprivation decreased NSG it also produced increasing amounts of the pleomorphic vesicles as well as resulting in the disappearance of the prominant synaptic vesicle accumulations seen in the normal animal. Vesicles the size and shape of the synaptic vesicles, occasionally occur in the dehydrated animals; however, the usual groups of synaptic vesicles seen in the control were not noted in dehydrated animals.

In an effort to distinguish further the differences between the typical synaptoid vesicles and the pleomorphic vesicles, measurements of membrane thicknesses, from center to center of the outer dark layers, were made. These measurements show that synaptoid vesicle membranes are about 50 Å wide. Pleomorphic vesicles have a membrane width of about 70 - 75 Å which is similar to that of the NSG.

In the four day dehydrated animals occasional nerve endings can be found densely packed with neurosecretion granules. A prominent feature of this group is the increased number of endings in the peri-

vascular space (figs. 32, 33, 34, and 36). Endings found in the perivascular space are not unusual but in the experimental animal there are more of them and their appearance changes. Where they were predominantly filled with dark granules in the controls, they now are filled with two types of structures. The pleomorphic vesicles already described are frequently found, some endings containing formed elements which appear to be either flattened or tubular. They are probably tubular since close examination of figs. 15 and 31 shows that when these structures are viewed in longitudinal sections the triple-layered membrane structure is not easily visible but is quite consistently seen in cross sections. This would lead one to conclude that they are tubules instead of flattened vesicles since the trilaminar structure is seen best when the angle of section is not longitudinal with the membrane but rather at right angles to it. It is not certain that these are neural endings. Figure 34 illustrates that in the 2½ day animal there is a blending of the normal vesicular structures of neurons with the tubular structures within the same cell. This would imply that the other cells exhibiting these tubular structures are neurons.

Occasionally one sees endings in the perivascular space containing densely packed dark neurosecretion granules. The usual components of the PVS such as collagen fibers and fibroblast processes are also seen. The processes with tubular structures can also be found in the parenchyma of the gland i.e. outside the PVS.

Another unusual finding is the occasional appearance of a nerve ending with a dark matrix which makes the clear vesicles prominent.

The significance of this type of ending is unknown (figs. 28 and 29).

The clear pleomorphic vesicles were not readily distinguishable in the control animals. Table XI summarizes their occurrence for the various groups. In the 2½ day animal they accounted for 26.2% of the total area counted and in the 7 day water deprived animal they occupied 25.7% of the area.

Pituicytes

The pituicytes exhibit no significant variation in number or volume as indicated by the quantitative studies. In the four day water-deprived animal an unusual pituicyte was encountered. This cell is shown in fig. 35. It is identified as a pituicyte on the basis of the normal cytoplasmic constituents. This cell appears to be undergoing mitosis and might be in metaphase although this is difficult to determine because the section is thin and its orientation is unknown. Nuclear fragments are shown and upon close examination microtubules can be seen randomly scattered. The prominant feature of the cell is the extensive vesiculation within the cytoplasm which is not typical of normal pituicyte cytoplasm. This is the only such cell encountered in the 60 animals examined.

Perivascular Space

Counts were made of the processes found in the spaces surrounding the capillaries. The results of these counts are found in table VIII where the percentage of PVS occupied by cell processes with dark granules is shown. The striking scarcity of dark vesicles in the

TABLE VI

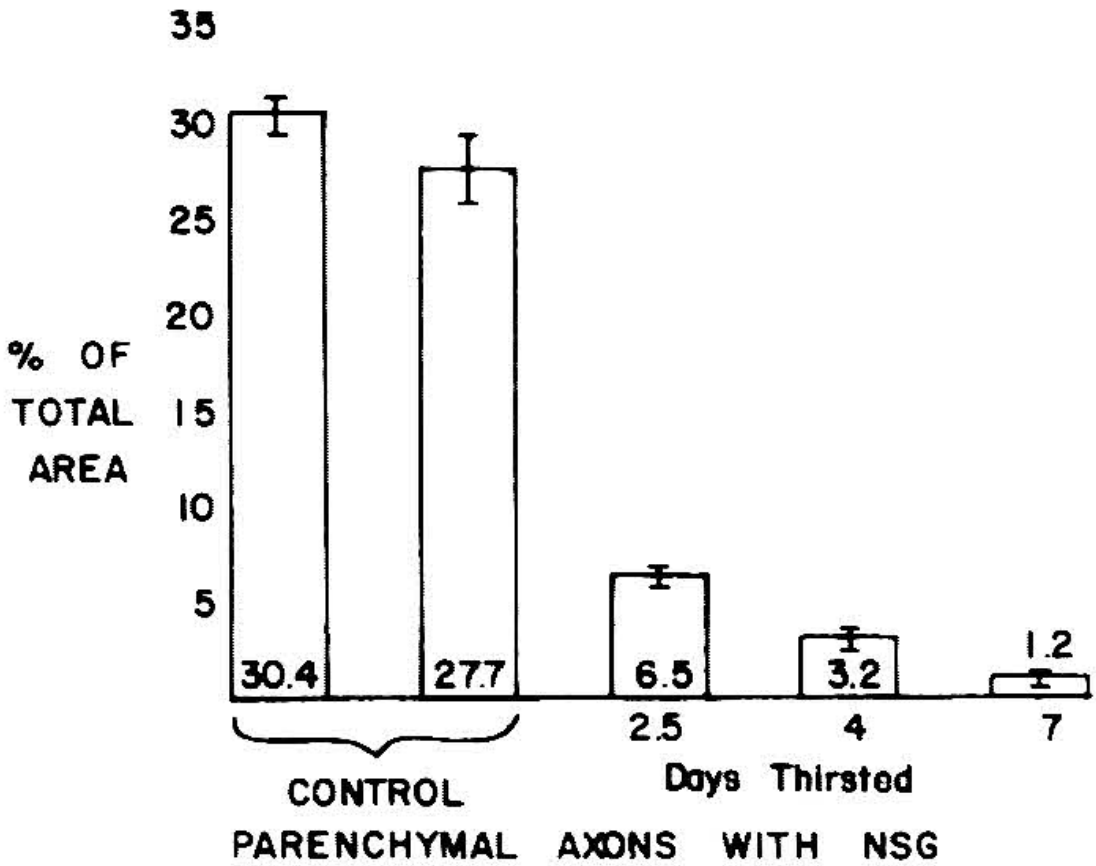

35
30
25
20
15
10
5
% OF TOTAL AREA
30.4
27.7
6.5
3.2
1.2
2.5
4
7
CONTROL
Days Thirsted
PARENCHYMAL AXONS WITH NSG

TABLE VII

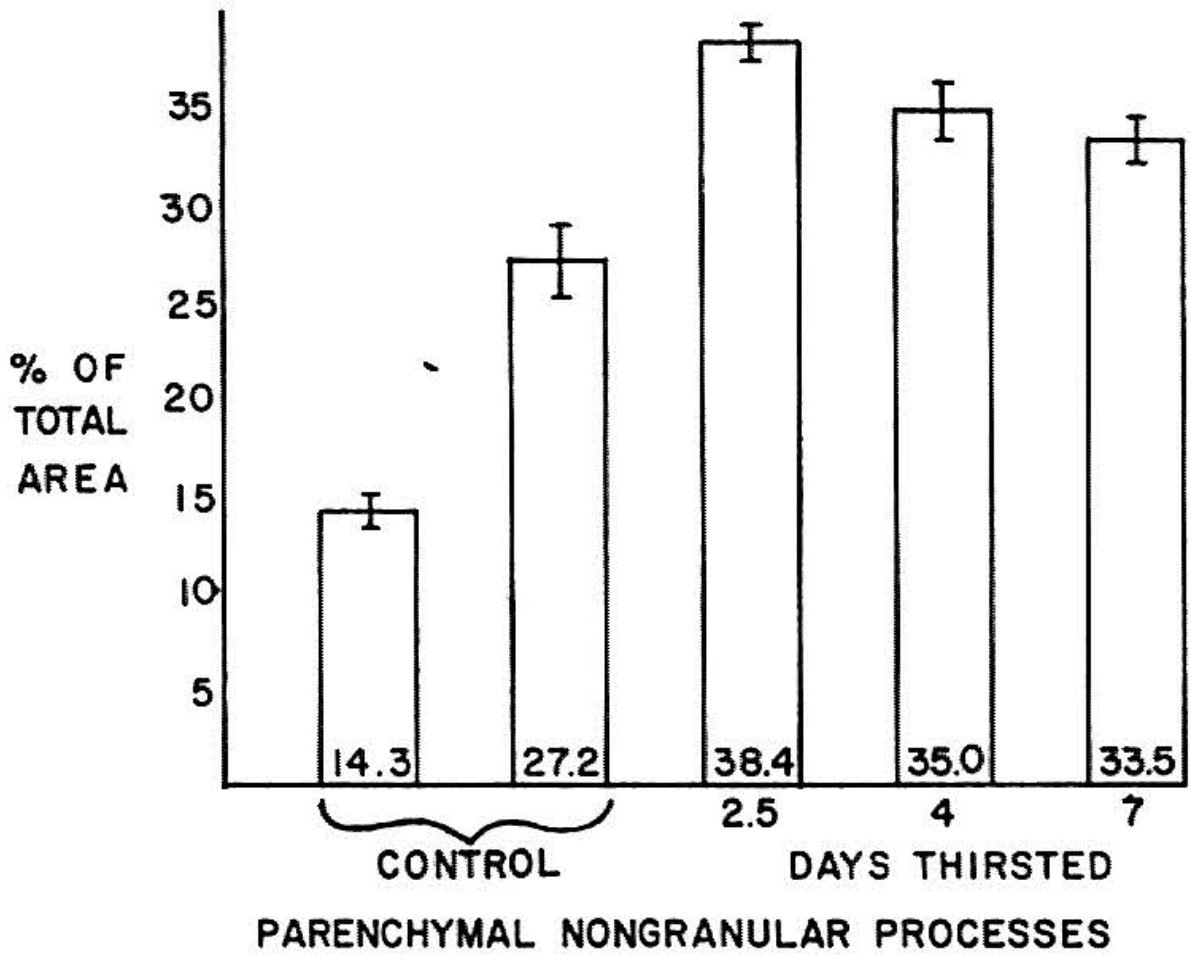
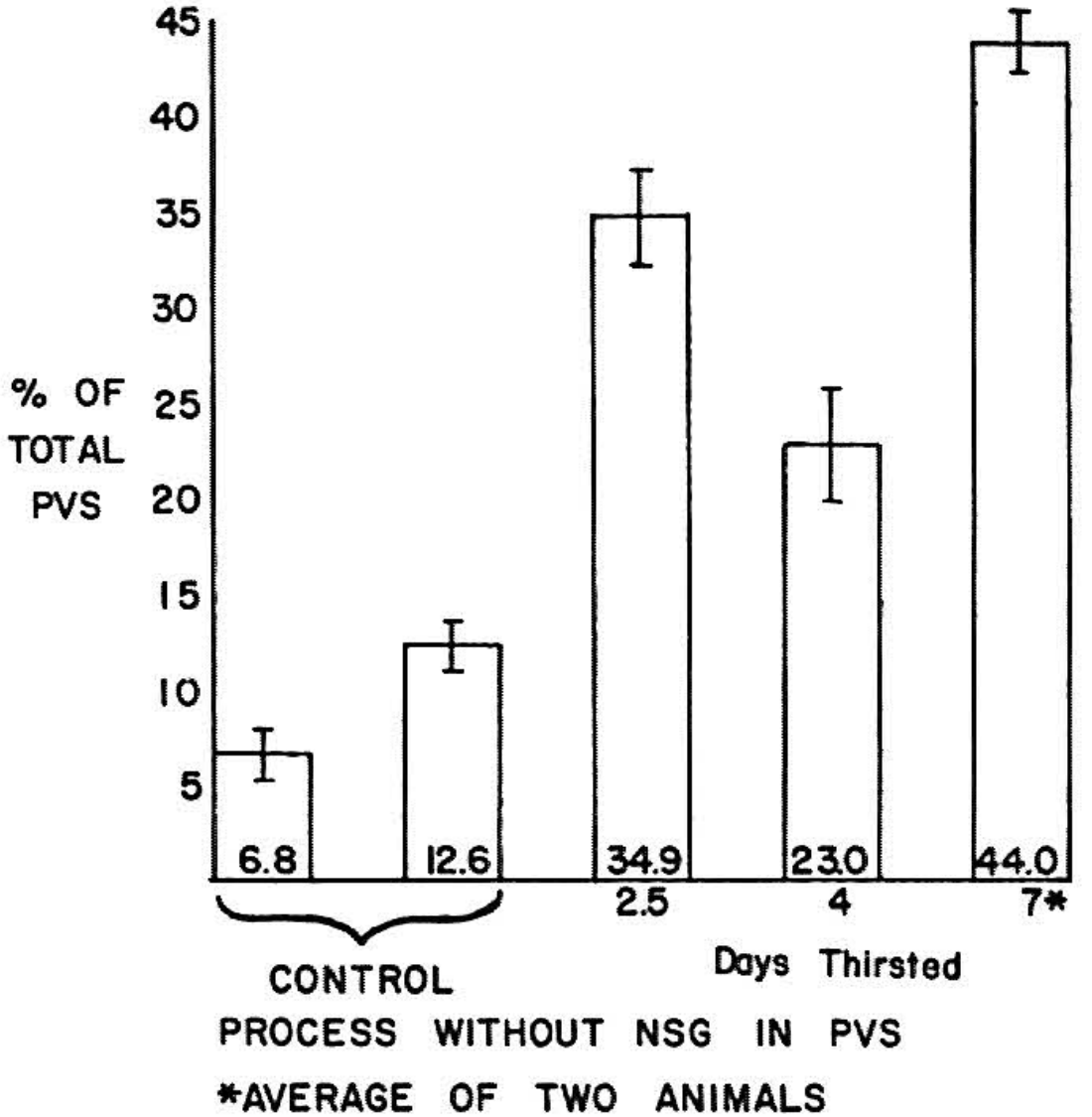

% OF TOTAL AREA
35
30
25
20
15
10
5
14.3
27.2
38.4
35.0
33.5
CONTROL
2.5
4
7
DAYS THIRSTED
PARENCHYMAL NONGRANULAR PROCESSES

TABLE IX



PROCESS WITHOUT NSG IN PVS

*AVERAGE OF TWO ANIMALS

TABLE X

SUMMARY OF PARS NERVOSA MEASUREMENTS

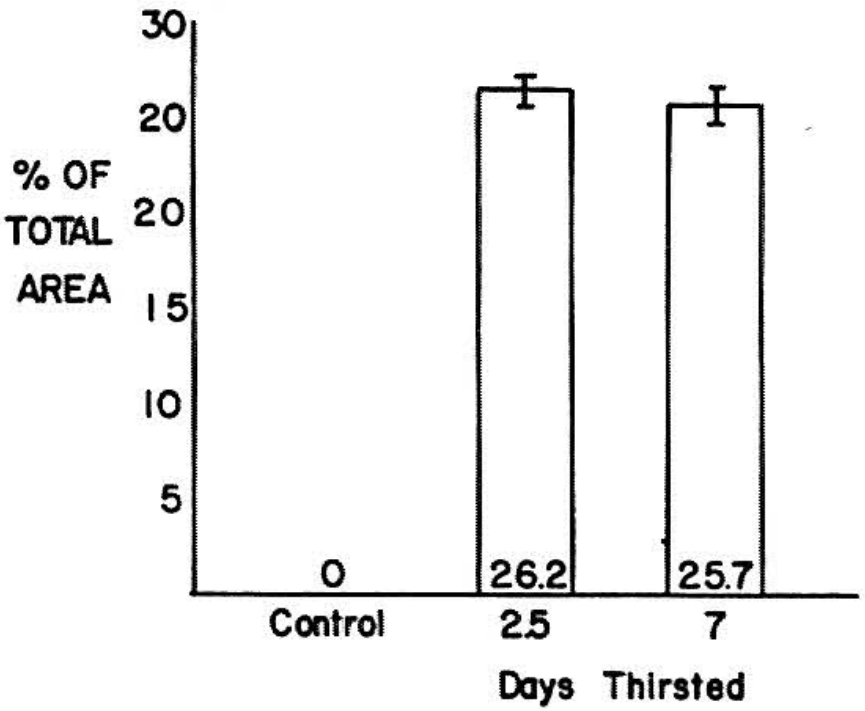
n = total number of counts
m = number of counts
s = standard deviation

		High Power Low Power	n	%	m	s	n	%	m	s	n	%	m	s	n	%	m	s	n	%	m	s
PVS	Free Space	x	354	69	245	.025	332	61.4	204	.026	398	51.5	205	.025	200	65.5	131	.034	282	47	132	.030
PVS	Processes with NSG	x	354	13.3	47	.018	332	11.4	38	.011	398	1.3	5	.006	200	2	4	.010	282	3.3	9	.010
PVS	Processes with no NSG	x	354	6.8	24	.013	332	12.6	42	.012	398	34.9	139	.024	200	23	46	.030	282	44	124	.030
PVS	Fibroblasts	x	354	10.7	38	.016	332	14.5	48	.013	398	12.3	49	.016	200	9.5	19	.021	282	6.1	18	.015
Total Tissue	Processes with no NSG	x	2038	14.3	291	.008	2287	27.2	623	.018	2909	38.4	1117	.009	1335	35	467	.013	1395	33.5	469	.013
Total Tissue	Extracellular Space	x	2038	14.1	288	.008	2287	14.4	330	.013	2909	10.6	309	.006	1335	20.5	274	.011	1395	13.0	181	.009
Total Tissue	Axons with NSG	x	2038	30.4	619	.010	2287	27.7	633	.018	2909	6.5	189	.005	1335	3.2	43	.005	1395	1.2	17	.003
Total Tissue	Pituicyte	x	2038	13.6	278	.008	2287	9.6	220	.011	2909	15.8	461	.007	1335	16.7	223	.010	1395	15.4	213	.010
Total Tissue	Total PVS	x	2038	17.4	354	.008	2287	14.5	332	.013	2909	13.7	398	.006	1335	14.9	200	.010	1395	20.6	282	.011
Total Tissue	Vessel + PVS	x	2038	27.7	562	.010	2287	21.0	481	.016	2909	28.6	833	.008	1335	24.5	327	.012	1395	36.7	511	.013
Total Tissue	Nerve Endings with NSG	x	2193	44.7	980	.010					3338	16.1	536	.006					2331	10.9	255	.006
Total Tissue	Extracellular Space	x	2193	19.3	424	.008					3338	12.9	430	.006					2331	30.4	708	.010
Total Tissue	Pleomorphic Vesicles	x	2193	0	0	.000					3338	26.2	876	.008					2331	25.7	600	.009
Total Tissue	Synaptoid Vesicles	x	2193	5.7	124	.005					3338	1.9	64	.002					2331	8.5	199	.006
Total Tissue	Nerver Fibers with NSG	x	2193	16.4	360	.008					3338	20.3	678	.007					2331	12.8	300	.007
Total Tissue	Pituicyte	x	2193	12.7	278	.007					3338	22.2	740	.007					2331	11.2	262	.007

no measurements available

no measurements available

TABLE XI



ENDINGS CONTAINING PLEOMORPHIC VESICLES

PVS of the water-deprived animals is demonstrated in this table. Table IX shows the occurrence in the PVS of processes without dark granules and indicates a marked increase in fibers which contain no dark granules. This category included those nerve processes which contain pleomorphic vesicles as well as those which contain the flattened or tubular structures. The micrographs counted for this comparison were of low magnification and therefore differentiation between the various types of clear vesicles was not feasible.

The PVS area occupied by fibroblasts did not vary significantly with the osmotic state nor did the free space of the PVS. The total figures for all the counted parameters are found in table X. Total volume for the PVS did not change significantly with changing conditions nor did the pituicyte volume. The extracellular space was found to vary slightly but this was probably due to the sampling variations previously discussed, rather than to any experimental procedures.

ACUTE HYPEROSMOLALITY EXPERIMENT

There were virtually no changes observed in the specimens of the pars nervosa which had been subjected to acute osmotic stress. This absence of change is discussed later.

DISCUSSION

Experimental Methods

Chronic. The experimental procedures employed with chronic osmotically stressed animals are similar to those used by Enestrom (1967), Howe and Jewell (1959), Zambrano and DeRobertis (1967) and others for the study of the supraoptico-neurohypophysial system. Similar results could have been achieved using other methods of dehydration such as infusion of hypertonic saline or by hypertonic drinking water. The latter might be preferable since it would limit the stress to hyperosmolality rather than other features of the response, such as increased blood volume.

One advantage of the method used was that the state of the animals could be documented by daily weight determination (table V).

Acute. The method used for acute osmotic response was a relatively rapid infusion of hypertonic saline solution through the femoral vein. While it is true that a number of factors are known to cause vasopressin release (see Sawyer, 1966), blood volume and osmotic pressure of the plasma are the most important. However, since the osmotic pressure of the blood produces the strongest response (Sawyer, 1966) an acute response was elicited using this method. Table II shows that there was an osmotic stimulus as evidenced by the serum osmotic checks taken at perfusion. Thus, it can be reasonably assumed that animals in the acute experiments were experiencing increased vasopressin release.

The fact that there was neither obvious depletion of NSG nor occurrence of pinocytosis in the acute experimental group indicated that

these are not the usual methods for accomplishing relatively minor adjustments in body osmolality. This inference is tenable only if one assumes that increased vasopressin release was occurring.

The disadvantage of this acute method is that while the osmotic load is being increased rapidly, so is the blood volume which would have the opposite effect on the system. But since control animals were infused with equal volumes of isotonic saline, this effect was discounted. Further proof of the net effect was the increase in serum osmolality which verified the osmotic stress (table II).

Quantitative Methods

In studies such as this, few attempts have been made by electron microscopists to quantitate morphological data. In this report, however, quantification has been employed to facilitate comparisons.

The error inherent in the sampling methods used has been evaluated by two methods. It must be remembered that standard error calculations used in the graphs and listed in table X represent only an estimation of the error that is present in the counting technique. This does not take into consideration such errors as sample and biological variations. Although the standard errors are not absolute, they serve as useful indices for comparison.

The Hormones

While the present study has not attempted to elucidate problems concerning the biochemistry or pharmacology of neurohypophysial hormones, the following material is presented as a background for the discussion

of the data and findings of this paper.

Isolation of two highly purified, physiologically active substances from mammalian posterior pituitary (see Sawyer, 1961) has precipitated an intensification of research activity on the pharmacology and physiology of neurohypophysial hormones.

Heller (1966) has compiled a comparative chart showing the phyletic distribution and chemical composition of the variations of these hormones. Their chemistry has been the subject of intense investigation. The active principles are known to be polypeptides with a molecular weight of about 1,000. These hormones can be extracted from the gland in combination with a protein of molecular weight about 30,000 (Sawyer, 1961). It is not known if the hormones are released from granules bound to the carrier protein, or in a free state. The latter, however, is usually assumed.

Van Dyke, et. al. (1941) felt that the protein which he isolated contained vasopressin and oxytocin. Acher (1956, 1957) and coworkers later showed that it was more likely that the protein represented the "carrier substance" to which the peptides were bound in a relatively loose association during transport and storage (Sawyer, 1961).

Numerous morphological studies, for example those of Palay (1957), have indicated that vasopressin is largely contained within membrane-bound vesicles. Sachs (1963) has recently verified these findings. He studied the occurrence of vasopressin among various cell organelles and found that the greatest biological potency, as measured by bioassayable pressor activity in the rat, was located in the neuro-

secretory granules. The activity of the granules from the hypothalamo-median eminence complex (3-5 units/mg) was much less than that observed for the neurosecretory particles isolated from homogenates of neural lobe tissue (14-24 units/mg).

Interesting developments have been made recently with respect to the hormone carrier proteins. Chauvet, et. al. (1960) have investigated in several species of mammals the inactive portion of the van Dyke protein which they have termed "neurophysin". They found that vasopressin and oxytocin can combine with neurophysin regardless of species origin of the peptides or neurophysin. Later, various workers recognized the fact that with starch-gel electrophoresis of neurophysin, several different proteins could be separated (Hope, et. al., 1964, Frankland, et. al., 1966). Interest was further intensified by findings in 1964 (Gaitan, Cobo and Mizrachi) that the two polypeptide hormones were released separately in man. This led to speculation that there was a separate carrier protein for each hormone. Dean and Hope (1966, 1967) isolated two different protein constituents from neurosecretion granules of bovine posterior pituitary glands. Similar proteins were found in extracts of acetone-dried powder of the same tissue. Hollenberg and Hope (1968) analyzed and characterized the extracted material and Dean and Hope (1968) verified the results with highly purified NSG, accomplished by sucrose density gradients at 145,000 g for up to 5 hours. They found comparable amino acid constituents in proteins examined by both methods. Neurophysin-II was distinguished from neurophysin-I by the absence of histidine. It was found that neurophysin-I and oxytocin are stored

together in neurosecretory granules which are different than those in which neurophysin-II and arginine vasopressin are stored (Dean, Hope and Kazic, 1960). These findings were the result of density gradient centrifugation.

Bindler, Labella, and Sanwal (1967) also working with the bovine posterior pituitary separated nerve endings into three fractions. They found that each was a nearly homogeneous population of "neurosecretosomes", and that they differed from each other in their vasopressin oxytocin (VP/OT) ratios. VP was localized in denser and OT in lighter neurosecretosomes. This corroborated earlier studies in which similar observations had been made in subfractions of neurosecretory granules. There were, however, no apparent morphological differences among the three subfractions. For some time it had been felt that there were particles specifically containing either OT or VP (Labella, Beaulieu and Reiffenstein, 1962) and now this idea seemed confirmed.

More recent studies (Hollenberg and Hope, 1968) showed that "neurophysin-I binds three molecules of either vasopressin or oxytocin whereas neurophysin-II binds only two molecules of each hormone per molecule of protein. Complexes containing two molecules of oxytocin and one molecule of 8-arginine vasopressin per molecule of protein are formed by neurophysin-I and -II; both proteins appear to possess three polypeptide-binding sites per molecule."

Now, however, it appears that there may even be a third neurophysin, at least in the bovine posterior lobe. Using ion-exchange chromatography three protein peaks have been obtained. The first and

third are neurophysin-I and -II respectively. The protein producing the intermediate peak was identified electrophoretically as the minor soluble-protein component of NSG (Rauch, Hollenberg, Hope, 1968). This protein apparently can bind hormone and its presence suggests the possibility of a third peptide that might be associated with the third neurophysin.

Hormone Release

The problem of mechanisms by which the neurohypophysial hormones are released is one which has received much attention recently. Douglas and Poisner (1964a and 1964b) have described a mechanism of release of vasopressin which involves depolarization followed by a calcium-dependent link. They visualize a system in which an action potential arrives at the pars nervosa from hypothalamic centers and this impulse stimulates an uptake of calcium in the endings. They also point out that sometimes the uptake of calcium and the secretion of hormone fail to parallel each other. The depolarization step is implicated by experiments in which high K levels and electrical stimuli caused vigorous hormone secretion and Ca uptake.

These authors (Douglas and Poisner, 1964b; Poisner and Douglas, 1968) are among many who have noted similarities between the neurohypophysial system and adrenal medullary hormone release mechanisms. Further studies (Poisner and Douglas, 1968) indicate that ATP and ATPase may participate in the release of hormones.

Ginsburg (1968) presents a detailed discussion of the "enhanced

diffusability" theory of hormone release. Two possible ways in which Ca^{++} could act to cause release are, first, by converting the hormones to a more readily diffusible form or, secondly, by promoting an exocytic or reverse pinocytotic process involving the fusion of granular and neuronal membranes. The implications of each of these theories on the basis of other known factors are discussed in detail (Ginsburg, 1968).

Dyball (1968) has recently reported examination of physiologic factors affecting the release of vasopressin under carefully controlled experimental conditions. He found the three most effective stimuli (hemorrhage vagal stimulation, and intracarotid injection of $Ca\ Cl_2$) released both VP and OT but in different proportions.

Bodian (1965, 1966) has described morphological evidence for a neuroapocrine method of secretion in the neurohypophysis of the monkey. According to his description the neurosecretory endings are liberated intact into the PVS and then into the vessel lumen. The evidence for this is based on breaks in the basement membranes and in the endothelial wall itself. The feasability of such a mechanism is difficult to consider and breaks such as those reported are frequently caused by inadequate preparation techniques. To use this evidence for the proposal of such a mechanism of release seems unwarranted. The present study does not indicate the existence of such a mechanism in the rat.

Neuronal Considerations

Synaptoid Vesicles. Many workers have observed that with increased osmotic stress a depletion of the neurosecretory granules (NSG)

and an increase of small electron-lucid vesicles occurs (Gerschenfeld, et al, 1960; Bern, et al, 1966; Hartmann, 1958; Palay, 1957; Barer and Lederis, 1966; Daniel and Lederis, 1966; Palay, 1955; Monroe, 1967). These vesicles appear identical to those which have been described as cholinergic (DeRobertis and Bennett, 1954). These synaptic-like vesicles have been the subject of much debate among researchers, some feeling that they contain neurotransmitter substances such as acetylcholine (Gerschenfeld, et al, 1960) while others have suggested the possibility that these vesicles are seen as a result of release of hormone and represent remnants or fragments of the neurosecretory granules (Knowles and Bern, et al, 1966; Scharrer and Kater, 1969).

Synaptic-like vesicles ranging in size from 300-500 A in diameter have not been proven to contain cholinergic neurotransmitter. Therefore, in this paper they will be termed synaptoid.

In an effort to elucidate the role of cholinergic neurotransmitters in the pars nervosa considerable work has been done by LaBella, Bindler and coworkers. Their finding of cholineacetylase in the posterior pituitary (LaBella, 1968) could be indicative of a cholinergic mechanism. Its enzyme activity, however, relative to that in brain and other known cholinergic tissues, was of a low order and a relatively large proportion was of the nonspecific or butryl variety (see LaBella, 1968). LaBella (1968) reported that acetyl cholinesterase has been found in the pars nervosa but at about 1/10th the enzymatic activity of brain and that butryl cholinesterase is also present at about the same level as the brain. From centrifugation studies LaBella indicated that these

substances may be associated with a membranous component. The presence of acetylcholine in the bovine posterior lobe has also been shown (LaBella, 1968) and is estimated to be 1/5 to 1/10 that found in beef cerebral cortex. It appeared to be localized in the fractions containing neurosecretory and microvesicular components, thus providing strong support for the hypothesis that the small clear vesicles in the endings are "synaptic" and that they do contain acetylcholine.

The findings concluded that the "cholinergic trinity" i.e. acetylcholine, acetylcholinesterase, and cholineacetylase, were all present in the bovine posterior pituitary and that their presence was approximately in the same proportions as in the brain but at about 20% of their concentrations in brain.

The assumption that smaller vesicles in the nerve endings contain this cholinergic system, was based on the fact that small clear synaptoid vesicles were present and provided a logical place for the acetylcholine to be located.

LaBella (1968) did not agree with the findings of DeRobertis and coworkers (Gerschenfeld, 1960) that the microvesicles (synaptoid vesicles) probably contained cholinergic transmitter substance. Instead, he hypothesized the existence of separate specific cholinergic nerve fibers in the posterior lobe for the acetylcholine which he found to be present in fractions containing the vesicles. His conclusions were the result of observations of synaptoid vesicles in tissue sections and subcellular fractions of the bovine posterior pituitary. He felt that the vesicles referred to by most as synaptic vesicles in the endings

with the NSG were instead "ghosts" of the NSG. In centrifugation fractions he observed apparent vesiculations of 40-80 millimicron diameter. These were occurring in NSG which had lost their electron opacity and exhibited membrane breaks.

The present work supports in part, several interpretations. The small clear vesicles seen in the endings are believed to be synaptoid (Gerschenfeld, et al, 1960) and the clear vesicles are membrane remnants or "ghosts" (Holmes and Knowles, 1960; Knowles, 1963; Lederis, 1963; LaBella, 1968). Gerschenfeld, et al (1960) have assumed that the numerous vesicles they saw in osmotically stressed animals were the same as those in the normal. The findings in this study show that they are different, in size, membrane width and packing density (figs. 9, 19, 30, and 41). It is the opinion of this writer that the increased vesicles seen after depletion of the NSG are not the same as those which are seen in the normal pars nervosa. Thus the findings of (Holmes and Knowles, 1960) Knowles (1963) and Lederis (1963) would seem to be valid when the fact of this difference is considered.

The hypothesis of LaBella (1968) that cholinergic components are in a separate nerve ending would seem completely unfounded histologically since there is no available evidence suggesting the existence of nerve fibers in the pars nervosa which are separate from the pituicyte and the NSG-containing fibers. Such fibers were not seen in the present study nor have they been described elsewhere. The occurrance of small endings filled only with synaptoid vesicles would be expected to occur occasionally since some sections would inevitably cut through part of an

axon ending or through a swelling where the NSG were not present at that point. The actual finding of such an ending in the normal animal has been much less frequent in this study than might be expected even assuming that they are all components of the same type nerve ending.

The findings here support those of Gerschenfeld and coworkers (1960) for the toad in which they reported synaptic vesicle membrane widths of 40 - 50 Å. The membranes in this study were found to be 50 Å as compared to 70 - 75 Å for the membrane width of the NSG and the cell plasma membranes. The fact that these membranes differ in width and staining intensity strongly suggests that they are separate structures and that the synaptic vesicles are not merely fragmentations of the NSG. The possibility of NSG dissolution with reconstitution of the membranes into the synaptic vesicles cannot be excluded but seems extremely unlikely. This study agrees with Holmes and Kiernan (1964) that the synaptic vesicles probably represent a distinct class of vesicles separate from the NSG.

Resolution of the problem might be pursued by various means. The number of pleomorphic vesicles could be counted to see if they outnumber the amount of NSG that would have occupied a given ending in the normal state. One could also calculate the membrane surface area of the NSG in the control animal and the pleomorphic vesicles in the dehydrated animals and compare total membrane area to see if a correlation could be made concerning the origin of the pleomorphic vesicles.

Neither of the above techniques was employed since one would expect a constant flow towards the distal ends of neurons with a

continual buildup of pleomorphic vesicles, if the axoplasmic flow theory which forms the basis for the concept of migration of NSG from the hypothalamus is correct. If one accepts the hypothesis (Daniel, Lederis, 1966; Lederis, 1965; Barer, 1965) that there are swellings along the axons, these could conceivably be involved in releasing hormones. Thus calculations of distal endings would be meaningless.

Pleomorphic Vesicles. Pleomorphic vesicles were found to vary in maximum diameter from 325 Å to 1500 Å or more. As can be seen in table IV most were in the range of 500 Å to 700 Å. This might suggest that these vesicles were undergoing a process of shrinking and that the 2½ day interval was the time delay which displayed them at the 500 - 700 Å size. Checks were done in the 7 day animals and the size of the pleomorphic vesicles was found to be similar to the 2½ day animals. This probably means that these pleomorphic vesicles either rapidly shrink from 1500 Å to the 500 - 700 Å size, or that the disappearance of the depleted vesicles does not occur until these vesicles have remained for a while. The fact that they are not found in sizes smaller than 325 Å could be due to the fact that they are not recognized as vesicles or that they do not shrink further before they disappear. In future experiments this area will be investigated. Tentatively it is planned to dehydrate and then rehydrate the animals and study the fate of pleomorphic vesicles.

Kobayashi, et al (1961) have also noted vesicles of intermediate size in parakeets. According to their measurements, synaptic vesicles were 390 Å in diameter and these intermediate vesicles, 490 Å.

They felt that the ovoid intermediate vesicles could be derived either from swelling of synaptic vesicles or from vesiculation and breakdown of the large pale vesicles which had already discharged their neurosecretory contents. Intermediate vesicles are slightly smaller than those reported in this study but they corresponded with those observed by other investigators (Bern, et al, 1966). The explanation for this is not obvious but one must realize that these studies were done with earlier fixation and embedding techniques. Palay (1957), for example, found the synaptic vesicle and NSG membranes to be 30 Å and 50 Å respectively in thickness while in this study they are 50 Å and 70 Å. The differences found in membrane thickness due to various fixation variables are discussed in detail by Karlsson (1966) and Lillibridge (1968). Karlsson also showed that there are differences in membrane width dependent on direction of tissue sectioning.

The interesting finding here is that the pleomorphic vesicles have a membrane width of 70 - 75 Å which corresponds to that found surrounding the NSG. This suggests that they are made of similar membranes and it is possible that they are derived from the NSG.

The concept that synaptoid vesicles are a distinct class of vesicles is strengthened by the following observations. The dark NSG in normal animals and the pleomorphic vesicles of stressed animals are found all along the axons, and synaptic vesicles are found only in terminals of the neurons. A factor that strengthens the idea that these pleomorphic vesicles are actually NSG "ghosts" or residuals is the recent finding that vesicles of 400 - 800 Å and the larger NSG membrane fragments

are localized in the same density-gradient fraction of bovine secretory endings (LaBella, 1968).

Axonal Swellings. Barer and Lederis (1966) have described swellings at intervals in nerve fibers of the rabbit neurohypophysis. Lederis (1965) has also reported finding them frequently in the human pars nervosa. They enclose the neurosecretory granules, mitochondria and a few microtubules, and are connected by narrow nerve fibers containing primarily neurotubules.

The only instance of similar swellings found in the rat, in the more than 1000 micrographs used in this study, was that shown in fig. 14. The observed swellings are connected by a length of a narrow fiber similar to those described by Barer and Lederis. It is apparent, however, that these structures are not typical of axons pervading the pars nervosa in the rat.

Tubular structures such as that seen in fig. 31, 32, and 33 have not been described previously. They are found more frequently in cell processes of the perivascular space than in those of the parenchyma, but their presence in the latter cannot be precluded (figs. 27, 28, and 41). The significance of these unusual structures is unknown. They are very similar to the smooth endoplasmic reticulum seen in the pituicyte (fig. 15). It is conceivable that they represent the same organelle in the axonal endings of the posterior lobe. One would expect, however, to see this endoplasmic reticulum more evenly dispersed within the axons. It appears, instead, to be confined to occasional fibers and is densely packed. The reason for its frequent occurrence in the PVS is not clear.

It is conceivable that the increased numbers of small vesicles seen in stimulated animals could be a result of reorganization and synthesis of membrane material. The smooth endoplasmic reticulum would be expected to be present if TPNH-producing systems were needed in lipid synthesis for membranes. The finding of tubular endings in the PVS but not in the parenchyma of normal animals will be discussed in a later section.

Multilamellar Structures. The observation of multilamellar structures (figs. 17 and 37) is not original with this study. Bodian (1963), Holmes and Kiernan (1964), Daniel and Lederis (1966), and Lederis (1965) have reported such structures. Lederis has suggested that the synaptic vesicles seen might originate from these concentric membranes. Similarly, Holmes and Kiernan (1964) have suggested that these structures give rise to membranous vesicles and used this idea as a basis for postulating the existence of a synthetic process in the distal axon of the hedgehog. Knowles has demonstrated similar distal vesicle formation in the pericardial organ of a crustacean, Squilla mantis (Knowles, 1964). Dellmman (1969) has attributed these multilamellar structures in the bovine posterior lobe to the degeneration of axons in which they are located. According to his views these structures represent one step in the physiological process of degeneration due to disuse. Daniel and Lederis agree with this concept (1966).

Multilamellar structures seen in the present investigation differ from those mentioned above. The concentric lamellae are tightly packed and more closely resemble myelin formation. This can be explained

on the basis of fixation procedures since immersion fixation, as already mentioned, is inferior to the procedures employed here. The findings in this study support the explanation of Dellmman (1969) in that multilamellar structures are invariably found within areas whose organelles are typical of degenerating fibers (figs. 17 and 37).

It is conceivable that with degeneration, the extensive membranes within the fibers undergo dissociation and this material becomes oriented in a lamellar fashion. It is well known that phospholipids change their molecular organization in different environments (Glauert, 1967).

Release Nonspecificity. Since this experimental study utilized only osmotic stimuli and since the pars nervosa is the release sight of two hormones, one of which has no known bearing on osmotic stimuli, one might expect to find some of the neurons depleted of material and some still containing their full complement of neurosecretory material. Instead, all neurons appear to be releasing hormone even though some of them represent cells whose perikarya lie in hypothalamic nuclei which are not primarily involved in osmotic control, e.g. the paraventricular nuclei. This apparent paradox has long been recognized and it is known that all stimuli bring about a simultaneous release of vasopressin and oxytocin (Barer, et al, 1963), but in a variable ratio depending on the stimuli. The complete dynamics of the releasing factors are not known. For a discussion of work done in this area the reader is referred to Barer, et al (1963) and LaBella (1968). It is generally accepted that the supraoptic nucleus is mainly concerned with the production of

vasopressin and the paraventricular nucleus with the production of oxytocin. It is also assumed that there are no interruptions of the nerve fibers in their course to the pituitary. Thus, one can expect a given neural ending to contain one or the other of the hormones.

NSG Specificity. The evidence that the NSG are associated with the hormones produced by the posterior pituitary comes from several sources. Light microscopic studies showing stainable inclusions can be correlated with the NSG of electron microscopy. It can be demonstrated that they appear and disappear together. Also, density-gradient centrifugation experiments (LaBella, Reiffenstein, and Beaulieu, 1963) showed that fractions containing hormonal activity were the fractions that contained the NSG. Further studies (Bindler, LaBella, and Sanwal, 1967; LaBella, Beaulieu and Reiffenstein, 1962) showed that the hormones were predominantly found in separate granules in the neurohypophysis. The vasopressin/oxytocin ratio increased progressively from the lighter to the more dense fractions, indicating that vasopressin is bound to heavier granules than is oxytocin (see LaBella, et al, 1963).

The logical question is whether there are any visible morphological distinctions between endings that would reflect the hormonal content of a particular ending. Bindler and coworkers (1967) have found no such differences in their centrifugation separations using electron microscopy, and their findings agree with those of others (Barer, Heller, Lederis, 1963) using thin sections of conventionally prepared material.

No convincing morphological evidence for such differences has been observed in this study although there was a suggestion of variation

in the tissues whose fixative contained small amounts of ethyl ether or acetone. The result of these additions to the fixative was decreased electron opacity. In some sections certain nerve swellings appeared more pale than adjacent processes but the findings were inconclusive. Further studies in this area might be informative.

Blood Vessels

The existence of fenestrated capillary endothelium in the pars nervosa was first described by Duncan (1956) in the chicken, Gallus domesticus. Its fenestrations are different than those found in other organs such as the kidney (Pease, 1955). Palay (1957) has suggested that membranous diaphragms described in the rat, (figs. 12 and 16) are formed by the fusion of the inner and outer plasmalemmae of the endothelial cells. These layers are not easily seen and Hartmann (1958) has pointed out that one cannot be certain of the origin of the diaphrams on the basis of their appearance. The present work supports this view, in that at no time was this bridge between gaps of the endothelium resolvable into 2 membrane layers. Bargmann and Knoop (1957) report the absence of pores in the endothelium of the neurohypophysis of the cat and dog.

Capillary endothelium in the present study resembles that described by others (Duncan, 1956; Palay, 1957; and Hartmann, 1958). The clear vesicles in the endothelium (figs. 23, 32, and 34) have been described as representing pinocytotic activity (Palade, 1953) and have been seen to occur more frequently with increasing secretory activity (Hartmann, 1958). This increase in clear vesicles was not seen to be

significant in this study and therefore the pinocytotic theory is questioned.

Perivascular Space

While the presence of parenchymal cell processes in the perivascular space is well known in the anterior pituitary (Rinehart and Farquhar, 1955), Palay (1957) has found that neurosecretory substance is never seen in the perivascular space of the neurohypophysis of the rat. Hartmann (1958) observed neuronal endings in the PVS but they were always surrounded by basement membrane. Fujita and Hartmann (1961) saw no secretory granules in the PVS of rabbit neurohypophysis in normal or stimulated animals.

The findings in this study of free endings in the space is not unprecedented since Wittkowski (1967) describes neuronal processes in this space in the guinea pig where they are seen to contain synaptic vesicles and the usual electron-dense neurosecretory granules. They are not surrounded by a basement membrane. Wittkowski feels they make synaptic connections with the "adventitial" cells in the perivascular space.

Such synaptic connections were not seen in this study nor were neuro-glial synaptic contacts seen in the other areas of the pars nervosa as reported by Wittkowski (1968). These have not otherwise been reported and the pictures showing synapses are not convincing. Fujita and Hartmann (1961) saw a few nerve endings in the PVS of adrenaline-injected rabbits and assumed this to be the result of PVS enlargement following

injection of adrenaline.

An interesting finding in this investigation was the quantitative increase of endings found within the perivascular space of osmotically stressed animals (see table X). Not only were there more of them, but their inclusion elements were different. This variation was associated with the changing inclusions of axons outside the PVS. They appear to change together, indicating that they are portions of the same cells. With osmotic stress they lost the dark NSG which were replaced with two types of structures. Pleomorphic vesicles similar to those found in the axons are frequently seen in endings within the PVS. The other structures are tubular shaped. The latter are similar in appearance to those seen in the pituicyte of fig. 15 but are probably neuronal elements. The occurrence of these tubular vesicles in the PVS of a typical nerve axon is taken as evidence that these vesicles or tubules when found in the PVS are in neurons rather than in pituicytes.

The fact that there was no significant change in the number and appearance of the cell types in the PVS in the acute osmotically stressed animals may indicate that the appearance of these endings in the PVS is not part of the usual acute release of hormones. Instead it is possibly a secondary result of the continued release of the hormones. If it were a significant method for their routine release, one would expect that the acute animals would demonstrate many protrusions of axonal processes into the PVS. It is evident that these protrusions are in some way concerned with the increase in hormone release. As has already been discussed, they are quite probably of the same cell type as

that found in the parenchyma of the gland. The relative amount of tubular inclusion does not seem to be proportionate inside and outside the perivascular space. This would indicate that the presence of the endings in the PVS is not just a simple result of overpopulation or hypertrophy.

Wittkowski (1967) has suggested that the presence of axons in the PVS may reflect the action of some type of regeneration process and that these axons are extruded into the PVS as a result of excessive growth.

The endings found in the perivascular space evidently get there by protruding through the basement membrane and becoming isolated in the PVS. A simple overproduction of the fibers in a response similar to regenerative growth (Wittowski, 1967) does not seem to be adequate to produce this protrusion since the space is no doubt filled with a fluid. With increased pressure outside the basement membranes this space could be changed and a protrusion would not inevitably result. There have been no micrographs in this study which clarify whether or not the endings found in the space are still attached to axons outside the space or are, subsequent to protrusion, pinched off and exist in the space as simple bags or sacs. The latter would not be likely since an open cell process is usually found to have a loss of background matrix density, and these exhibit no such loss. Instead they appear to be typical neuronal axons. Serial section studies are contemplated for the future to clarify questions as to the actual size, shape and attachment of these swellings. Micrographs in this study (figs. 10, 25, 28, and 29) show what appear to

be cell protrusions into the space. Wittkowski felt that he saw similar occurrences in the guinea pig. It is highly probable that these axonal processes in the PVS represent some as yet undescribed physiological process related to hormone release.

Barer (1966) was one of the first to speculate on the functional significance of the wide and ubiquitous perivascular space. He considered it a vast spongework of mucopolysaccharide or mucoprotein complex in which the secreted hormones can pool. The extensiveness of the PVS has been verified by this study. Large montages have been prepared in which 10 to 12 capillaries have been found to be completely interconnected by an extensive perivascular space.

Barer (1966) suggests that this extensive space provides a plausible explanation consistent with the findings that hormone depletion of the gland is not a concomitant of decreased electron-dense vesicles (Daniel and Lederis, 1966).

It has been pointed out (Daniel and Lederis, 1966) that the total hormone content of the gland probably represents the algebraic sum of release and rates of synthesis and transport.

The mucopolysaccharide spongework Barer envisages would be a possible pool for hormone whose electron opacity has been lost due to chemical transformations involved with release.

It was possible to follow the basement membrane much farther than the few microns reported by Barer (1965). It has been followed in this study for several hundred microns. Its continuity was demonstrated wherever the space was seen to ramify. This achievement was attributed

to the degree of preservation attained.

Palay (1957) has described macrophages as being a cellular component of the PVS in addition to the fibroblasts. These have not been evidenced in the present study. Their presence might help to explain the function or fate of axonal endings found free in the PVS.

The Pituicyte

Before the theory of neurosecretion was postulated it was widely assumed that the pituicyte was the site of hormone production (see Hartmann, 1958). An historical account of this theory is outlined by Fujita and Hartmann (1961). These authors described three types of pituicytes in the rabbit. One of these corresponded to the astrocyte as described in cerebral cortex by Farquhar and Hartmann (1957) and Schultz, Maynard, and Pease (1958). Another was similar to the microglia (Farquhar and Hartmann, 1957; Schultz, et al, 1958). The third was an atypical astrocyte with increased numbers of cytoplasmic organelles. Wittkowski (1968) distinguishes between fibrous and protoplasmic glia cells in the rat neurohypophysis. Such differentiation was not possible in the present study. Some pituicytes do appear to have more lipid material in them than others and occasionally the lipid material varies in electron opacity, but these differences could not be attributed to various types of pituicytes.

Some investigators have found that there are always pituicyte processes located between the axonal endings and the outer limits of the perivascular space. This has been described as a glial "cuff" (Bern,

et al, 1966) in the white-crowned sparrow. Glial interposition has also been reported in the parakeet (Kobayashi, et al, 1961), and the rat (Palay, 1957). The significance of this arrangement is not clear to these investigators, but since pituicyte processes exist between the secretory cells and the blood stream, it is assumed "highly probable that glia mediate" hormone release (Bern, et al, 1966). This relationship was not observed in the rat pars nervosa in this study (figs. 4, 5, 8, and 24) although glial cells were occasionally observed directly adjacent to the basement membrane. The method by which this mediation takes place, or to what extent it is important in the physiological control of release, is not known. It is obvious that these cells are metabolically active and recent findings have augmented information concerning them. Sunde, et al (1969) have shown that osmotically stressed rats show an increase in uptake of ^{3}H-uridine into RNA. Radioautography showed that the label was localized primarily in the nuclear region of pituicytes in control and osmotically stimulated animals. This increase with stimulation did not occur in similarly treated cerebral cortex or hypothalamic tissue slices. Thus, a stimulus producing increased vasopressor secretion also affects RNA metabolism in pituicytes.

It is generally assumed that free ribosomes are associated with endogenous protein production and ribosomes associated with the endoplasmic reticulum (rough ER) are involved with exogenous protein synthesis (Hicks, et al, 1969; Birbeck and Mercer, 1961). The pituicytes have both (fig. 15). It is possible that there is a vital metabolic interrelationship between axons and pituicytes much the same as that

existing between Schwann cells and peripheral nerve axons. Sjostrand has hypothesized a similar relationship between neurons and glia in the central nervous system (Sjostrand, 1960). It is conceivable that pituicytes provide substrates and/or enzymes needed by neurons and that they work together as one metabolic unit. It might be helpful in this respect to determine whether they function normally when isolated.

Since the pituicytes are seen to have close relationships to the perivascular space and the outer basement membrane (Kobayashi, et al, 1961; Bern, et al, 1966; figs. 37, 40, and 41), it is possible that they are involved in metabolism of carrier proteins, perhaps releasing their enzymes into the PVS where the substrate is also released.

The nerve endings, which are in close proximity to the pituicytes, are known to have a highly active hexosemonophosphate shunt, as evidenced by a high C-1/C-6 glucose oxidation ratio (Krass and LaBella, 1965). Because elevated C-1/C-6 ratios have been found in all endocrine tissues (see LaBella, 1968), and because this high ratio is present in the pars nervosa but not in the paraventricular nucleus (Krass and LaBella, 1965) it appears that the pentose shunt is concerned with hormone storage and/or secretion rather than hormone synthesis.

The significance of the extensive distribution of free ribosomes in the pituicytes is not known. However, since free ribosomes are indicative of endogenous protein synthesis (Hicks, et al, 1969; Birbeck and Mercer, 1961) this finding would fit in with others indicating the metabolic activity of these cells. The lipid granules which undoubtedly represent sites of active lipid turnover would necessitate endogenous

enzymes. It has been shown recently that free polysomes can be involved in membrane synthesis (Ragnotti, et al, 1969).

Wittkowski (1968) has reported seeing synaptic contacts in the rat neuroglial appositions. These have also been shown in the eel pars nervosa (Knowles and Vollrath, 1965). The micrographs showing synapses in the eel are much more convincing than those in the rat. Further evidence is needed to substantiate the existance of electrical connection between rat pituicyte and the axons.

The existence of lipid granules in rat pituicytes is well documented (Palay, 1957; Hartmann, 1958) although these droplets were not seen in the rabbit pituitary (Fujita and Hartmann, 1961). The exact composition of the granules is not known except that they contain neutral unsaturated lipids (Gersh, 1939). They do not appear to be present in significantly different amounts in osmotically-stressed animals although there was some suggestion of this in 7 day animals.

The explanation of the presence of these lipid granules in the pituicytes is unknown. The fact that their surface is the site of accumulation of many small dense particles is, of itself, interesting. These particles appear to be the same size as, and resemble free polysomes. Well-formed Golgi membrane complexes are often observed in close proximity to clumps of these lipid droplets. It is noteworthy that the Golgi apparatus is frequently associated with polysaccharide production (Neutra and LeBlond, 1966). The possibility exists that these lipid granules may in fact be glycolipids or lipoproteins. The occurrence of the dark halos at the periphery of the lipid granules and areas of

greater density is an interesting finding but of unknown significance.

The finding of a cilium (fig. 11) in a pituicyte process is unusual and unprecedented. Similar structures have been found in the neurons of the lateral geniculate body in rats (Karlsson, 1966). Schultz (1969) has observed such a cilium in a sub pial astrocyte in the cerebral cortex. The functional implications of such structures in these locations are obscure.

Rodrigues, et al (1969) have observed dense-core microtubules in glial cells. Similar glial tubules 600 A in diameter have been described in the white-crowned sparrow (Bern, et al, 1966). Dense-core microtubules were not seen in this study.

The finding in fig. 15 of an elaborate smooth endoplasmic reticulum system in a glial cell is interesting. It closely resembles that seen in adrenal cortex and testes (Fawcett, 1966). The functional implications of this feature of the pituicyte are not clear but it is known that smooth endoplasmic reticulum can be associated with enzymes of lipid synthesis. Also, enzymes of the pentose-phosphate shunt may be associated with the smooth endoplasmic reticulum (Altman and Chayen, 1966). This is considered an indication of the metabolic nature of the pituicytes.

The existence of a pituicyte undergoing mitosis (fig. 35) was another unexpected finding. The occurrence of this in an animal thirsted for 2½ days might be related to the metabolic activity. This would be another indication that pituicyte activity is increased with increasing neuronal activity.

Extracellular Space

One unusual feature was the extraordinary amount of extracellular space found in the pars nervosa. In control animals this was found to vary between 14.1% and 14.8% of the total volume of the tissue as estimated by the point sampling method described earlier. The presence of so much space is surprising for two reasons. First, the cerebral cortex and other parts of the central nervous system in similarly fixed animals (Karlsson, Schultz, 1964) are found to contain virtually no extracellular space and most adjacent cell processes are found to be apposed with tight junctions. In several animals in this study cerebral cortex was also examined and found to contain little or no extracellular space. Secondly, in no other study of the neurohypophysis has ECS been emphasized. Indeed, the pictures in virtually all other studies show relatively closely apposed plasma membranes and little extracellular space. The amount of ECS found in the neural lobe in this study tends to fit with that which physiologists calculate should be present in the nervous system in order to explain the extracellular ion pool.

The explanation for the ECS is difficult but it is well known that the amounts of space can be changed by varying fixation procedures (Schultz and Karlsson, 1965). One must evaluate fixation techniques on the basis of several criteria and it has been found in this study that the techniques used resulted in relatively "good" fixation. Since there were rarely indications of poor fixation it can be assumed that the amounts of space found in this study more closely resemble the living

condition than those reported previously. Indeed, others show that virtual absence of extracellular space results with the poor fixation techniques they employed, such as immersion of the tissue into the fixative instead of perfusion of the fluid through the vascular system.

The findings support observations of others that at no time does one find NSG or vesicles in the ECS, but that they are at all times surrounded by cellular plasms membranes.

The possibility cannot be ruled out that the extracellular space seen in this study could represent a pathway for the release of hormone. It is conceivable that the hormone could diffuse out of the cell processes at sites other than juxta-capillary or pericapillary locations and then molecularly disperse and be secreted thru the peri-capillary space and endothelium into the lumen. This possibility is supported by the continuity that exists between the ECS and the 500 Å - 2000 Å gap present between the cell processes and the outer basement membrane of the PVS. The extensive space found in this study would represent the anatomical basis for such a pathway.

Another possible explanation for the finding of such extensive ECS is the consideration of the fact that the neurohypophysis is one part of the nervous system which does not exhibit the usual blood-brain barrier (Barer, 1965). It has been pointed out by Barer that while the neurohypophysis is derived from nervous tissue, its vascular organization is quite unlike that of other parts of the nervous system and closely resembles that of other endocrine organs. On this basis it might be assumed that the extracellular space of the posterior lobe of the

pituitary might also react differently to fixation and that their extracellular space in life may be entirely different from the central nervous system.

SUMMARY AND CONCLUSIONS

In an electron microscopic study of the posterior pituitary gland, 60 Sprague-Dawley rats were used. Morphological evidence of hormone release was studied and quantitative methods, using the point-counting technique, were also employed. One experimental group consisted of animals chronically stressed by water deprivation for varying periods of time. A second group of animals was acutely stressed by slowly infusing hypertonic saline into the femoral vein.

The animals were sacrificed by perfusion fixation with phosphate buffered 3% glutaraldehyde and the pars nervosa was postfixed in phosphate buffered 1% osmium tetroxide. Following embedding in Vestopal, tissues were sectioned and stained with lead citrate and uranyl acetate and examined with a Siemens IA electron microscope.

In the chronic experimental group, a marked depletion of neurosecretory granules (NSG) occurred beginning between 1½ and 2½ days after withdrawal of drinking water. At the end of 7 days of thirst the number of nerve endings containing NSG dropped to 5% of the normal level.

Accompanying the drop in dark NSG in the chronic animals was a concomitant increase in the electron-lucent vesicles which are smaller than the NSG. These pleomorphic vesicles are irregular in size and shape.

Evidence is presented to support the view that the small synaptic-like vesicles present in the normal animal are a distinct class of vesicle and are different from the NSGs and the pleomorphic vesicles.

Observations indicate that the pleomorphic vesicles seen after hormone release are NSG-residual vesicles and represent "ghosts" or

remnants of the hormone-containing NSG and are probably not synaptic vesicles. Endings containing pleomorphic vesicles are rarely found in normal animals but in chronically dehydrated animals they account for 26% of the total area.

Occasionally tubular cisternae, probably of smooth endoplasmic reticulum, were seen in the axons of both normal and chronically stressed animals. These irregular tubular structures appeared more frequently in stressed animals and were present in axons in the PVS as well as in the parenchyma. They were rarely seen within processes containing other vesicular components. It was concluded that they represent a previously undescribed metabolic phase of hormone release, since they were much more numerous in stressed animals.

The existence of numerous axonal swellings as described for the rabbit and human has not been observed, with only one instance of possible axonal swelling seen.

The perivascular space (PVS) in normal and experimental animals is interposed between two basement membranes. Each membrane is continuous, rarely exhibiting breaks or discontinuities, and can be followed without interruption for several hundred microns.

The PVS is known to contain fibroblasts and collagen. This study showed that axonal processes are also present and have no sleeve of basement membrane around them. In normal animals the majority of these processes contain dark NSG. Fibers with these granules account for about 12% of the total PVS. In chronically stressed animals the NSG-containing fibers occupy only approximately 2% of the PVS while the total for axonal

processes increased from 22% in normal animals to 33%. The importance of this finding is discussed in relation to hormone release.

The PVS in both normal and experimental animals was found to interconnect extensively, forming an elaborate network. The percentage of total area occupied by PVS did not change significantly with osmotic stress. The functional implications of such an extensive space are discussed.

The pituicytes occupied about 16% of the total area of the posterior lobe in normal animals and this figure did not change significantly with chronic osmotic stress. The glial cuffs reported by some investigators to lie between axonal endings and the PVS were seldom seen.

The role pituicytes play in hormone release remains obscure, but several indications of metabolic activity in these cells were observed.

An unexpected finding was the existence of about 14% extracellular space which did not vary consistently with experimental conditions. Tissue from the cerebral cortex of these same animals was examined and contained very little extracellular space. These findings are discussed.

BIBLIOGRAPHY

Acher, R. 1966 Chemistry of neurohypophysial hormones in The Pituitary Gland. Ed. by G. W. Harris. University of California Press, Los Angeles, p. 269.

Altman, F. P. and J. Chayen 1966 The significance of a functioning hydrogen transport system for the retention of 'soluble' dehydrogenases in unfixed sections. J. Royal Mic. Soc., 85: 175.

Barer, R. 1965 The ultrastructure of small blood vessels of the posterior pituitary gland in relation to neurosecretion. Bibl. Anat., 7: 304.

Barer, R., H. Heller, K. Lederis 1963 The isolation, identification and properties of the hormonal granules of the neurohypophysis. Proc. Roy. Soc. (London Series B), 158: 388.

Barer, R. and K. Lederis 1966 Ultrastructure of the rabbit neurohypophysis with special reference to the release of hormones. Z. Zellforsch., 75: 201.

Bargmann, W. and A. Knoop 1957 Elektronenmikroskopische beobachtungen an der neurohypophyse. Z. Zellforsch., 46: 242.

Bargmann, W., A. Knoop and A. Thiel 1957 Elektronenmikroskopische studie an der neurohypophyse von Tropidonotus natrix (mit berucksichtigung der pars intermedia), Z. Zellforsch., 47: 114.

Bern, H. A. and F. G. W. Knowles 1966 Neurosecretion in Neuroendocrinology. Ed. by L. Martini and W. F. Ganong. Academic Press, New York, p. 139.

Bern, Howard A., Richard S. Nishioka, L. Richard Mewaldt and Donald S. Farner 1966 Photoperiodic and osmotic influences on the ultrastructure of the hypothalamic neurosecretory system of the white-crowned sparrow, Zonotrichio leucophrys gambelii. Z. Zellforsch., 69: 198.

Bindler, Elliot, Frank S. Labella and Madhu Sanwal 1967 Isolated nerve endings (neurosecretosomes) from the posterior pituitary. J. Cell Biol., 34: 185.

Birbeck, M. S. C. and E. H. Mercer 1961 Cytology of cells which synthesize protein. Nature, 189: 558.

Bodian, David 1951 Nerve endings, neurosecretory substance and lobular organization of the neurohypophysis. Bull. Johns Hopkins Hosp., 89: 354.

Bodian, David 1963 Cytological aspects of neurosecretion in opposum neurohypophysis. Bull. Johns Hopkins Hosp., 113: 57.

Bodian, David 1965 Herring bodies: a mechanism of neuroapocrine secretion. Anat. Rec., 151: 326.

Bodian, David 1966 Herring bodies and neuroapocrine secretion in the monkey. Bull. Johns Hopkins Hosp., 118: 282.

Cammermeyer, Jan 1960 The post-mortem origin and mechanism of neuronal hyperchromatosis and nuclear pyknosis. Exper. Neurol., 2: 379.

Chauvet, J., M. T. Lenci, R. Acher 1960 L'ocytocine et la vasopressine du mouton. Reconstitution d'un complexe hormonal actif. Biochim. biophys. Acta, 38: 266.

Christ, J. F. 1966 Nerve supply, blood supply and cytology of the neurohypophysis in The Pituitary Gland, Vol. 3. Ed. by G. W. Harris. University of California Press, Los Angeles, p. 62.

Daniel, A. R. and K. Lederis 1966 Effects of ether anesthesia and haemorrhage on hormone storage and ultrastructure of the rat neurohypophysis. J. Endocr., 34: 91.

Dean, C. R. and D. B. Hope 1966 Protein constituents of neurosecretory granules isolated from the posterior lobes of bovine pituitary glands. Biochem. J., 101: 17P.

Dean, C. R. and D. B. Hope 1967 The isolation of purified neurosecretory granules from bovine pituitary posterior lobes. Biochem. J., 104: 1082.

Dean, C. R. and D. B. Hope 1968 The isolation of neurophysin-I and -II from bovine pituitary neurosecretory granules separated on a large scale from other subcellular organelles. Biochem. J., 106: 565.

Dean, C. R., D. B. Hope and T. Kazic 1968 Evidence for the storage of oxytocin with neurophysin-I and of vasopressin with neurophysin-II in separate neurosecretory granules. Brit. J. Pharmacol., 34: 192P.

Dellmann, Horst-Dieter and Patricia Ann Owsley 1969 Ultrastructure of Herring bodies in the bovine neurohypophysis. Anat. Rec., 163: 176.

DeRobertis, E. and H. Stanley Bennett 1955 Some features of the submicroscopic morphology of synapses in frog and earthworm. J. Biophys. Biochem. Cytol., 1: 47.

Douglas, W. W. and A. M. Poisner 1964a Stimulus-secretion coupling in a neurosecretory organ: The role of calcium in the release of vasopressin from the neurohypophysis. J. Physiol., 172: 1.

Douglas, W. W. and A. M. Poisner 1964b Calcium movement in the neurohypophysis of the rat and its relation to the release of vasopressin. J. Physiol., 172: 19.

Duncan, Donald 1956 An electron microscopic study of the neurohypophysis of a bird, Gallus domesticus. Anat. Rec., 125: 457.

duVigneaud, V. 1956 Trail of sulfur research: from insulin to oxytocin. Science, 123: 967.

Dyball, R. E. J. 1968 Stimuli for the release of neurohypophysial hormones. Br. J. Pharmac., 33: 319.

Enestrom, Sverker 1967 Nucleus supraopticus, a morphological and experimental study in the rat. Acta Patholog et Microbiol. Scandin. Suppl., 186: 1.

Eranko, Olavi 1955 Estimation of relative volume in Quantitative Methods in Histology and Microscopic Histochemistry. Ed. by Little, Brown, and Company, Boston, p. 61.

Farquhar, M. G. and J. F. Hartmann 1957 Neuroglial structure and relationships as revealed by electron microscopy. J. Neuropath. Exp. Neurol., 16: 18.

Farrell, Gordon, Louis F. Fabre and Elizabeth W. Rauschkolb 1968 The neurohypophysis. Ann. Rev. Physiol., 30: 557.

Fawcett, Don W. 1966 The Cell. W. B. Saunders Co., Philadelphia, p. 161.

Frankland, B. T. B., M. D. Hollenberg, D. B. Hope and B. A. Schacter 1966 Dissociation of oxytocin and vasopressin from their carrier protein by chromatography on Sephadex G-25. Brit. J. Pharmacol., 26: 502.

Fujita, Hisao and J. Francis Hartmann 1961 Electron microscopy of neurohypophysis in normal, adrenaline-treated and pilocarpine-treated rabbits. Z. Zellforsch., 54: 734.

Gaitan, E., E. Cobo and M. Mizrachi 1964 Evidence for the differential secretion of oxytocin and vasopressin in man. J. Clin. Invest., 43: 2310.

Ganong, William F. 1967 Review of Medical Physiology, 3rd Ed. Lange Medical Publications, Los Altos, California

Gauer, O. H. and J. P. Henry 1963 Circulatory basis of fluid volume control. Physiol. Rev., 43: 423.

Gersh, I. 1939 The structure and function of the parenchymatous glandular cells in the neurohypophysis of the rat. Am. J. Anat., 64: 407.

Gerschenfeld, H. M., J. H. Tramezzani and E. DeRobertis 1960 Ultra-structure and function in neurohypophysis of the toad. Endocrinology, 66: 741.

Ginsburg, M. 1968 Molecular aspects of neurohypophysial hormone release. Proc. Roy. Soc. (Biol.), 170: 27.

Glauert, Audrey M. 1967 Electron microscopy of lipids and membranes. J. Royal Microscop. Soc., 88: 49.

Gorbman, Aubrey and Howard A. Bern 1962 A Textbook of Comparative Endocrinology. John Wiley and Sons, Inc., New York.

Ham, Arthur W. 1965 Histology. J. B. Lippincott Co., Philadelphia, p. 226.

Hartmann, J. F. 1958 Electron microscopy of the neurohypophysis in normal and histamine treated rats. Z. Zellforsch., 48: 291.

Heller, H. 1966 The hormone content of the vertebrate hypothalamo-neurohypophysial system. Brit. Med. Bull., 22: 227.

Hicks, Sonja J., J. W. Drysdale, H. N. Munro 1969 Preferential synthesis of ferritin and albumin by different populations of liver polysomes. Science, 164: 584.

Hollenberg, M. P. and D. B. Hope 1968 The isolation of the native hormone-binding proteins from bovine pituitary posterior lobes. Biochem. J., 106: 557.

Holmes, R. L. 1966 The neurohypophysis of the foetal monkey. Z. Zellforsch., 69: 288.

Holmes, R. L. 1968 The infundibular process of rodents of the genus Meriones. Z. Zellforsch., 85: 256.

Holmes, R. L. and J. A. Kiernan 1964 The fine structure of the infundibular process of the hedgehog. Z. Zellforsch., 61: 894.

Holmes, R. L. and F. G. W. Knowles 1960 "Synaptic vesicles" in the neurohypophysis. Nature (London), 185: 710.

Hope, D. B., B. A. Schacter and B. T. B. Frankland 1964 Dissociation of oxytocin, vasopressin and neurophysin by gel filtration. Biochem. J., 93: 7P.

Howe, A. and P. A. Jewell 1959 Effects of water deprivation upon the neurosecretory material of the desert rat (Meriones meriones) compared with the laboratory rat. J. Endocrin., 18: 118.

Karlsson, Ulf 1966 Comparison of the myelin period of peripheral and central origin by electron microscopy. J. Ultrastructure Res., 15: 451.

Karlsson, Ulf 1966 Three-dimensional studies of neurons in the lateral geniculate nucleus of the rat. I. Organelle organization in the perikaryon and its proximal branches. J. Ultrastruct. Res., 16: 429.

Karlsson, Ulf and R. L. Schultz 1964 Plasma membrane apposition in the central nervous system after aldehyde perfusion. Nature, 201: 1230.

Karlsson, Ulf and R. L. Schultz 1965 Fixation of the central nervous system for electron microscopy by aldehyde perfusion. I. Preservation with aldehyde perfusates versus direct perfusion with osmium tetroxide with special reference to membranes and the extracellular space. J. Ultrastruct. Res., 12: 160.

Karlsson, Ulf and R. L. Schultz 1966 Fixation of the central nervous system for electron microscopy by aldehyde perfusion. III. Structural changes after exsanguination and delayed perfusion. J. Ultrastruct. Res., 14: 47.

Kleeman, C. R. and R. E. Cutler 1963 The neurohypophysis. Ann. Rev. Physiol., 25: 385.

Knowles, F. G. W. 1964 Vesicle formation in the distal part of a neurosecretory system. Proc. Roy. Soc., B160: 360.

Knowles, Francis and Lutz Vollrath 1965 Synaptic contacts between neurosecretory fibres and pituicytes in the pituitary of the eel. Nature (London), 206: 1168.

Kobayashi, Hideshi, Howard A. Bern, Richard S. Nishioka and Yasuko Hyodo 1961 The hypothalamo-hypophyseal neurosecretory system of the parakeet, Melopsittacus undulatus. Gen. Comp. Endocrinology, 1: 545.

Kobayashi, Hideshi, Tetsuya Hirano and Yoshihiko Oota 1965 Electron microscopic and pharmacological studies on the median eminence and pars nervosa. Arch. d'Anatomie Microscopique, 54: 277.

Koelle, G. B. 1961 A proposed dual neurohumoral role of acetylcholine: its functions at the pre- and post-synaptic sites. Nature, 190: 208.

Krass, M. E. and F. S. LaBella 1965 Oxidation of 14-C-1 and 14-C-6 glucose by hormone synthesizing and hormone secreting portions of neurohypophysial neurons. Mol. Pharmacol., 1: 306.

Kurosumi, K., T. Matsuzawa and S. Shibasaki 1961 Electron microscopic studies on the fine structures of the pars nervosa and pars intermedia and their morphological interrelation in the normal rat hypophysis. Gener. Comp. Endocr., 1: 433.

Kurtz, Stanley M. 1961 A new method for embedding tissues in Vestopal W. J. Ultrastruct. Res., 5: 468.

LaBella, Frank S. 1968 Storage and secretion of neurohypophyseal hormones. Canad. J. Physiol. Pharmacol., 46: 335.

LaBella, F. S., G. Beaulieu and R. J. Reiffenstein 1962 Evidence for the existence of separate vasopressin and oxytocin-containing granules in the neurohypophysis. Nature, 193: 173.

LaBella, F. S., R. J. Reiffenstein and G. Beaulieu 1963 Subcellular fractionation of bovine posterior pituitary glands by centrifugation. Arch. Biochem. Biophys., 100: 399.

Lederis, K. 1963 A preliminary report on the ultrastructure of the human neurohypophysis. J. Endocr., 27: 133.

Lederis, K. 1964 The fine structure and hormone content of the hypothalamo-neurohypophysial system of the rainbow trout (Salmo irideus) exposed to sea water. Gen. Comp. Endocr., 4: 638.

Lederis, K. 1965 An electron microscopic study of the human neurohypophysis. Z. Zellforsch., 65: 847.

Lillibridge, Clinton B. 1968 Electron microscopic measurements of the thickness of various membranes in oxyntic cells from frog stomachs. J. Ultrastruct. Res., 23: 243.

Liss, L. 1958 Die perivaskularen strukturen des menschlichen neurohypophyse. Z. Zellforsch., 48: 283.

Monroe, Barbara 1967 A comparative study of the ultrastructure of the median eminence, infundibular stem and neural lobe of the hypophysis of the rat. Z. Zellforsch., 76: 405.

Monroe, Barbara and David E. Scott 1966 Ultrastructural changes in the neural lobe of the hypophysis of the rat during lactation and suckling. J. Ultrastruct. Res., 14: 497.

Neutra, M. and C. P. Leblond 1966 Radioautographic comparison of the uptake of galactose-H^3 and glucose-H^3 in the Golgi region of various cells secreting glycoproteins or mucopolysaccharides. J. Cell. Biol., 30: 137.

Oliver, G. and E. A. Schafer 1895 On the physiological action of extracts of pituitary body and certain other glandular organs. J. Physiol., 18: 277.

Oota, Y. 1963a Electron microscopic studies on the region of the hypothalamus contiguous to the hypophysis and the neurohypophysis of the fish, Oryzias latipes. J. Fac. Sci. Univ. Tokyo, 10: 143.

Oota, Y. 1963b Fine structure of the median eminence and the pars nervosa of the mouse. J. Fac. Sci. Univ. Tokyo, 10: 155.

Oota, Y. 1963c Fine structure of the median eminence and the pars nervosa of the turtle, Clemmys japonica. J. Fac. Sci. Univ. Tokyo, 10: 169.

Oota, Y. and H. Kobayashi 1962 Fine structures of the median eminence and pars nervosa of the pigeon. Anat. Zool. Jap., 35: 128.

Oota, Y. and H. Kobayashi 1963 Fine structure of the median eminence and the pars nervosa of the bullfrog, Rana catesbeiana. Z. Zellforsch., 60: 667.

Palade, G. E. 1953 The fine structure of blood capillaries. J. Appl. Physics, 24: 1424.

Palay, S. L. 1955 An electron microscopic study of the neurohypophysis in normal, hydrated and dehydrated rats. Anat. Rec., 121: 348.

Palay, S. L. 1957 Ultrastructural and Cellular Chemistry of Neural Tissue. Ed. by H. Waelsh. Hoeber-Harper, New York, p. 31.

Pease, D. C. 1955 Electron microscopy of the vascular bed of the kidney cortex. Anat. Rec., 121: 701.

Poisner, A. M. and W. W. Douglas 1968 A possible mechanism of release of posterior pituitary hormones involving adenosine triphosphate and an adenosine triphosphatase in the neurosecretory granules. Mol. Pharmacol., 4: 531.

Porter, K. R. and F. Kallman 1953 The properties and effects of osmium tetroxide as a tissue fixative with special reference to its use for electron microscopy. Exp. Cell Res., 4: 127.

Ragnotti, G., G. R. Lawford and P. N. Campbell 1969 Biosynthesis of microsomal NADP-cytochrome C reductase by membrane-bound and free polysomes from rat liver. Biochem. J., 112: 139.

Rauch, R., M. D. Hollenberg, D. B. Hope 1968 Isolation of a third neurophysin from bovine pituitary posterior lobes. Biochem. J., 110: 38P.

Reynolds, Edward S. 1963 The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell. Biol., 17: 208.

Rinehart, J. F. and M. G. Farquhar 1955 The fine vascular organization of the anterior pituitary gland; an electron microscopic study with histochemical correlations. Anat. Rec., 121: 207.

Rodriquez Echandia, E. L., R. S. Piezzi and E. M. Rodriquez 1969 Dense-core microtubules in neurons and gliocytes in the toad Bufo arenarum hensel. Am. J. Anat., 122: 157.

Rodriquez, E. M. and J. LaPointe 1969 Histology and ultrastructure of the neural lobe of the lizard, Klauberina riversiana. Z. Zellforsch., 95: 37.

Roth, L. M. and S. A. Luse 1964 Fine structure of the neurohypophysis of the opossum. J. Cell. Biol., 20: 459.

Rothballer, A. B. 1966 Pathways of secretion and regulation of posterior pituitary factors. Res. Publ. Assoc. Res. Nervous Ment. Dis., 43: 86.

Sachs, Howard 1963 Studies on the intracellular distribution of vasopressin. J. Neurochem., 10: 289.

Sawyer, Wilbur H. 1961 Neurohypophysial hormones. Pharmacol. Rev., 13: 225.

Sawyer, Wilbur H. 1966 Biological assays for neurohypophysial principles in tissues and in blood in The Pituitary Gland, Vol. 3. Ed. by G. W. Harris. University of California Press, Los Angeles.

Sawyer, Wilbur H. and Elliott Mills 1966 Control of Vasopressin secretion in Neuroendocrinology, Vol. I. Ed. by Luciano Martini and William F. Ganong. Academic Press, New York.

Scharrer, Berta and Stanley B. Kater 1969 Neurosecretion. XV. An electron microscopic study of the corpora cardiaca of Periplaneta americana after experimentally induced hormone release. Z. Zellforsch., 95: 177.

Scharrer, Ernst and Berta Scharrer 1954 Hormones produced by neurosecretory cells. Recent Progr. Hormone Res., 10: 183.

Schultz, R. L., E. A. Maynard and D. C. Pease 1957 Electron microscopy of neurons and neuroglia of cerebral cortex and corpus callosum. Amer. J. Anat., 100: 369.

Schultz, R. L. and Ulf Karlsson 1965 Fixation of the central nervous system for electron microscopy by aldehyde perfusion. II. Effect of osmolarity, pH of perfusate, and fixative concentration. J. Ultrastruct. Res., 12: 187.

Schultz, R. L. 1969 Personal communication.

Scott, D. E. 1968 Fine structural features of the neural lobe of the hypophysis of the rat with homozygous diabetes insipidus (Brattleboro strain). Neuroendocrinology, 3: 156.

Share, L. 1967 Vasopressin, its bioassay and the physiological control of its release. Am. J. Med., 42: 701.

Shinhoj, E. and O. B. Paulson 1969 Carbon dioxide and cerebral circulatory control. Arch. Neurol., 20: 249.

Sjostrand, F. 1960 Topographic relationship between neurons, synapses and glial cells in The Visual System· Neurophysiology and Psychophysics. Springer-Verlag, Berlin.

Sjostrand, F. S. 1967 The preparation of specimens by chemical fixation in Electron Microscopy of Cells and Tissues, Vol. 1. Academic Press, New York.

Sunde, D. A., D. D. Anthony, J. Osinchak and H. Sachs 1969 Effect of osmotic stimulus on synthesis of RNA in the rat neurohypophysis. Fed. Proc., 28· 317.

Thorn, Niels A. 1958 Mammalian antidiuretic hormone. Physiol. Rev., 38· 169.

Van Dyke, H. B., B. F. Chow, R. O. Greep, and A. Rothen 1941 The isolation of a protein from the pars neuralis of the ox pituitary with constant oxytocic, pressor and diuresis-inhibiting effects. J. Pharmacol., 74· 190.

Van Dyke, H. B., K. Adamsons, Jr. and S. L. Engel 1955 Aspects of the biochemistry and physiology of the neurohypophysial hormones. Rec. Progr. Hormone Res., 11· 1.

Watson, Michael L. 1958 Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol., 4· 475.

Wittkowski, W. 1967 Kapillaren und perikapillare raume in hypothalamus-hypophysen-system und ihre beziehungen zum nervengewebe. Z. Zellforsch., 81: 344.

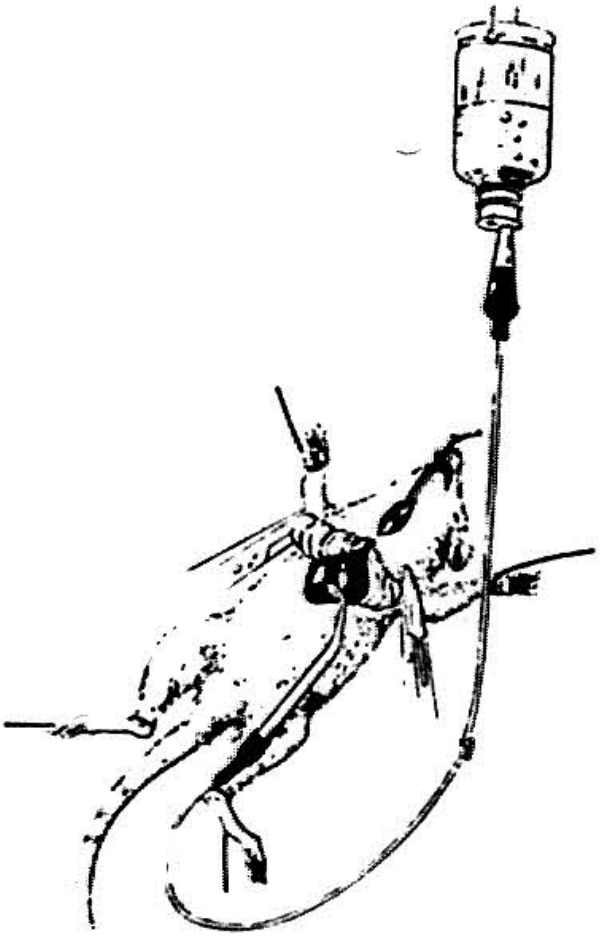
Wittkowski, W. 1968 Zur funktionellen morphologie ependymaler und extraependymaler glia im rahmen der neurosekretion. Z. Zellforsch., 86: 111.

Zambrano, David and Eduardo deRobertis 1967 Ultrastructural aspects of the inhibition of neurosecretion by puromycin. Z. Zellforsch., 76: 458.

KEY TO EXPLANATION OF FIGURES

A	axons	La	lamellar structures
B	basal corpuscle	M	mitochondria
BM	basement membrane	NP	neuronal process
C	capillary	NSG	neurosecretion granules
Ch	chromatin material	NT	neurotubules
Ci	cilium	P	pituicyte
Co	collagen	Pl	pleomorphic vesicles
E	endothelium	PVS	perivascular space
ECS	extracellular space	R	free ribosomes
F	fibroblast	RE	rough endoplasmic reticulum
Fe	fenestration	S	synaptoid vesicles
H	Herring body	T	tubular structures
L	lipid droplets	X	unusual feature

Figure 1. Drawing of perfusion apparatus. Modified from Sjostrand (1967) after Karlsson and Schultz.



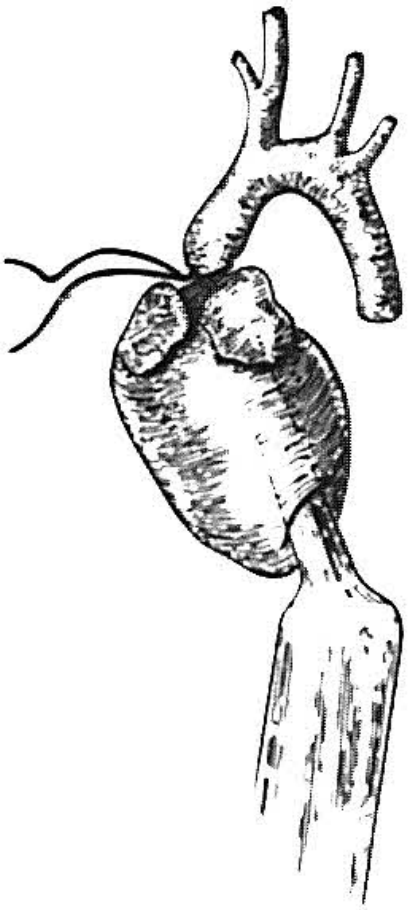


Figure 2. Normal pars nervosa X 2,600

A low power micrograph demonstrating the extensive extracellular space (ECS) and perivascular space (PVS). The small dark granules within the axons and their endings are hormone-containing neurosecretion granules.

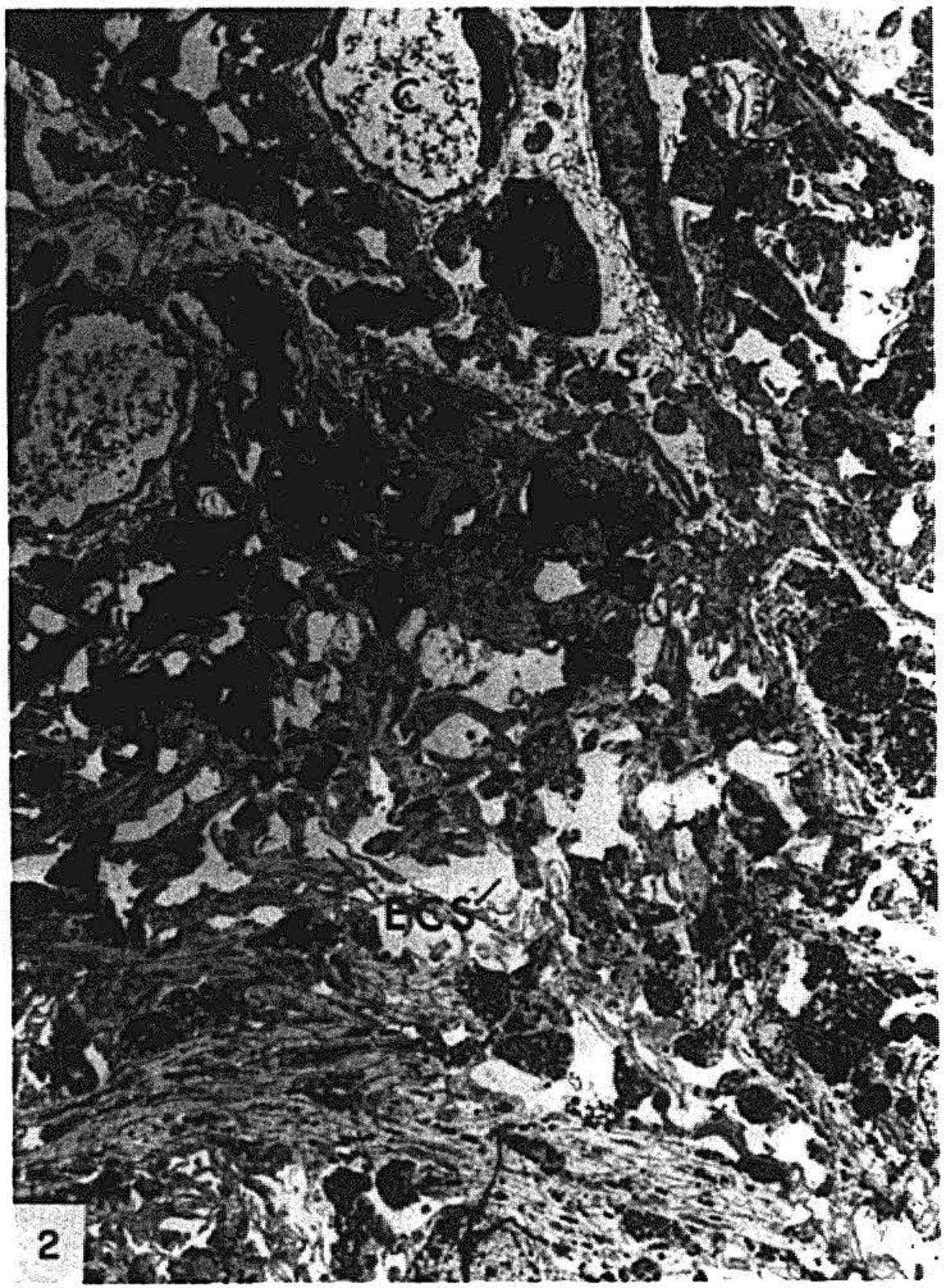

EGS
2

Figure 3. Normal pars nervosa X 2,400

Five capillaries are demonstrated which are all interconnected by the perivascular space (PVS). The pituicyte (P) demonstrates numerous lipid droplets (L) within its cytoplasm. Notice the fibroblast (F) and its nucleus in the upper right hand corner located within the perivascular space. Axonal endings containing neurosecretion granules (arrow) are distributed within the perivascular space.

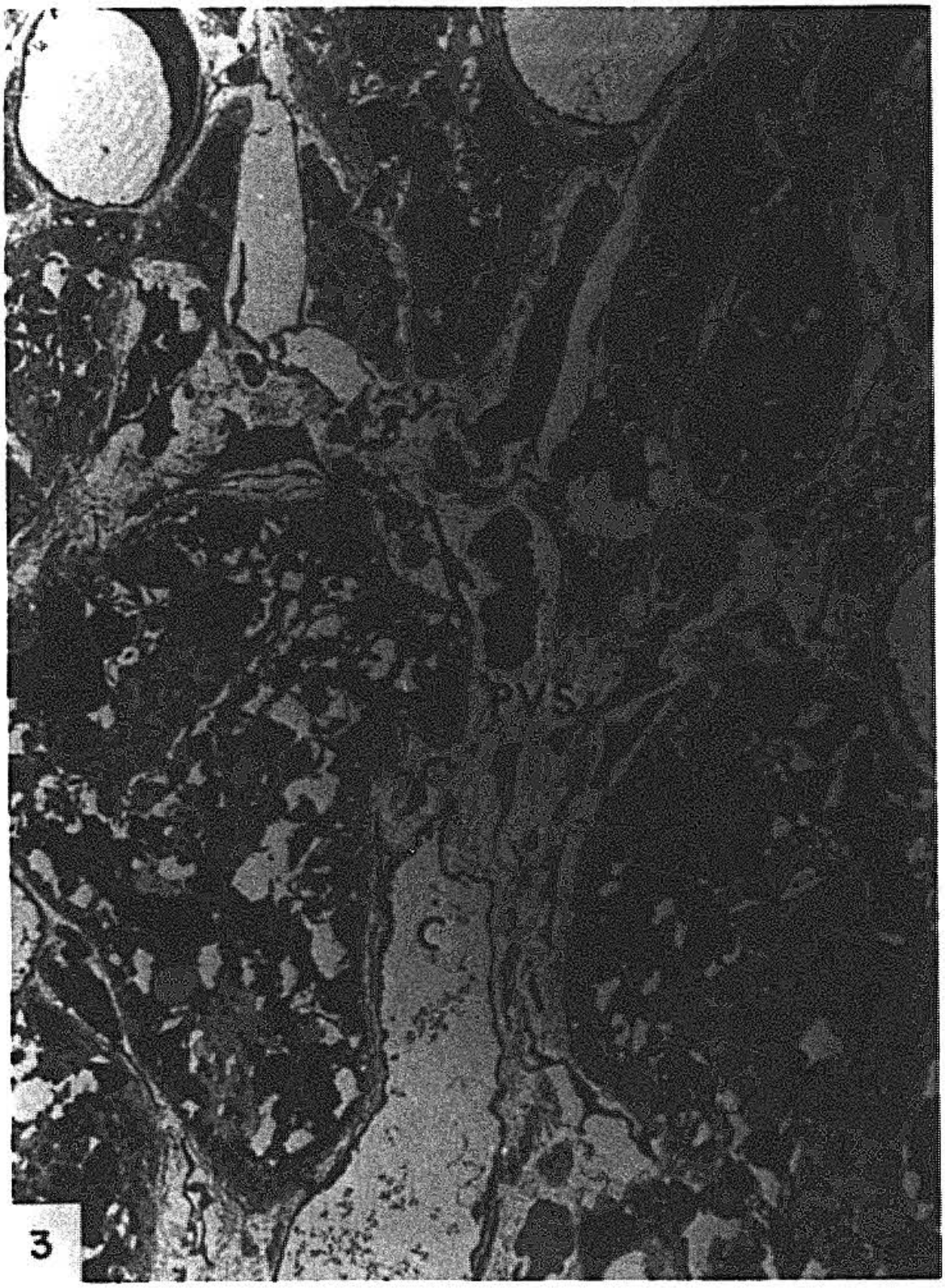


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Figure 4. Normal pars nervosa X 41,200

A nucleus of an endothelial cell is seen at lower left (E) surrounded by an inner basement membrane (BM) which forms the border of the perivascular space (PVS). The outer edge of the PVS is always bounded by an outer basement membrane (BM). Note the absence of a glial "cuff" separating the axonal endings from the outer basement membrane. The synaptoid vesicles (S) are seen to be densely packed and regular in size, appearance and shape. The NSG are osmiophilic and are usually clumped together rather than distributed among the synaptoid vesicles. Notice the collagen fibers seen in cross section within the PVS.

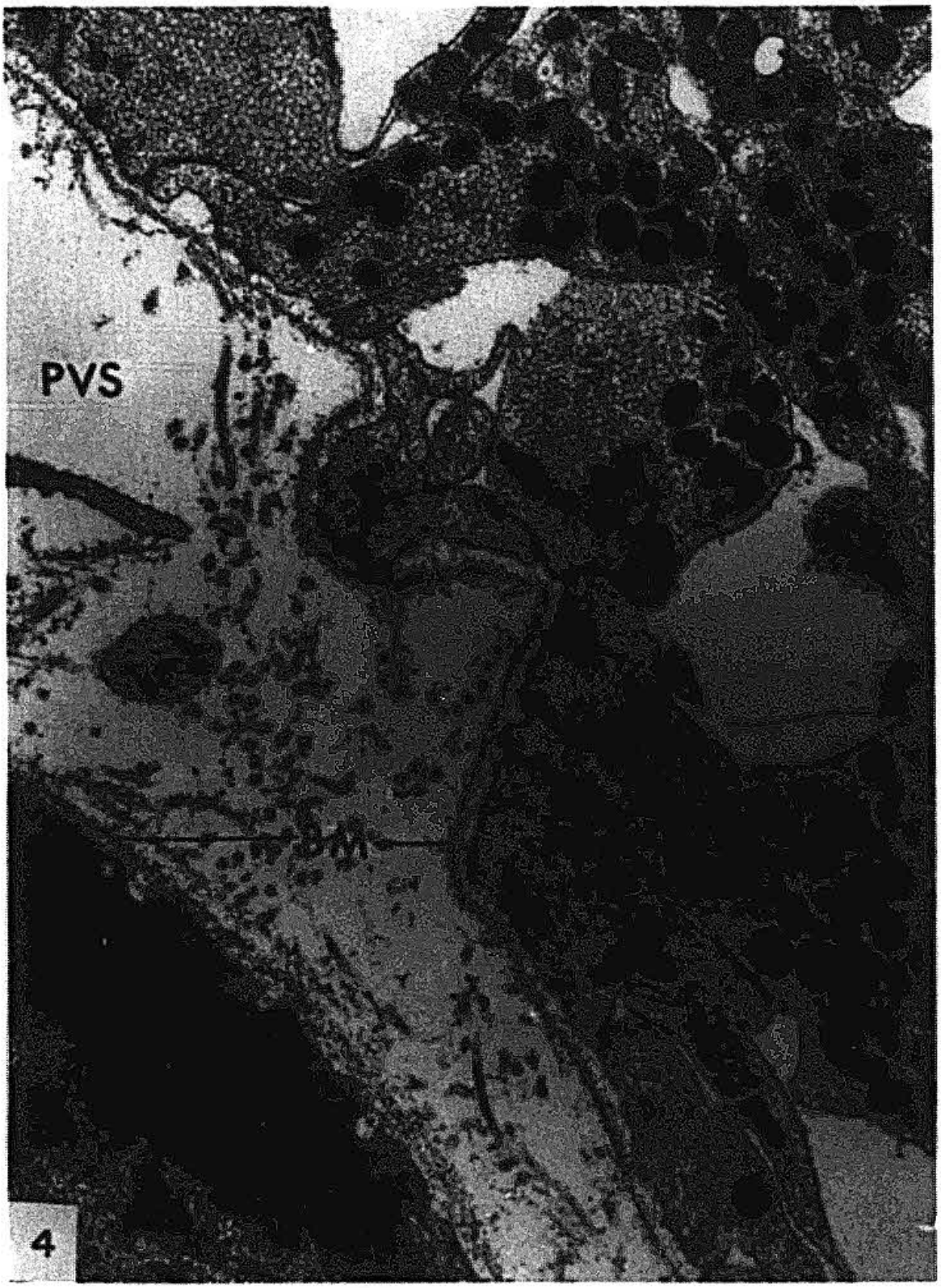
PVS
BM
4

Figure 5. Normal pars nervosa X 106,800

A high power view of a neuronal ending adjacent to the PVS. A longitudinally sectioned collagen fiber demonstrates its periodicity (Co). The synaptoid vesicles (S) are densely packed and regular in size and shape and have a membrane thickness of about 50 Å. These membranes differ in size and staining quality from the membranes of the NSG seen below. Two mitochondria are seen at the right.

5

Figure 6. Normal pars nervosa X 75,800

The perivascular space (PVS) demonstrates a thin fibroblast process and collagen fibrils in cross section. The gap seen between axonal endings and the PVS is a consistent finding. Notice the myelin-like formation (arrow) within the axonal ending. Synaptoid vesicles are seen at the right (S) as well as a mitochondrion (M).

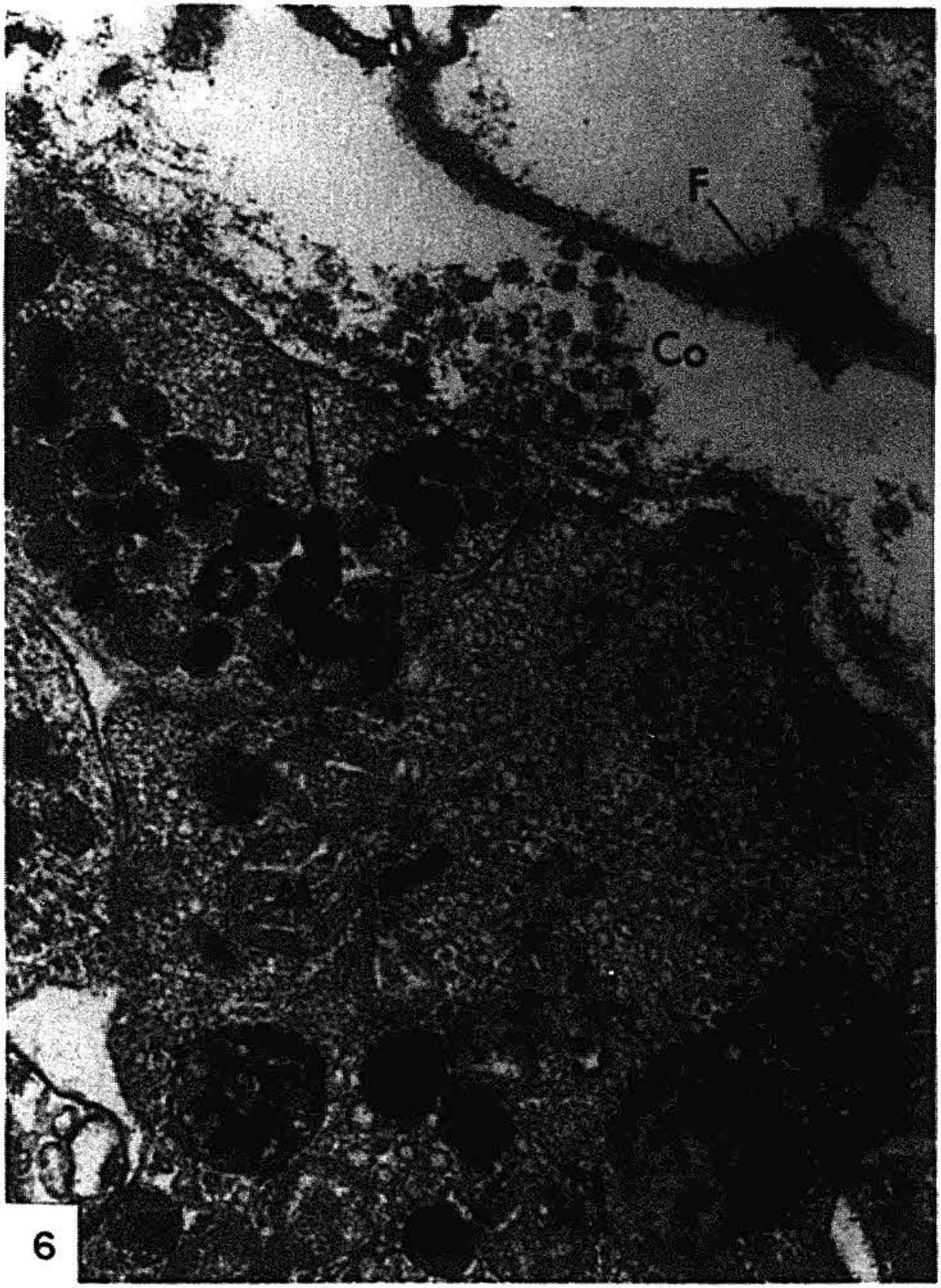

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Figure 7. Normal pars nervosa X 36,500

A pituicyte is seen containing smooth endoplasmic reticulum (arrow), mitochondria, and randomly scattered polysomes. The axon running diagonally (A) is seen to be filled with longitudinally disposed neurotubules (NT). Occasionally neurosecretion granules are seen (NSG) as they migrate toward the axonal ending. Numerous pituicyte processes are seen at upper right (P).

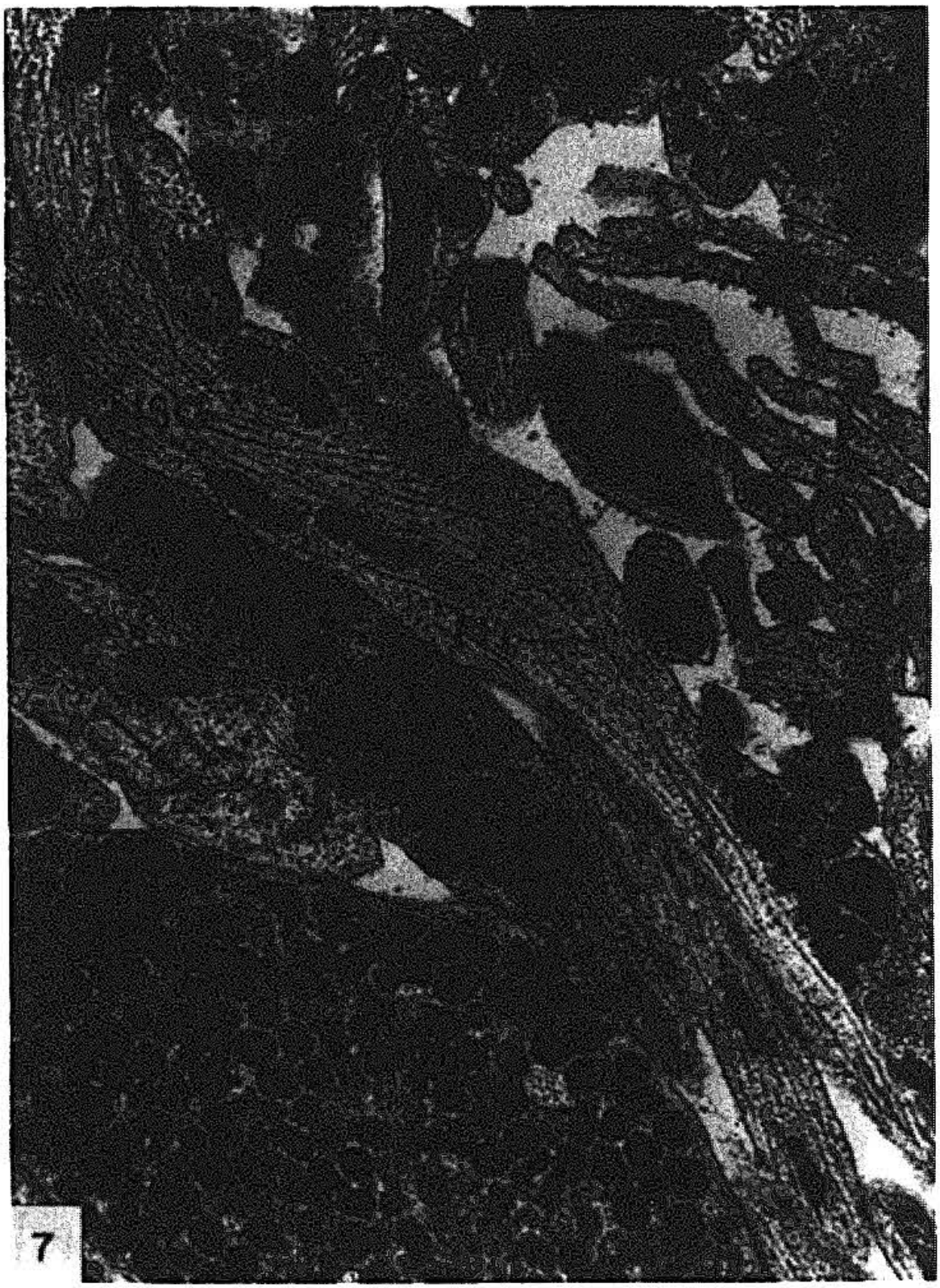
7

Figure 8. Normal pars nervosa X 18,100

Axonal endings surrounding the outer basement membrane of the PVS seem to be devoid of NSG as they terminate against the basement membrane. Within the PVS are found axonal endings, some containing neurosecretion granules (NSG) and others with tubular structures (T).

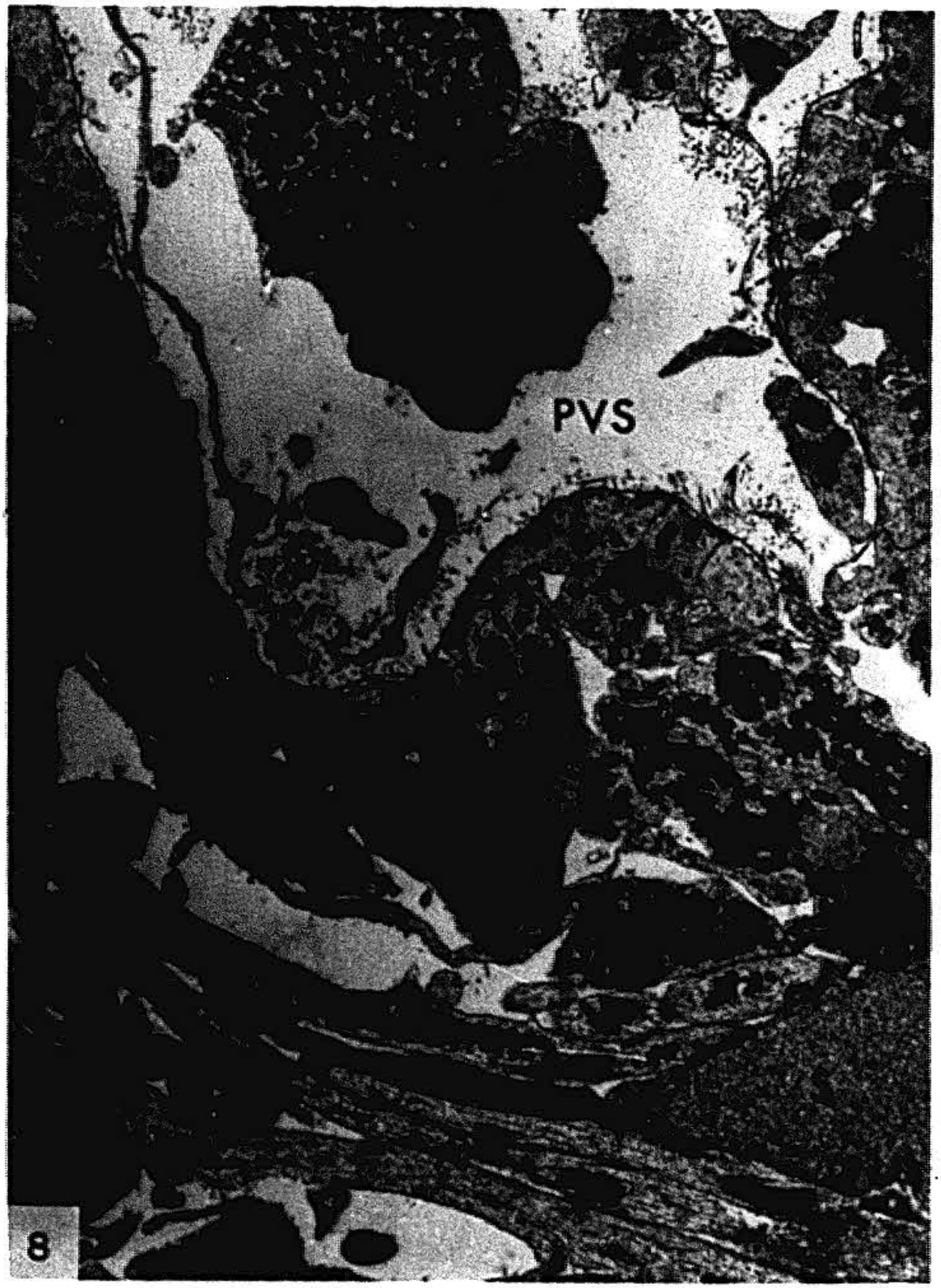
PVS
8

Figure 9. Normal pars nervosa X 33,800

Synaptoid vesicles are densely packed (S) and can demonstrate a hexagonal array (arrow). A pituicyte process with lipid droplets and endoplasmic reticulum is seen at right (P).

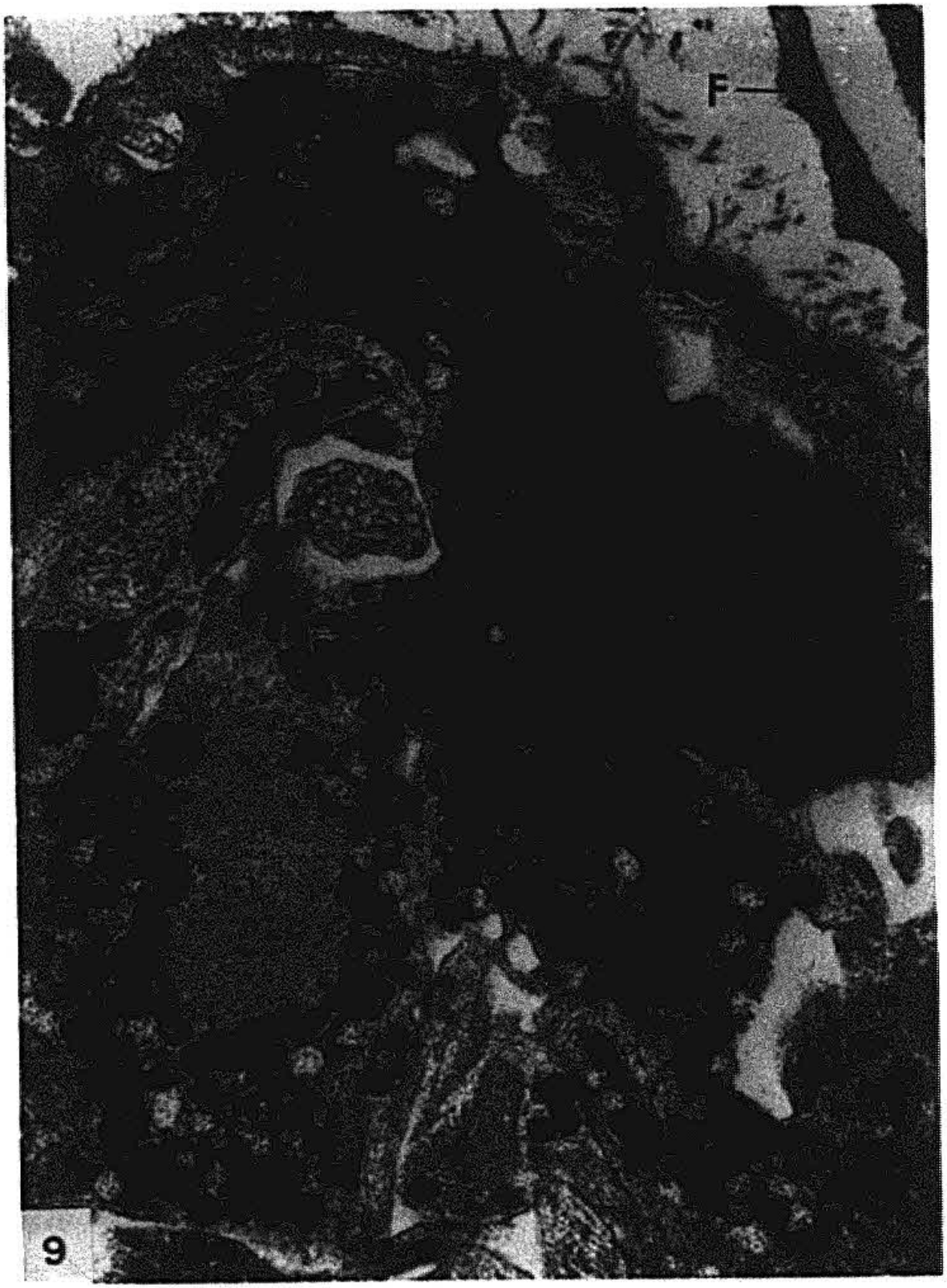
F
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Figure 10. Normal pars nervosa X 19,300

The perivascular space surrounds a capillary (C) and contains cellular elements including the fibroblast (F) and neuronal processes (NP). The arrow indicates the terminal portion of a fiber seemingly penetrating into the PVS.

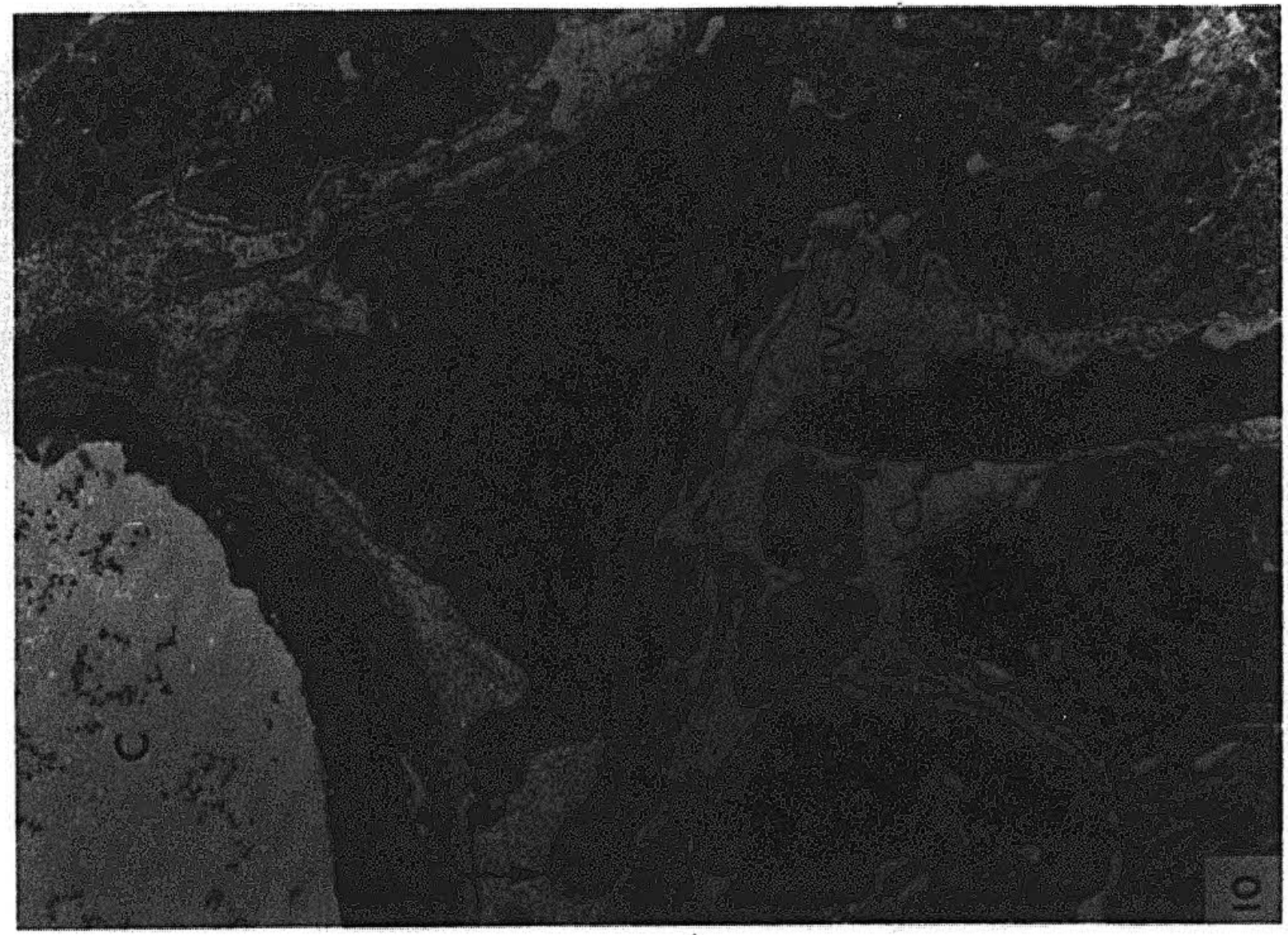

Figure 11. Normal pars nervosa X 30,600

The finding of a cilium (Ci) within the pars nervosa has not been reported elsewhere. The shaft of the cilium is cut in longitudinal section and reveals longitudinally disposed filaments. The basal corpuscle (B) is shown. It appears that the cilium is located within a pituicyte process.

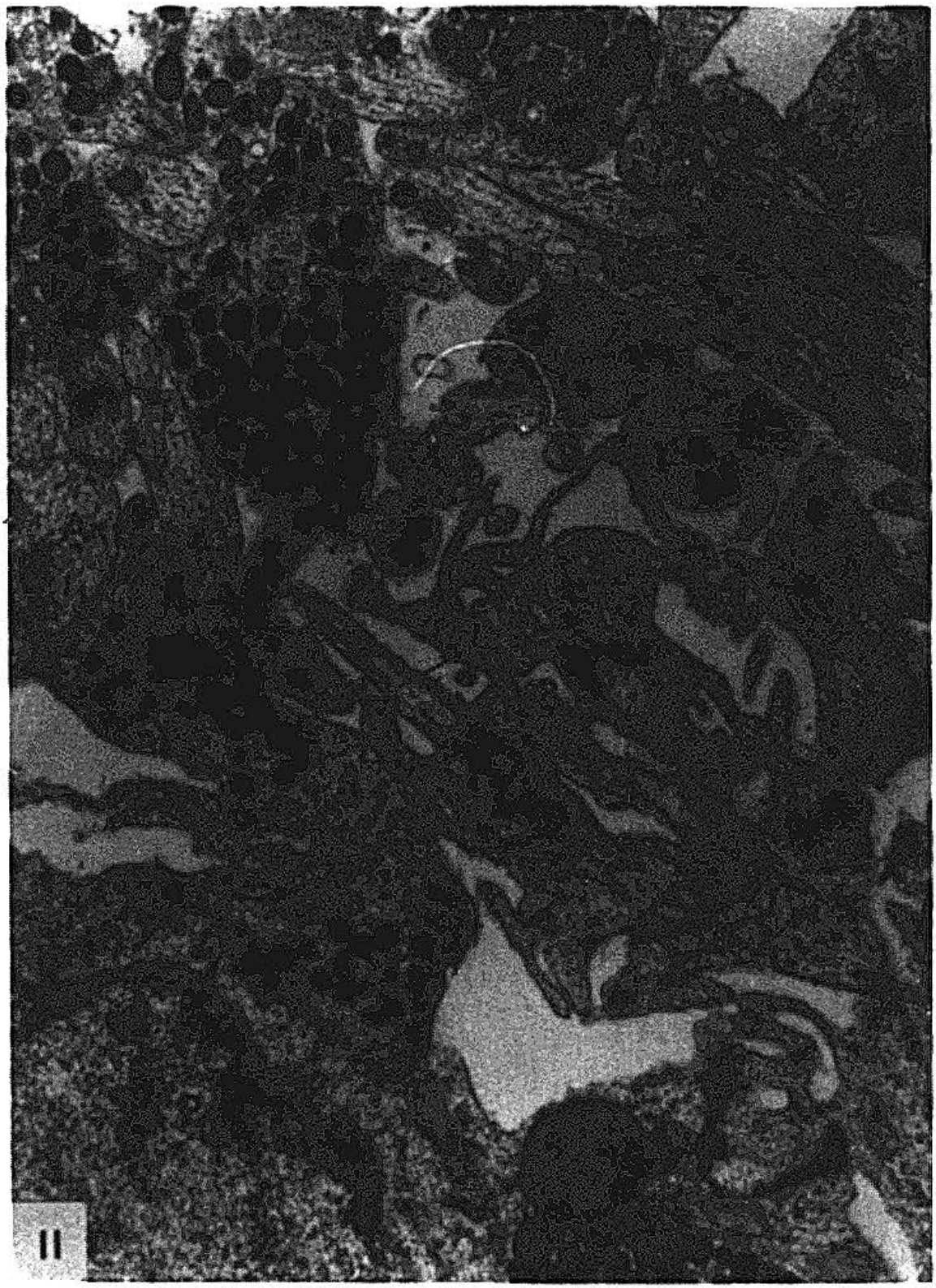
II

Figure 12. Normal pars nervosa X 35,000

An example of cellular protrusion into the PVS is seen. Notice the two processes of the axon penetrating the basement membrane (bm). The inner and outer basement membranes are plainly visible and the diaphram bridging a fenestration in the endothelium is plainly visible (Fe). Sparce collagen material in cross and longitudinal section can be observed in the PVS.

Figure 13. Normal pars nervosa X 34,800

The sheet-like nature of the basement membrane is demonstrated as it has been sectioned tangentially across this basement membrane. Notice the fibrillar material of which this membrane is made.

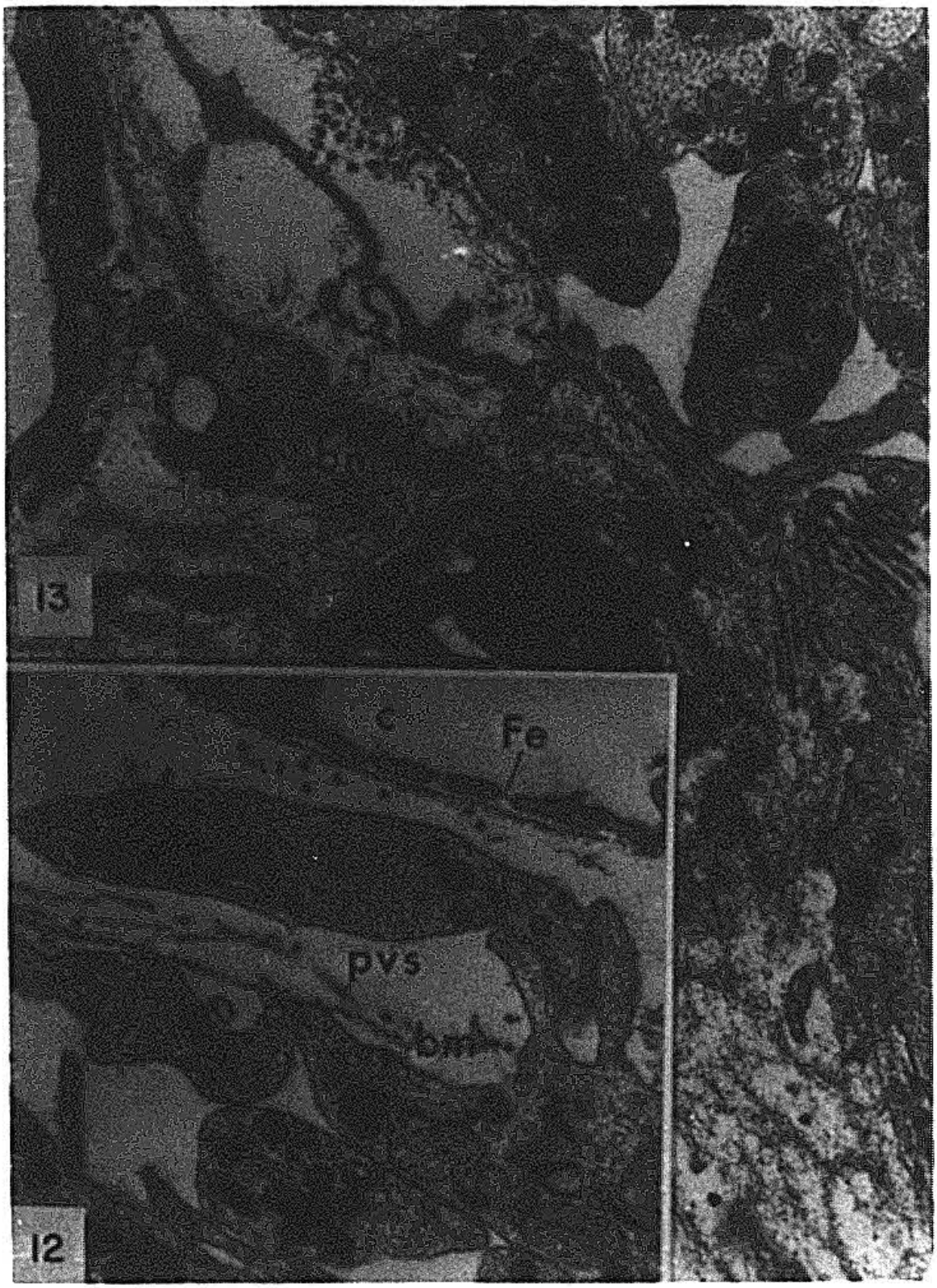
13
Fe
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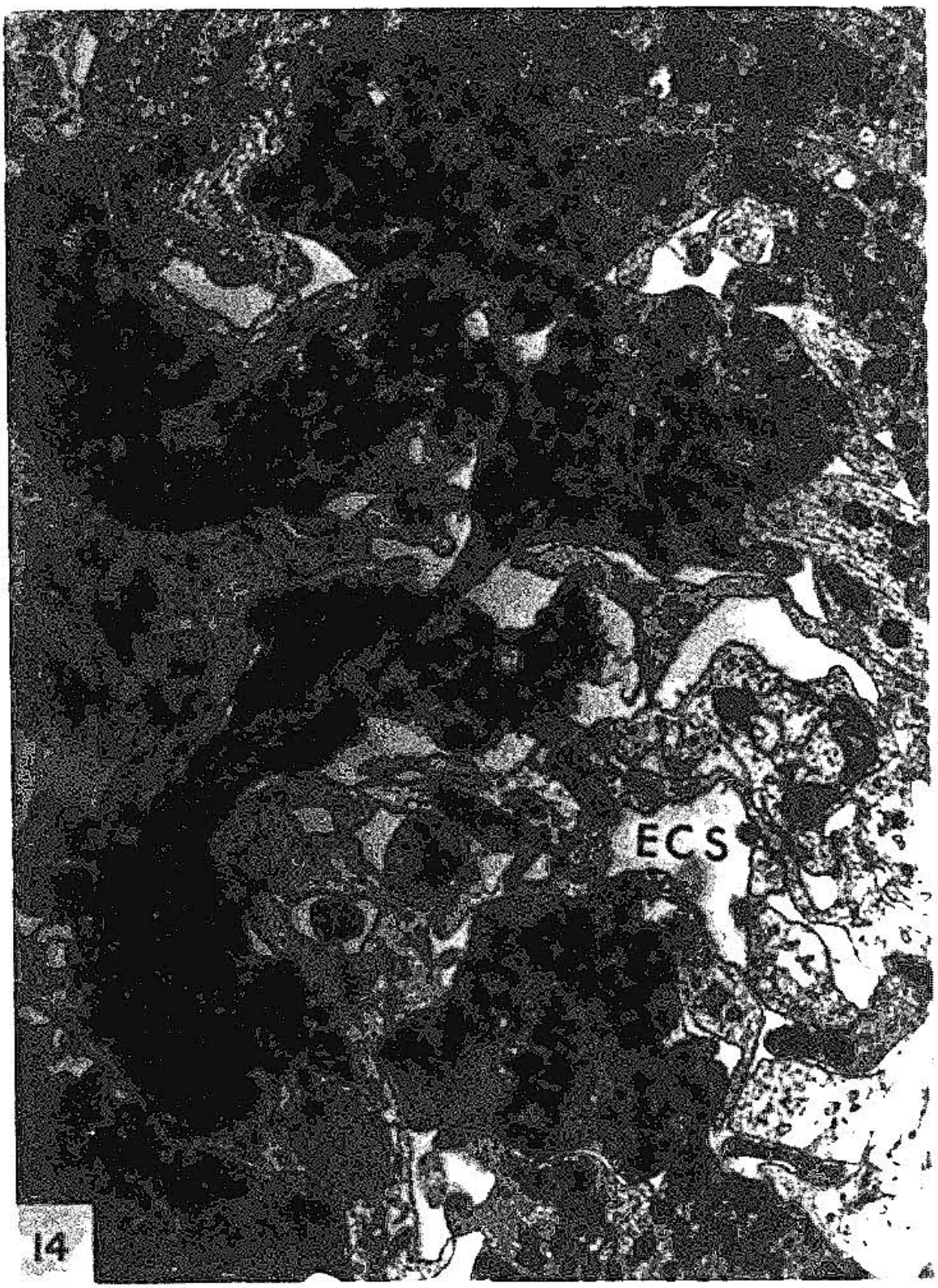
Figure 14. Normal pars nervosa X 30,300

The verification of neuronal swellings is shown in this micrograph. Notice that one neuronal process (NP_1) is connected to another neuronal process (NP_2) by an axon (A). This indicates that there are swellings along the axons and that all dilated processes are not necessarily terminal processes.



14

Figure 15. Normal pars nervosa X 36,400

The predominant cell process seen in this micrograph is a pituicyte as evidenced by the free ribosomes (R), the lipid droplets (L), as well as the background matrix density. Also seen is rough endoplasmic reticulum (RE). The predominant feature of the cytoplasm is the abundant tubular cisternae seen pervading the entire figure (arrow). This appears typical of smooth endoplasmic reticulum.

15

Figure 16. Normal pars nervosa X 6,100

This unusual micrograph was sectioned tangentially through the edge of a capillary (C). The vessel is surrounded by the usual perivascular space containing collagen (Co) and fibroblasts (F). The unusual feature is that cell processes are located within the endothelial cells of the capillary wall (arrow). These appear to be neuronal in nature and might represent innervating nerve cells within the vessel wall. The unusual vesicular and lobulated structures seen at X are not readily identifiable and similar structures have not been seen elsewhere. Those vesicular structures which seem to be contained within the endothelium (X_1) suggest pinocytosis and are an unusual finding.

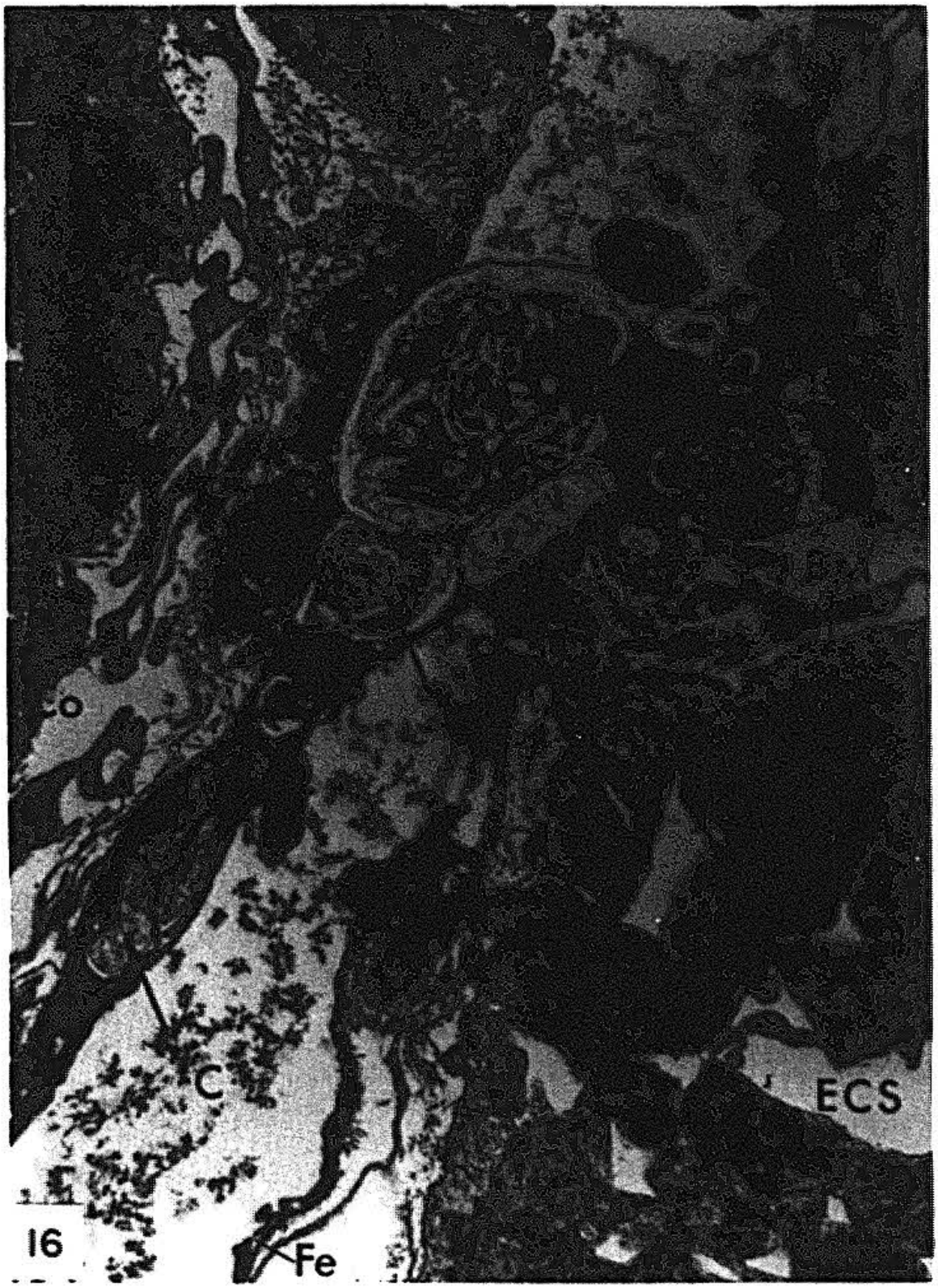
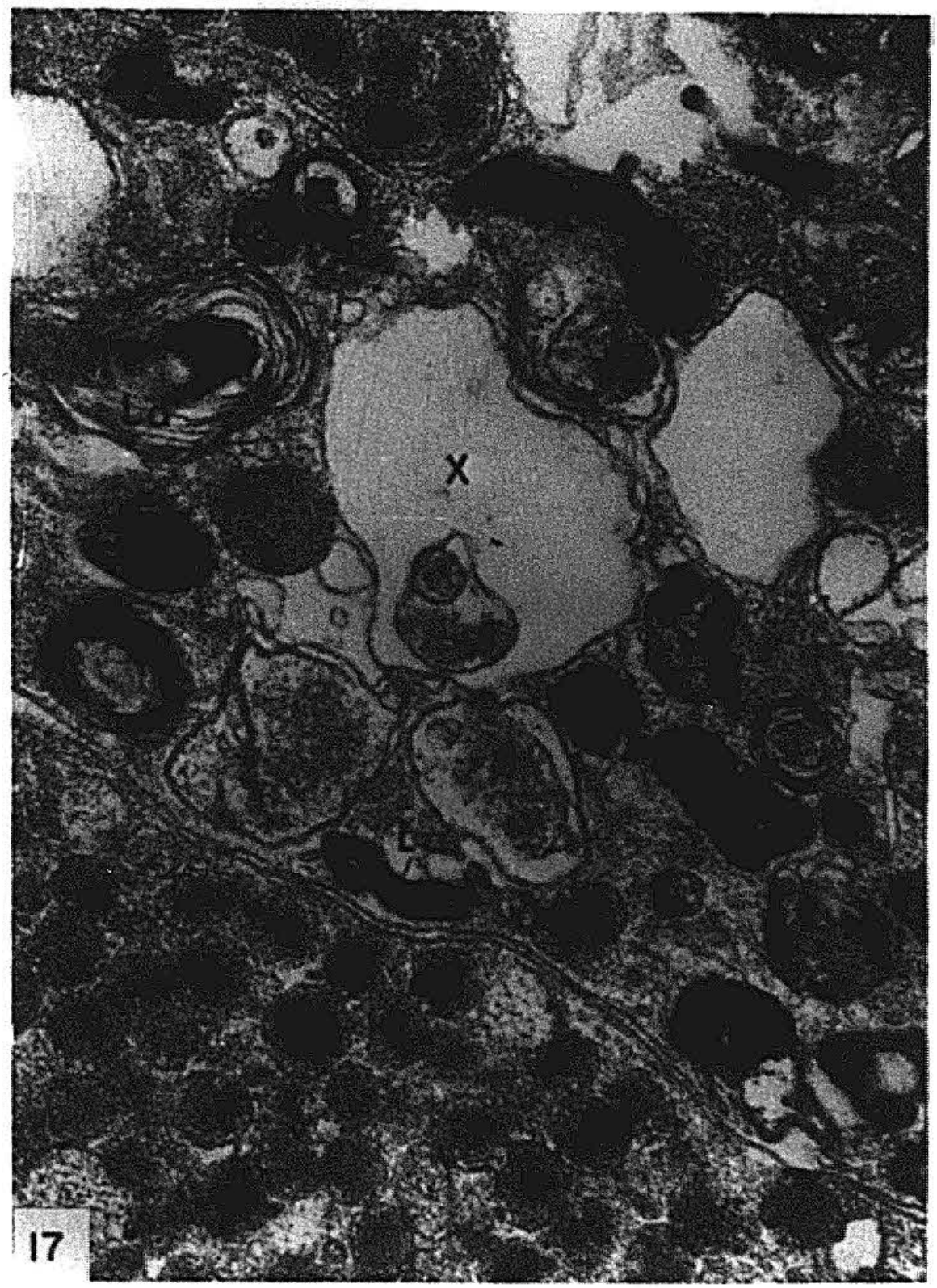

O
C
C
ECS
16
Fe

Figure 17. Normal pars nervosa X 76,000

A high power micrograph of a degenerating neuronal process shows large spaces (X) and numerous indications of degenerating processes. Notice the lamellar bodies present (La), seen to be in various stages of formation.

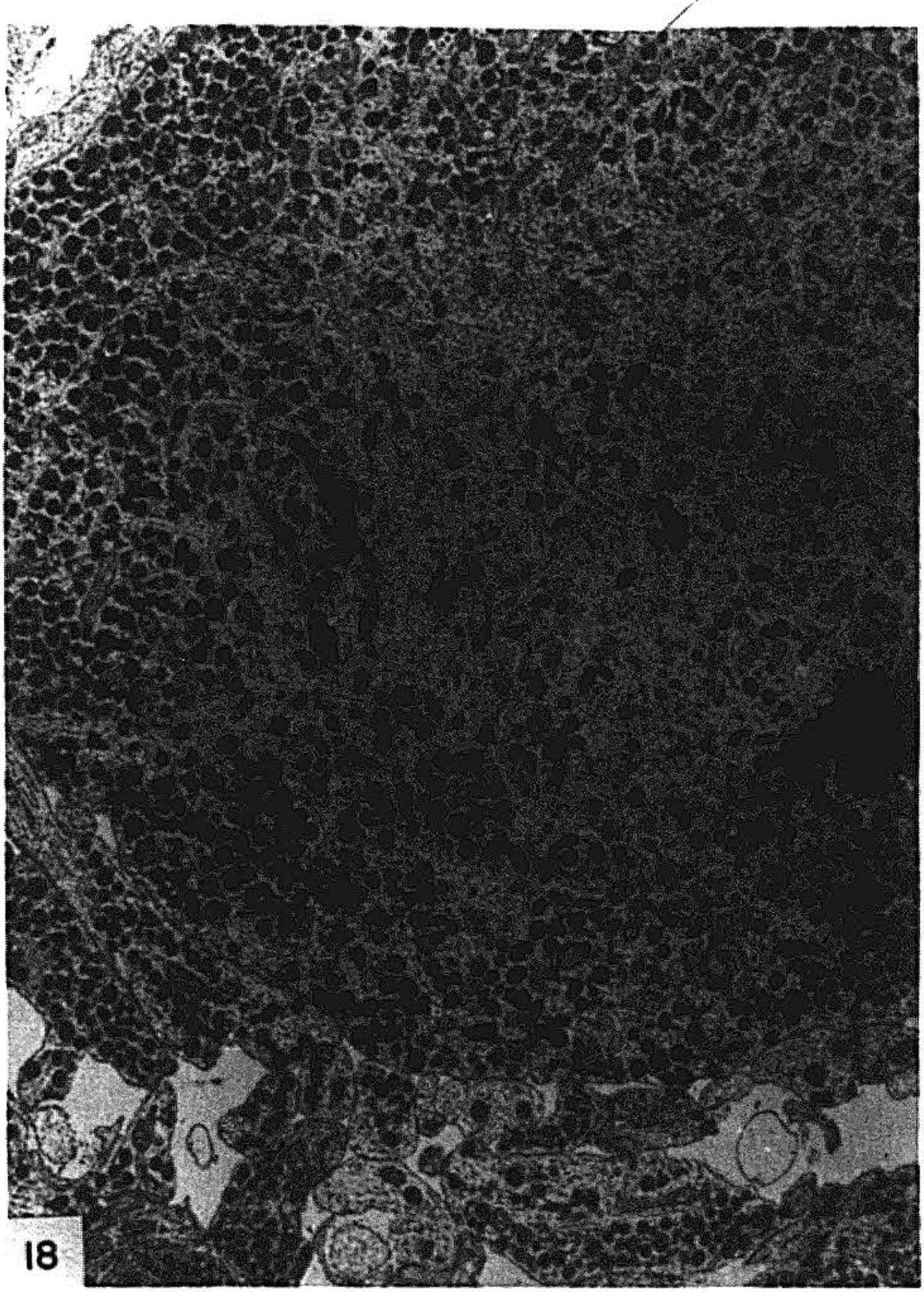


17

Figure 18. Acute experimental animal, pars nervosa (E1, see table II)

X 20,400

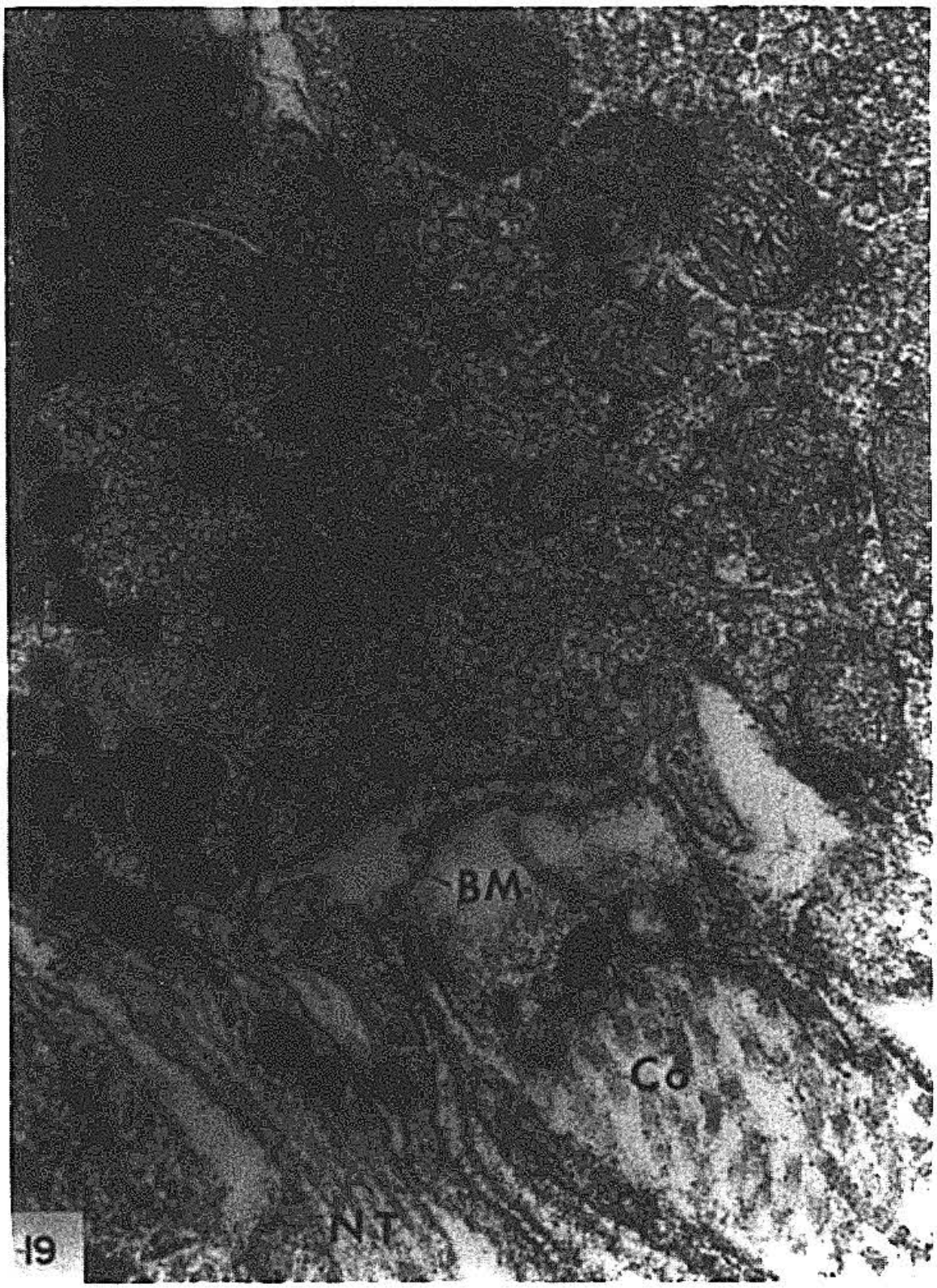
A Herring body is observed containing randomly disposed neurotubules (NT) with the center of the process containing much less densely packed NSG.



18

Figure 19. Normal pars nervosa X 76,300

Normal constituents of a hydrated animal are seen. Notice the basement membrane (BM) outlining the perivascular space in which collagen is found (Co). Neurosecretion granules (NSG) and synaptoid vesicles (S) predominate.



19

Figure 20. Acute experimental series, pars nervosa (E1, see table II)
X 87,200

A pituicyte process is seen at lower left (P). The neuronal processes contain numerous synaptoid vesicles (S) as well as neurosecretion granules (NSG). The NSG are surrounded by membranes with distinct layers and the difference between these membranes and those enclosing the synaptoid vesicles is obvious. This difference is both in thickness and in staining quality.

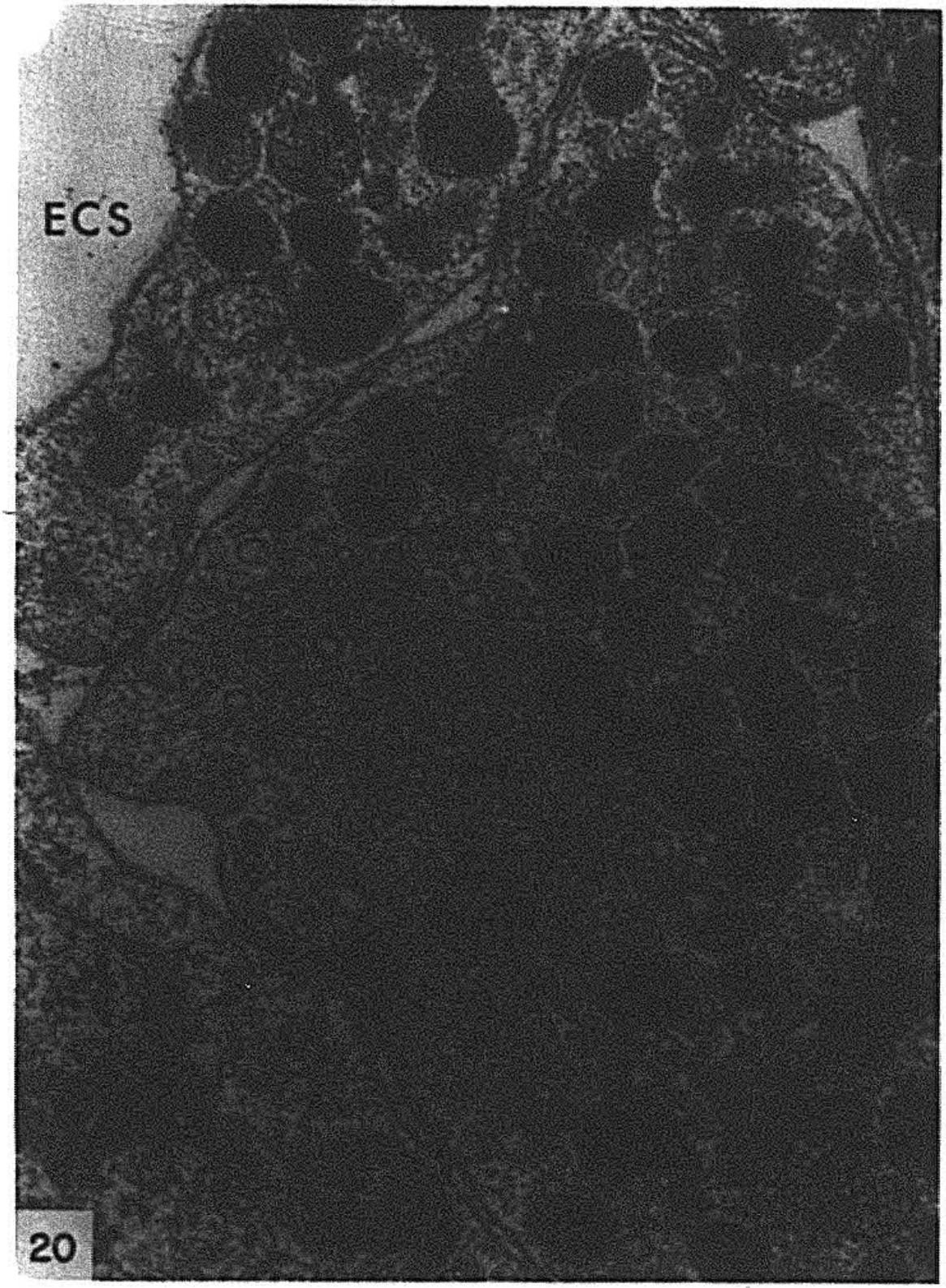

ECS
20

Figure 21. Acute experimental series, pars nervosa (E1, see table II)

X 87,200

The typical appearance of the perivascular space (PVS) containing collagen fibers and surrounded by the basement membrane (BM) is demonstrated. The gap between the basement membrane and the neuronal processes is variable in width. The neurotubules (NT) of an axon are seen in cross section.

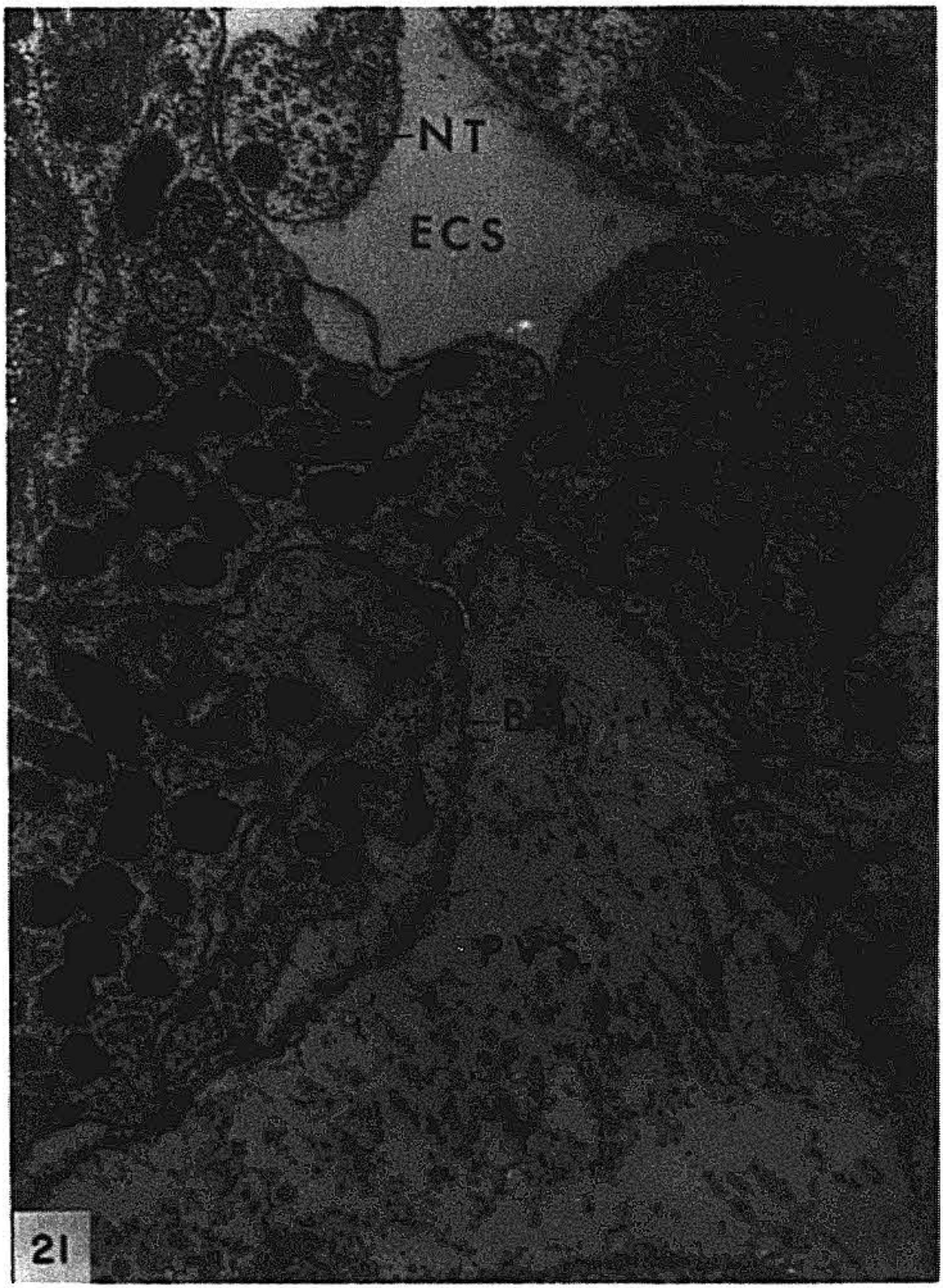

NT
ECS
21

Figure 22. Acute experimental series, pars nervosa (E2, see table II)

X 35,900

An example of the intimate connection between the pituicyte processes (P) and the neuronal processes. Note the dilations in the extracellular space (ECS) which suggest the presence of channels between axonal processes. Note the neurosecretion granules and the unusual lamellar enclosure (arrow) of these granules.

22

Figure 23. Acute experimental series, pars nervosa (E3, see table II)

X 35,900

The perivascular space is surrounded by the usual basement membrane (BM) and contains a neuronal process not surrounded by a sleeve of basement membrane (NP). Endings abutting against the outer basement membrane appear typical of the normal pars nervosa with synaptoid vesicles (S) and mitochondria (M) and an occasional area suggesting pleomorphic vesicles (Pl).

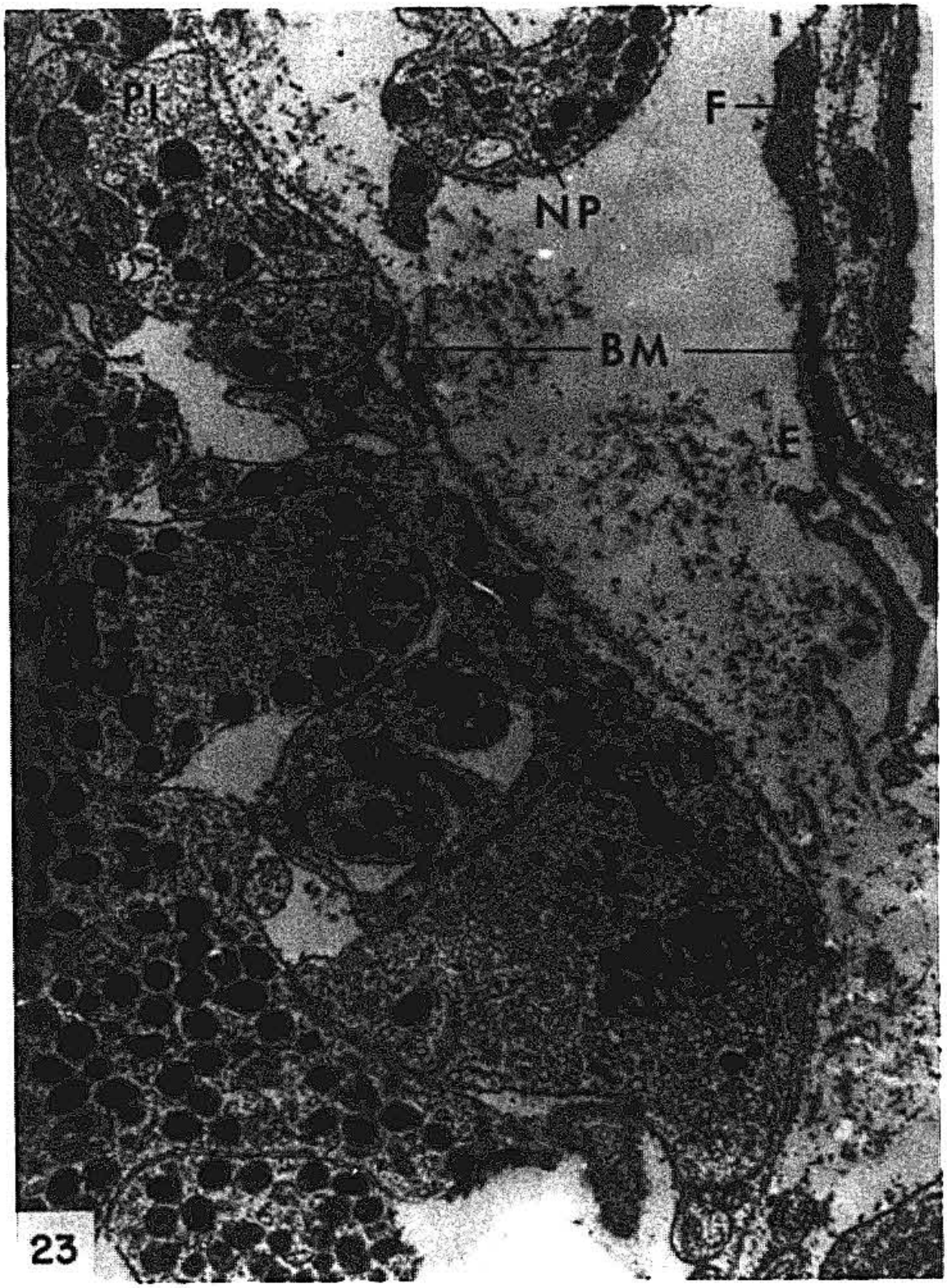
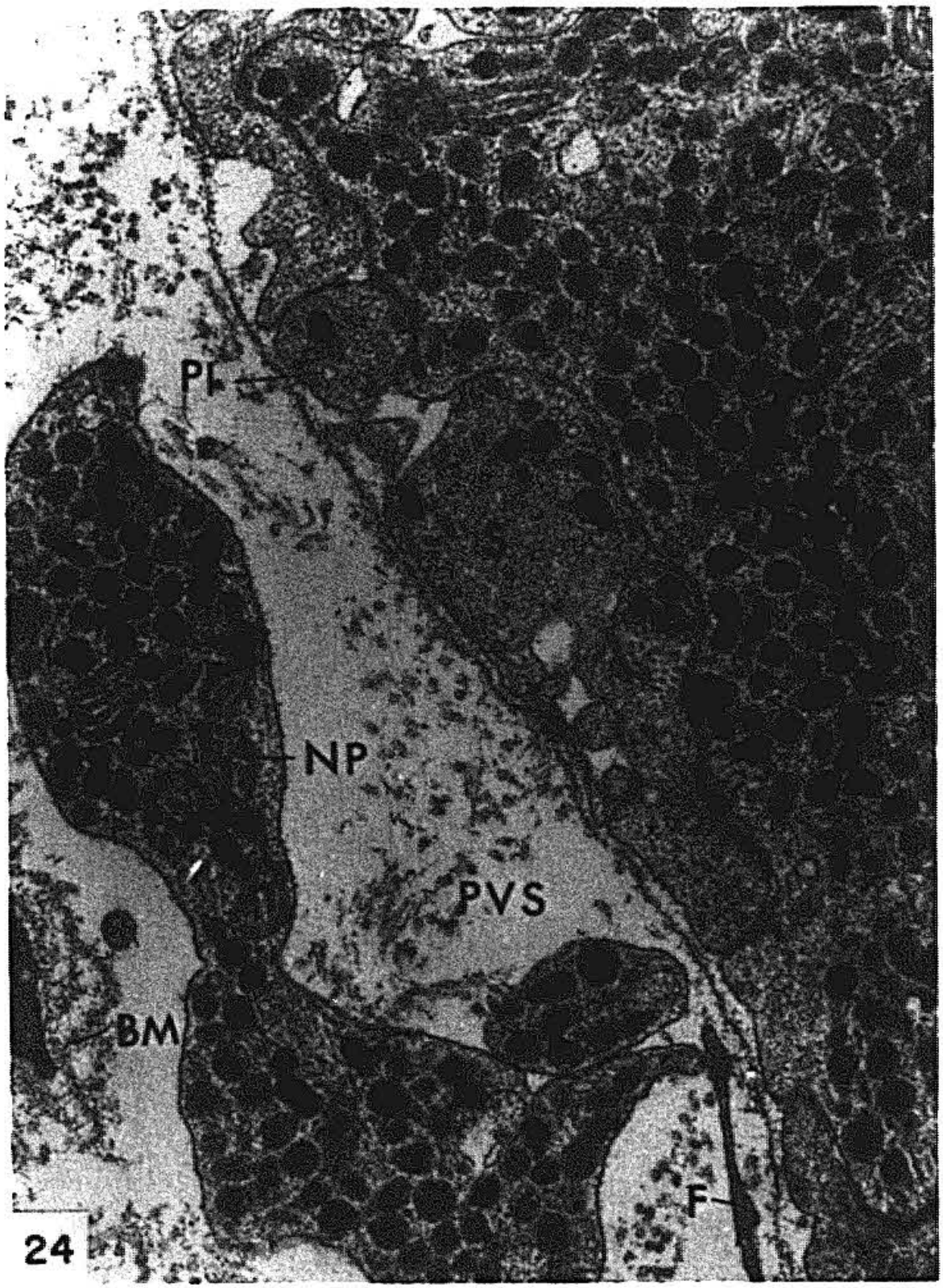

PL
F
NP
BM
E
23

Figure 24. Acute experimental series, pars nervosa (E5, see table II)

X 35,900

A relatively wide perivascular space (PVS) is seen containing a large neuronal process (NP) and basement membrane sectioned slightly tangentially (BM). The usual neuronal elements are seen (S, NSG, Pl).



24

Figure 25. Acute experimental series, pars nervosa (E10, see table II)

X 2,300

A low power micrograph indicating relative frequency of pituicyte cells (P). The perivascular space (PVS) is seen to contain neuronal processes (NP) with an example of a possible protrusion into the PVS indicated (arrow). A Herring body can be observed (H). Note the rather extensive extracellular space between cellular components.

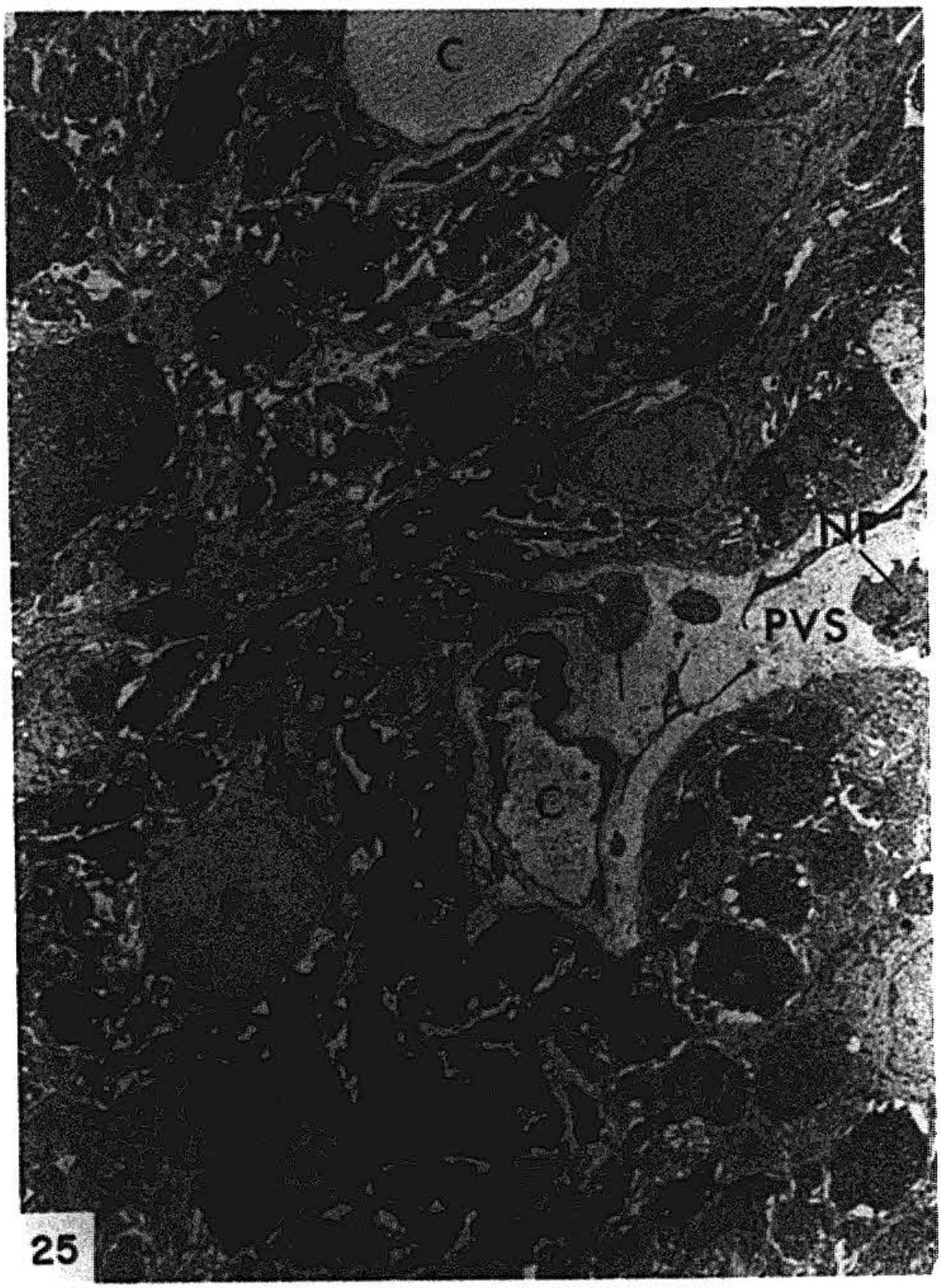

C
IN
PVS
25

Figure 26. Acute experimental series, pars nervosa (E10, see table II)

X 2,200

A low power micrograph demonstrating the relationships of incoming axons (A) to neuronal dilations. The perivascular space can be seen to surround the capillaries (C).

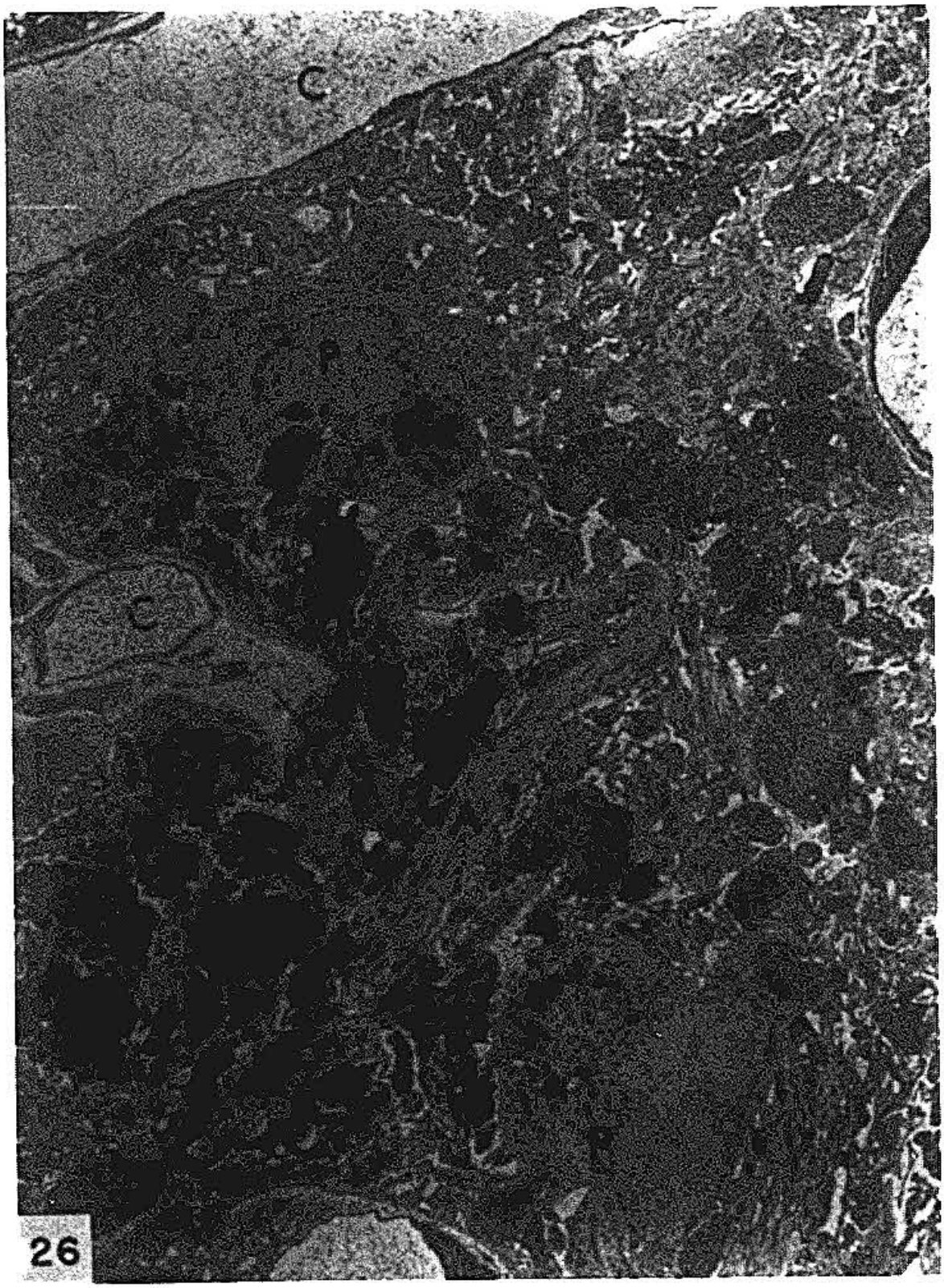
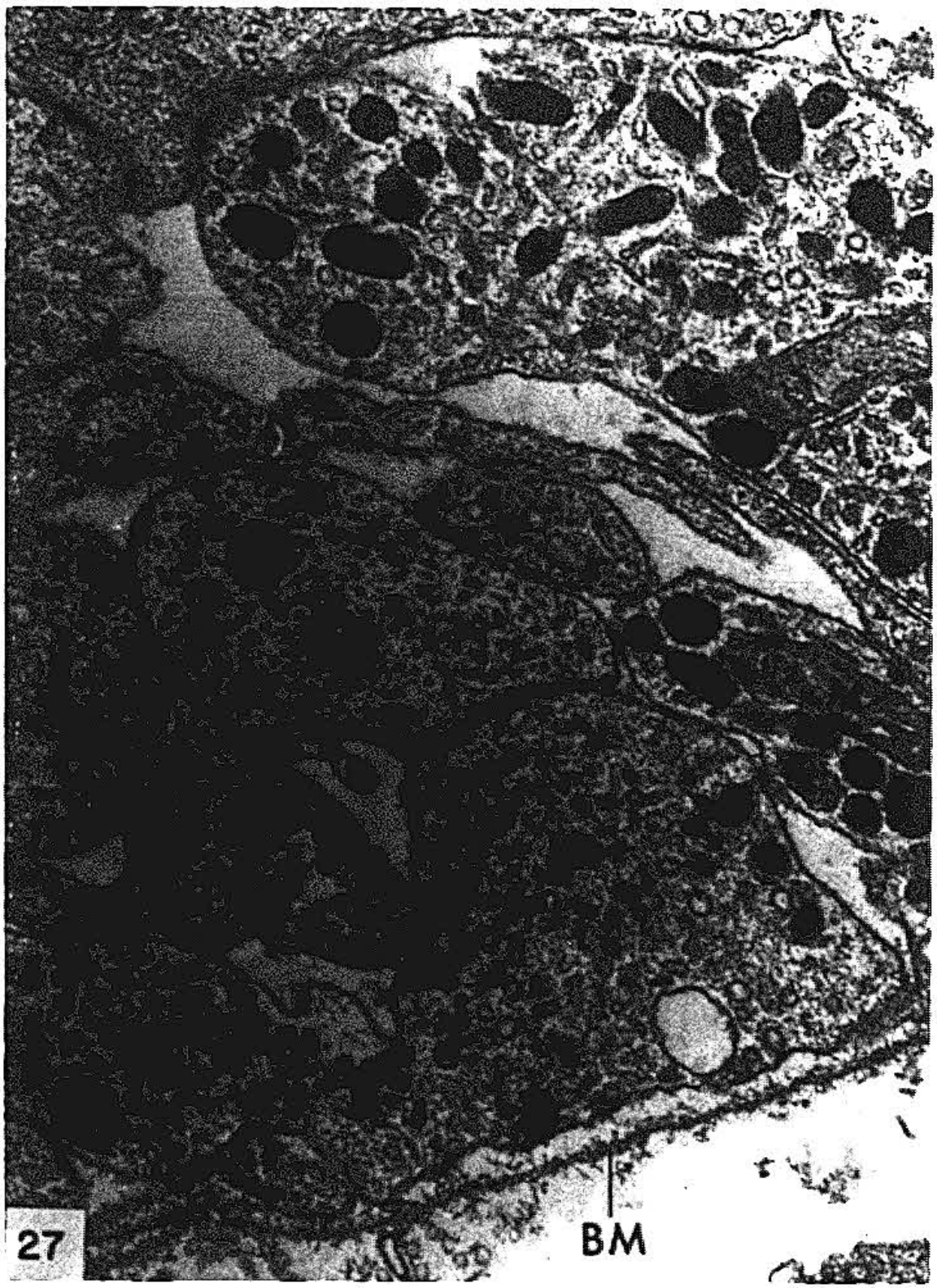

C
P
C
P
26

Figure 27. 2½ day dehydrated animal, pars nervosa X 54,200

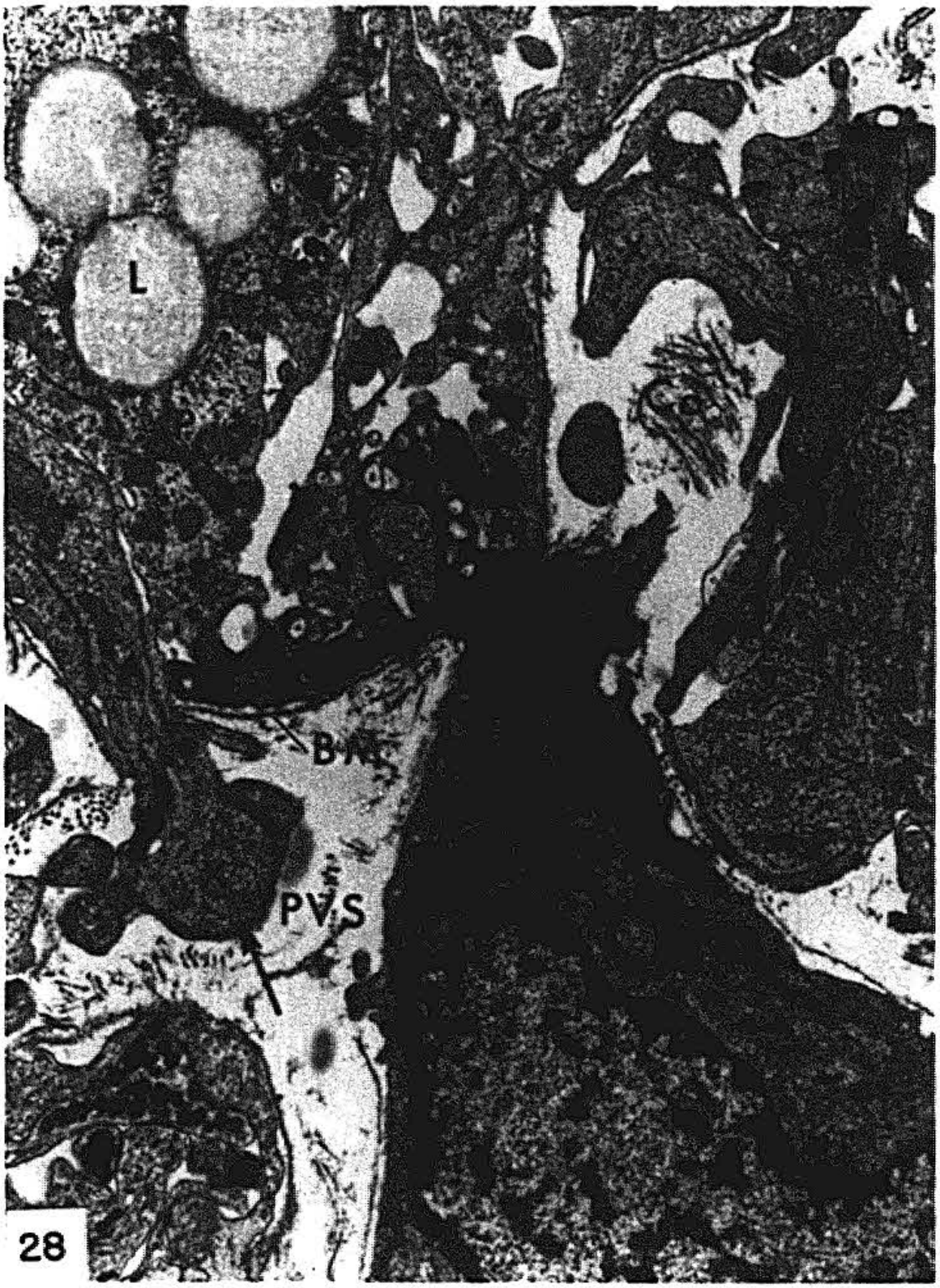
The size variation in pleomorphic vesicles can be seen in this micrograph showing large oval-shaped vesicles (Pl_1) as well as small shrunken vesicles (Pl_2). These presumably result from loss of hormone content and represent "ghosts" or residual vesicles of the neurosecretion granules (NSG).



27

Figure 28. 2½ day dehydrated animal, pars nervosa X 19,900

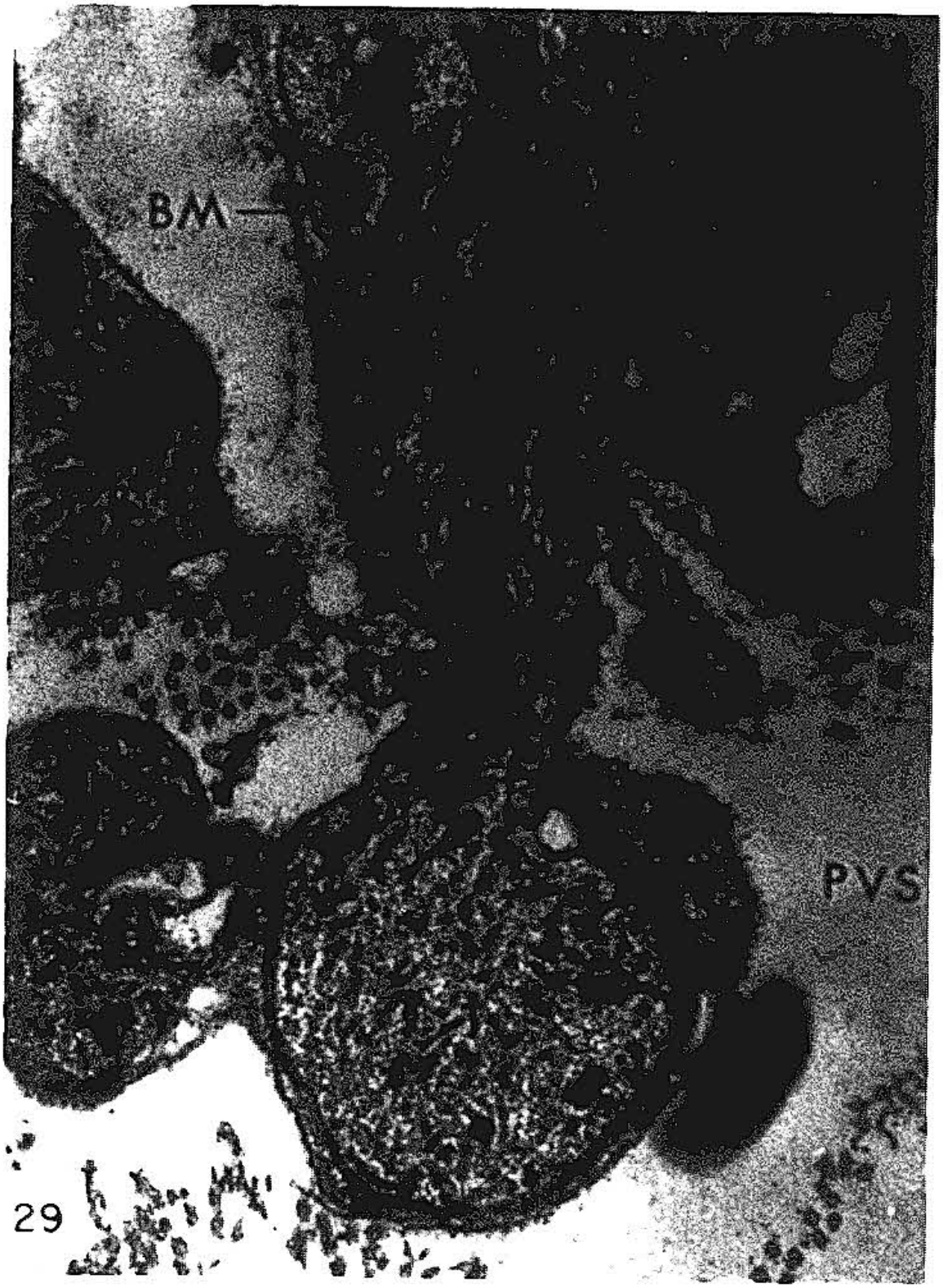
The extensive cytoplasm of a fibroblast is seen at lower right (F) within the perivascular space (PVS). Neuronal processes with tubular cisternae are readily visible (X). The arrow indicates the probable site of protrusion of an axon through the basement membrane (BM) into the PVS.



28

Figure 29. 2½ day dehydrated animal, pars nervosa X 56,900

A higher power micrograph of fig. 28 demonstrates the protrusion into the perivascular space (PVS). Note the neurotubules (NT) and mitochondria (M) blending in with the tubular structures in the nerve endings. The basement membrane is not seen surrounding the terminal portion of the axon within the PVS.



29

Figure 30. 2½ day dehydrated animal, pars nervosa X 113,100

A high magnification micrograph showing pleomorphic vesicles (Pl) of varying sizes. Note that the membrane thickness of these pleomorphic vesicles corresponds closely to that of the NSG membrane.

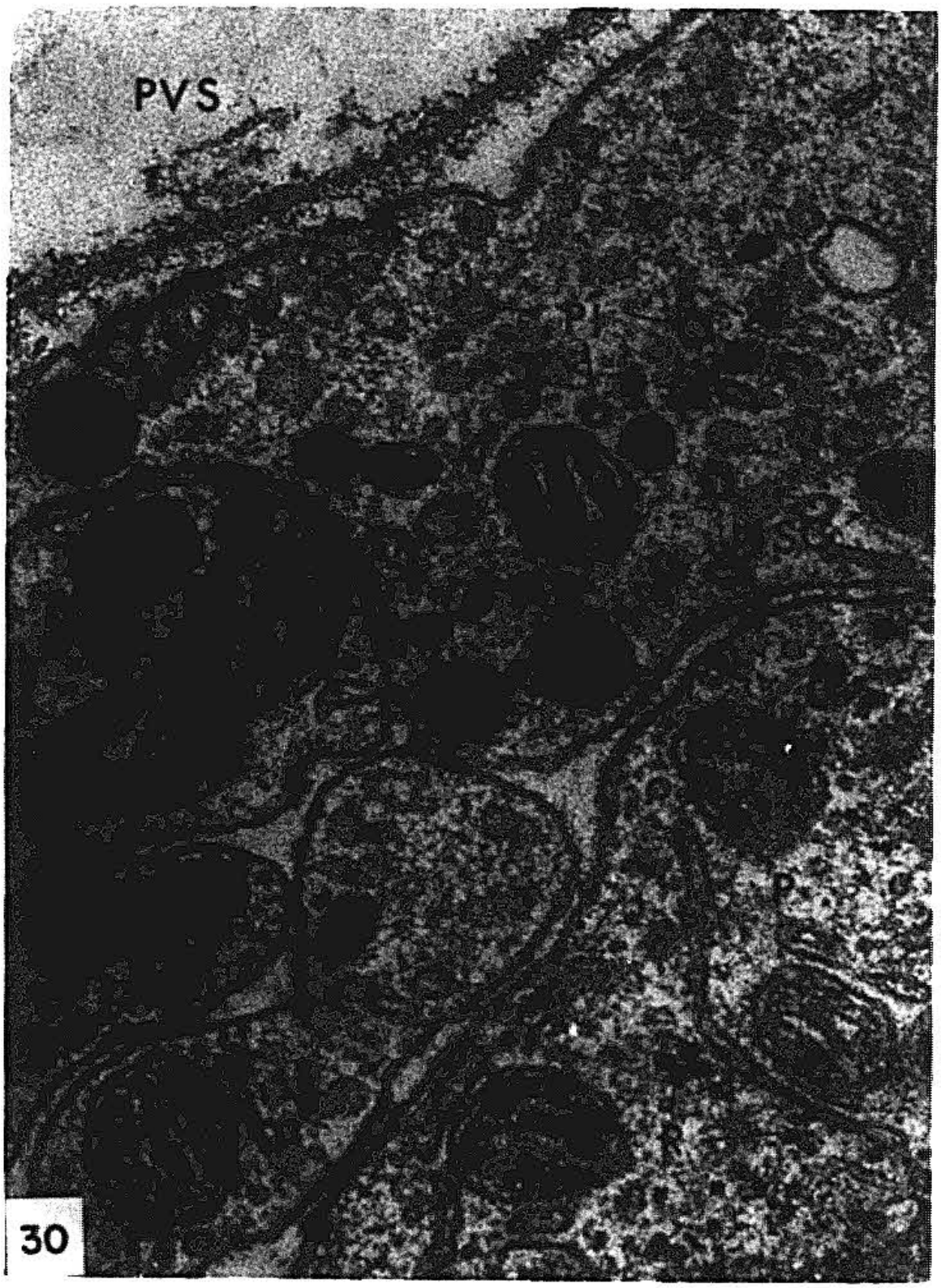
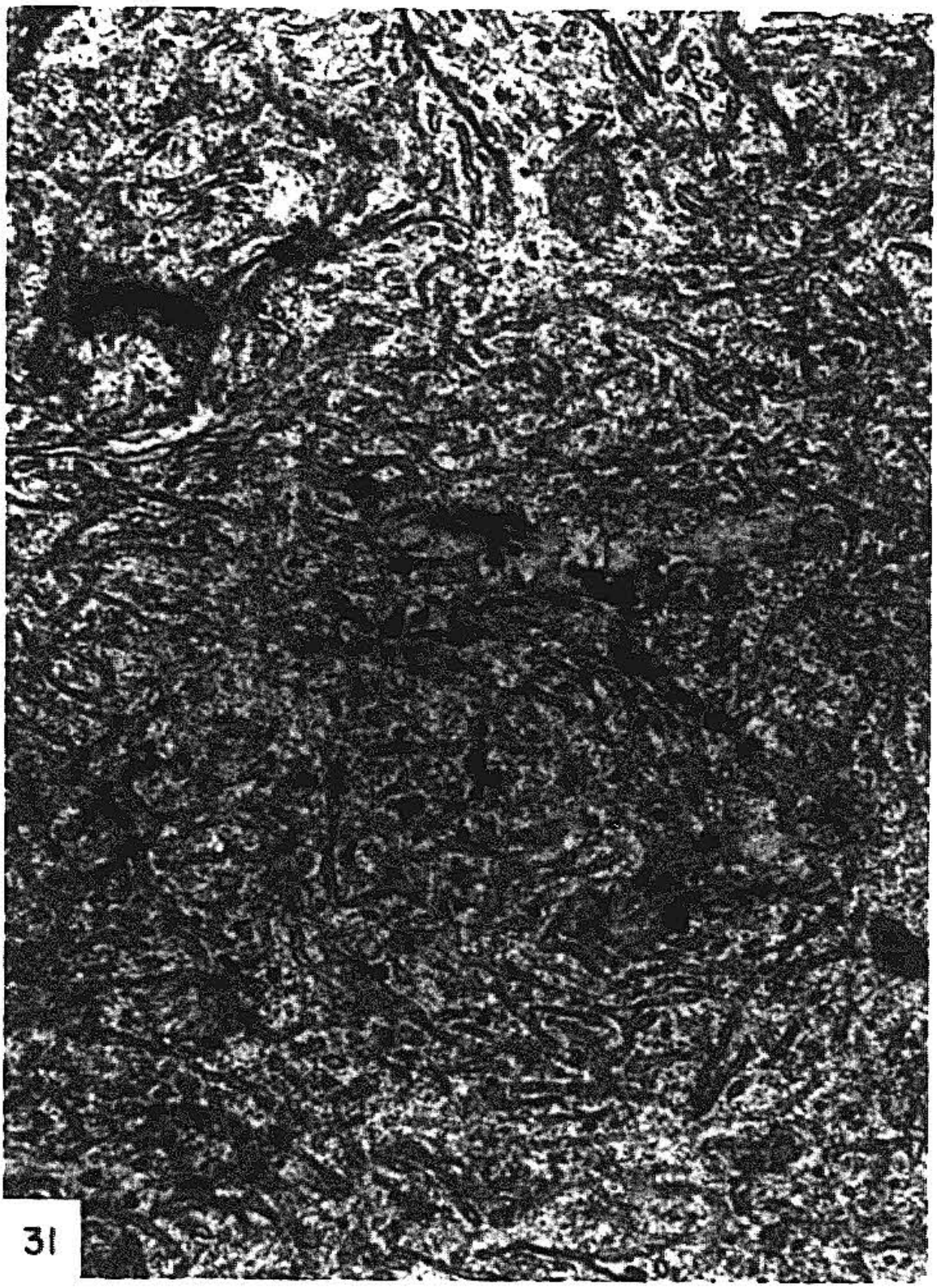

PVS
30

Figure 31. 4 day dehydrated animal, pars nervosa X 65,000

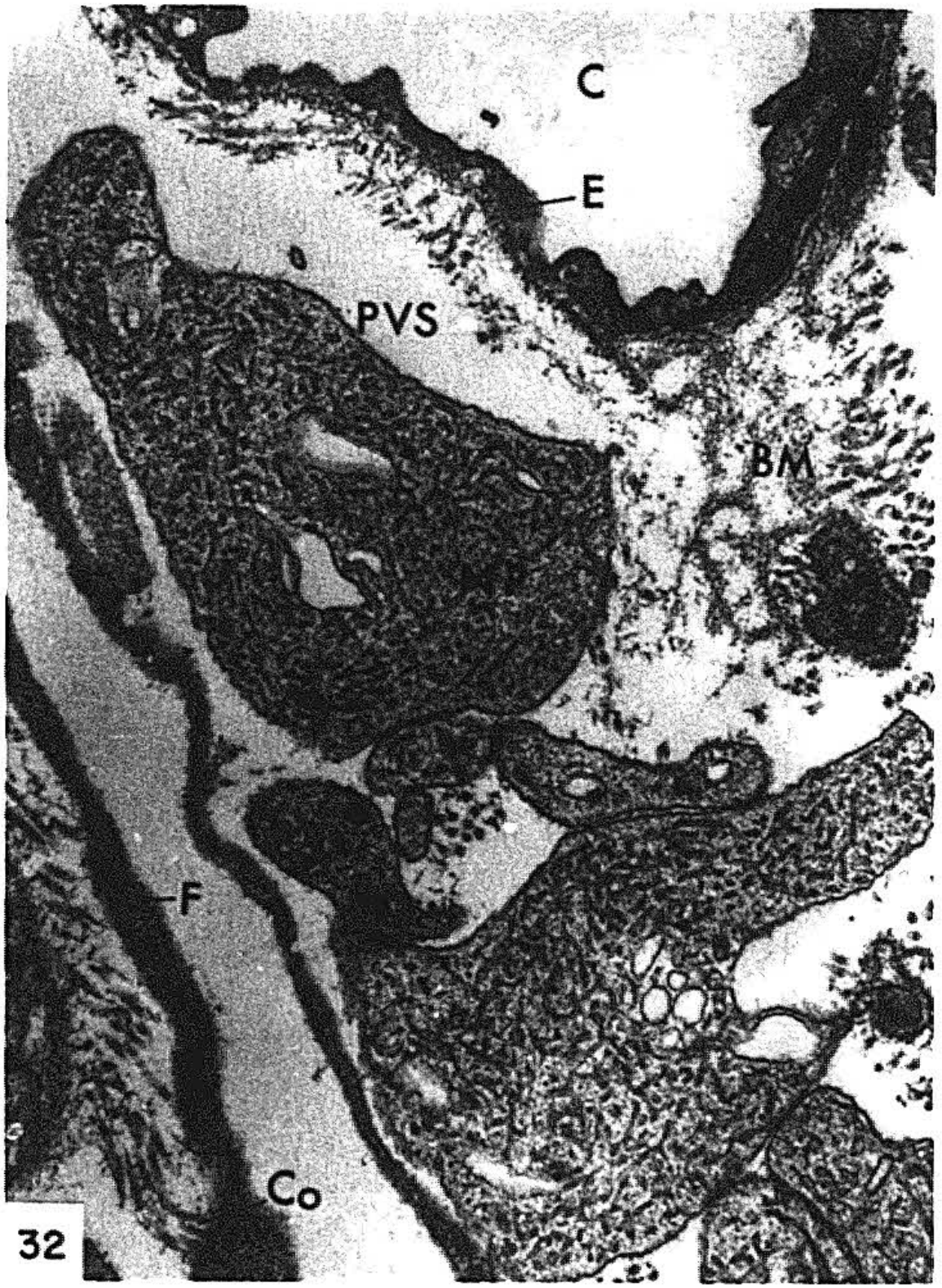
A neuronal process demonstrating numerous tubular cisternae reminiscent of smooth endoplasmic reticulum. Notice the triple layer membrane structure and the random orientation of the tubules.



31

Figure 32. 4 day dehydrated animal, pars nervosa X 36,400

The perivascular space is seen to contain numerous neuronal processes (NP) with tubular structures present. Note the long fibroblast process (F) with the collagen polarized to one side of this process. The collagen (Co) seems to be extruded from the left side of the fibroblast and is perpendicular to the cell surface at the point of attachment.



32

Figure 33. 4 day dehydrated animal, pars nervosa X 23,300

The perivascular space contains numerous large neuronal processes densely packed with tubular structures. Small axons are seen containing neurotubules and mitochondria (NT). Other processes demonstrate tubules adjacent to vesicular structures within the same endings (arrow). The random orientation of collagen fibers is seen at lower right.

33

Figure 34. 2½ day dehydrated animal, pars nervosa X 28,100

A process within the perivascular space is seen to contain both vesicular structures (Pl) and tubular cisternae (T). Since these two structures are within a neuronal process, it is assumed that the tubular structures seen elsewhere are also within neuronal cells. Compare matrix density with the adjacent fibroblast (F). Also to be observed is a capillary fenestration (Fe) and an endothelial extension into the lumen of the capillary (C). An axon with neurotubules is seen within the PVS (A).

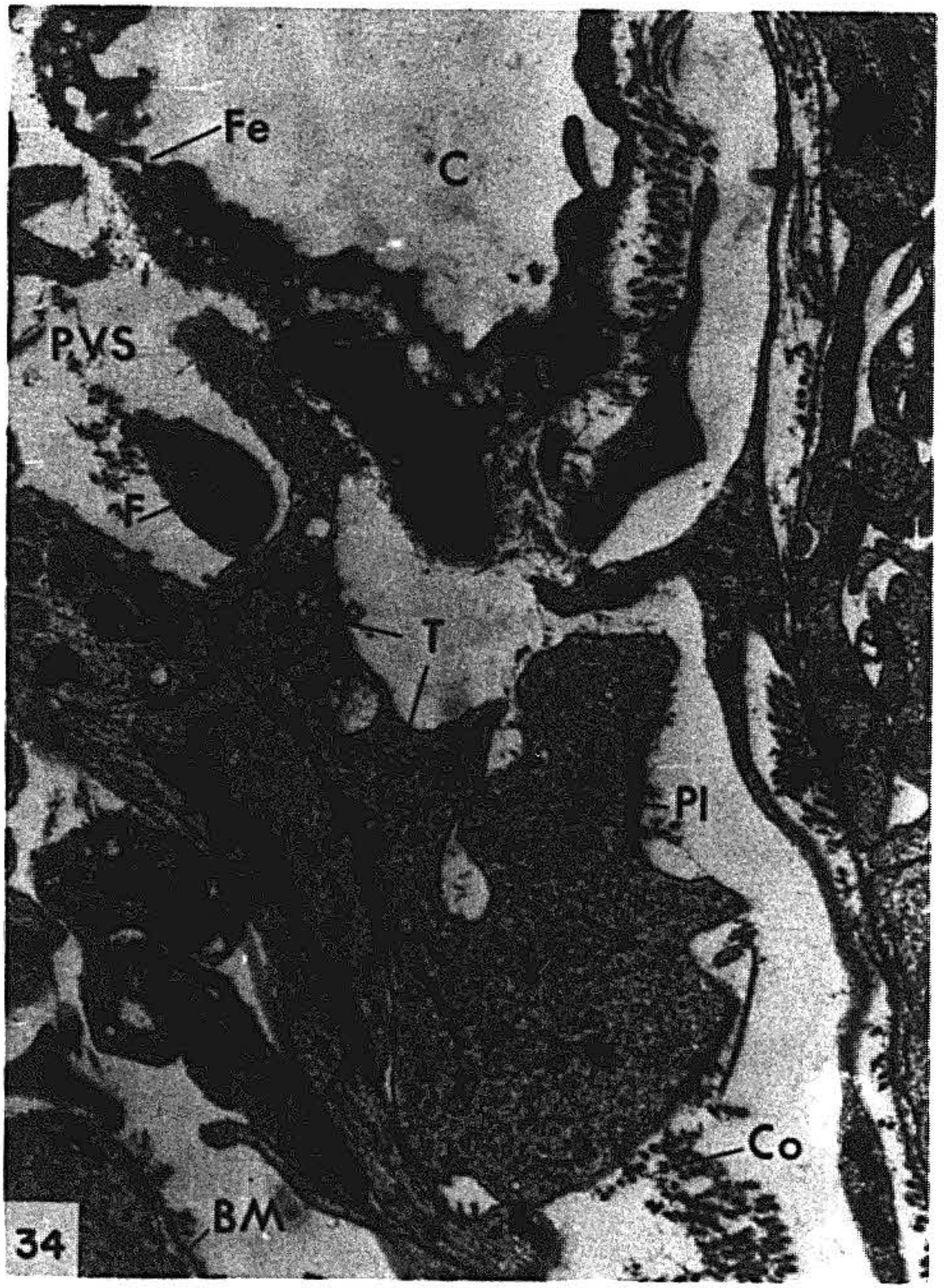

Fe
C
PVS
F
T
Pl
Co
BM
34

Figure 35. 4 day dehydrated animal, pars nervosa X 12,000

This pituicyte appears to be undergoing mitosis. The lipid droplets (L), the diffusely scattered ribosomal particles, and the long mitochondria all indicate that this is a pituicyte. Notice the nuclear chromatin material scattered throughout the cell (Ch) and upon close examination one can see numerous filaments randomly distributed within the cytoplasm. A prominent feature is the marked vesiculation within the cytoplasm. Also present in the center are several lysosome-like structures.

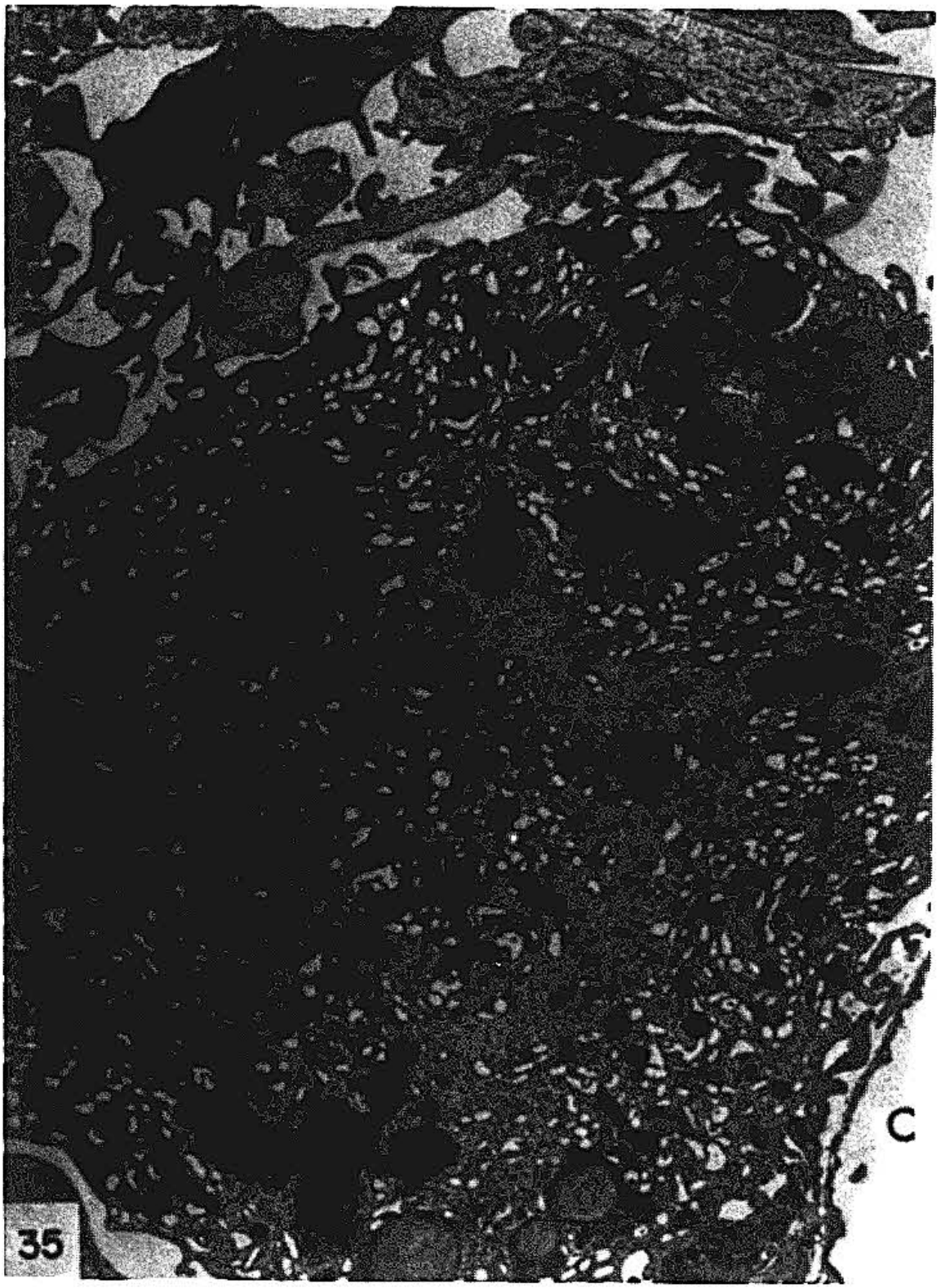
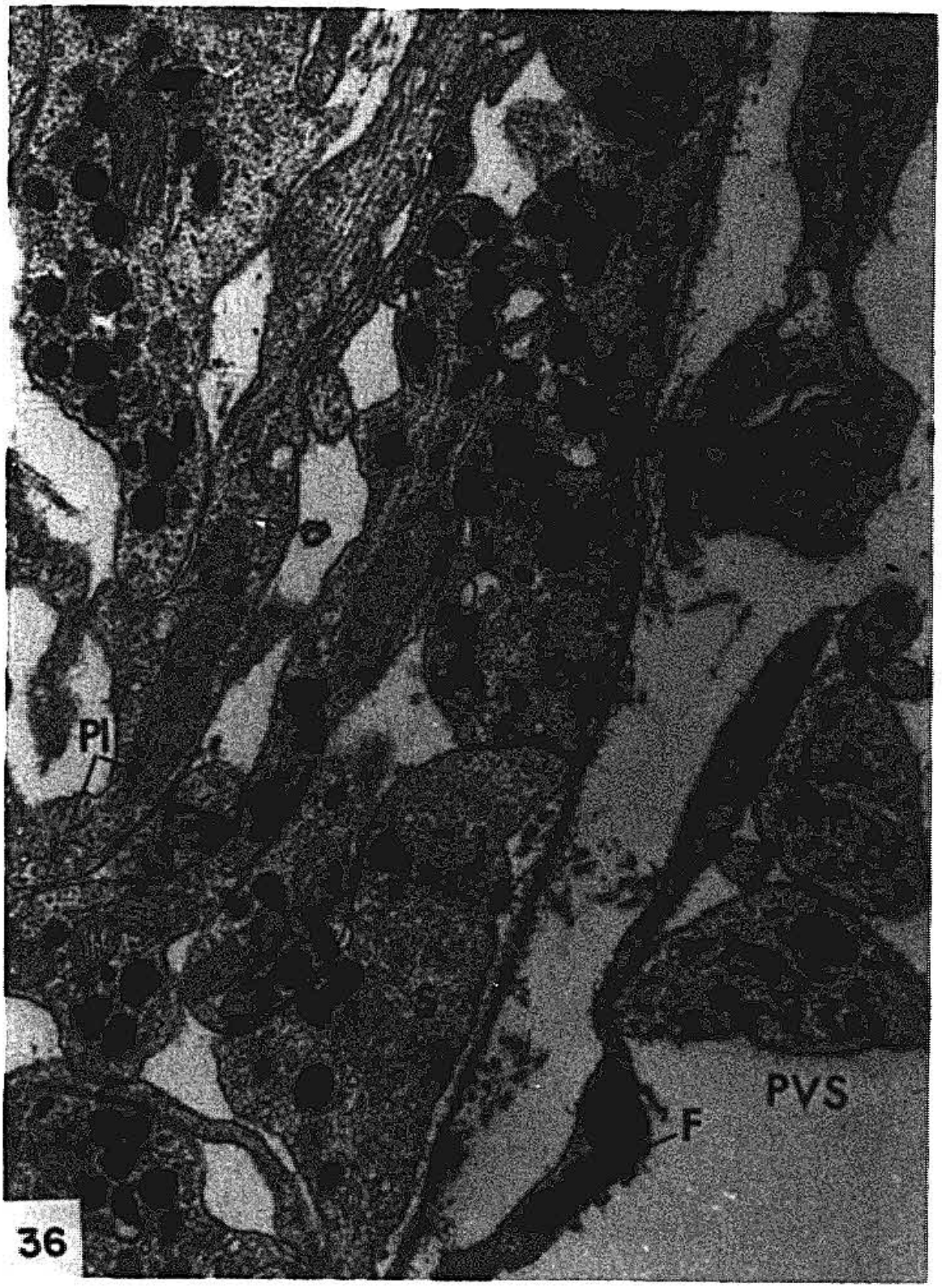

C
35

Figure 36. 4 day dehydrated animal, pars nervosa X 36,100

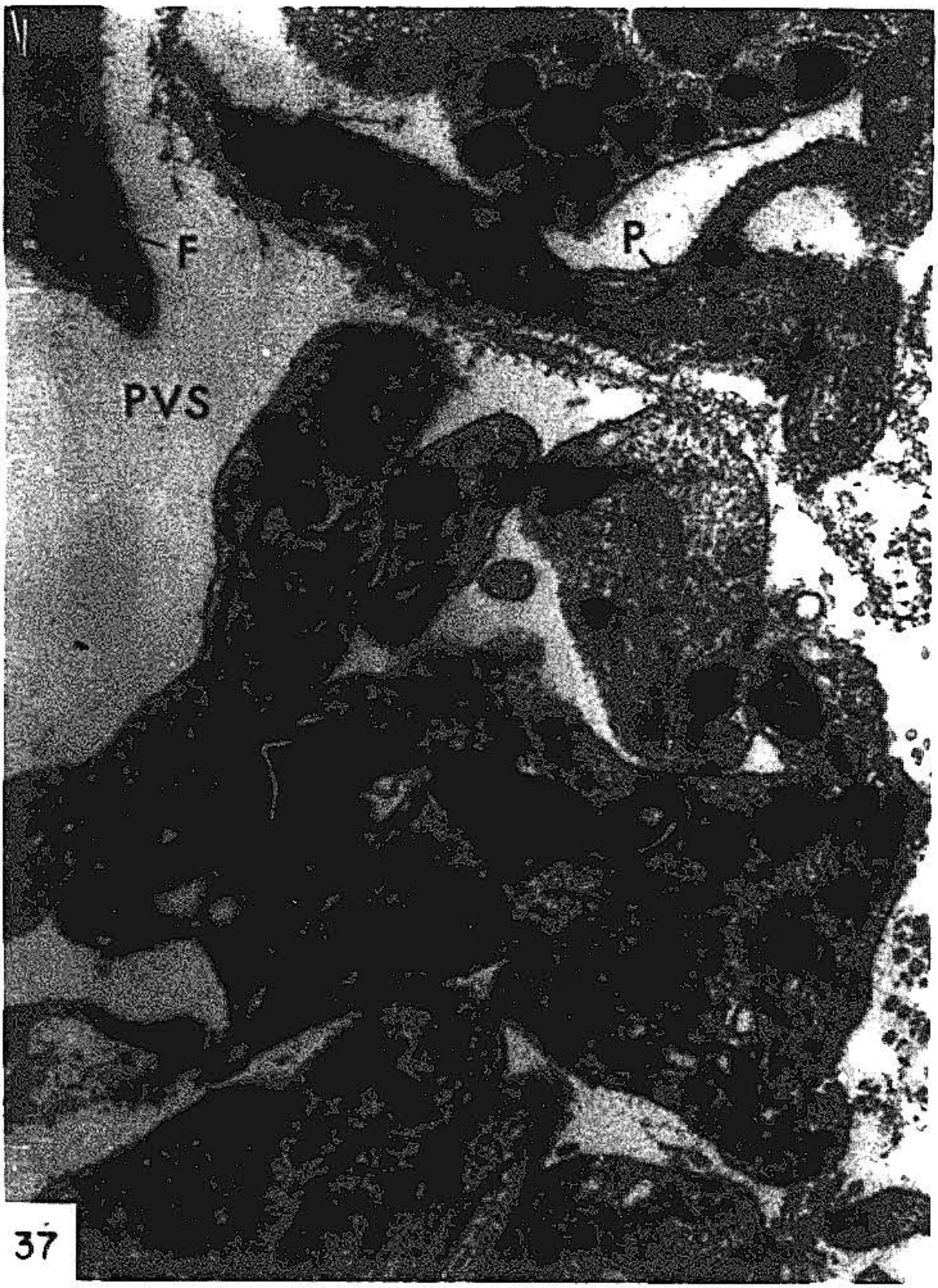
The palisade zone is seen to consist of nerve terminals with fewer NSG than are seen in normal animals. Numerous small vesicles are observed (Pl) (S). The perivascular space (PVS) contains nerve processes (NP) filled with tubular structures as well as occasional vesicles. A fibroblast is also seen (F).



36

Figure 37. 4 day dehydrated animal, pars nervosa X 54,200

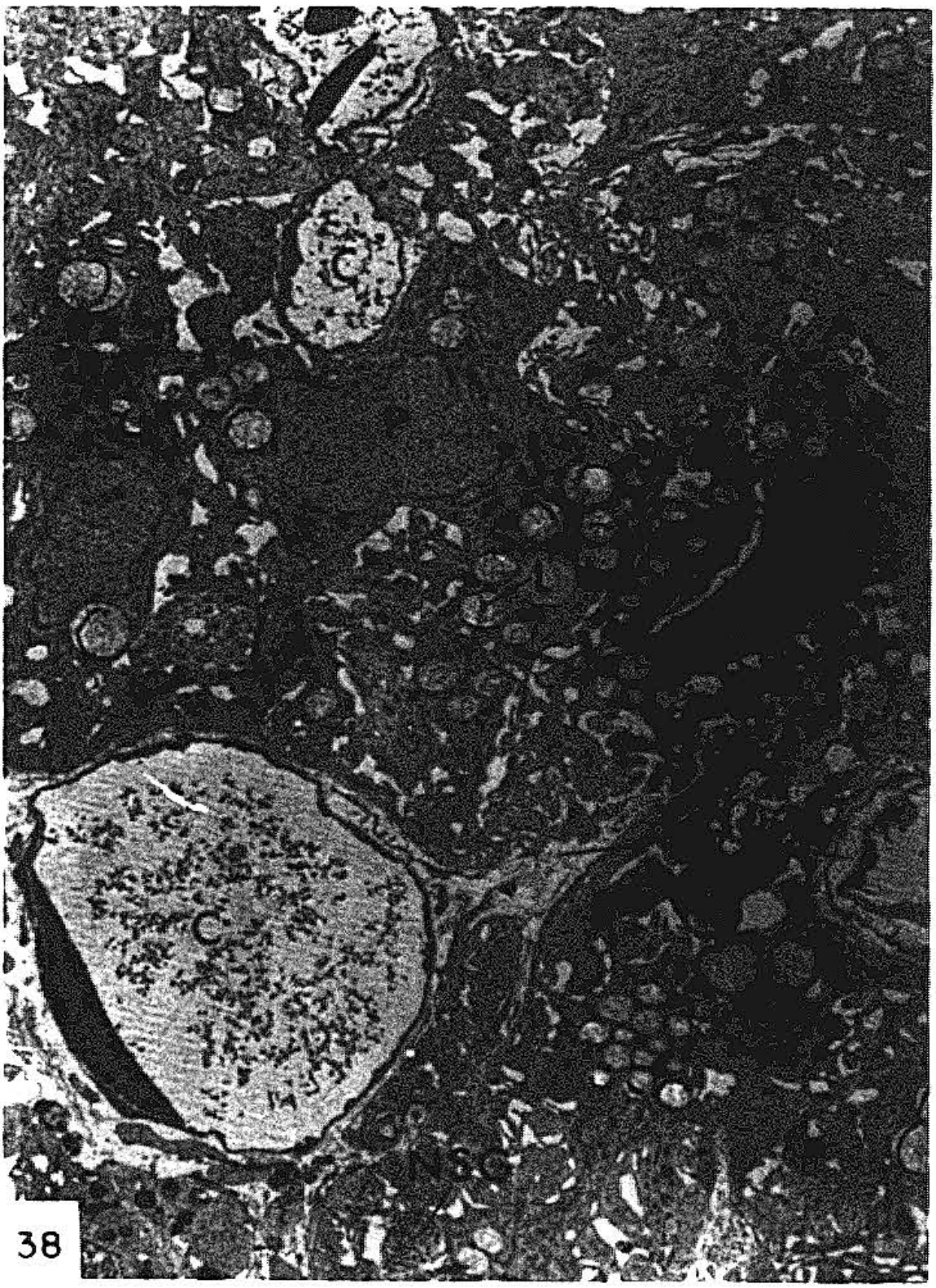
A fiber containing evidence of degeneration is seen within the PVS. Notice the prominent myelin-like lamellar arrangement (La).



37

Figure 38. 7 day dehydrated animal, pars nervosa X 2,500

A low power micrograph showing the dramatic paucity of dark neurosecretion granules (NSG). The dark crescents within the lipid granules (L) are sectioning artefacts. Also notice that the extracellular space appears to be about the same as in the normal animal. Pituicytes also appear similar to that seen in hydrated animals and demonstrate no marked changes.



38

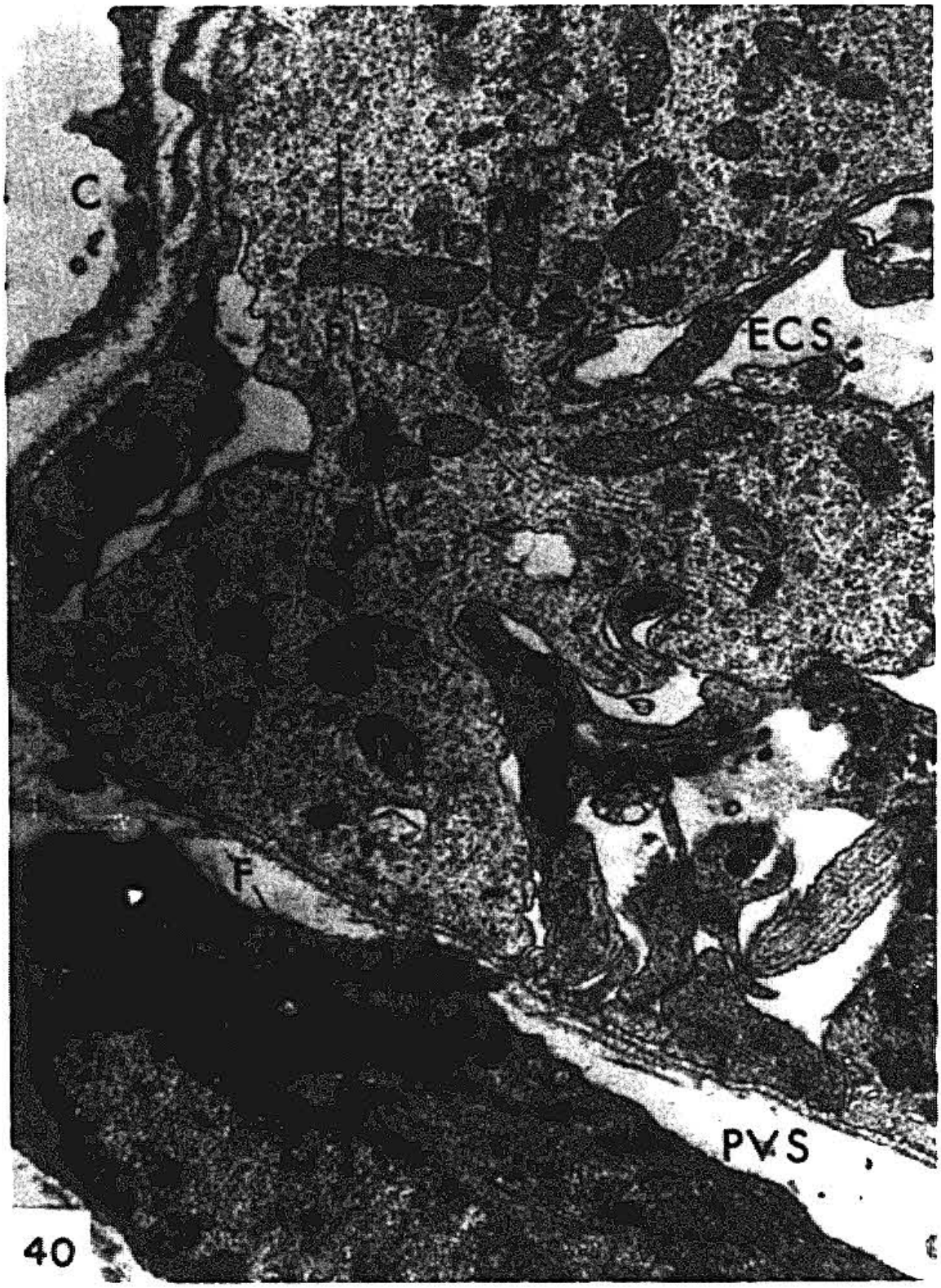
Figure 39. 7 day dehydrated animal, pars nervosa X 2,500

A capillary (C) is seen to be surrounded by extensive PVS. This space contains nerve processes with dark NSG (NP_1) and many processes without dark NSG (NP_2). The latter contain tubular structures as well as clear vesicular structures.

39

Figure 40. 7 day dehydrated animal, pars nervosa X 27,900

A marked loss of NSC is seen in animals deprived of drinking water for 7 days. Notice the dispersed and numerous small pleomorphic vesicles (Pl) seen within the nerve ending and the numerous mitochondria (M). The pleomorphic vesicles appear less densely packed than synaptoid vesicles (S) and are quite irregular in shape.



40

Figure 41. 7 day dehydrated animal, pars nervosa X 46,600

A frequent finding of the 7 day dehydrated animals are the tubular structures (T). A few pleomorphic vesicles are seen near the release area adjacent to the basement membrane (bm). The tubular structures are similar to smooth endoplasmic reticulum.

41