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Quantitative and histomorphological studies on age-correlated changes in canine and porcine hypophysis

by

Lakshminarayana Das

Volume 1 of 2

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
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DOCTOR OF PHILOSOPHY

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Dean of Graduate College

Iowa State University
Ames, Iowa

1971

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INTRODUCTION

Throughout its life span, every organism manifests certain dynamic processes which reveal certain specific roles when they are distributed among the age intervals. In earlier stages of the life span, these processes mainly contribute towards the goal of attaining functional and reactive abilities leading to reproduction of the species. This stage commonly termed development and/or growth period is followed by one which denotes the period of maximum functional ability, both in reproduction and reactance to the environment. During the latter stage, different tissues and organ systems of the body are often referred to as being normal. Terms such as adult, sexually mature, matured and fully grown are often used to denote this stage. The next stage is evidenced by changes undergone by tissues and organ systems in the body as a result of the dynamics of life's processes with the passage of time. These morphological and functional deviations reduce the functional ability of organ systems, decrease the adaptability of the organism and may contribute towards its death. This stage is commonly referred to as old age or senescence.

In earlier days of gerontological research, the term age changes was applied in a limited sense to the morphological and functional changes that are evident during senescence (Strehler, 1962). Since most visible changes

with advancement of age are evinced by organs of the reproductive system, the main cause of such changes in the process of ageing was thought to be the decreased activity of gonads. This concept has since been modified. The process of ageing is no longer considered to be limited to changes during senescence but is envisaged to involve all basic underlying changes in structure and function that lead to the gradual development and subsequent dysfunction of various systems in the body (Strehler, 1962; Getty, 1966). Thus, the science of gerontology incorporates the changes that occur in the organism during its post-natal life, growth, maturity and senescence.

Many of the changes in structure and function represent the sum of changes which are of extrinsic and/or intrinsic origin. Since the process of ageing is considered as a fundamental property of the organism itself and not of the environment in which it lives, basic age-correlated changes should be characterized from those of extrinsic and pathological origin. Strehler (1962) emphasized that any age-associated change should belong to the criteria of universality (occur in all members of the species), intrinsicality (even though widespread, should be of intrinsic origin), progressiveness (should resemble a process but not a sudden event) and deliteriousness (should contribute towards death of the organism). Thus, it is needless to emphasize that

gerontological research should involve animals maintained with the least possible variations in management and environment.

Modern gerontological research aims at elucidating basic changes in structure and function with a view to delineate effectively causes and effects of the ageing process. Such a knowledge will contribute towards advances in human geriatrics and animal population. In the former, gerontology is applied to many social and psychological problems, such as treatment of the aged persons, prevention of disease and disability and preservation of vigor and good health (Getty, 1962). In the latter case, gerontology has been envisaged to enhance the reproductive and productive period in the life span of an animal.

In modern biology, the science of gerontology has achieved considerable appreciation due to its wide range of application as a basic as well as applied science. Modern cell physiology has been elucidated to such a great extent that an understanding in basic age-correlated changes is necessary for a differential study of the physiological and pathological changes. Since these changes follow a definite pattern in a particular species, study of the ageing process in a wide variety of species will contribute much towards such differential studies.

Since earlier periods, role of endocrine glands in the process of ageing has attracted the attention of gerontolo-

gists. As growth and functional ability of many organs are influenced by the endocrine glands, ageing of the entire organism has been considered by some authors in terms of age-associated changes in these glands (Carlson, 1952; Verzar, 1966). With the advancement of knowledge in the field of neuroendocrinology, more emphasis is being laid at present on the neuroendocrine integration mechanism than on the endocrine system itself. Shock (1966) considered the breakdown of neural and endocrine integrative functions in the individual as one of the major causes of age-correlated impairments in performance of the organism. With the hypophysis being the key organ associated with the mechanism of neuroendocrine integration, age-correlated changes in it have been considered as major contributing factors towards ageing of the individual (Verzar, 1966). Furthermore, several physiological disturbances in the body which are characteristic of advanced age, are also manifested in conditions of decreased activity in some pituitary cells, independent of age of the animal. Hypophysectomy leads to certain peculiar phenomena which are also manifested during senility (Verzar, 1966). Thus, age-correlated changes in the structure of the hypophysis will elucidate a basic understanding of similar changes in other endocrine glands as well as the animal as an entity.

There is considerable variation in histomorphology of the hypophysis, and a comparative study of age-correlated

changes in more than one species will contribute much towards the understanding of biological ageing in this organ. With this view in mind, hypophyses of the dog and the hog have been incorporated in the research project reported herein. The beagle dog, with only two heat periods annually and the hog with an estrous cycle of 21 days also represent breeds with diverse reproductive behavior. As body sizes of dogs vary greatly among the different breeds, study of this species was limited to the beagle. The objectives of the research reported herein are: 1) to contribute to the information available on the morphology of the hypophysis from birth to senescence in the dog and hog; 2) to determine quantitative changes in the hypophysis and their correlations with age and body weight; and 3) to elucidate the possible role of these changes in the process of ageing of the animal. As part of a comprehensive gerontological program, this study is also envisaged to contribute towards a better understanding of similar age-associated changes in other endocrine glands of both species.

REVIEW OF LITERATURE

Nomenclature

Nomenclature that has been used to describe the morphology of the hypophysis in different species is very divergent. Not only is there variation in the adoption of identical terms to designate similar cell types in various species of mammals, but many a time different terms have also been utilized to denote the same cell type in a particular species. Often, terms have been coined following tinctorial affinity of various cell types as a result of which many new terms have been added, every time a new staining technique has been formulated. Another factor that has led to such diversity is the species variation among the human, domestic animals and laboratory animals in the histomorphology of the hypophysis. All of these have contributed towards the present state of confusion in hypophysis nomenclature and have hindered its standardization.

The hypophysis is derived embryologically from two sources: the buccal evagination is termed the Rathke's Pouch and the ventral evagination from the diencephalon is termed the Saccus Infundibuli. The former gives rise to all parts collectively termed as glandular lobe or adenohypophysis, while the saccus infundibuli is transformed into the neurohypophysis (Nelson, 1930, 1932; Hanstrom, 1966). The adenohypophysis is subdivided into a proximal part which is

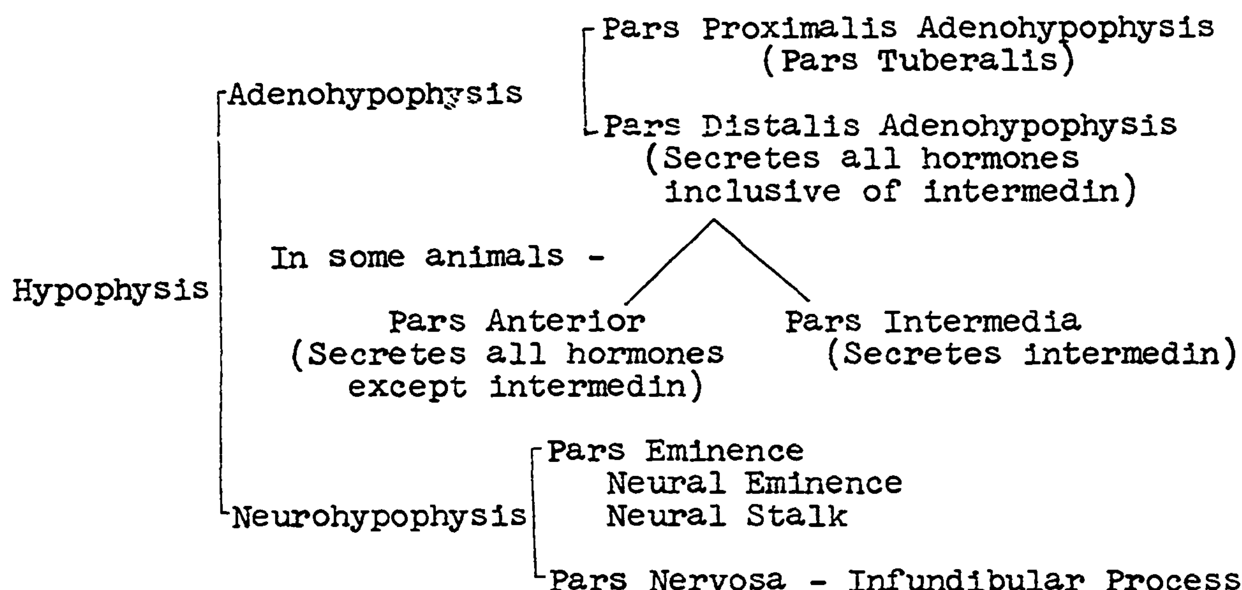
called pars tuberalis, a rostro-ventral part called pars distalis and a caudo-dorsal part termed pars intermedia, which remains separated from pars distalis by the hypophysial cleft. The neurohypophysis is likewise differentiated into three parts. The median eminence is the proximal part that forms part of the diencephalic floor. The distal part of neural lobe is termed infundibular process while the intermediate connecting part is termed the infundibulum or infundibular stem (Hanstrom, 1966).

In common usage, the terms anterior and posterior lobes came into vogue. In human, the pars intermedia does not occur as a separate part and the hypophysis lies in a vertical plane to the base of the skull (Romeis, 1940). Hence, the term anterior lobe is appropriate to designate the pars distalis. The term posterior lobe in human denotes the infundibulum and infundibular process (Severinghaus, 1938; Romeis, 1940). In species, where the hypophysis is oriented obliquely or parallel to the base of the skull and in those, where a separate pars intermedia is present, adoption of these terms in a general sense led to confusion.

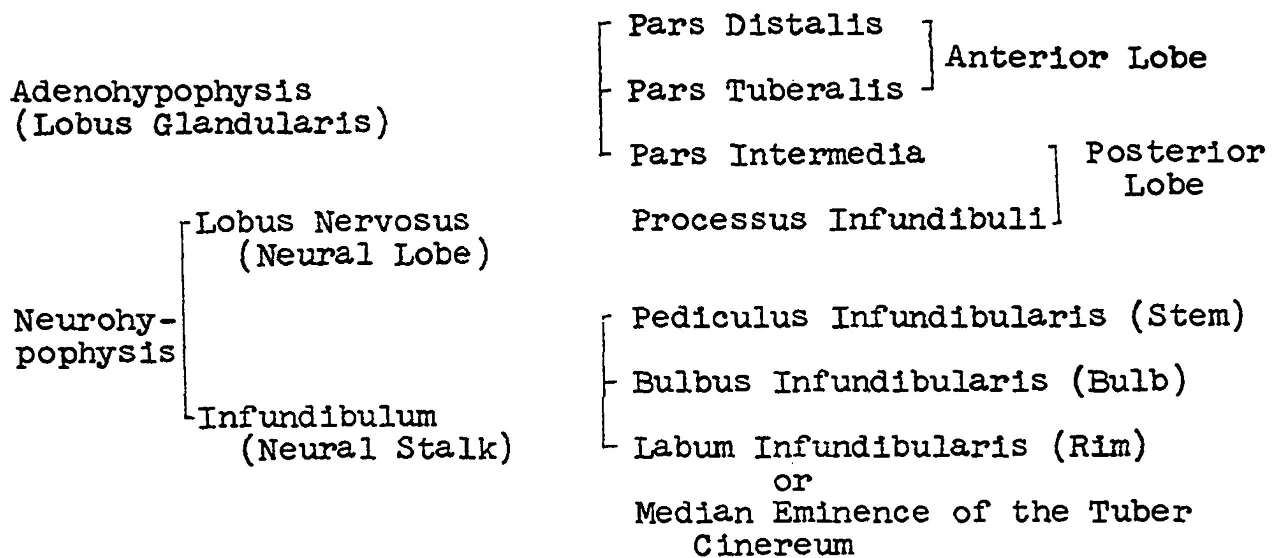
In domestic animals, Trautmann (1911) used the term anterior lobe to denote pars distalis and pars tuberalis and the term posterior lobe to denote pars intermedia, infundibulum and infundibular process. Bloom and Fawcett (1968) followed an identical classification as Trautmann (1911) but

applied the term anterior lobe with reference to pars distalis only.

Purves (1961, 1966) suggested a standardized nomenclature based on a functional basis for both human and animals. The classification advocated by the author can be summarized as follows:

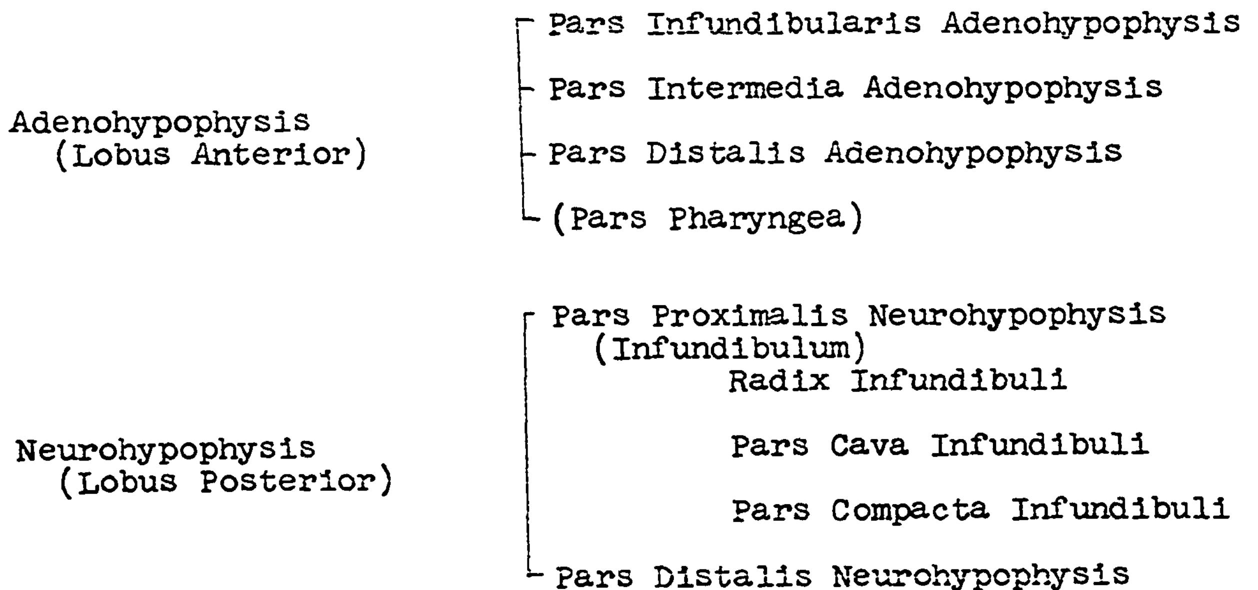


Basing on recommendations of the International Commission on Anatomical Nomenclature (ICAN, 1966), Daughaday (1968) used the term anterior lobe to designate pars tuberalis and pars distalis and the term posterior lobe to denote pars intermedia and processus infundibuli. The complete division of the hypophysis, as has been suggested by the author, is as follows:



The Commission (ICAN, 1966) has originally suggested the term pars infundibularis to denote pars tuberalis and the term posterior lobe to describe, specifically, the neurohypophysis.

The International Committee on Veterinary Anatomical Nomenclature (NAV, 1968) recommended the following classification:



There is no indication to use the term median eminence in this classification. Probably, the term radix infundibuli denotes the former. The Committee (NAV, 1968) has also suggested the terms recessus neurohypophysialis (recessus infundibuli) and cavum hypophysis to replace the terms infundibular cleft and hypophysial cleft, respectively.

In the present study, terminology recommended by the International Committee on Veterinary Anatomical Nomenclature (NAV, 1968) has been adopted throughout. In the chapters on Review of Literature and Discussion the equivalent terms suggested by the Committee have been used with the original terminology of authors being indicated in parenthesis, wherever any ambiguity has been visualized.

In designating various cell types of the hypophysis, Flesch (1884, cited by Peterson and Weiss, 1955) used the terms chromophobe and chromophil to denote, respectively, the glandular cell types in the adenohypophysis which possessed no stainable cytoplasmic granules, and which contained such granules. By combined use of alum hematoxylin and eosin stains, Schonemann (1892) successfully differentiated two types of chromophil cells in human pars distalis and designated them as acidophils or eosinophils and cyanophils. But not until 1900, when Mallory introduced his trichrome technique using acid fuchsin, aniline blue and orange G, could the terms acidophils and cyanophils be

generalized for all species (Landing, 1954). Thom (1901) substituted the term basophil for cyanophil which has since been commonly followed. Since acid dyes such as orange G and aniline blue were used to differentiate between acidophil and basophil cell types, Bailey (1928) advocated the use of terms alpha and beta in place of acidophil and basophil cells. The original Mallory technique as well as its modifications, in some species, displayed several additional colorations of cells, in some species. Thus, the idea of more than two chromophil cell-types comprising the structure of adenohypophysis was thus initiated.

Dawson and Friedgood (1938) successfully used the azocarmine-orange G technique to differentiate between two types of acidophil cells in the cat, which they designated as orangeophil (staining yellow with orange G) and carminophil (retaining the azocarmine dye). Later on, such differentiation was achieved in many other species, which established the two terms orangeophil and carminophil (Dawson, 1963). Orangeophils are the stable cell-type, while carminophil cells are the reactive type, undergoing changes according to the reproductive cycle. However, in some species like the rat and human, orangeophils are the reactive type (Purves, 1961).

Romeis (1940) was the first to contradict the classical theory of two cell-types by the use of his kresazan staining

technique. He distinguished five different types of cells in the human pars distalis and adopted Greek letter system to designate them. In his classification, the cells were termed as alpha, beta, gamma, delta, and eta. The cells which become evident in adenohypophysis of pregnant women were termed epsilon cells. Specific secretion of any hormone was not assigned to any cell type, but changes manifested by the cells during different physiological states were described for each type. As human pars distalis incorporated cells of pars intermedia, it became difficult to adopt Romeis's(1940) nomenclature in other species. Moreover, kresofuchsin was not easily available and did not give uniform results, which made it difficult for the reproduction of Romeis's (1940) results (Herlant, 1965; Purves, 1966).

Halmi (1950, 1952b) modified Romeis's (1940) kresazan method by substituting Gomori's (1950) aldehyde-fuchsin stain in place of kresofuchsin and obtained differentiation between two types of basophil cells in the hypophysis of the rat. He designated the cell-types as beta (aldehyde-fuchsin positive) and delta (aldehyde-fuchsin negative). Purves and Griesbach (1951b, 1952) demonstrated, by physiological experimentation, that the aldehyde-fuchsin positive beta cells are thyrotropic cells and the delta cells are gonadotropic cells. Thus, a classification based on function of the cells became available.

Catchopole (1949), Herlant (1949) and Pearse (1949) applied the periodic acid-Schiff technique of McManus (1946, 1948) to rat and human hypophysis and distinguished a group of PAS positive cells and another group of PAS negative cells. The former proved to be classical basophil cells with glyco- or mucoproteinaceous granules, while the latter represented acidophil cells with granules composed of simple protein. Thus, a firm classification based on histochemical reaction of the glycoprotein content of secretory product of cells came into use. Pearse (1953) named PAS-positive cells of the adenohypophysis as mucoid cells, while Herlant (1949, 1960) designated such cells as mucoproteinaceous cells and the PAS-negative cells as serous cells.

In human, Adams and Swettenham (1958) also termed the basophil cells as mucoid cells. By the use of alcian blue-periodic acid-Schiff-orange G technique, with prior oxidation in performic acid, they further differentiated mucoid cells into two categories: S cells which were stained blue with the alcian blue dye and R cells which were stained magenta by leuco fuchsin. Adams and Pearse (1959) studied the distribution of S and R cells in different physiological and pathological cases and attributed the secretion of thyrotropin to R cells and corticotropin to S cells. Pearse and Van Noorden (1963) combined cytological, cytochemical and immunohistological techniques in their study of the

cell-types of the human hypophysis. In contradiction to the views of Adams and Pearse (1959), the authors subscribed that S cells could be subdivided into two types: S 1 cells and S 2 cells. The former was described as a small cell staining blue with performic acid-alcian blue-periodic acid-Schiff technique and was stipulated to be associated with gonadotropin secretion. The large S 2 cell was stained purple in the same method and was designated as the thyrotropic cell. The authors further claimed that a sub-group of R cells, R 2 cells, were responsible for corticotropin secretion and another subgroup, R 1 cells were concerned with the secretion of follicle stimulating hormone. Purves (1966) observed that R cells, described by Pearse and Van Noorden (1963) in human adenohypophysis, were probably melanotropic cells.

Ezrin and Murray (1963) applied the aldehyde-thionin-periodic acid-Schiff-orange G technique, after permanganate oxidation, to human hypophysis and described five different types of mucoid cells. They designated the cell-types as beta 1, beta 2, beta 3, delta 1 and delta 2. This terminology is somewhat akin to that adapted by Halmi (1950), but is different from all other Greek letter nomenclature. The authors ascribed corticotropin secretion to beta 1 cells, thyrotropin secretion to beta 2 cells, follicle stimulating hormone secretion to delta 1 cells and luteinizing hormone

secretion to delta 2 cells. The authors postulated beta 3 cell-type as an additional source of corticotropin secretion. Conklin (1968) designated the mucoid cells of human adenohypophysis, with Roman numerals, as types III, IV, V, VI and IX, which is an entirely new system. He claimed that these cell-types are associated with corticotropin, thyrotropin, interstitial cell stimulating hormone, follicle stimulating hormone and melanocyte stimulating hormone, respectively. From these reports, it becomes evident that the drawing of homologies between two studies on the same species is a difficult task.

Herlant (1960) applied the erythrosin-orange G-aniline blue-acid alizarine blue technique, after fixation of the hypophysis in Bouin-Hollande, to differentiate various cell-types in the adenohypophysis of several species of mammals. He was able to achieve differentiation of five different cell-types, which were termed as alpha, epsilon, beta, gamma and delta, respectively. The first two cell-types represented acidophils or serous cells and the remaining three types belonged to the category of basophils or mucoproteinaceous cells. With the use of the alcian blue (pH 0.2)-periodic acid-Schiff technique, after permanganate oxidation, the author demonstrated that beta cells were stained violet and were associated with secretion of follicle stimulating hormone; gamma cells were colored brick red and

were responsible for secretion of interstitial cell stimulating hormone; and, delta cells were stained deep blue and represented thyrotropic cells. Thus, the Greek letter nomenclature adopted by Herlant (1960) deviated from similar systems adopted by other authors (Halmi, 1950; Ezrin and Murray, 1963).

In later studies, Herlant (1964, 1965) favored the adoption of functional nomenclature in preference to Greek letter system or any other morphological names. The functional terminology and the corresponding morphologically distinct cell-types advocated by the author are as follows:

STH cell	Alpha cell
LTH cell	Eta cell
ACTH cell	Epsilon cell
FSH Gonadotropic cell	Beta cell
LH Gonadotropic cell	Gamma cell
TSH cell	Delta cell

The International Committee for Nomenclature of the Adenohypophysis (Oordt, 1965) suggested the use of functional as well as morphological nomenclature, according to suitability. The terminology, recommended by the Committee, based on functional classification, designated the cell-types as follows:

Somatotropic or STH Cells

Lactotropic or LTH Cells

Corticotropic or ACTH Cells

Gonadotropic or GTH Cells

FSH Cells
ICSH Cells

Thyrotropic or TSH Cells

Melanotropic or MSH Cells

No standard terminology was offered for morphological classification in order not to restrict the number of techniques employed for such studies. The Committee suggested the use of a morphological name instead of a functional name in cases where the function of a cell-type remains unknown or doubtful (Oordt, 1965).

Purves (1966) adopted a morphological classification based on the tinctorial affinity of different types of cells. The classification adopted by the author is as follows:

Acidophil Cells:

Aurantiphil - Orange after staining with acid dyes, orange G and erythrosin or azocarmine; somatotropes.

Erythrophil - Retain the red dye in the above sequence; equivalent to carminophil cell; lactotropes.

Basophil Cells:

Cyanophil - Aldehyde-fuchsin positive (AF 1); alcian blue positive (AlB 1); stain deep blue with acid dyes; thyrotropes.

Amphophil - Show marked tendency to retain the red dye and to appear purple; two types in most species

Amphophil, AlB 2 - Purple after Ox-AlB-PAS-Or; folliculotrope (gonadotrope I)

Amphophil, AlB 3 - Brick red after Ox-AlB-PAS-Or; Interstitiotrope (gonadotrope II)

Neutrophil - Little affinity for acid dyes and not strongly or specifically stained by any method; corticotrope.

From the foregoing, it becomes evident that there is great deal of variation in the description of similar cell-types of the adenohypophysis among different species. Due to lack of any consistent classification of adenohypophysial cells based on morphological grounds alone, and the fact that it is difficult to draw homology of cell-types with other species unless reference is made with regard to the function of the cell-types, the functional classification recommended by the International Committee for Nomenclature of the Adenohypophysis (Oordt, 1965) has been adopted in this study. Following the suggestions of the Committee, detail morphological description of cell-types and their staining affin-

ities in different techniques have been furnished. The terminology used in this study for the dog corresponds to that adopted by Carlon and Stahl (1964) and Carlon (1967) for the same species. The terminology that has been adopted in the present study for the hog is identical to that used by Bugnon (1963b) and Bugnon and Racadot (1963) in the same species. Dubois and Herlant (1968) have followed the same nomenclature in their study on adenohypophysis of the bovine.

General Morphology

In all species, the hypophysis is situated on the floor of the cranial cavity in a depression termed sella turcica (Hanstrom, 1966). The latter is a complex of bony structures which are constituent parts of the basisphenoid bone. The cranial surface of the basisphenoid bone contains an oval depression--the hypophysial fossa. The fossa is bounded rostrally by the tuberculum sellae which represents the junction between presphenoid and basisphenoid bones (Miller et al., 1964). The caudal limit of the fossa is denoted by a bony process, the dorsum sellae, which is flanked on either side by the caudal clenoid processes. The hypophysial fossa provides lodgment to pars distalis adenohypophysis, pars intermedia adenohypophysis and pars distalis neurohypophysis.

In the hypophysial fossa, the organ remains partially enclosed by a circular fold of dura mater termed diaphragma sellae. The latter prevents the hypophysis from coming into

direct contact with the brain and contains the cavernous and intercavernous sinuses. Rostrally, the diaphragma sellae is attached to the sphenoidal crest and caudally to the caudal clenoid process (Wislocki, 1937a; Miller _et al_., 1964). The dura (of the diaphragma sellae) splits into two layers. The superior lamina extends up to the pars infundibularis adenohypophysis and the inferior lamina passes down into the depths of sella turcica around the hypophysis. Both laminae become blended with the capsule of the hypophysis (Schwartz, 1936; Wislocki, 1937a; Boyd, 1960). Neither the subdural space nor the subarachnoid space extends so deep into the sella turcica as to invest the body of hypophysis (Wislocki, 1937b; Boyd, 1960). The investiture of superior lamina, arachnoid and pia-arachnoid around the pars infundibularis adenohypophysis constitutes the central opening of the diaphragma sellae.

Orientation of hypophysis to the base of the skull differs among species (Herlant, 1951; Krolling and Grau, 1960; Hanstrom, 1966). In some animals, e.g., dog, horse, rat, mice and elephant, the longitudinal axis of the hypophysis is almost parallel to the horizontal axis of the cranial cavity, so that the organ lies almost flat in the sella turcica (Hanstrom, 1966). In the human, camel, tiger, African rhinoceros, opossum and lion, the hypophysial axis is parallel to the vertical plane of the cranial cavity

(Romeis, 1940; Hanstrom, 1966). In other species such as cattle, pig, sheep, goat and cat, the hypophysis is oriented along an oblique plane to the horizontal axis of the cranial cavity (Trautmann, 1911; Krölling and Grau, 1960).

Though, in all mammalian species, the gross divisions of the hypophysis are identical, their degree of development differs (Legait, 1963). The pars distalis adenohypophysis occupies the rostral or rostro-ventral part depending on orientation of the hypophysial axis. In some species (e.g., dog, horse and cat) a dorsal extension over the pars distalis neurohypophysis completely invests the latter lobe (Herlant, 1951). The pars intermedia adenohypophysis always intervenes between the pars distalis adenohypophysis and the pars distalis neurohypophysis, with respect to the extension of the former. In the elephant, whale, flying lemur, monkey and human, the pars intermedia adenohypophysis is either absent or very rudimentary (Hanstrom, 1966). In the opossum, the pars intermedia adenohypophysis occurs in the form of a unicellular layer (Herlant, 1951; Hanstrom, 1966).

The cavum hypophysis occurs as a wide cleft between pars intermedia and pars distalis adenohypophysis depending on their extent. The cavity is reduced to a mass of cysts in some primates, flying lemur and human, and is totally absent in the elephant and rabbit (Purves, 1966). In the whale, a connective tissue septum intervenes between the pars distalis

adenohypophysis and the pars distalis neurohypophysis (Hanstrom, 1966; Purves, 1966). The pars infundibularis adenohypophysis occurs as a cellular collar around the pars proximalis neurohypophysis abutting the tuber cinereum. It terminates distally at the junction of pars distalis and the pars intermedia adenohypophysis. The pars distalis adenohypophysis can be separated from the rest of the organ up to this point. In some species (e.g., pig, rabbit and monkey) a transitory zone of basophil cells, called the zona tuberalis or pars paraneuralis (Romeis, 1940) occurs at the junction of the pars infundibularis and the pars distalis adenohypophysis (Dawson and Friedgood, 1938; Dawson, 1948; Hanstrom, 1952; Purves, 1961).

The neurohypophysis is generally smaller in animals with extensive body surface area (e.g., elephant and whale) and is comparatively larger in small animals (Green, 1951; Legait, 1963). It remains embedded in a groove formed by the pars distalis adenohypophysis. The radix infundibuli and pars distalis infundibuli vary little among different species. All higher species, starting with amphibia, possess a well developed radix infundibuli (Green, 1951). The pars cava infundibuli and the pars compacta infundibuli may occur in some species (e.g., cattle and camel) as two component parts of pars proximalis neurohypophysis or one of the two may constitute the entire neural stalk depending

on the caudal extension of the infundibular recess (Green, 1951; Hanstrom, 1966). In some species of mammals (cat, sheep, tiger and lion), the recessus infundibuli even extends up to variable depths into the pars distalis neurohypophysis (Green, 1951; Herlant, 1951; Hanstrom, 1966).

Depending on the morphology, hypophyses of all vertebrate species other than fishes can be categorized into one of the three following types (Purves, 1966):

Type 1: Pars distalis adenohypophysis and pars intermedia adenohypophysis are both present with the latter being adherent to pars distalis neutrohypophysis. The cavum hypophysis may be present (e.g., dog, pig, cattle, sheep, rat and mice) or may be absent (horse and rabbit).

Type 2: The pars intermedia adenohypophysis does not occur as a separate lobe and pars intermedia cells are intermingled with those of pars distalis adenohypophysis. The latter is not adherent to the pars distalis neurohypophysis and the cavum hypophysis is absent (e.g., birds, elephant and whale).

Type 3: The same condition as type 2 is present with the exception that the lobes, pars distalis adenohypophysis and pars distalis neurohypophysis, are adherent to each other (e.g., human, gorilla and chimpanzee).

The hypophysis is enclcsed in a thin capsule which is pierced by blood vessels. In the deeper part of sella turcica the capsule is fused with the periosteum of presphenoid bone and dorsally it blends with the dura (Wislocki, 1937a). Not only the histological structure of the three component parts of the adenohypophysis is different, but structure of the same part also varies among species (Bloom and Fawcett, 1968).

Pars distalis adenohypophysis

The cells of pars distalis adenohypophysis are arranged in thick irregular cords. Each cord is composed of more than one cell-type (Herlant, 1951). The granulated cells usually occupy the periphery of the cord, while chromophobe cells lie in its central part (Bloom and Fawcett, 1968). The cells are supported by delicate reticular fibers on their peripheral margins. The parenchymal stroma consists of collagen fibers which intervene among the cell cords. Wide vascular channels lined with large endothelial cells and collagen fibers occupy a considerable amount of space in the parenchyma (Herlant, 1951). These are branches of the portal vessels and are predominantly oriented in the sagittal plane (Szentagothai *et al.*, 1968). The cell cords are usually oriented parallel to the portal vessels.

During earlier days, three main types of cells were recognized in the pars distalis adenohypophysis--chromophobes,

acidophils and cyanophils or basophils (Schonemann, 1892; Rasmussen, 1929). Some authors considered the chromophobes as mother cells which gave rise to both acidophils or basophils depending upon the physiological requirement of the body (Rasmussen, 1933, 1936; Severinghaus, 1937). But, lack of bigranulated cells in any species, distinctive position of Golgi apparatus in acidophil and basophil cells and involvement of only one cell-type in a particular type of hypophysial adenoma provoked the idea that chromophobes are resting and degranulated cells and become transformed, only, into either acidophils or basophils depending on predetermined status (Severinghaus, 1938; Jubb and McEntee, 1955). The latter idea is currently in vogue (Purves, 1961, 1966; Herlant, 1964).

By application of complex staining techniques employing several acid dyes in combination, six different cell types have been differentiated in the pars distalis adenohypophysis of many vertebrates (Herlant, 1964, 1965; Holmes, 1964; Purves, 1961, 1966). The somatotrope or STH cells belong to orangeophil or aurantiphil type of the acidophil class (Purves, 1966). The cells can be stained selectively with luxol fast blue (Shanklin _et al_., 1959). They constitute the largest part of the cell population and are more numerous in lateral regions of the lobe than in the centromedial region (Herlant, 1964). The cells are oval,

occur singly or in small groups and do not form clumps. A well distinct Golgi apparatus is often discernible in the juxtanuclear region of these cells (Atwell, 1932; Purves, 1961). Their secretion granules are large and are densely distributed throughout the cytoplasm. In fresh state they are highly refractile (Purves, 1966). The somatotrope cells become degranulated in thyroid deficiency and their granule content increases with thyroxin treatment (Herlant, 1965; Purves, 1966). The cells do not undergo any change with the reproductive cycle except in human and rat (Purves, 1961).

The lactotropic or LTH cells belong to the second group of acidophilic cells. They constitute the carminophils (Dawson, 1946, 1963) or the eythrophils (Herlant, 1960, 1964; Purves, 1966). The lactotrope cells are comparatively larger than somatotrope cells and possess large sized granules (Herlant, 1963, 1964; Purves, 1966). They usually occur in areas where somatotropic cells are scarce and often in small masses or clumps (Racadot and Herlant, 1958). Their form is generally irregular and the cells increase in number, strikingly, during lactation (Herlant, 1964). They multiply in response to reserpine treatment, which stimulates the secretory activity of the mammary gland, and predominate in hypophysial grafts (Purves, 1961). In hyperactive state, their cytoplasm becomes crowded with RNA particles which confer upon them a strong basophilia (Holmes, 1964). Their

Golgi zones become enlarged and nuclei become lobulated. During weaning, they undergo degranulation and often become indistinguishable from chromophobes (Herlant, 1964).

The thyrotropic or TSH cells are a group of basophils which form a small percentage of the cell population (Herlant, 1964). They are uniformly distributed throughout the centromedial region of the pars distalis adenohypophysis and occur singly (Purves and Griesbach, 1956, 1957b). Their form is variable, often oval with distinct cellular outlines (Halmi, 1950; Purves and Griesbach, 1951b). They may also possess angular or rounded contours. The cells are usually situated away from capillary walls. The thyrotropic cells stain specifically with aldehyde-fuchsin, alcian blue and aldehyde-thionin (Pearse and Van Noorden, 1963; Swope *et al*., 1970) and are the classical cyanophils (Purves, 1966). A negative image of the Golgi complex is distinctly visible in the central part of the cell (Atwell, 1932; Purves, 1961). The cells possess fine granules which are distributed in clumped masses around the periphery (Herlant, 1963, 1964). They undergo hypertrophy during exposure of animals to cold and in thyroprivic state (Halmi, 1950; Purves and Griesbach, 1951a, b). In chronic conditions of the latter, the cells become deprived of granules and become transformed into vacuolated cells termed thyroidectomy cells (Halmi, 1952b; Purves and Griesbach, 1956, 1957b).

The FSH gonadotropic cell is a comparatively larger cell than the thyrotrope. Both occur in almost equal proportions in mammals (Herlant, 1964). The cell has variable outline in different species and often remains in contact with the capillary wall (Purves and Griesbach, 1954, 1955). It occurs predominantly in the rostromedian region of the pars distalis adenohypophysis forming the basophil zone (Purves and Griesbach, 1952, 1954; Racadot, 1955). The FSH gonadotropic cell stains purple due to its affinity for both red and blue acid dyes (Herlant, 1960; Holmes, 1964). It is, thus, amphoteric in its staining property (Purves, 1966). The granules of these cells are very fine, but form small clumps due to fixation (Herlant, 1963, 1964). As such, their cytoplasm often appears vacuolated (Herlant, 1964). A negative image of Golgi complex occurs distinctly in these cells and lies away from the nucleus (Purves, 1961). In castration, they undergo hypertrophy and vacuolation giving rise to characteristic castration cells (Purves and Griesbach, 1954, 1955).

The third type of typical basophil cells includes the ICSH gonadotropic cells. The latter constitute variable percentage of the cell population among species (Purves and Griesbach, 1955; Dubois and Herlant, 1968). They are usually distributed singly, most commonly in the central region (Purves and Griesbach, 1954). However, they are not

normally as precisely localized as FSH cells. Their usual form is round or oval with distinct peripheral margins (Purves and Griesbach, 1954, 1955; Herlant, 1964). The cells show little affinity for acid dyes and are strongly stained with periodic acid-Schiff technique as a result of which they are colored brick red in many techniques (Heath, 1964; Herlant, 1965). The brick red color manifested by the gamma cells is attributable to the fact that the polysaccharides that they contain are limited to a strongly acidophilic protein substrate (Herlant, 1960). Species differences in staining character of this cell are evident (Dubois and Herlant, 1968). The granules are fine and densely distributed throughout the cytoplasm so that the staining is homogeneous (Herlant, 1963). They are constantly present in some mammalian species, while in others they are only evident during the course of certain phases of the sexual cycle (Herlant, 1964). In various animals with periodic genital activity, the ICSH cells are only evident during pregnancy and at the time of rut in males. They are comparatively more numerous in females than in males and in sexually mature animals than in immature animals (Purves and Griesbach, 1954, 1955; Heath, 1964). In female animals there is direct correlation between active ICSH cells and presence of corpus luteum in the ovary (Herlant, 1964).

The corticotropic or ACTH cells are still open for

discussion though they have been identified in many mammalian species (Holmes, 1964; Herlant, 1965; Purves, 1966). Racadot (1963), Herlant (1964, 1965) and Carlon (1967) classified them as one type of acidophils (eta cells), while Purves (1961, 1966), Pearse and Van Noorden (1963) and Baker _et al_. (1970) considered them as one type of basophil cells. Siperstein (1963), Dubois and Herlant (1968) and Siperstein and Miller (1970) claimed that the source of corticotropin is a chromophobe cell with distinct morphological and cytological properties of its own. There seems to be less controversy in the morphology of this cell-type. The corticotropic cells are chromophobic, possess very fine granules and less cytoplasmic volume (Siperstein and Miller, 1970). They have little affinity for acid dyes and do not stain specifically by any of the conventional methods (Holmes, 1964; Purves, 1966). A periodic acid-Schiff positive reaction of these cells has been observed in all species (Racadot, 1963; Baker _et al_., 1970). The cells are suitably demonstrated by means of immunofluorescent techniques (Pearse and Van Noorden, 1963; Hachmeister and Kracht, 1965; Baker _et al_., 1970). They occur in small groups in the central part of pars distalis adenohypophysis. They are stellate shaped and have a tendency to send cytoplasmic processes towards the capillaries around neighboring cells (Baker _et al_., 1970; Siperstein and Miller, 1970). Their usual

location site is in the center of the cell-cords. They constitute a small percentage of the total cell population and are larger in the female than in the male (Herlant, 1965; Baker et al., 1970). In adrenalectomy, they become hypertrophied and alterations in their size are related directly to changes in corticotropin content of the hypophysis (Nakayama et al., 1969; Siperstein and Miller, 1970).

The acidophil and basophil cells, as groups, show distinct difference in the location and shape of their Golgi complex (Atwell, 1932; Severinghaus, 1938; Purves, 1961). In acidophils, the Golgi apparatus is small, appears as a shallow cup and is located close to the nucleus (Severinghaus, 1938). The negative image of the Golgi complex is especially conspicuous in basophil cells. In the latter, Golgi apparatus occurs as an incomplete spheroidal or ovoid shell in the cytoplasm some distance from the nucleus (Atwell, 1932; Purves, 1961). The cytoplasm of basophil cells enclosed by the Golgi region is more deeply stained than the rest of cytoplasm (Purves, 1961, 1966).

Pars intermedia adenohypophysis

The pars intermedia adenohypophysis constitutes a variable proportion of the hypophysis volume in different species (Racadot, 1948; Legait, 1963). It is more voluminous in mammalian species which are nonaquatic (Legait, 1963).

The part is extensively lobulated, the lobules being separated by connective tissue septa (Wingstrand, 1966b). In that part of pars intermedia which faces the cavum hypophysis a single layer of ependyma-like undifferentiated cells (termed marginal cells) usually line the pars intermedia, while in those parts where the cavum hypophysis is obliterated, the cells of pars intermedia and pars idstalis adenohypophysis intermingle with each other (Wingstrand, 1966b).

Some cells of the pars intermedia adenohypophysis are usually slender and polygonal or prismatic, while other cells are spherical in outline (Racadot, 1953; Wingstrand, 1966b). The former cells usually possess vacuolated cytoplasm and intranuclear vacuoles while the latter type has a dense basophilic cytoplasm (Racadot, 1953). The nuclei of both types are vesicular. The pars intermedia cells generally resemble basophils of the pars distalis adenohypophysis. They are periodic acid-Schiff positive and are also weakly stained with aldehyde-fuchsin, and alcian blue (Racadot, 1953; Purves and Bassett, 1963). Species variations in the affinity of these cells for certain dyes have been observed (Purves and Bassett, 1963). Their granules are very small and many cells are scarcely populated with them (Bloom and Fawcett, 1968).

The stroma of pars intermedia is very scanty. Apart from a few connective tissue septa, there are very few

capillaries present. The caudal part is less lobulated and vascular than the rostral part of the lobe (Wingstrand, 1966b).

In some species of mammals and lower forms of life, the cells of pars intermedia secrete melanocyte stimulating hormone (Wingstrand, 1966b). This hormone causes dispersion of melanin pigment synthesized by melanocytes (Bloom and Fawcett, 1968). In higher mammalian species, role of this hormone is not specifically known (Daughaday, 1968). However, in all mammals pars intermedia involutes after water deprivation, continuous illumination, during lactation and in all other states of hypothalamic hyperactivity (Legait, 1963). On the other hand, sectioning of the hypophysial stalk or during states of hypothalamic hypoactivity the pars intermedia undergoes hyperplasia (Legait, 1963).

Pars infundibularis adenohypophysis

The degree of development of this lobe varies among different species (Severinghaus, 1938). It is better developed in human than in other mammals (Atwell, 1936; Bloom and Fawcett, 1968). In all species, however, it occurs as a cellular collar around the neural stalk extending between the tuber cinereum and pars distalis adenohypophysis (Green, 1948). Its rostral part is better developed than the caudal. The lobe is almost equal in volume to the pars intermedia (Purves, 1961, 1966).

The pars infundibularis adenohypophysis is composed of

a network of cell cords oriented parallel to the longitudinal axis of the infundibulum (Severinghaus, 1938; Dawson, 1948). In many mammals, the cells are also arranged in the form of alveoli, follicles or acini (Atwell, 1936). The parenchymal cells are chromophobic and are either cuboidal or columnar in shape. The cells contain fine granules which show no affinity for any type of stain (Purves, 1966). Some small basophils invariably occur among the chromophobic cells of pars infundibularis, but acidophil cells are rarely present (Purves, 1961, 1966).

The interstices of the cell-cords are occupied by longitudinal capillaries which have a thin endothelial lining as their walls (Szentagothai et al., 1968). The capillaries form a rich plexus in all mammalian species and finally drain into the portal vessels of pars distalis adenohypophysis (Daniel, 1966; Bloom and Fawcett, 1968). The functional significance of the rich vascular plexus and the parenchymal cells has not been elucidated in any species (Purves, 1966).

Radix infundibuli (median eminence)

The radix infundibuli represents the junctional zone between pars parainfundibularis tuberis of the tuber cinereum and infundibulum of the neurohypophysis. The sulcus tuberoinfundibularis which denotes the line of demarcation between infundibulum and tuber cinereum is not distinct in all species. A section passing through the radix infundibuli

reveals the following layers in medial to lateral direction: 1) ependymal layer, 2) zona interna, 3) zona externa, 4) mantel plexus and 5) pars infundibularis adenohypophysis (Kobayashi et al., 1966; Monroe, 1967; Szentagothai et al., 1968). The layer, zona externa inclusive of the mantel plexus, is also termed as zona palisadica, while the zona interna is termed as zona fibrillaris (Szentagothai et al., 1968).

The ependymal cells which border the third ventricle within the limits of radix infundibuli are characterized by microvilli and cilia at their luminal surface. Nonmyelinated nerve fibers occur in many species in the interstices of ependymal cells (Rinne, 1966). The zona interna is mainly composed of nonmyelinated nerve fibers belonging to supraopticohypophysial, paraventriculohypophysial and tuberohypophysial tracts. Fibers of the latter tract run in transverse direction, while those of other two tracts run in a parallel direction. Thus, the fibers of the tracts criss-cross each other in this zone (Szentagothai et al., 1968). Some myelinated fibers occur in this zone (Monroe, 1967; Kobayashi et al., 1967). Herring bodies resembling in character with those of the pars distalis neurohypophysis are present in this zone along with chrome alum hematoxylin stained material (Duffy and Menefee, 1965; Rinne, 1966). Glial cells resembling oligodendroglia and processes of

ependymal cells are associated with the nerve fibers of zona interna (Rinné, 1966). There are few capillaries in the zona interna and structurally they resemble typical capillaries. No nerve terminals are present in the vicinity of these vessels (Duffy and Menefee, 1965; Kobayashi _et al._, 1966).

The zona externa is comparatively narrower and contains relatively few nonmyelinated nerve fibers. Myelinated fibers are not present in this zone (Oota, 1963; Kobayashi _et al._, 1967). Bulbous axon terminals occur in the vicinity of capillaries throughout their extent in this zone (Monroe, 1967). The neuroglia cells of the zona externa possess vesicular nuclei, relatively pale cytoplasm and branched processes. They resemble astrocytes present in other locations of the central nervous system (Rinne, 1966). The capillaries form a rich network which is especially very extensive between zona externa and pars infundibularis adenohypophysis (mantel plexus; Szentagothai _et al._, 1968). These capillaries are very tortuous and open into the primary capillary bed of the hypothalamo-hypophysial portal system (Cummings and Habel, 1966; Daniel, 1966). Axon terminals of the tuberoinfundibular tract are most numerous in the vicinity of capillaries of the mantel plexus. Neurosecretory material that can be stained with chrome alum hematoxylin has not been observed in the zona externa of any species (Gabe, 1966; Szentagothai _et al._,

1968).

<u>Pars cava infundibuli and pars compacta infundibuli</u>

The structure of the infundibulum does not vary much among different species (Green, 1948, 1951). However, the infundibular recess terminates at different levels in various species, so that the pars cava infundibuli is quite variable in its length (Green, 1951). Structurally, the pars cava infundibuli and pars compacta infundibuli are identical except for the presence of ependymal cells which line the infundibular recess in the former (Green, 1948, 1951). Both parts contain a thin external zone and a rather thick internal zone (Akmayev, 1969). The latter is mainly composed of longitudinally oriented nonmyelinated nerve fibers of the supraopticohypophysial and paraventriculohypophysial tracts. These nerve fibers contain chrome alum hematoxylin positive neurosecretory material as well as Herring bodies (Green, 1948; Gabe, 1966). Glial cells resembling pituicytes of the pars distalis neurohypophysis occur in between the nerve fibers (Romeis, 1940; Green, 1948). Axon terminals are not present in the vicinity of capillaries within this zone (Monroe, 1967).

The external zone contains a rich network of capillaries (Green, 1948; Szentagothai *et al*., 1968; Akmayev, 1969). These vessels enter the infundibulum from the pars infundibularis adenohypophysis, primary capillary plexus

and the junctional zone between pars distalis adenohypophysis and pars intermedia adenohypophysis. Monroe (1967), Szentagothai *et al*. (1968) and Akmayev (1969) observed that some of these axon endings in this region belong to fibers of the tuberohypophysial tract. In addition, collaterals from fibers of the supraopticohypophysial and paraventriculohypophysial tracts and few axons themselves terminate in close proximity to the vessels (Green, 1948; Gabe, 1966; Akmayev, 1969). Neurosecretory material is discernible in all these fibers and the axon terminals (Bloom and Fawcett, 1968; Akmayev, 1969). Barer and Lederis (1966), Gabe (1966) and Akmayev (1969) concluded that the release of neurohypophysial hormones occurs normally from the nerve terminals located in the external zone of pars infundibuli and the quantity released may be enough to maintain the basal physiological level. Many pituicytes occupy spaces between the nerve fibers. Postganglionic sympathetic fibers form a continuous network around the capillaries of this zone (Green, 1948; Christ, 1966).

Pars distalis neurohypophysis

The nonmyelinated nerve fibers of the supraopticohypophysial and paraventriculohypophysial tracts spread out as soon as they reach the rostral and proximal parts of pars distalis neurohypophysis (Christ, 1966; Gabe, 1966). As a result, nerve fibers seem to be widely distributed in this

part. The nerve fibers become finer towards the caudal end of the neurohypophysis and their content of neurosecretory material also increases (Bodian, 1951; Gabe, 1966). The terminal arborizations of axons abut the basement lamina lining the perivascular spaces. In many species such arborizations are seen in discrete areas giving rise to a rosary pattern (Bodian, 1951; Gabe, 1966). Groups of such rosaries constitute a lobule, separated from others by connective tissue septa (Bodian, 1951).

Dense bodies, highly variable in size, shape and staining intensity with chrome alum hematoxylin occur randomly distributed throughout the pars distalis neurohypophysis. These are the Herring bodies (Christ, 1966; Monroe and Scott, 1966; Bloom and Fawcett, 1968). Axon terminals containing neurosecretory material also occur among the epithelial cells lining the recessus infundibuli in species, where the latter is present (Bodian, 1951). Such axon terminals also penetrate into the pars intermedia adenohypophysis (Gabe, 1966). Myelinated fibers containing neurosecretory material occur among the nonmyelinated fibers (Barer and Lederis, 1966). Postganglionic sympathetic fibers are also present along the course of blood vessels (Christ, 1966). These fibers are derived from the autonomic plexus, which follows the caudal hypophysial artery (Christ, 1966).

The pituicytes are glial cells of the neurohypophysis.

They resemble astrocytes of the central nervous system. Usually two types of pituicytes are present in most mammals (Christ, 1966). The fibrous pituicytes possess long cytoplasmic processes but are poor in cytoplasmic volume. The protoplasmic pituicytes are polymorphic cells with much cytoplasm and many branching processes. Several intermediate types may also be evident (Romeis, 1940). The pituicytes may be scattered individually or two or three of them may occur in close proximity to each other. They are frequently associated with capillaries and their processes about the perivascular space (Gabe, 1966).

The pars distalis neurohypophysis has a rich vascular bed (Bodian, 1951; Christ, 1966). In addition to several branches derived from the caudal hypophysial artery, many veins of small and medium caliber also occur throughout the lobe. The vascularity of the junctional zone with the pars intermedia is especially prominent (Christ, 1966; Gabe, 1966). In many species, the two lobes have a common vascular supply (Green, 1951).

Stains and Staining Reactions

The dyes, employed in histomorphological studies, belong either to the acid or basic group. The distinction between these two categories depends on whether the significant part of the dye is anionic or cationic and bears no direct relation to the reaction of any solution of the dye (Lillie, 1969).

Further, the nature of the salt-forming groups of the dyes, the auxochromes, determines the specific category of a dye (Pearse, 1968a). The basic dyes possess the amino group ($-NH_2$) which yields hydroxyl ions and enables the compound to ionize and act as a cation in forming salts. On the other hand, acid dyes have carboxyl group (-COOH) which is capable of furnishing hydrogen ions by electrolytic dissociation (Gurr, 1962; Lillie, 1969). A basic dye can be converted into an acid dye by sulfonation (Pearse, 1968a). The sulfonic group-SO_3H is a salt-forming group of strong acid character which not only changes an otherwise basic dye into an acidic one but renders it soluble in water (Pearse, 1968a; Lillie, 1969). In general, sulfonated (acid) dyes appear to become more solidly fixed in tissues which they stain and are not completely removed by ordinary solvents (Gurr, 1962).

The acid (anionic) dyes impart coloration to the cytoplasm due to salt unions between them and histidine, guanidine arginine, and perhaps with citrulline groups while coloration of nuclei with basic (cationic) dyes is attributed to nucleic acids and to the presence of sulfuric acid esters of the aminopolysaccharides (Pearse, 1968a; Lillie, 1969). When two dyes of the same class compete with each other, their color fastness is determined by their molecular weights (Gurr, 1962).

Uptake of both anionic and cationic dyes by various

tissue elements is greatly influenced by pH of the staining solution (Peterson and Weiss, 1955; Gurr, 1960, 1962). In general, acid dyes stain intensely at pH 4 to 5 while basic dyes stain intensely at pH 8 to 9 (Peterson and Weiss, 1955). At low pH levels (pH 1 or lower), only, sulfuric and sulfonic residues take up cationic dyes; phosphoric acid residues begin to stain at pH 1.5 and over; and, carboxylic acid residues become reactive at pH 3 to 4 (Lillie, 1969). At pH 3 or lower, vigorous staining of most tissues with anionic dyes is evident even after deamination (Lillie, 1969).

In a staining sequence where interaction between the basic dye already present and the acid dye is to be avoided, the latter should preferably be used in alcoholic solution (Lillie, 1969). In its use as a cytoplasmic stain, where it precedes the basic dye, the acid as well as the basic dye should be in aqueous solution for better interaction between the two (Lillie, 1945, 1969). Acid and basic dyes in alcoholic solution do not form insoluble reaction products (Gurr, 1960, 1962). The dyes which have been employed in the present study to achieve differentiation between various cell-types of the adenohypophysis are reviewed below.

Acid alizarine blue (BB) is an acid dye of the hexahydroxy anthraquinone group. It is a sulfonated member of the group with two sulfur atoms which furnish it the acid character (Gurr, 1960; Lillie, 1969). It forms a

soluble lake with aluminum sulfate, which is responsible for the coloration imparted by the dye (Racadot, 1962c). Gurr (1960) pointed out that the final staining solution of pH 2.9 gives the sharpest and cleanest coloration.

Acid fuchsin is a magenta-red acid dye of the triphenyl-methane group consisting of sodium salts of the sulfonic acids of basic fuchsin (Gurr, 1960; Lillie, 1969). Like the latter, it is also a mixture and is among the most commonly used plasma stains. Acid fuchsin possesses the peculiar property of uniting chemically through its amino groups with another acid dye, light green (Gurr, 1960, 1962).

Alcian blue 8GX is a phthalocyanine dye. It is an amphoteric dye with a chemical composition similar to chlorophyll with $C_6H_4 \cdot C_2N_2$ radicals surrounding a central copper atom (Steedman, 1950). Though the dye is not strictly specific to stain mucin selectively, it can be used so that only mucins take the stain (Gurr, 1960). The dye has an affinity for acids as well as for carbohydrates at acid pH (Pearse, 1968a). Lison (1954) employed alcian blue to obtain coloration of the mucopolysaccharide components of connective tissue. Feeney and McEwen (1956) and Herlant (1960) confirmed the dye as a general stain for acid mucopolysaccharides. Adams and Sloper (1956) employed alcian blue in order to demonstrate cystine in neurosecretory material of the neurohypophysis. In this

reaction, prior oxidation in performic acid converts cystine into cystic acid which becomes stained by alcian blue at a strongly acid pH (0.2). Adams and Swettenham (1958) concluded that alcian blue stains the cystine component of certain mucoid cells in the hypophysis and claimed that affinity for alcian blue after performic acid oxidation reveals the presence of S-S groups oxidized into sulfydryl groups. Herlant (1964) believed that the sialic acid component in the mucoproteins of these cells becomes stained with the sulfate radicals of the dye.

Aniline blue (water soluble) is an acid dye of the triphenyl-methane series (Gurr, 1960). It is a mixture of two acid dyes, water blue I and methyl blue I. The latter has two sulfonic groups and thus, is responsible for the strong acidic character of aniline blue (Lillie, 1969). Aqueous solutions of the dye especially in low percentages become decomposed rapidly due to air-borne bacteria (Gurr, 1960). Acidification of the aqueous solution with trace of acetic acid intensifies the color of the dye solution. Mallory (1900) used aniline blue as one of the constituents of his trichrome stain for connective tissue. Rona and Morvay (1956) employed aniline blue in conjunction with orange G and Gomori's (1941) chrome alum hematoxylin for differentiation of cells in adenohypophysis and pancreatic islets. Aniline blue has also been found useful for

staining of collagen fibers and in the rhodocyanin technique, a one-step method for the pars distalis adenohypophysis (Lillie, 1969).

Azocarmine is an acid phenylrosinduline aposaframin dye of the azine group. The dye is available in two forms (Lillie, 1969). Azocarmine G is the monosodium salt of the disulfonation product of phenylrosinduline, which is the parent basic dye. Azocarmine B is the disodium salt of the trisulfonation product of the same basic dye. There is little chemical difference between the two dyes; but the former is comparatively less soluble in water (Gurr, 1960; Lillie, 1969). Azocarmine is mostly used in Heidenhain's azan technique (Gurr, 1962) and, in conjunction with Mallory's (1900) aniline blue-orange G stain. Dawson and Friedgood (1938) employed azocarmine to differentiate two types of acidophil cells in the pars distalis adenohypophysis.

Basic fuchsin is a mixture of three basic dyes, pararosanilin, rosanilin (magenta I) and magenta II (Lillie, 1969). It belongs to the triaminotriphenyl-methane series. Pararosanilin is least intense in color; rosanilin (magenta I) is more intense in color, with a bluish shade due to its methyl group; and, magenta II is more bluish in shade due to its content of three methyl groups (Gurr, 1960). The final combination imparts magenta-red coloration. It is a powerful nuclear dye and stains mucin, elastic fibers and

fuchsinophil granules (Lillie, 1969). In Weigert's elastic tissue stain, basic fuchsin is combined with resorcinol and ferric chloride by heat. Binding of resorcin-fuchsin does not convey any information concerning the protein moiety of tissue structures, but apparently demonstrates compounds associated with proteins, presumably polysaccharide esters (Lillie, 1945).

Hotchkiss (1948) employed leuco fuchsin (Schiff reagent) in conjunction with periodic acid oxidation to demonstrate the polysaccharides. Schiff's reagent is prepared by the action of sulfurous acid on basic fuchsin. Sulfurous acid is generally produced by the action of hydrochloric acid on potassium metabisulfate and unites with basic fuchsin in such a way as to disrupt the resonance system of the latter, thereby bringing about its decolorization (Pearse, 1968a). The periodic acid-Schiff technique depends on the fact that periodic acid oxidizes glycols with vicinal OH group (CHOH-CHOH) converting them into dialdehydes (CHO·CHO). It also oxidizes carbonyl compounds in which the carbonyl group is adjacent to a hydroxyl group, resulting in the production of dialdehydes. The dialdehydes unite with leuco fuchsin (decolorized fuchsin-sulfurous acid) forming a new compound characterized by its red coloration (McManus, 1946; Pearse, 1968a). Water insoluble aldehydes (trans-hydroxyls) require a longer period of time than water soluble aldehydes (cis-

oriented hydroxyls: Pearse, 1968a; Lillie, 1969). Carbohydrate complexes (glycoproteins and mucoproteins) are among other substances which show periodate clevage oxidation (Holmes, 1964). After treatment of the tissue with periodic acid, all glucose and fucose residues and most galactose and mannose residues are eliminated and, therefore, these monosaccharides must be present in such a form that their 1, 2-glycol groups are free for oxidation with periodic acid (Leblond et al., 1957).

The difference in color in the periodic acid-Schiff reaction of different carbohydrates has been interpreted as the result of formation of different types of Schiff-aldehyde addition compounds, depending on the technique used and on the steric arrangement of the aldehyde groups (Barka and Ornstein, 1960). Leblond et al. (1957) observed that the hexose content of tissues parallels the intensity of periodic acid-Schiff staining. Pearse (1968a) subscribed that the amount of color developed by the reaction is dependent primarily on the amount of reactive glycol structure present in the tissues. Since certain adenohypophysial hormones (follicle stimulating hormone, luteinizing hormone and thyrotropic hormone) are glycoproteins and contain a carbohydrate moiety, the positive periodic acid-Schiff reaction in certain adenohypophysial cells has been regarded as an indication

of the cellular localization of such hormones (Purves and Griesbach, 1951a; Pearse, 1952a; Holmes, 1964).

Another important use of basic fuchsin in hypophysial cytology is through its derivative aldehyde-fuchsin. Gomori (1950) first reported that basic fuchsin in the presence of strong mineral acids forms intensely purplish dyes with certain aldehydes, like formaldehyde, paraldehyde, propionaldehyde and benzaldehyde. Among the latter, paraldehyde contributed the best results. Aldehyde-fuchsin is prepared by gradual polymerization of paraldehyde at 25°C in the presence of an acid catalyst (hydrochloric acid). This reaction yields acetaldehyde, a highly reactive aldehyde which combines with the open amino groups in the basic fuchsin dyes to form azomethines (Schiff's bases). This results in the addition of unsaturated groups to the basic fuchsin molecules with resultant change in color (Gabe, 1953). Eventually upon ageing, the paraldehyde-fuchsin dye solution gains an absorption spectrum, similar though not identical, to that of methyl violet 2B and of crystal violet (Gabe, 1953; Lillie, 1969). Aldehyde-fuchsin stains elastic fibers (Gomori, 1950) and thyrotropic cells (Halmi, 1951; 1952b; Purves and Griesbach, 1951a, 1951b; Elftman, 1959a; Pearse and Van Noorden, 1963).

Scott and Clayton (1953) reported that the tissue elements which are stained most intensely by aldehyde-

fuchsin without prior oxidation are those which have been found to contain, or to be associated with, highly sulfated mucopolysaccharides. Elements which contain high amounts of cystine react strongly with aldehyde-fuchsin after oxidation but do not react with Schiff reagent. Since the sulfated mucopolysaccharides possess sulfuric groups and the dithio bonds of cystine are converted to sulfonic groups on oxidation, it has been suggested that these groups may be responsible for the staining of tissue elements by aldehyde-fuchsin when the Schiff reaction is negative (Scott and Clayton, 1953). The authors concluded that the reactivities of aldehyde-fuchsin and Schiff reagent are similar in that they both show an affinity for aldehyde groups, but differ in that aldehyde-fuchsin seems to possess an affinity for strong sulfur acids which the Schiff reagent does not.

Ortman _et al_. (1966) stated that the selective staining of thyrotropic cells by aldehyde-fuchsin is due to carboxyl groups in the tissue which react with an intermediate metastable species formed in the dye solution. The nature of this intermediate is unknown. Involvement of tissue hydroxyl or aldehyde groups in this staining reaction has been ruled out. It was subscribed that acid groups are involved in the selective staining reaction in hypophysial tissue. Halmi and Davis (1953) and Elftman (1959a) demonstrated that, after prior oxidation, aldehyde-fuchsin stains granules of

thyrotropic cells, gonadotropic cells, neurosecretory material and colloid. Purves (1966) observed that factors like concentration of the dye, oxidation and postchromation affect the staining behavior of aldehyde-fuchsin. Pearse (1968a) reported that, in the staining reaction of elastic fibers, aryl, hydroxyl or amino groups of the dye molecule are required for staining and a reaction based on the formation of hydrogen bonds takes place with the sphingomyelin component of the tissue.

Eosin is a rose-pink acid dye of the xanthene series belonging to the fluorone group (Gurr, 1960; Lillie, 1969). It is one of the most common plasma stains employed in routine histological work. Aqueous solutions of the dye are decomposed on long storage by air-borne bacteria (Gurr, 1960). The aqueous solution of the dye has a lucid color-shade when pH of the solution is on the alkaline side (Lillie, 1969).

Erythrosin B (or bluish) is a cherry-red acid dye of the xanthene group and is a tetraiodo compound corresponding to the tetrabromo compound of typical eosin (Lillie, 1969). Gurr (1960) claimed that aqueous solutions of the dye are less liable for decomposition with air-borne organisms and the stain is not readily removed from sections by alcohol.

Another acid dye of the xanthene series is phloxine B. It is a bluish-red acid dye possessing four instead of two

chlorine atoms in the phthalic acid residue of the molecule (Gurr, 1960). Among the three acid dyes, eosin is strongly acidic followed by erythrosin and phloxine B in that order. The color depth of the dyes follow the reverse order. The latter character is attributed to the number of halogen atoms present in the dye molecule (Gurr, 1960, 1962). Lillie (1969) observed that the use of these three acid dyes should depend on the necessity in a particular staining procedure. As a general counterstain, the one with diffuse staining properties and a color showing good contrast for the nuclear stain should be preferred. The more acid and lighter color dyes in the series are suitable for such use.

Hematoxylin is a natural coloring substance obtained from the wood of the tree <u>Caesalpina campechianum</u> (hematoxylon). Hematoxylin has no staining properties of its own but owes its tinctorial properties to the formation of one of its oxidation products, hematin (Gurr, 1960). In order that the oxidized product, hematin, be formed in solution, hematoxylin requires lengthy periods of ripening. Ripening can be hastened by the addition of small quantities of oxidizing agents such as hydrogen peroxide, chloral hydrate, potassium permanganate or sodium iodate (Gurr, 1960).

Some form of mordanting is generally required when solutions of hematoxylin are used for staining (Lillie,

1969). The lakes formed with these mordants carry a strong positive charge and accordingly behave as strongly basic dyes (Gurr, 1960). The ferric lake (iron hematoxylin) is intensely blue-black and has a tendency to accumulate in sites, where there is dense stainable material. As such, iron hematoxylin stains chromosomes intensely and is used as a convenient nuclear stain (Lillie, 1969). Hematoxylin, with the aluminum mordant ammonia alum and the oxidizing agent sodium iodate in an acid medium, forms Mayer's acid hemalum which also serves as a suitable nuclear stain (Gurr, 1960; Lillie, 1969). With phosphotungstic acid (Luna, 1968) hematoxylin is used for staining of reticulum in Mallory's phosphotungstic acid-hematoxylin method. In Verhoeff's elastin stain, hematoxylin is combined with ferric chloride and Lugol's iodine (Pearse, 1968a). Gomori's chrome alum hematoxylin has been employed to stain specifically neurosecretory material, in the neurohypophysis (Bargmann, 1950; Bargmann and Scharrer, 1951; Scharrer, 1954).

Light green SF (yellowish) is an acid dye of the diamino-triphenyl-methane class. It is a derivative of brilliant green which is sulfonated, and is therefore an acid dye (Lillie, 1969). The powder form of the dye is hygroscopic and the stain fades if exposed to bright light (Gurr, 1960). Fast green FCF is another acid dye of the triphenyl-methane series which is similar to light green SF in its staining properties. Fast green has been

claimed to be advantageous because of its color fastness when exposed to bright light (Gurr, 1960; Lillie, 1969). Lillie (1945) used light green in preference to aniline blue in his modification of Masson's (1929) trichrome stain, which was subsequently employed by Halmi (1950) for the adenohypophysis. Goldberg and Chaikoff (1952a) reported that fast green brings about better contrast between thyrotropic and gonadotropic cells than aniline blue.

Luxol fast blue MBS is a diarylguanidine salt of a sulfonated copper phthalocyanine dye. It is, thus, an acid dye and possesses the character of good light fastness (Lillie, 1969). The dye is only soluble in methanol, ethanol and propanols and is totally insoluble in water and hydrocarbons (Gurr, 1960). The effectiveness in staining is partly attributed to its peculiar solubility (Gurr, 1960). Kluver (1944) and Kluver and Barrera (1954) employed luxol fast blue to stain myelin sheaths in the peripheral and the central nervous systems. The authors claimed that the staining reaction of luxol fast blue is due to the porphyrin content of the tissue elements. On the other hand, Pearse (1955) attributed staining reaction of the dye to an ion-association reaction that terminates by linking the base-$Cu \cdot SO_3H$ (lupric form) to the free oxygen atom of the phospholipid phosphate. Since then, Pearse (1968a) has concluded that, after paraffin embedding, luxol fast blue stains the lipoprotein

reactants of the tissue.

Shanklin et al. (1959) claimed that luxol fast blue is highly specific in selective staining of acidophil (alpha) cells in the hypophysis. Herlant (1964) observed that, after paraffin embedding, luxol fast blue reveals the presence of lipoproteins in the somatotropic cells of pars distalis adenohypophysis. Salthouse (1965) concluded that luxol fast blue stains collagen due to the fact that the molecular arrangement of the dye in methanol is able to complex with collagen. Pearse (1968a) observed that copper phthalocyanin dyes stain elastic tissue due to the presence of sphingomyelin and a mechanism of hydrogen bonding is involved in this reaction.

Methyl blue is an acid dye, which forms one of the component parts of aniline blue. It is strongly acidic on account of two sulfonic groups (Lillie, 1969). Methyl blue has been in use since a long time for simple differentiation of eosinophil and cyanophil cells in the hypophysis (Gurr, 1960). Methyl blue in conjunction with periodic acid-Schiff procedure has been used by Wilson and Ezrin (1954) and Rennels (1957) to differentiate basophil cells in the adenohypophysis of rat and human. Herlant (1964) subscribed that the granules of FSH gonadotropic cells in mammalian species possess selective affinity for methyl blue. This selective staining character may be attributed to the

formation of hydrogen bonds between the unsaturated amino groups of the dye and the hydroxyl radicals in the substrate (Herlant, 1964, 1965).

Orange G is one of the commonly employed dyes in hypophysis histology. It is a monoazo dye having two sulfonic groups and is, thus, strongly acidic (Lillie, 1969). Gurr (1960) suggested the use of an acidic solution of orange G with sulfuric acid which, the author claimed, enhances color fastness of the dye. Herlant (1964) observed that all amphoteric substrates have affinity for acid dyes and acidophil granules become stained by orange G due to their amphoteric properties.

Thionin is a basic dye and is a chloride salt of the thiazin base (Gurr, 1960; Lillie, 1969). It may occur either in the orthoguinoid form, paraguinoid form or combination of both. The salt formation of the dye takes place through the tetravalent sulfur. Though the dye is violet in dilute solutions, it imparts different colors to different histological and cytological structures due to its strong metachromatic properties (Gurr, 1960; Lillie, 1969). Aldehyde-thionin, a derivative of thionin, is commonly used to achieve differentiation between the three types of mucoid cells in human adenohypophysis (Conklin, 1968).

Paget (1959) used thionin to prepare aldehyde-thionin in a similar manner to Gomori's (1950) aldehyde-fuchsin.

Paget and Eccleston (1960) claimed that aldehyde-thionin with prior oxidation of the tissue in acidified potassium permanganate solution stains the same structures as aldehyde-fuchsin. The mechanism of staining of the dye has not been elucidated. Herlant (1964) observed that aldehyde-thionin stains only cells which are rich in sulfated (acid) mucopolysaccharides. Purves (1966) reported that aldehyde-thionin requires prior oxidation in all cases whereas aldehyde-fuchsin stains certain tissue elements without prior oxidation.

Peterson and Weiss (1955) reported that granules of both acidophils and basophils have amphoteric properties. The acidophil cells are stained intensely by acid dyes at low pH and are only moderately stained by basic dyes at high pH. Basic dyes stain basophil cells intensely at high pH while acid dyes stain them only moderately at low pH. Using ribonuclease digestion in conjunction with controlled pH staining, the authors demonstrated that basophilia of basophil cells is distinct from that of ergastoplasm.

Purves (1961) and Herlant (1965) subscribed that cells of the adenohypophysis exhibit their tinctorial affinities when adequate amounts of their specific products have accumulated within their cytoplasm, in the form of granules. The latter contain respective hormones in association with hormonally inactive proteins. Both the hormone and the

carrier protein differ among the various species (Purves, 1961; Herlant, 1964, 1965). Functionally equivalent cells may therefore have no specific component common to each other despite the fact that they serve identical functions in both species. The inconsistency in staining reactions of the granules of functionally equivalent cells from species to species has been attributed to the above cause (Purves, 1961; Herlant, 1964; Holmes, 1964).

In case of acidophil granules (Purves, 1961, 1966; Herlant, 1964, 1965) and the neurosecretory product (Bargmann, 1966; Gabe, 1966; Pearse, 1968a) staining reactions of the granules have been envisaged as being due to a product distinct from the hormonally active substance. Purves (1961) and Purves and Bassett (1963) have also concluded that the staining affinity of basophilic mucoproteinaceous cells of the pars intermedia adenohypophysis is due to the carrier protein only. The staining affinity of the basophilic mucoproteinaceous cells in pars distalis adenohypophysis has been postulated to be due to the hormonally active product itself (Purves, 1961; Holmes, 1964).

In order to differentiate the two types of acidophil cells in the hypophysis of cat and rabbit, Dawson and Friedgood (1938) employed azocarmine in conjunction with orange G. In this procedure, the cells that still retained the red color of azocarmine after differentiation in aniline

oil are termed carmine cells while cells whose granules are stained by orange G are classified as true acidophils or orangeophils. The authors postulated that carmine cells are associated with secretion of interstitial cell stimulating hormone while the other type of acidophils elaborate somatotropin (Friedgood and Dawson, 1940). Subsequently, Dawson (1946) concluded definitely that the carmine cells in cat and rabbit are the lactotropic cells. Pearse (1952c), combining histochemistry with morphological observations, confirmed that the carmine cells contain granules of simple protein and are responsible for the secretion of lactogenic hormone. Distinction between two types of acidophil cells using azocarmine and orange G have also been revealed in rat (Dawson, 1954a), in monkey (Dawson, 1948, 1954b), in dog (Hartmann _et al_., 1946; Purves and Griesbach, 1957a), in human (Romeis, 1940; Pearse, 1952b, c), in rabbit (Dawson and Friedgood, 1938; Pearse, 1952c), in cat (Dawson, 1946; Dawson and Friedgood, 1938; Racadot and Herlant, 1958) and in cattle (Jubb and McEntee, 1955; Dawson, 1963).

Goldberg and Chaikoff (1952a) and Purves and Griesbach (1957a) employed Mallory's (1900) trichrome stain as modified by Martins (1933) and Crossman (1937), respectively, in the dog. Racadot and Herlant (1958) and Racadot (1961, 1962a) adopted the same technique for the hypophysis of the cat. In this technique, acid fuchsin and orange G are used

in a strong acidic medium to differentiate the acidophil cell-types. The authors obtained distinct separation between orangeophils which stained yellow with orange G and the carminophils which stained red with acid fuchsin. With simultaneous use of azocarmine in Heidenhain's azan technique (Gurr, 1962), Purves and Griesbach (1957a) were able to conclude that the red acidophils in the dog are analogous to Dawson's (1946) carmine cells in the rabbit and cat.

Cleveland and Wolfe (1932) employed a trichrome stain of erythrosin, orange G and aniline blue, in which the erythrosin and orange G dyes are used to stain the two types of acidophils in red and yellow, respectively. Using the above trichrome stain, distinct separation between the two types of acidophils has been reported in dog (Wolfe and Cleveland, 1932; Wolfe et al., 1933; Hartmann et al., 1946; Wolfe, 1959), in cat (Racadot and Herlant, 1958; Racadot, 1962a) and in pig (Cleveland and Wolfe, 1933; Bugnon, 1963b; Bugnon and Racadot, 1963). Hartmann et al., (1946) applied the trichrome stain of Cleveland and Wolfe (1932) along with azocarmine technique of Dawson and Friedgood (1938) and concluded that the erythrophil cells are identical to the carmine cells (Dawson and Friedgood, 1938). By staining consecutive sections in erythrosin and acid fuchsin, the authors also confirmed that the erythrophil cells possess equal affinity for the latter dye. Dawson (1963) observed

that the acidophils can be differentiated into two categories, red acidophils (acid fuchsin or azocarmine) and orange acidophils (orange G) in cat, dog, rat, rabbit, cow and sheep. Herlant (1964) has confirmed the observations of Dawson (1963).

Herlant (1960) advocated a tetrachrome stain using erythrosin and orange G, after fixation of the tissue in Bouin-Hollande solution saturated with sublimate. Using this tetrachrome method, Racadot (1961, 1962a) in cat, Bugnon (1963a, b) and Bugnon and Racadot (1963) in pig, Carlon and Stahl (1964), Purves (1966) and Carlon (1967) in dog and Dubois and Herlant (1968) in cattle have claimed distinct separation between erythrophils and orangeophils or aurantiphils. Racadot (1962a) and Purves (1966) observed that Cleveland-Wolfe's method (Wolfe et al., 1933), Mallory's procedure (Crossman, 1937) and Dawson and Friedgood's (1938) procedure bring about, in some species, the same differentiation as Herlant's (1960) tetrachrome technique.

Pearse (1955) reported that methazol blue (British marketed dye equivalent to luxol fast blue MBS) stains the granules of eosinophilic cells. Shanklin et al., (1959) by simultaneous staining of consecutive sections from the hypophysis of man, pig, dog, cat, cattle, horse, rat, rabbit and jackal with luxol fast blue-periodic acid-Schiff procedure and Masson's (1929) trichrome technique, demonstrated

that luxol fast blue stained selectively the acidophil cell granules (alpha granules). Paget and Eccleston (1960) confirmed these observations in rat and dog. Racadot and Herlant (1958) and Herlant (1964), by employing methazol fast blue in combination with orange G, demonstrated that the somatotropic or STH cells are stained blue after methazol fast blue while the lactotropic or LTH cells stained greenish-yellow after orange G. Herlant (1964) claimed that this combination of methazol blue-orange G not only differentiates somatotropic cells from lactotropic cells in mammals but is also equally favorable in nonmammalian vertebrates. Dubois and Herlant (1968) followed the procedure of methazol fast blue in conjunction with Herlant's (1960) tetrachrome technique and achieved differential staining of somatotropic and lactotropic cells in cattle. The former are stained green with methazol blue while the latter cells are stained with erythrosin.

In dog, Purves and Griesbach (1957a) observed that the lactotropic cells, which stained red with Mallory's trichrome stain (Crossman, 1937), manifested a slight affinity for periodic acid-Schiff reaction. Purves (1961, 1966) further subscribed that the weakly periodic acid-Schiff positive cells in some mammalian species represent the carminophils. Holmes (1964) reported similar observations in the ferret. Heath (1964) and Nayak _et al_. (1968) claimed

that the weak affinity of lactotropic cells for Schiff reagent can be effectively used in conjunction with orange G to isolate them from somatotropic cells in several species of domestic animals.

Barrnett _et al_. (1956) utilized differential protein solubility at various strengths of trichloroacetic acid, in combination with histochemical staining and bioassay, to differentiate the somatotropic and lactotropic cells. The authors reported that treatment of unpreserved hypophysis of the rat in one percent trichloroacetic acid retained only lactotropic hormone as revealed by bioassay. In such hypophysis, the only stainable cells were those which showed tinctorial affinities of typical lactotropic cells. Treating hypophysis in 2.5 and 4 percent trichloroacetic acid solutions, the authors were able to demonstrate further, corticotropin and somatotropin activity, respectively. In a later study, Barrnett _et al_. (1961) confirmed that only lactotropic acidophils remain active during staining of the hypophysis that has been treated with 0.5 percent trichloroacetic acid, while basophils, chromophobes and the remainder of acidophils are unreactive. Purves and Griesbach (1957a) reported that acidophil cells possess solid protein granules which are not soluble in aqueous buffers in the range from pH 4.0 to 8.0.

The classical basophil cells of the adenohypophysis

contain muco- or glycopolysaccharides which stain positively with the periodic acid-Schiff technique (Pearse, 1952a; Halmi and Davis, 1953; Purves, 1961; Holmes, 1964; Herlant, 1965). To bring out morphological differentiation between them, an additional primary stain is applied in conjunction with the periodic acid-Schiff technique.

Adams and Swettenham (1958) employed alcian blue in an acidic medium after oxidation of the sections in performic acid. The authors designated the alcian blue positive cells, which were stained deep blue, as S cells and alcian blue negative cells which stained red with Schiff reagent as R cells. Adams and Pearse (1959) modified the same technique slightly and believed that the alcian blue positive cells are associated with adrenocorticotropin activity which was later on modified by Pearse and Van Noorden (1963) and Pearse (1968a) to thyrotropin activity. Swettenham (1960) adopted the technique of Adams and Pearse (1959) and was successful in differentiating blue cells and red cells in human as well as in rat hypophysis. Herlant (1960) adopted the technique in several mammalian and nonmammalian species using acidified potassium permanganate solution instead of performic acid as the oxidizing agent, and ajusting pH of the alcian blue solution at 0.2. He claimed differentiation of three cell-types; strong alcian blue positive cells (delta cells); violet cells colored weakly with alcian blue as well as

periodic acid-Schiff procedure (beta cells); and, alcian blue negative and rose or red colored periodic acid-Schiff positive cells (gamma cells). Herlant's Greek system nomenclature of delta, beta and gamma cells denote, respectively, thyrotropic, FSH gonadotropic and ICSH gonadotropic cells. Employing the same technique, Racadot (1961, 1962a, 1962b), Racadot (1963), Bugnon (1963b), Bugnon and Racadot (1963), Purves and Bassett (1963), Herlant (1964), Carlon and Stahl (1964) and Carlon (1967) demonstrated three types of basophil cells in the cat, sheep, pig, bat, rat and dog.

Herlant (1965) observed that at pH 0.2, only the FSH gonadotropic cells (beta cells) show slight affinity for alcian blue in mammalian species whereas ICSH gonadotropic cells (gamma cells) are entirely negative. In cattle, Dubois and Herlant (1968) reported that the ICSH gonadotropic cells are reactive to both alcian blue and periodic acid-Schiff reaction; FSH gonadotropic cells are stained red with Schiff reagent alone; and, the thyrotropic cells manifest strong affinity for alcian blue in Herlant's (1960) alcian blue-periodic acid-Schiff technique.

Heath (1964, 1965) applied the alcian blue-periodic acid-Schiff technique of Adams and Pearse (1959), using alcian blue with performic acid oxidation, in several species of domestic animals (horse, dog, cow, pig, cat and sheep)

and observed three uniform types of basophil cells in all species. The author classified the cells as blue (alcian blue postive and periodic acid-Schiff negative), purple (positively stained with both alcian blue and Schiff reagent) and red cells (alcian blue negative and periodic acid-Schiff positive). No functional correlation has been offered for any of these cell-types. However, from his differential solubility tests with trichloroacetic acid, the author remarked that the red cells are probably associated with interstitial cell stimulating hormone secretion in the equine species.

Like alcian blue, aldehyde-fuchsin has also been extensively used in hypophysis histochemistry. Halmi (1950) applied the aldehyde-fuchsin technique as a counterstain to his azan technique and reported that the two types of basophils, beta cells (stained violet after aldehyde-fuchsin) and delta cells (stained blue to blue-green after light green) can be easily differentiated in rat. In a later study, the author reported differentiation between these two types of basophils in human, pig, horse, cattle, cat, dog and mouse (Halmi, 1951). Coupling aldehyde-fuchsin staining with physiological experimentation, Purves and Griesbach (1951b) showed that the beta cells of Halmi (1950, 1951) are thyrotropic cells and the delta cells are gonadotropic cells, which was confirmed by Halmi (1952a).

Aldehyde-fuchsin has been demonstrated to stain specifically the thyrotropic cells in pig (Bugnon, 1963b; Bugnon and Racadot, 1963; Heath, 1964), in cattle (Heath, 1964; Dubois and Herlant, 1968), in sheep (Racadot, 1962b; Heath, 1964), in dog (Carlon and Stahl, 1964; Heath, 1964; Carlon, 1967), in horse (Heath, 1964), in cat (Racadot, 1961; Heath, 1964) and in human (Halmi, 1951; Pearse and Van Noorden, 1963; Pearse, 1968b).

Halmi and Davis (1953) oxidized hypophysial tissue with sulfuric-permanganate, prior to aldehyde-fuchsin staining and reported that aldehyde-fuchsin also stains certain gonadotropic cells. Scott and Clayton (1953), Elftman (1959a) and Gabe (1953) also pointed out that oxidation of the hypophysial tissue allows aldehyde-fuchsin to stain not only thyrotropic cells but gonadotropic cells as well. Racadot (1963) identified these as the FSH gonadotropic cells (beta cells). Herlant (1964) and Dubois and Herlant (1968) concluded that, in addition to thyrotropic cells, the ICSH gonadotropic cells also show some affinity for aldehyde-fuchsin following prior oxidation.

Paget (1959) reported that the dye, aldehyde-thionin, stains specifically thyrotropic cells. Paget and Eccleston (1960) combined aldehyde-thionin with periodic acid-Schiff technique and demonstrated that the thyrotropic cells stained blue-black (after aldehyde-thionin) and the

gonadotropic cells red (after Schiff reagent) in the rat and dog hypophyses. Ezrin and Murray (1963), Pearse and Van Noorden (1963) and Conklin (1968) applied the same sequence to human hypophysial tissue and reported that aldehyde-thionin specifically stains basophil cells associated with thyrotropic activity. Three types of staining were reported: blue-black in strongly positive granules, purple in less intensely stained granules and red or magenta in aldehyde-thionin negative cells. Pearse and Van Noorden (1963) and Conklin (1968) considered the alcian blue positive cell and the aldehyde-thionin positive cell as being identical in human. Similar views have been expressed by Purves and Bassett (1963) and Purves (1966) for many other mammalian species. In domestic animals, Purves and Bassett (1963) observed that the strongly colored cell with aldehyde-thionin is identical to the cell which stains strongly with aldehyde-fuchsin. The authors further reported that one cell-type is stained with aldehyde-thionin after permanganate oxidation in case of the pig. Herlant (1964) and Purves (1966) concluded that the aldehyde-fuchsin, alcian blue and aldehyde-thionin positive cells are identical and secrete thyrotropic hormone in mammals. In cattle, Dubois and Herlant (1968) showed that the thyrotropic cell is strongly positive for aldehyde-thionin whereas the ICSH gonadotropic cell is stained purple after aldehyde-thionin and Schiff

reagent.

Wilson and Ezrin (1954) used methyl blue as a counterstain in association with periodic acid-Schiff technique and achieved differentiation of gonadotropic and thyrotropic cells in the adenohypophysis of human and rat. The former cells are stained purple while the latter cells are stained red. Rennels (1957) modified the same method by substituting methyl blue in saturated picric acid solution in place of aqueous methyl blue and reported that the gonadotropic cells of the rat hypophysis can be further subdivided into coarsely granulated FSH gonatotropic and diffusely granulated ICSH gonadotropic cells. The former cells are stained purple (methyl blue positive and periodic acid-Schiff positive) while the latter are colored red (methyl blue negative and periodic acid-Schiff positive). Hildebrand *et al*. (1957) and Hellbaum *et al*. (1961) confirmed the observations of Rennels (1957), by using rat hypophysial tissue.

Herlant (1960) employed a tetrachrome stain, which used orange G, erythrosin, aniline blue and acid alizarine blue as differentiating stains, after fixing the hypophysis in Bouin-Hollande saturated with sublimate. In this technique, the basophils are differentiated into three categories: FSH gonadotropic cells (beta cells), ICSH gonadotropic cells (gamma cells) and thyrotropic cells (delta cells). The FSH and ICSH gonadotropic cells are stained light blue after

aniline blue and violet after acid alizarine blue, while the thyrotropic cells are colored deep blue with aniline blue. The author obtained distinct separation of cells in the hypophysis of the bat and the rat. Racadot (1961, 1963) reported three distinct types of basophils in the cat, using Herlant's (1960) tetrachrome technique. Among other species, such distinction of basophil cell-types with the application of Herlant's (1960) tetrachrome stain has been reported in pig (Bugnon, 1963b; Bugnon and Racadot, 1963) and in the dog (Carlon and Stahl, 1964; Carlon, 1967). Adapting the same technique in cattle, Dubois and Herlant (1968) observed that the violet stained cells are FSH gonadotropic cells and the rose or lilac red colored cells are ICSH gonadotropic cells.

In the technique of Cleveland and Wolfe (1932), erythrosin, orange G and aniline blue are used, in combination, to differentiate the cell-types in the adenohypophysis. The authors reported that the basophils are stained light blue, dark blue and in various other intermediate shades. Though the authors did not mention the functional significance of these cells due to lack of contemporary literature, the light blue basophils are of identical to FSH gonadotropic cells, the dark blue cells are identical to thyrotropic cells and the purple rose cells, which the authors conceived as intermediate forms, are ICSH gonadotropic cells (Bugnon, 1963b; Bugnon and Racadot, 1963; Dubois and Herlant, 1968).

Barrnett *et al*. (1956) reported that treatment of unfixed hypophysis in 2.5 percent solution of trichloroacetic acid extracted FSH and TSH completely but did not remove ICSH, as determined by bioassay. Staining of such hypophysial tissue with periodic acid-Schiff and aldehyde-fuchsin techniques revealed the presence of very few periodid acid-Schiff positive cells which the authors believed as ICSH gonadotropic cells (Barrnett *et al*., 1961). Heath (1964) obtained identical results in equine hypophysis, and with the use of alcian blue-periodic acid-Schiff-orange G technique confirmed the nonextracted cells as ICSH gonadotropic cells. Purves (1966) adapted buffer extraction procedures (isotonic saline buffered to pH 4.0 with acetate buffer) and was able to extract FSH and TSH but not ICSH by using isotonic saline, buffered to pH 4.0 or 5.0 with acetate buffer. He concluded that the loss of follicle stimulating hormone and thyrotropin due to trichloroacetic acid treatment and buffer extraction should be ascribed to inactivation of the hormones rather than to complete extraction.

Severinghaus (1938) observed that the cells of pars intermedia adenohypophysis are basophilic in nature. Racadot (1953) applied McManus-Hotchkiss periodic acid-Schiff technique and reported that the cells of pars intermedia in the cat and the guinea pig vary in their reaction to Schiff reagent. Purves and Bassett (1963) studied the

staining character of pars intermedia cells in 12 different species including ox, cat, dog, horse, sheep, pig, rat and rabbit. In all species examined, the authors reported that the cells of the pars intermedia contain glycoprotein granules and thus are identical to basophil cells of the pars distalis adenohypophysis. These cells are also found to be aldehyde-fuchsin positive and aldehyde-thionin negative in all species. Their staining character to alcian blue, without prior oxidation, was variable. In pig, dog, horse and sheep, the pars intermedia cells were not stained with alcian blue while in ox, rat, rabbit and cat, positive staining was obtained. In the dog, cells of the pars intermedia are stained in Crossman's (1937) procedure instead of being stained blue as in other species (Purves and Bassett, 1963). Holmes (1964) reported variable periodic acid-Schiff stainability of pars intermedia cells in the ferret, while Wingstrand (1966b) subscribed that the pars intermedia cells contain glycoprotein and react positively to the periodic acid-Schiff procedure. Purves (1966) reported success in staining of the pars intermedia cells in cow, dog, horse and rat with resorcin-fuchsin.

Bargmann (1949, 1950) applied Gomori's (1941) chrome alum hematoxylin procedure to the hypothalamus and neurohypophysis of the dog and reported that the neurosecretory material is stained distinctly in the chrome alum

hematoxylin technique. Later, Bargmann and Scharrer (1951) demonstrated that hypophysis fixed in Bouin or Susa solution is preferable to formalin-fixed tissue. Gabe (1953), Halmi and Davis (1953) and Elftmann (1959a, 1959b) reported that, with prior oxidation in acidified potassium permanganate solution, neurosecretory material also becomes stained with aldehyde-fuchsin.

Sloper (1955) used histochemical techniques for cystine, in order to demonstrate neurosecretory material. Adams and Sloper (1956) used alcian blue at a very low pH after oxidation of the tissue in performic acid and advocated it as a histochemical technique as well as a routine stain for neurosecretory material. Gabe (1966) and Pearse (1968a) subscribed that Bargmann's (1950) chrome alum hematoxylin method as well as aldehyde-fuchsin is not specific for neurosecretory material. On the other hand, the performic acid-alcian blue technique of Adams and Sloper (1956) should be considered as specific for histochemical demonstration of neurosecretory material contained in the neurohypophysis (Pearse, 1968a).

Morphology of the Canine Hypophysis

Trautmann (1909, 1911) described the canine hypophysis as being constituted by three parts, pars distalis adenohypophysis (anterior lobe), pars intermedia adenohypophysis and pars distalis neurohypophysis. The

latter two lobes were collectively termed as the posterior lobe. The pars distalis neurohypophysis was completely enveloped by the pars intermedia and pars distalis adenohypophysis with the interposition of cavum hypophysis between them. The author also reported that the pars proximalis neurohypophysis was very short in dogs and the recessus infundibuli extended to the middle of the pars distalis neurohypophysis. Stockard (1941) reported the occurrence of a fourth subdivision, viz. the pars infundibularis adenohypophysis in the dog and observed that the hypophysis did not vary in its size among the different breeds. In his study of hypophyses collected from six purebreds and their breed-crosses, the author observed variations in the proportion of gross subdivisions of the organ, the most conspicuous being the dorsal extent of pars distalis adenohypophysis. In brachycephalic breeds, the hypophysis was thicker in its dorsoventral axis, while in dolichocephalic breeds the opposite case was observed.

Legait (1963) determined volumetric proportion of the gross subdivisions of canine hypophysis and found that the pars distalis adenohypophysis, pars intermedia and pars distalis neurohypophysis constituted, respectively, 66, 10 and 24 percent of the hypophysial volume. Krolling and Grau (1960) subscribed that the pars distalis adenohypophysis was more voluminous on its ventral and caudal parts

than on the dorsal aspect. Stockard (1941) and Hanstrom (1952, 1966) concluded that morphology of the hypophysis of domesticated dogs was very variable among different breeds as well as within the same breed.

The histological structure of the canine hypophysis, comprised of the three cell-types, viz. acidophils, basophils and chromophobes, has been dealt with in the publications by Benda (1900), Herring (1908) and Trautmann (1909, 1911). Wolfe and Cleveland (1932) were the pioneer authors to describe four different cell-types in the canine hypophysis. These cell-types were designated, respectively, type I, II, III and IV. Wolfe et al. (1933) concluded that the cell-types I, III, and IV resembled, respectively, the classical acidophils, basophils and chromophobes, while the new group of chromophil cells, type II, belonged to the basophil class. The new cell type was found to vary in its tinctorial affinity and proportion during different stages of the estrus cycle which led the authors to classify it as belonging to the basophil class. The percentages of these four cell-types were found to vary in different stages of the estrus cycle, during pregnancy and also with age. In each of these cases, variations among different breeds were perceptible.

Romeis (1940) reviewed the work of Wolfe et al. (1933) and supplementing with his own observations, stated that

both type I and type II cells of the latter authors belonged to acidophil class and types III and IV belonged, respectively, to basophil and chromophobe classes. Hartmann _et al._ (1946) applied the trichrome procedure of Cleveland and Wolfe (1932), the azocarmine technique of Dawson and Friedgood (1938) and the basic fuchsin mitochondrial technique to the canine hypophysis and arrived at the conclusion that type II cells of Wolfe and Cleveland (1932) and Wolfe _et al._ (1933) were lactotropic cells (erythrophils) identical to the carmine cells of Dawson and Friedgood (1938) and acid fuchsin cells observed in other species.

Goldberg and Chaikoff (1952a) applied the trichrome procedure of Martins (1933), periodic acid-Schiff procedure and the aldehyde-fuchsin technique of Gomori (1950) to the canine hypophysis and reported the occurrence of six cell-types. The cell-types were named alpha, epsilon, beta, delta, gamma and zeta. Alpha and epsilon cells were found to be acidophil cells, the former akin to somatotropic cells and the latter to lactotropic (erythrophil or carmine) cells. Both types were found to be negatively stained with periodic acid-Schiff technique. The gamma cells of Goldberg and Chaikoff (1952a) were the true chromophobe cells which possessed no specific tinctorial affinity. Among the cells containing glycoprotein granules, the beta cells were stained with aldehyde-fuchsin, while the delta cells

remained unstained. The authors showed that the zeta cell was neither periodic acid-Schiff positive nor aldehyde-fuchsin positive. Goldberg and Chaikoff (1952b) reported that the alpha cells disappeared after complete thyroid destruction by ^{131}I which was also reflected in a stunting of growth. This, along with similarity of tinctorial affinities between these cells and somatotropic cells of other species led the authors to attribute growth hormone secretion to alpha cells. In the same study, the authors also observed that the aldehyde-fuchsin positive beta cells varied with functional state of the thyroid gland and hence, they concluded that beta cells of the dog were concerned with thyrotropin secretion (akin to thyrotropic cells) and delta cells elaborated follicle stimulating and luteinizing hormones.

Mikami and Ono (1956) failed to differentiate between two types of basophil cells, beta and delta, in the canine hypophysis even though they adapted the same techniques as Goldberg and Chaikoff (1952a). They claimed that the somatotropic cells (alpha cells) were selectively stainable with azocarmine while the lactotropic cells (epsilon cells) possessed no affinity for this stain. The latter cell-type was reported to be periodic acid-Schiff positive. These findings were contradictory to those of Hartmann _et al._ (1946) and Goldberg and Chaikoff (1952a). Among other differences,

they also observed that the zeta cells were stained positively with aldehyde-fuchsin. Mikami and Ono (1957) carried out ablation of target organs, viz., thyroid and gonads, in conjunction with histomorphological studies of the canine hypophysis and found that a specific group of basophil cells reacted to a particular type of experimentation. They reported the occurrence of typical thyroidectomy and castration cells in the canine hypophysis. Mikami (1956) investigated the histomorphology of hypophyses collected from aderenalectomized dogs and demonstrated hypertrophy of the zeta cells accompanied with degranulation. From these experiments, the author concluded that zeta cells were the most influenced cell-type manifesting cytological changes as a result of adrenalectomy.

Purves and Griesbach (1957a) applied several experimental techniques and staining procedures to the investigation of the canine hypophysis. The authors distinguished five different types of cells which were functionally and tinctorially different from each other. The cell-types were designated as orange acidophils, red acidophils, blue basophils, purple basophils and pale cells. The orange acidophils were stained yellow with orange G and were considered as somatotropic cells. The red acidophils were stained selectively with acid fuchsin, azocarmine and Schiff reagent after periodate oxidation. Such cells were found

to be almost absent in immature animals and to undergo hypertrophy and hyperplasia as a result of estrogen treatment. The authors equated these cells with carmine cells of Dawson and Friedgood (1938) and pregnancy cells of Romeis (1940) and considered them as lactotropic cells.

The blue cells of Purves and Griesbach (1957a) manifested severe hypertrophy and degranulation in animals with hyperplastic goiter and were ladden with granules in animals which received thyroid tablets. The granules of these cells were stained strongly with periodic acid-Schiff technique as well as aldehyde-fuchsin and thus, the blue cells were considered as thyrotropic cells. The purple cells were equated with gonadotropic cells due to their scarcity in immature animals and their tinctorial affinity for Schiff reagent and aldehyde-fuchsin. The authors believed that the pale cells were akin to the zeta cells of Goldberg and Chaikoff (1952a) even though such cells showed positive reaction for periodid acid-Schiff as well as aldehyde-fuchsin techniques, both of which had been reported in the negative by Goldberg and Chaikoff (1952a). The number of pale cells was scarce in immature animals and the cells responded to estrogen treatment with hyperplasia and accumulation of granules in their cytoplasm. So the authors considered these cells as a type of basophil associated with gonadotropin secretion. Purves (1966), citing the work of Mikami (1956), observed that the pale

cells of Purves and Griesbach (1957a) and the zeta cells of Goldberg and Chaikoff (1952a) were neutrophils, probably, concerned with corticotropin secretion, as had been pointed out by Mikami (1956).

Wolfe (1959) reported that erythrophil cells of the canine hypophysis were stained moderately with periodic acid-Schiff technique and also, with aldehyde-fuchsin. He was able to differentiate three types of basophil cells viz., strongly positive with periodic acid-Schiff and aldehyde-fuchsin techniques; weakly periodic acid-Schiff positive and weakly aldehyde-fuchsin positive; and, weakly to moderately periodic acid-Schiff positive but aldehyde-fuchsin negative. No attempts were made by the author to draw homologies between these cell-types and those of other studies on the canine hypophysis. But the three types of basophil cells were identical to thyrotropic, FSH-gonadotropic and ICSH-gonadotropic cells, respectively.

Carlon and Stahl (1964) applied the tetrachrome and alcian blue-periodic acid-Schiff-orange G procedures of Herlant (1960) to the investigation of canine hypophysis. They demonstrated five different cell-types with specific tinctorial affinities. The authors adopted the functional nomenclature to designate these cell-types. Concerning corticotropic cells (epsilon cells), the authors observed that small chromophobe cells containing few erythrophilic

granules were found distributed throughout the pars distalis adenohypophysis, but were difficult to be revealed without resorting to such experimental conditions as administration of corticoid inhibitors or adrenalectomy.

The somatotropic cells (alpha cells) were stained yellow in both techniques and resembled in their morphological character to similar cells in other species (Carlon and Stahl, 1964; Carlon, 1967). The lactotropic cells (eta cells) were stained selectively with erythrosin and moderately with periodic acid-Schiff technique so that they were colored brick-red in periodic acid-Schiff-orange G procedure. The functional significance of these cells was proved by their scarcity in young animals, proliferation during pregnancy, hypertrophy and hyperplasia in female dogs during lactation, and their response to reserpine treatment by hypertrophy and degranulation (Carlon and Stahl, 1966b; Carlon, 1967).

Among the glycoproteinaceous cells, the thyrotropic cells (delta cells) showed selective affinity for aldehyde-fuchsin, alcian blue and Schiff reagent (Carlon and Stahl, 1964, 1966a). In the hypophyses of dogs treated with reserpine, the thyrotropic cells manifested hypertrophy and secretory activity as has been observed in several other species (Carlon and Stahl, 1966b; Carlon, 1967). Further functional significance of these cells was derived from the fact that they were the only type of glycoproteinaceous cells

observed in puppies (Carlon and Stahl, 1966a).

The FSH-gonadotropic cells (beta cells) and ICSH-gonadotropic cells (gamma cells) were differentiated by their specific coloration in the tetrachrome technique. The former cells were stained light blue after aniline blue while the latter were colored violet with acid alizarine blue (Carlon, 1967). The FSH-gonadotropic cells were reported to be stained positively with Schiff reagent and alcian blue at pH 0.2, so that a violet color was imparted on them in the alcian blue-periodic acid-Schiff-orange G technique (Carlon and Stahl, 1966a; Carlon, 1967). They were reported to be absent in puppies and to undergo hypertrophy and extensive degranulation as a result of castration (Carlon, 1967). The ICSH-gonadotropic cells manifested affinity for Schiff reagent and orange G, so that, they were colored brick-red in the periodic acid-Schiff orange G procedure. However, they were reported to be stained magenta after Schiff reagent in the alcian blue-periodic acid-Schiff-orange G technique, even though the cell possessed some affinity for alcian blue at pH 0.2 (Carlon, 1966, 1967). Their functional significance was derived from the facts that the cells were found to undergo hypertrophy and hyperplasia as a result of castration; they were selectively stained with lead hematoxylin; they were found to be absent in puppies; they were comparatively more numerous in female than in male dogs;

and, they underwent hypertrophy with accumulation of granules during gestation and reserpine treatment (Carlon, 1966, 1967).

These morphological and experimental studies proved that each of the adenohypophysial cell-types of the dog was associated with the elaboration of a specific hormone (Purves and Griesbach, 1957a; Purves, 1966; Carlon, 1967). The cell types exhibited certain morphological characteristics and tinctorial affinities by means of which they could be differentiated from one another and correlated with similar cell-types of other species. Purves and Griesbach (1957a) and Carlon (1967) subscribed that cell-types in the pars distalis adenohypophysis of the dog were functionally and morphologically identical with cell-types that have been demonstrated in other species.

Severinghaus (1938) reported the occurrence of large colloid-filled cysts in pars distalis adenohypophysis of the dog. He observed two types of lining cells in such cysts: ciliated columnar cells akin to simple stomodeal epithelium; and at times, chromophobe, acidophil and basophil cells. Stockard (1941) found such cysts in pure-breds as well as in breed-crosses of the canine species and concluded that the persistent stalk of Rathke's pouch was the source of these cysts. The author found no diminution in any function of the hypophysis due to the presence of such cysts. Hanstrom (1952, 1966) observed colloid-filled

cysts in the pars distalis adenohypophysis of wild as well as domesticated canine species. In one specimen, persistence of the epithelial stalk in the form of an open canal (canalis craniopharyngeus) extending between pharynx and hypophysis was observed (Hanstrom, 1966). Hanstrom (1966) considered the cysts as embryonic vestigial structures. Kagayama (1965) studied the structure of follicular cells in the pars distalis adenohypophysis of the dog with the help of electron microscope. He demonstrated that follicular cells, lining the colloid-containing cavities, possessed cilia as well as microvilli on their luminal surfaces. He also reported the presence of small number of granules, 150-200 millimicrons in diameter, in the cytoplasm of the follicular cells subjacent the following surface.

The cells in the pars intermedia adenohypophysis of the dog were investigated by Purves and Griesbach (1957a) and Purves and Bassett (1963). Purves and Griesbach (1957a) demonstrated that the cells could be stained positively by both periodic acid-Schiff as well as aldehyde-fuchsin procedures. The cells possessed more affinity for acid fuchsin than for aniline blue, as was revealed by their purplish-red coloration in the trichrome technique. The authors concluded that the cells belonged to a single specific type in the dog. Rothballer and Skoryna (1960) and Hanstrom (1966) noticed ramifications of the cavum hypophysis at the

peripheral extremities so that the junctional zone of pars intermedia was subdivided into columns and strands of parenchymatous cells. Stockard (1941) reported the extension of such diverticula of the cavum hypophysis into the pars infundibularis adenohypophysis in several breeds of dogs.

Rothballer and Skoryna (1960) described the pars infundibularis adenohypophysis of the dog as a cuff-like cellular mass which encircled the zona externa throughout its extent. They observed arrangement of the moderately basophilic cells in the form of small single layered acini, short strands and tubules. Like other species, presence of abundant blood vessels in the pars infundibularis adenohypophysis of the dog was observed (Rothballer and Skoryna, 1960; Szentagothai et al., 1968).

The occurrence of a well developed pars paraneuralis or zona tuberalis, in the dog, has been reported by Mikami and Ono (1957) and Rothballer and Skoryna (1960). In the two studies, the term seemed to have been used with different contexts. Mikami and Ono (1957) applied the term to denote the ventromedian area of the pars distalis adenohypophysis where basophils were predominant. The same area was referred to as the basophil zone by Giroud and Desclaux (1947a), Racadot (1955) and Purves (1961, 1966). Rothballer and Skoryna (1960) observed that the pars paraneuralis or zona tuberalis was that portion of the adenohypophysis which

contacted the neurohypophysis, and extended between the pars infundibularis adenohypophysis, proximally and the pars intermedia adenohypophysis, distally. The authors further reported that cells of this part were arranged in irregular strands and resembled those of pars intermedia, with whom they blended freely. New fully granulated basophil cells were also observed among the parenchymatous cells in this part. This definition of pars paraneuralis by Rothballer and Skoryna (1960) was in conformity with that of Romeis (1940) and Hanstrom (1966).

The hypothalamohypophysial system of the dog has been extensively studied by many authors. Bargmann (1966) and Gabe (1966) reviewed most of the papers concerned with neurosecretory phenomenon in their exhaustive publications. It was in the dog that Bargmann (1949, 1950) first demonstrated neurosecretory material of a mammalian system with the application of Gomori's (1941) chrome alum hexatoxylin technique. Bargmann and Scharrer (1951), Laqueur (1954) and Scharrer (1954) studied the hypothalmohypophysial system of the dog in order to establish the site of synthesis of the neurohypophysial hormones and axonal flow.

In dogs, Laqueur (1954) demonstrated that the fibers of the paraventriculohypophysial and supraopticohypophysial tracts descended in the zona interna of the radix infundibuli and pars compacta infundibuli. Chrome-

hematoxylin stainable material was evident in these fibers all along their course as well as in the perikaryon. Scharrer (1954) reported that the stainability of the neurosecretory material with chrome hematoxylin, in the hypothalamohypophysial system of the dog, was highly selective and revealed even small amounts of the substance. Scharrer and Frandson (1954) reported that the quantity of hormone present in the neurohypophysis was directly related to the amount of stainable neurosecretory material present in the pars distalis neurohypophysis. The authors observed stainable neurosecretory material inside the blood vessels of the neurohypophysis and concluded that, under normal conditions, the neurosecretory substance passed through the wall of the vessels in granular form, presumably, to be dissolved in the blood.

Laqueur (1954), Scharrer (1954) and Bargmann (1966) subscribed that the amount of stainable neurosecretory material in hypothalamohypophysial tract and pars distalis neurohypophysis of the dog was much higher than that present in any other mammalian species. However, Sloper (1955) concluded that neither Gomori's chrome alum hematoxylin technique nor the aldehyde-fuchsin procedure, after preliminary oxidation, stained the neurosecretory material of the dog specifically. Other substances like lipofuchsin pigment, Nissl granules and connective tissue fibers were

also stained in both techniques.

Rothballer and Skoryna (1960) described the morphological structure of the radix infundibuli and pars cava infundibuli of the dog, both of which resembled those of other mammalian species. In the zona externa of the radix infundibuli, the authors observed few nerve fibers in which the neurosecretory material could be stained with the chrome alum hematoxylin technique. The number of such fibers in the zona externa was found to have increased considerably in animals with long standing stalk resection. Based on these observations, the authors advanced the hypothesis that a limited but definite number of Gomori-positive neurosecretion bearing axons occurred normally in the zona externa of the dog. Such fibers have not been reported in any other mammalian species (Gabe, 1966; Szentagothai *et al.*, 1968).

Presence of axon terminals and collaterals from the fibers of the hypothalamohypophysial tract ladden with neurosecretory material have been reported in the vicinity of the capillary vessels of the pars cava infundibuli (Scharrer, 1954; Rothballer and Skoryna, 1960; Akmayev, 1969). Scharrer (1954) stated that such localization of neurosecretory material in the infundibular stalk, though occurred in all adults, was very conspicuous in young animals. Akmayev (1969) concluded that discharge of neurosecretory material from the fibers of the

hypothalamohypophysial tract into the vascular system occurred regularly in the infundibulum to maintain the basic requirements, while depletion from pars distalis neurohypophysis occurred only in conditions of excessive requirements.

Stockard (1941) reported the extension of pars intermedia cells into the pars distalis neurohypophysis in form of cell-cords, in several breeds of dogs. In the latter lobe, the cell-cords were transformed into follicles, many of which contained colloid. Occurrence of such adenohypophysial tissue in the pars distalis neurohypophysis was found to be especially prominent in adult dogs. Another morphological deviation, observed in adult dogs, was indentation of pars intermedia into the pars distalis neurohypophysis at several places, some of these being deep enough to carry with them diverticula of the cavum hypophysis (Stockard, 1941).

Morphology of the Porcine Hypophysis

Compared to that of the dog, publications dealing with the hypophysis of the pig were few in number. References regarding the structure of porcine hypophysis were available in the papers by Trautmann (1909, 1911), Oboussier (1943), Herlant (1951) and Green (1951). Trautmann (1909) observed that the pars intermedia adenohypophysis was firmly adherent to the pars distalis neurohypophysis and lined only its rostroventral surface. The pars distalis

adenohypophysis was reported to be thicker caudally and was separated from the pars intermedia by a wide hypophysial cavity which contained colloid in many instances. The author further reported that the infundibular recess was encountered, many times, extending into the pars distalis neurohypophysis. A similar description was furnished by Krolling and Grau (1960).

Oboussier (1943), Herlant (1951) and Krolling and Grau (1960) subscribed that the pars infundibularis adenohypophysis completely encircled the infundibulum of the pig and the infundibular recess terminated at the proximal end of the infundibulum. Green (1951) reported that the external shape and structure of the hypophysis was remarkably different in the new-born pig as compared to that of the adults. In his illustration, the author depicted the pars intermedia adenohypophysis as completely encircling the pars distalis neurohypophysis, a fact which was not encountered in other publications. Giroud and Desclaux (1947a) made a comparative study of the topographical distribution of cell-types in the pars distalis adenohypophysis of domestic animals. The authors reported that, among all domestic animals, the pig possessed the unique character of distinct cell zones in its adenohypophysis. The acidophil cells occupied the caudolateral areas while the basophil cells predominated the median region of the rostroventral surface. Racadot

(1955) also reported the occurrence of such basophil and acidophil zones in the pig.

Reference as to the existence of three basic cell-types, *viz*., chromophobes, acidophils and basophils in the pars distalis adenohypophysis of the pig, was available in the publications of Trautmann (1909, 1911), Oboussier (1943) and Herlant (1951). Maurer and Lewis (1922) demonstrated the presence of a third chromophil cell-type in addition to eosinophil and basophil cell-types. The authors termed the new cell-type as type III. The latter occurred predominantly in the rostroventral median region and extended proximally to the infundibulum. The cells of type III were large, spherical and presented an irregularly vacuolated appearance.

Cleveland and Wolfe (1933) applied the trichrome procedure (Cleveland and Wolfe, 1932) to the porcine hypophysis and were able to differentiate three types of cells: type I, type III and type IV. The authors observed that type I included the somatotropic cells identical to those of other mammals. Type IV cells belonged to the chromophobe class as had been observed in the dog (Wolfe and Cleveland, 1932). Type III cells were gonadotropic cells which were believed to be identical to the type II and type III cells of the canine adenohypophysis (Wolfe *et al*., 1933). The authors were able to subdivide type III cells into three groups: cells filled with granules; cells with clumped granules;

and, cells with no granular material or partially granulated. These three groups of cells behaved differently in their tinctorial affinities (Cleveland and Wolfe, 1933). The granules of fully granulated cells were stained violet or orange red; clumped-granules of type III cells were stained blue; and, granules of partially granulated cells were stained purple in the trichrome procedure.

Racadot (1955) reported the occurrence of two types of basophil cells <u>viz</u>. gonadotropic (beta) and thyrotropic (delta) cells in the porcine adenohypophysis and subscribed that gonadotropic (beta) cells were more numerous in the rostromedian region and represented the major part of basophil cells. The caudolateral regions of the pars distalis adenohypophysis contained somatotropic (alpha) cells and few thyrotropic (delta) cells. The gonadotropic cells and remainder of the thyrotropic cells occupied the centromedian region of pars distalis adenohypophysis. Purves (1961) referred to the paucity of chromophobe cells in the pars distalis adenohypophysis of the pig and remarked that the basophil zone contained 2 to 4 times more corticotropin that the caudolatel acidophil zones.

The functional significance of acidophil and basophil cells from different cell zones was investigated by Marshall (1951) and Giroud and Desclaux (1947a, 1947b). Corticotropin activity was found to be primarily localized in the basophil

cells of the centromedian region, as was evident by the application of the fluorescent antibody method (Marshall, 1951). Highest concentration of growth hormone was obtained from the acidophil zone in the caudolateral regions of the pars distalis adenohypophysis, where alpha or somatotropic cells formed the predominant cell-type. Giroud and Martinet (1952a, 1952b) observed the localization of cells postulated to contain thyrotropic activity in the rostromedian basophilic zone of the pars distalis adenohypophysis. Giroud and Martinet (1948b) proved that implants of cells from the central basophil zone caused follicular growth in the ovary of immature rats whereas implants from other areas of the pars distalis adenohypophysis failed to achieve such results. Corte and Biondi (1964) reported that beta cells of the centromedian region of pars distalis adenohypophysis showed immunofluorescence reaction for FSH antibodies, indicating the presence of follicle-stimulating hormone in the cells. By the same implantation technique, Giroud and Martinet (1948a) showed that the concentration of corticotropin was higher in the rostromedian zone than in the caudolatel acidophil zone.

Bugnon (1963a, 1963b) and Bugnon and Racadot (1963) studied the cell-types of porcine hypophysis by adapting several cytological staining procedures. The variations in different cell-types and functional significance of each

were derived by examination of hypophyses collected from pregnant, lactating and castrated animals. The authors reported that the alpha cells occupied the caudolateral areas of the pars distalis adenohypophysis and possessed tinctorial affinity for the orange G stain, in the trichrome procedure of Cleveland and Wolfe (1932) as well as tetrachrome technique of Herlant (1960). The cells stained negatively with periodic acid-Schiff technique as well as with alcian blue-periodic acid-Schiff procedure (Bugnon, 1963b; Bugnon and Racadot, 1963). The authors subscribed that alpha cells of the pig were concerned with the secretion of growth hormone and were identical to the somatotropic cells of other species.

The eta cells were found to occupy the caudolateral as well as the central and ventral regions of the pars distalis adenohypophysis (Bugnon and Racadot, 1963). The cells possessed specific affinity for erythrosin and were colored moderately with the periodic acid-Schiff technique. Bugnon (1963a) reported that the eta cells manifested extensive hyperplasia and hypertrophy during lactation and gestation. Their number was found to have increased in castrated animals, too. Due to their morphological alteration during gestation and lactation, and their tinctorial affinity for Schiff reagent and erythrosin, Bugnon (1963a, 1963b) and Bugnon and Racadot (1963) considered eta cells as being akin to the lactotropic cells of other species. Bugnon (1963a)

claimed that the lactotropic (eta) cells were the source of pregnancy cells in the pig.

The delta cells of the porcine hypophysis were described as small spherical cells with eccentric nuclei and clumped granular material in the cytoplasm (Bugnon, 1963b; Bugnon and Racadot, 1963). These cells manifested selective staining affinity for aldehyde-fuchsin and alcian blue (pH 0.2), so that they were colored blue or blue-black when these dyes were employed in conjunction with periodic acid-Schiff procedure (Bugnon, 1963b). The cells were strongly periodic acid-Schiff positive and were stained deep blue after aniline blue in tetrachrome and trichrome procedures. They predominated the rostromedian basophilic zone of the pars distalis adenohypophysis. Bugnon (1963b) equated the delta cells of the pig with the thyrotropic cells of other spcies, and claimed that they were akin to the cells in which Giroud and Martinet (1952a, 1952b) found the localization of thyrotropic activity.

Bugnon and Racadot (1963) reported that the gonadotropic cells in the porcine adenohypophysis possessed specific tinctorial affinities by which the cell-types could be differentiated. The beta cells were colored light blue with tetrachrome (Herlant, 1960) and trichrome procedures (Cleveland and Wolfe, 1932). The cells were stained with Schiff reagent as well as alcian blue at the same time so as to be colored

violet. Their granulation was sparse and the cells occupied the basophil zone in the rostroventral region of the lobe (Racadot, 1955; Bugnon, 1963b). Bugnon and Racadot (1963), in their investigation of the hypophyses from castrated animals, encountered hypertrophic and vacuolated beta cells akin to castration cells. In hypophyses of pregnant and lactating sows, the beta cells were also found to be hypertrophic (Bugnon, 1963b). Thus, Bugnon (1963b) and Bugnon and Racadot (1963) claimed that the beta cells were a homologue of the FSH-gonadotropic cells of other species.

The gamma cell was much smaller in the pig and contained granules which were uniformly and densely distributed throughout its cytoplasm. The cells occupied the centromedian region of the pars distalis adenohypophysis (Bugnon, 1963b). The granules of the gamma cell were found to be periodic acid-Schiff positive and alcian blue (pH 0.2) negative. In the tetrachrome technique of Herlant (1960) they were colored violet and in the periodic acid-Schiff-orange G procedure, they were colored brick-red due to their affinity for Schiff reagent and orange G (Bugnon and Racadot, 1963). The cells manifested hypertrophy and degranulation during gestation and following castration. Bugnon and Racadot (1963) identified the gamma cells as ICSH-gonadotropic cells.

Bugnon (1963a) and Racadot (1963) observed large number of chromophobic cells in the centromedian, centrolateral and

rostromedian regions of the porcine adenohypophysis. The cells were named epsilon cells. The latter possessed faintly stained erythrophilic granules and manifested hypertrophy and hyperplasia during gestation in sows and during conditions of stress in both sexes (Bugnon, 1963b). The authors considered the epsilon cells as being identical to corticotropic cells.

The morphology of the pars intermedia adenohypophysis of the pig was studied by Maurer and Lewis (1922), Legait (1963) and Purves and Bassett (1963). Maurer and Lewis (1922) observed two types of cells in the intermediate lobe of the pig, which they designated as follicle (colloid producing) cells and grandular cells. Legait (1963) reported that the pars intermedia of the pig constituted 7 percent of the total volume of the hypophysis whereas the pars distalis adenohypophysis and pars distalis neurohypophysis constituted, respectively, 81 percent and 12 percent of the volume. Purves and Bassett (1963) demonstrated that the pars intermedia cells were stained by aldehyde-fuchsin and periodic acid-Schiff technique but remained unstained after alcian blue and aldehyde-thionin dyes.

Zeitz (1965) studied the ultrastructure of the hypothalamohypophysial system of the pig and reported the occurrence of Herring bodies, exclusively, in the zona interna of the radix infundibuli. He also observed myelinated fibers

without any content of neurosecretory material in the zona interna. Osmophilic granules resembling those of the supraopticohypophysial tract were encountered in the zona externa of the radix infundibuli of the pig (Zeitz, 1965).

Ultrastructure of the Hypophysis

Ultrastructure of the adenohypophysis has been studied extensively in several mammalian species (Barnes, 1962, 1963; Herlant, 1963, 1964; Kurosumi, 1968). Apart from general cytological character, specific topics such as structure of the portal vessels, mechanism of granule formation, and mode of secretion have also been dealt with in many publications (Kurosumi, 1961; Herlant, 1963; Racadot _et al._, 1965; Smith and Farquhar, 1966). Such studies elucidated many ambiguous facts regarding hypophysial cytology which could not have been resolved at the level of optical microscope and, at the same time, contributed to the differentiation of various cell-types. At the ultrastructure level, differentiation of the cells of the adenohypophysis was achieved by electron density, size, and location of secretion granules; by the structural character of cell organelles; by subjecting animals to different experimental states such as ablation of target organs and administration of hormones; by the study of organs from animals at various physiological conditions, _viz._, lactation and gestation; and, by correlated electron microscopy and bioassay of granule fraction (Barnes,

1962; Herlant, 1963; Kurosumi, 1968; Tesar et al., 1969). Herlant (1963) and Kurosumi (1968) subscribed that ultrastructure of the adenohypophysial cells was basically similar in all species, with the exception of few minor details.

In all mammalian species studied so far, the somatotropic cells constituted the more abundant cell-type (Herlant, 1963, 1964). Their granules were uniformly electron-dense and occurred evenly distributed throughout the cytoplasm. The diameter of these granules measured about 350 millimicrons. The granules were enclosed by a distinct membrane and were found to be less variable in their size and shape (Kurosumi, 1968). The Golgi apparatus was well developed and consisted of many vesicles and vacuoles. The rough-surfaced endoplasmic reticulum was randomly oriented and was composed of slightly dilated cisternae. The mitochondria were stout and contained moderate number of cristae, but scarcely any mitochondrial granules. Kurosumi (1968) stated that the number of secretory granules were few in somatotropic cells of young animals, and that they were arranged along the cell surface. After thyroidectomy, extensive degranulation of the somatotropic cells was observed (Herlant, 1963; Kurosumi, 1968).

Unlike the somatotropic cells, the granules of lactotropic cells were variable in their shape and size (Herlant, 1964). The granules of these cells were either

spherical and smaller in size than granules of the somatotropic cells, e.g., *Myotis myotis* (Herlant, 1963) and mouse (Barnes, 1962) or were irregular and larger in size as in most mammals (Kurosumi, 1968). The size of the granules in latter animals was reported to be as large as 700 millimicrons in diameter (Herlant, 1964; Kurosumi, 1968). In the normal state, the rough-surfaced endoplasmic reticulum and Golgi apparatus were small and were localized on one part of the cell (Barnes, 1962, 1963; Herlant, 1963). In the functionally active cell, these organelles manifested hypertrophy. Barnes (1962) reported that the number of prolactin cells was much less in sexually immature mice and that castration before attainment of sexual maturity inhibited development of these cells. Their number was also reported to be much less in adult male animals than in females of the same age (Barnes, 1963; Herlant, 1963; Kurosumi, 1968). Herlant (1963, 1964) and Kurosumi (1968) demonstrated extensive hypertrophy of the endoplasmic reticulum accompanied with degranulation, in lactotropic cells, following estrogen administration. Administration of prolactin was reported to bring about the opposite effect (Kurosumi, 1968). Studies on the adenohypophyses of female mice during different stages of the sexual cycle and pregnancy also revealed their hypertrophic character (Barnes, 1963). In the mole, Herlant (1963) observed hyperactivity of lactotropic cells during

lactation.

The thyrotropic cell, in electron microscopy, was found to be angular in outline. The secretory granules of this cell measured 100-150 millimicrons in diameter (Herlant, 1964; Kurosumi, 1968). The granules occurred characteristically along the peripheral margin of the cytoplasm and the cell itself was usually encountered away from the vicinity of blood vessels (Barnes, 1962, 1963). The cell organelles such as endoplasmic reticulum, Golgi apparatus and mitochondria were poorly developed in the normal state (Herlant, 1963). The functional significance of these cells was established by their vacuolar hypertrophic-response as a result of thyroidectomy, radiothyroidectomy and administration of thyroprivic drugs (Barnes, 1962; Kurosumi, 1968). A characteristic feature exhibited by thyrotropic cells after thyroidectomy was the occurrence of electron-dense granules within the dilated cisternae of their rough-endoplasmic reticulum (Kurosumi, 1968).

The FSH-gonadotropic cells contained granules which were quite variable insize. Their average diameter was approximately 200-250 millimicrons but granules ranging in diameter from 150 to 250 millimicrons were observed in some species (Kurosumi, 1968). The endoplasmic reticulum of the cell occurred in the form of a series of small dilated sacs. Another distinguishing feature was the peculiar granular

texture and comparatively less electron density of their secretory material (Herlant, 1963). The follicle stimulating potency of the cells was demonstrated by their peculiar response to castration in both sexes. Kurosumi (1968) detailed the various stages which led to the formation of castration cells in castrated rats. According to the author, the endoplasmic reticulum formed excessive dilations due to accumulation of an amorphous material. As this progressed, the cisternae coalesced and gave rise to a large vacuole in the central part of the cell. The nucleus and other cell organelles were pushed towards one side and the appearance of the cell, thus, resembled a signet-ring.

It was reported that, after daily injection of estrogen to new-born female rats for 30 days, a condition of persistent diestrus could be initiated where the FSH-gonadotropic cells decreased in number and became atrophic (Kurosumi, 1968). ICSH-gonadotropic cells were not affected appreciably in such animals, which facilitated distinction between the two types of gonadotropic cells (Kurosumi, 1968). Barnes (1963) demonstrated that, during proestrus in the mouse, the FSH-gonadotropic cells underwent hypertrophy due to accumulation of granules. Extensive degranulation was evident in these cells during estrus.

The ICSH-gonadotropic cells differed from the FSH cells by possessing granules which were less variable in

size and electron-density. The size of the granules varied from 200-250 millimicrons in diameter (Herlant, 1964; Kurosumi, 1968). The accumulation of granules at one pole of the cell facing the blood capillary was also encountered (Kurosumi, 1968). The endoplasmic reticulum of this cell always occurred as a series of dispersed cisternae (Herlant, 1963). Barnes (1963) reported that the cytoplasmic-nuclear ratio was smallest in ICSH cells as compared with that of any other cell-type in the mouse adenohypophysis. Barnes (1963), Herlant (1963) and Kurosumi (1968), by independent observations in different species, showed that the ICSH-gonadotropic cells manifested a certain degree of hyperactivity following castration, but the changes occurred slowly and never proceeded beyond the stage of hypertrophy. In rats with persistent estrus, caused by injections of small amounts of estrogen for 5 consecutive days after birth, the ICSH cells showed an extraordinary accumulation of secretory granules, while FSH cells remained unchanged. During late stages of pregnancy as well as following administration of progesterone, the secretory granules of ICSH-gonadotropic cells accumulated in the cytoplasm (Barnes, 1963; Kurosumi, 1968). Herlant (1963) reported that the ICSH-gonadotropic cells became fully degranulated immediately after parturition.

As in optical microscopy, cytological structure of cells secreting corticotropin had not been clearly elucidated at

the ultrastructure level. Recent studies by Kurosumi (1968) and Siperstein and Miller (1970) helped a great deal in solving this problem. Working with the rat hypophysis, both groups of authors showed that the corticotropic cell was stellate-shaped and its protoplasmic processes extended out towards the capillary wall insinuating between other cells. These cells were usually encountered in the center of the cell-cords. Following adrenalectomy or severe stress, the cells were found to contain many granules measuring 150-200 millimicrons in diameter. Kurosumi (1968) claimed that these granules were electron-lucent with only a small dense-core located in their central part. Siperstein and Miller (1970) reported striking accumulation of secretory granules in both adrenalectomy cells of adrenalectomized rats and normal corticotropic cells.

In addition to these six cell-types, another type which contained microvilli and cilia, but no secretion granules, had also been reported (Kagayama, 1965; Kurosumi, 1968). These cells were named follicular cells. Their number was reported to be very small. Kurosumi (1968) reported the presence of some undifferentiated cells in the pars distalis adenohypophysis and subscribed that the great numerical difference between chromophobe cells of light microscopy and the undifferentiated cells of electron microscopy was suggestive of the fact that the chromophobe cells included

undifferentiated cells as well as other types of granulated cells in their degranulated or immature stages.

Barnes (1963) stated that the age-correlated changes in the adenohypophysis at the ultrastructure level consisted of an increase in the granule content of both types of gonadotropic cells in the male and female mice, increased tendency for vacuolation during hyperactivity of cells and a decrease in the number of well-granulated somatotropic cells which were especially prominent in the female.

Electron microscopic studies revealed that the rough-surfaced endoplasmic reticulum and Golgi apparatus were the two cell organelles involved in the synthesis of the secretory material (Kurosumi, 1961; Racadot *et al*., 1965). It was also substantiated that elaboration of the secretory material in adenohypophysial cells occurred in the same way as that taking place in all other protein secreting cells (Racadot *et al*., 1965; Smith and Farquhar, 1966). Many studies revealed budding of small vesticles from the cisternae of rough-surfaced endoplasmic reticulum towards the Golgi apparatus (Kurosumi, 1961, 1968). In many cases, continuity between the extremities of Golgi apparatus and the endoplasmic reticulum was also evident (Herlant, 1963). Not until the secretory product had been condensed by the Golgi apparatus, did it become discernible at the ultrastructure level (Kurosumi, 1961; Barnes, 1963). The

first granules to appear occurred near one end of the Golgi apparatus in the form of spherical electron-dense vesicles, completely filling the enclosing membranous capsule. Herlant (1963) reported that, in hypertrophied lactotropic cells, such electron-dense granules even became evident within the dilated cisternae of the Golgi apparatus.

A second method of granule formation was described by Herlant (1963) in the lactotropic cells of the mole and ICSH-gonadotropic cells of the bat. In these instances it was observed that small groups of microvesicles which were detached from the Golgi apparatus became enclosed by a membrane giving rise to multivesicular bodies. The microvesicles within such multivesicular bodies became progressively agglomerated into a dense mass which was finally transformed into a secretion granule.

Racadot _et al_. (1965) injected labelled leucine into rats and followed the radioautographic reaction in different cell organelles of the adenohypophysis. They found the reaction on Golgi apparatus and on the secretory granules in the course of elaboration after a lapse of 30 minutes. The reaction was subsequently observed in the granules, formed after the injection and scattered throughout the cytoplasm, after 90 minutes. From these experiments, the authors concluded that the secretory granules were formed in Golgi apparatus from the immediately prior synthesized

proteins by the rough-surfaced endoplasmic reticulum.

Smith and Farquhar (1966) suggested that lysosomes took an active part in regulation of the secretory process in the adenohypophysial cells by providing a mechanism whereby overproduction of secretory products was inhibited. The authors contemplated that the mature secretion granules were destroyed by being transformed into dense lytic bodies, while immature granules were converted into multivesicular lytic bodies. Following a state of hyperactivity and hypertrophy, the rough-surfaced endoplasmic reticulum underwent sequestration and was converted into autophagic vacuoles. The lytic bodies, _viz_., dense bodies, multivesicular bodies and autophagic vacuoles merged together by entering into the lysosomal system of the cell and the combined lytic body, thus formed, was termed the myelinated body. In subsequent steps, the content of the myelinated body was progressively degraded and the myelinated body was transformed into the vacuolated dense body. The vacuole of the latter increasingly protruded out of the dense body and eventually became separate from the latter, resulting in a free lipid droplet and a dense body. The former was believed to be again utilized by the cell through the action of appropriate lipid-splitting enzymes. These findings explained the regulatory mechanism of secretion as well as the appearance of vacuoles in otherwise

normally active cells of the adenohypophysis.

Different views were expressed regarding the excretion mechanism of secretory products from different types of adenohypophysial cells. Farquhar (1961) described the excretory mechanism from adenohypophysial cells as a process of reverse pinocytosis. Herlant (1963) reported two modes of excretion occurring in all cells. He demonstrated that the secretion granules first became attached to the plasma membrane and then contents of the granules either escaped through a temporary opening on the cell membrane or underwent *in situ* diffusion, leaving an empty vacuole. Kurosumi (1968) subscribed that *in situ* or diacrine mode of excretion occurred only in FSH-gonadotropic cells and reverse pinocytosis or merocrine type of excretion took place in all other cell-types of the adenohypophysis.

Apart from the mode of synthesis of secretory products and the excretory mechanism, ultrastructural studies of the adenohypophysis made a valuable contribution towards elucidation of the relationship between the parenchymal cells and blood vessels. The apical poles of the acinar cells in the pars distalis adenohypophysis remained separated from the vascular lumen by a number of barriers (Farquhar, 1961). The capillaries were lined with a continuous endothelium which rested upon a basement membrane. The nucleus and other cell organelles of the endothelial cells were localized

on the basal part of the cell while the outer part of the cytoplasm was very thin and contained several fenestrations. The latter were bridged by diaphragma. The basement membrane was a continuous layer of moderate density and variable thickness with relatively ill-defined limits (Farquhar, 1961; Herlant, 1964). A similar basement membrane was also found around the parenchymal cells. A comparatively large pericapillary space intervened between the two basement membranes (Herlant, 1963, 1964). At places, this intervening space was not discernible and the two basement membranes appeared to be in contact with each other (Herlant, 1964). The pericapillary space contained an amorphous ground substance. Farquhar (1961) believed that the latter was composed of a mucopolysaccharide. In the pericapillary space, macrophages, unit collagen fibers and a few fibroblasts were localized (Farquhar, 1961; Herlant, 1964). Intact secretory granules were neither observed in the pericapillary space nor inside the capillaries (Herlant, 1964).

Duffy and Menefee (1965), Kobayashi *et al.* (1966), Monroe (1967) and Szentagothai *et al.* (1968) observed such capillaries with fenestrations in the zona externa of radix infundibuli, too. Duffy and Menefee (1965) further reported that the capillaries of the zona interna resembled typical capillaries without fenestrations. In the pars compacta infundibuli and pars distalis neurohypophysis, Lederis

(1965), Zeitz (1965), Barer and Lederis (1966) and Rinne (1966) observed such fenestrated capillaries surrounded by pericapillary spaces. Barer and Lederis (1966) hypothesized that the pericapillary space, around the capillaries of pars distalis neurohypophysis, contained a mucopolysaccharide sponge-work which provided a protective mechanism that prevented sudden and excessive increases in blood hormone level on the one hand and an accessory store of readily diffusible hormone on the other.

Oota and Kazumasa (1966) and Rinne (1966) described the ultrastructure of the pars infundibularis adenohypophysis in the rat. Oota and Kazumasa (1966) classified the cells of this part into two categories, on ultrastructure basis. The nongranulated cells were found to constitute the major part of cell population. These cells possessed microvilli and cilia, which extended into the interstitial space. The granular cells possessed many dark granules measuring 150 millimicrons in diameter. In order to investigate a possible secretory role of these granulated cells which the authors believed to be akin to the mucroprotein secreting-cells of the pars distalis adenohypophysis, Oota and Kazumasa (1966) carried out several physiological experiments but failed to induce any change in these cells. Rinne (1966) claimed that the granulated parenchymal cells of the pars infundibularis adenohypophysis contained electron-dense granules with a

mean diameter of either 133 millimicrons or 200 millimicrons. The author contemplated that cells with granules of the former type resembled gonadotropic cells of pars distalis adenohypophysis and cells with granules of the latter type were akin to corticotropic cells. A few ciliated nongranulated cells were also observed (Rinne, 1966).

The ultrastructure of the pars intermedia adenohypophysis was studied in the rat (Kurosumi _et al._, 1961; Howe and Maxwell, 1968) and in the ferret (Vincent and Anand Kumar, 1968; Vincent, 1969). Two types of cells were found in the parenchyma of the lobe. The predominant type was pear-shaped and possessed well developed cell organelles and lobulated nucleus (Kurosumi _et al._, 1961; Vincent and Anand Kumar, 1968). The granules of these cells measured 200-500 millimicrons in diameter and possessed uniform electron-density. Many empty vacuoles which were membrane bound and were of the same order of size as the dense-granules were also found in these cells (Howe and Maxwell, 1968). The other type of cells possessed rounded nuclei and well developed cell organelles. They were stellate-shaped and contained many electron-lucent membrane-bound granules of the same order as the former cell-type (Howe and Maxwell, 1968; Vincent, 1969).

Howe and Maxwell (1968) demonstrated the presence of an intercommunicating system of channels in the interstices of

parenchymal cells of the pars intermedia. The channels were continuous, on one hand, with intercellular dilatations that were evident at junctional zones between cells and, on the other hand, opened on the basal lamina at the junction of pars intermedia and pars distalis neurohypophysis. These intercommunicating channels were believed to be involved in diffusion of nutrients from blood capiallaries which were not present among the parenchymal cells.

In the rat, Kurosumi et al. (1961) observed two types of nerve terminals embedded deeply within the cytoplasm of parenchymal cells of the intermedia. One type of terminal was found to contain mitochondria and small dense elementary granules which led the authors to believe that some parts of the neurosecretory axons had penetrated into the parenchyma of pars intermedia. The second type of nerve terminal constituted normal axon terminals with neurofilaments and synaptic vesicles. Howe and Maxwell (1968) reported occurrence of only the second type of nerve terminals in the male rat. Vincent and Anand Kumar (1968) observed profuse innervation of the parenchymal cells in the ferret and described the neuroglandular junctions as being formed by a synaptoid type of union. They encountered two types of nerve terminals forming such junctions with each cell. One type of terminal possessed mitochondria, neurofilaments and electron-lucent synaptic vesicles measuring 25-40 millimi-

crons. Electron-dense granules measuring 60-90 millimicrons in diameter were also present in these nerve terminals. The other type of axon terminal contained neurofilaments, mitochondria, electron-lucent vesicles and electron-dense granules measuring over 100 millimicrons in diameter. By hypophysial stalk resection in ferrets, Vincent (1969) demonstrated that both synthetic and secretory activities of the cells increased after denervation, suggesting that the influence of the nerve fibers in the pars intermedia was inhibitory in nature.

From his histochemical and ultrastructural studies, Pearse (1968b) derived the conclusion that the cells of pars intermedia (melanotropic or MSH cells), the corticotropic cells of pars distalis adenohypophysis, the light cells of the thyroid gland and C cells of the ultimobranchial bodies resembled each other very closely in their ultrastructure as well as cytochemistry. He designated these cells as the APUD series of cells in mammals.

Considerable attention has been paid towards the electron microscopic study of the neurohypophysis in several species of animals. Many of these studies not only have elucidated the morphology of the hypothalamohypophysial system but have contributed valuable information regarding the mechanism of synthesis and release of neurosecretory material. Studies in various species, e.g., rat (Hartmann,

1958; Kobayashi *et al.*, 1966; Kobayashi *et al.*, 1967; Monroe, 1967), rabbit (Duffy and Menefee, 1965; Barer and Lederis, 1966), opossum (Bodian, 1966), mouse (Oota, 1963), human (Lederis, 1965), monkey (Bodian, 1966), pig (Zeitz, 1965) and dog (Bargmann, 1966; Szentagothai *et al.*, 1968) revealed the fact that there was little structural difference among various species at the ultrastructure level. These studies also delineated the course of supraopticohypophysial and paraventricuohypophysial tracts through the zona interna of radix infundibuli and through the pars compacta infundibuli. They also revealed the course and distribution of fibers of the tuberoinfundibular tract in the zona externa of the radix infundibuli (Monroe, 1967; Szentagothai *et al.*, 1968). In addition, confirmation as to the synthesis of neurosecretory material by the neurosecretory neurons of the hypothalamus and their transport to the pars distalis neurohypophysis was achieved through ultrastructure studies (Hartmann, 1958; Oota, 1963).

Electron microscopic studies of the neurohypophysis revealed the fact that the neurosecretory material was discernible in the form of spherical electron-dense granules with an average diameter of 100 millimicrons. The granules were termed elementary granules. Ultrastructural studies have also revealed the fact that Herring bodies were localized inside the unmyelinated axons of the

hypothalamohypophysial tract and were composed of large accumulations of elementary granules and neurofilaments (Bargmann, 1966; Monroe, 1967). The axon terminals were found to contain elementary granules, electron-lucent vesicles of the same order as elementary granules, mitochondria and typical synaptic vesicles (Oota, 1963; Duffy and Menefee, 1965; Lederis, 1965). Such axon terminals were encountered in the vicinity of blood capillaries throughout the pars distalis neurohypophysis as well as in certain parts of the pars compacta infundibuli.

In spite of several efforts, the morphological aspect of the mechanism of release of the neurosecretory material has not yet been clearly elucidated. Hartmann (1958) was the first author to report the possible mechanism of *in situ* diffusion of the contents from elementary granules. In this diffusion process acetylcholine, contained in the synaptic vesicles, was envisaged to play a role in enhancing membrane permeability (Oota, 1963; Kobayashi *et al.*, 1966). Views on the contrary were expressed by Barer and Lederis (1966) and Monroe (1967). In the opossum, Bodian (1966) reported osmotic depletion of the elementary granules by *in situ* diffusion of material, leaving behind empty membrane bound vesicles. However, during periods of excessive secretion of the neurohypophysial principles, it was observed that entire vesicles were thrown out due to rupture of the outer

membrane of the axon swellings and Herring bodies. The author further observed that groups of such vesicles entered the lumen of capillaries through dissolution of the basement membrane and rarefaction of the capillary endothelium. In the monkey, vascular extrusion was found to be the primary mechanism of release, while in situ diffusion served as a relatively less important mechanism of release (Bodian, 1966).

Development of Hypophysis

Apart from standard text descriptions, development of the hypophysis has been chosen as the theme of several extensive studies involving human and domestic animals. Romeis (1940) dealt exhaustively about development and morphogenesis of the human hypophysis. Among other studies, those of Gilbert (1935), Tilney (1938), House (1943), Falin (1961) and Villee(1969) elucidated the correlation between development and functional significance of the hypophysis. Miller (1916), Nelson (1930, 1932) and Shanklin (1944) studied the development and histogenesis of glandular cells of the porcine hypophysis. Kingsbury and Roemer (1940) undertook a similar study in the dog. Gilbert (1935) used pig and dog fetuses to compare certain developmental characteristics with those of the human. Cytogenesis in the pars distalis of the horse was studied by Harrison and Shryock (1940) and that of the cattle was studied by House (1943). Gorbman and Bern (1962) and Wingstrand (1966a) combined

extensive review of several publications with their own observations in several species to study evolution of the hypophysis. Green (1951) studied the development of hypophysis in a wide variety of species to formulate evolutionary relationships in the hypothalamohypophysial portal circulation.

The origin of the hypophysis from the lowest vertebrates to human was found to be constant in its fundamental constituents and mode of development (Tilney, 1938; Gorbman and Bern, 1962). The organ took its origin from two specialized sources of ectoderm, viz., the somatic ectoderm in or about the region of the mouth and the neural ectoderm in the floor of the third ventricle. The former was termed Rathke's pouch and the latter was termed saccus infundibuli (Wingstrand, 1966a). Divergent views were expressed as to the mode of formation of these two anlagen. Miller (1916), Tileny (1938), Rahn (1939), Romeis (1940), Hanstrom (1966) and Wingstrand (1966a) contributed to the theory that both, Rathke's pouch and saccus infundibuli, developed in all species as separate and independent evaginations. A constant attraction was envisaged to exist between the two evaginated parts during development which resulted in the approximation of the two anlagen to form a single composite structure.

Brahms (1932), Nelson (1932), Gilbert (1935), Kingsbury and Roemer (1940) expanded a second school of thought.

According to these authors, the primordium of the hypophysis was not derived from two separate anlagen but was initiated due to a firm adherence between the roof-part of the stomodeal ectoderm and the floor-part of the neural tube, in the region of the cranial flexure of the head. The adherence between stomodeal and neural epithelia developed during closure of the anterior neuropore as a result of the peculiar manner of development of the median prechordal part of the neural plate (Brahms, 1932). Beginning with very early stages of development, the ventral surface-ectoderm of the head remained closely adherent to the floor of the neural tube over a median area lying just rostral to the oral membrane. As the neural tube expanded cephalad and then ventrad around the rostral end of the foregut, the ventral surface of the embryonic head was swung ventrad and caudad. This resulted in an acute flexion involving the surface of the head and the oral membrane. The angle thus formed was named the hypophysial angle (Brahms, 1932; Gilbert, 1935; Kingsbury and Roemer, 1940). The rostral wall of the hypophysial angle was formed by that area of ectoderm which was adherent to the floor of the brain. As this relatively mesenchyme-free area became filled with the migrating mesenchymal tissue, the pharynx and forebrain were gradually separated. But, due to the neuro-ectodermal adherence of the two parts at the hypophysial angle, the Rathke's pouch was formed by pulling

away of the stomodeal wall.

The latter theory implied that the development of the hypophysis was an expression of the correlative growth of the neural wall, stomodeal epithelium and adjacent mesoderm (Kingsbury and Roemer, 1940). Gorbman and Bern (1962) observed that, in reptiles, birds and mammals, the hypophysial angle was so acute that, from the time it was first formed, the Rathke's pouch remained in contact with the infundibulum. In fish and amphibia, Rathke's pouch originated as an independent anlage of solid cellular mass. Since the head-fold in these species was not very pronounced as compared to other vertebrates, Rathke's pouch extended in a rostrodorsal direction to reach the infundibulum (Gorbman and Bern, 1962).

Once the two anlagen were differentiated, development and morphogenesis of the hypophysis followed an identical pattern in all species (Atwell, 1936; House, 1943; Wingstrand, 1966a). As development of the hypophysis progressed, the proximal end of Rathke's pouch became constricted and ultimately, the lumen at this end became completely obliterated. This part of Rathke's pouch constituted the epithelial stalk (Wingstrand, 1966a). The original cavity of Rathke's pouch, also, became constricted in its middle giving rise to two principal parts: the one nearer to the mouth was termed the oral lobe and the one further from it was termed the aboral lobe. It was the

aboral lobe that remained in contact with the saccus infundibuli and became expanded at the top. From near the junction of oral and aboral lobes, a lateral evagination took origin from the wall of Rathke's pouch on either side. These outgrowths constituted the lateral lobes (Brahms, 1932; Gorbman and Bern, 1962). They grew upwards curving towards the eminentia of the diencephalon. In many species, an unpaired anterior process took origin as a diverticulum of the oral lobe and extended rostrad up to the floor of third ventricle. According to Wingstrand (1966a), the mammalian hypophysial anlage was deficient in this anterior process. Nelson (1932) contended that the lateral lobes of pig embryos extended rostrad in the form of a cellular process and behaved identically as the anterior process of other species. After these subdivisions were delineated, the stalk of Rathke's pouch became detached from the wall of the stomodeum.

In the pig, Miller (1916) and Nelson (1932) reported that the hypophysial anlage was discernible at the stage of 5.5 mm crown-rump length of the embryo. The saccus infundibuli appeared as an outgrowth of the neural floor, for the first time, in the 9 mm embryo. The lateral lobes became visible at 10 mm stage. The epithelial stalk was detached from the stomodeum, and the Rathke's pouch was transformed into the Rathke's pocket at the stage of 15 mm. Kingsbury and Roemer

(1940) observed the same pattern of development in dog embryos.

Wingstrand (1966a) demonstrated that, after detachment and shrinkage of the epithelial stalk, Rathke's pocket underwent rotation, caused by growth of the infundibulum and surrounding structures. This rotation caused the distal parts of lateral lobes to come in contact with the infundibular region, and with the superior and rostral extremities of Rathke's pocket. During development and differentiation of Rathke's pouch, the saccus infundibuli also increased in size. Depending upon the species involved, the aboral lobe, which was in contact with the saccus infundibuli expanded on either side of the latter in an effort to encircle it. Miller (1916) and Nelson (1932) reported that the saccus infundibuli became so large in the pig embryo that the aboral lobe was unable to encircle it completely. On the other hand, the saccus infundibuli invaded the entire superior and caudal surfaces of the aboral lobe, the result of this was that contact between the two lobes was limited to the rostroventral surface of the saccus infundibuli (Shanklin, 1944). In the dog embryo, Kingsbury and Roemer (1940) found that the saccus infundibulum was comparatively much smaller so that it became completely surrounded by lateral outgrowths from the aboral lobe.

Miller (1916), Tilney (1938) and Wingstrand (1966a)

subscribed that growth of the saccus infundibuli through active proliferation of its distal end led to the formation of the adult neurohypophysis. Brahms (1932), Nelson (1932), Gilbert (1935) and Kingsbury and Roemer (1940) showed that the cells of saccus infundibuli did not contain any mitotic figures throughout the course of development, whereas mitosis and proliferation were abundant in the neighboring diencephalic floor. The authors, therefore, contended that formation of the pars neuralis occurred as a result of the reaction of growth processes taking place in the surrounding regions of the brain. The infundibular region of the brain floor and adjacent wall of Rathke's pocket underwent a rotation from dorsoventral to a cephalocaudal plane (Gilbert, 1935). This rotation incorporated a small segment of the diencephalic floor where mitoses were evident. From this incorporated region, the radix infundibuli (median eminence), pars compacta infundibuli (infundibular stem) and pars distalis neurohypophysis (infundibular process) were derived. Once the saccus infundibuli grew out, the infundibular recess of the third ventricle receded superiorly to variable distances in different species. Nelson (1932) and Kingsbury and Roemer (1940) demonstrated that the infundibular recess became obliterated in both, pig and dog fetuses, at an early age.

In course of development, the sac-like simple form of

the saccus infundibulum was lost and the part was thrown into a series of folds and papillae of different degrees of complexity (Shanklin, 1944). In some species, e.g., opossum (Bodian, 1951), teleosts, elasmobranchs and lungfish (Gorbman and Bern, 1962), the lobular character of the saccus infundibuli persisted to the adult stage. In birds, mammals and some reptiles, fusion of these folds and papillae produced the thick knob-like organ that was typical of the pars nervosa of higher vertebrates. In the adult form of these species, the pattern of original folding was considered to be reflected in the distribution of fibrous connective tissue and blood vessels (Nelson, 1932; Kingsbury and Roemer, 1940).

Final differentiation into the adult parts followed a set-pattern in all species (Wingstrand, 1966a). The contact surface of the aboral lobe was transformed into the pars intermedia adenohypophysis. The variability in size of this part was attributed to the extent of development of the saccus infundibuli and lateral alae of the aboral lobe which invested it (Atwell, 1932; Gorbman and Bern, 1962). In the pig fetus, the pars intermedia was smaller (Nelson, 1932), while in the dog fetus it gained great proportions and surrounded the saccus infundibuli on all sides (Kingsbury and Roemer, 1940). The growth of the pars intermedia of the dog was considered as appositional rather than proliferative.

The adult pars distalis adenohypophysis was derived from the caudal wall of aboral lobe, and the entire oral lobe. The original cavity of Rathke's pocket became reduced to a cleft (*viz.*, cavum hypophysis) between pars intermedia and pars distalis adenohypophysis. The pars infundibularis adenohypophysis was formed by union of the two lateral lobes and their investiture of the infundibulum. The saccus infundibuli and adjacent part of the diencephalic floor, which became incorporated in the former due to the rotation process, were transformed into the neurohypophysis of adult form.

Miller (1916) reported the participation of entodermal cells in the morphogenesis of the hypophysis in addition to ectodermal and mesodermal contributions. He contended that the notochord pulled with it a mass of mesodermal cells from the foregut into the extrapharyngeal space, just above the hypophysial angle, during its caudal retraction. With the detachment of the epithelial stalk, this entodermal mass reportedly spread along the caudal surface of Rathke's pocket and later on, moved to a medullary position due to rotation of the hypophysis. The author believed that chromophil cells of the adenohypophysis were derived from the entodermal component and chromophobe cells took their origin from the ectodermal component of Rathke's pocket. Tilney (1938) and Wingstrand (1966a) subscribed that partici-

pation of entodermal cells in morphogenesis of the hypophysis was questionable whereas Nelson (1932) denied the involvement of any entodermal tissue. In many species, it was observed that the contributions of different segments, viz., part of the aboral lobe, oral lobe and proximal portions of the lateral lobes in order to constitute the pars distalis adenohypophysis in its final form, were reflected in the adult in shape of well delimited zones of specific cell-types (Gorbman and Bern, 1962).

Racadot (1948) studied the relative volume of different lobes of the hypophysis during early, middle and late stages of fetal development in the pig. He found that the pars intermedia and pars distalis neurohypophysis maintained a proportion of almost 1:1 throughout the course of development while the pars distalis adenohypophysis varied in its proportion from 3 times in the 4 mm pig embryo to 10 times that of other lobes in the full-term fetus.

Varied results have been published regarding the morphogenesis of cell-types in the pars distalis adenohypophysis of different species. Similar contradictory views have also been expressed by several authors regarding the functional state of these cell-types at various stages of development. Maurer and Lewis (1922) reported the differentiation of granular cells in the pars intermedia of pig fetuses measuring 17.5 cm in crown-rump length.

Rumph and Smith (1926) demonstrated the occurrence of few basophil cells with aniline blue stainable granules at 14 cm stage of pig embryos. The authors stated that eosinophil cells were not differentiated until after the 16 cm stage. Nelson (1930, 1932), using pig fetuses, reported that the cells of pars distalis adenohypophysis assumed epithelioid nature and became arranged in cords at 4 cm stage. Basophil cells were reportedly discernible at 5-6 cm stage and eosinophils at 7-8 cm stage. Nelson (1932) also observed that eosinophil cells did not form an appreciable proportion until the 16 cm stage.

Racadot (1949) studied histogenesis of hypophysial cell-types in cow, sheep and pig fetuses by the application of Heidenhain's azocarmine and Mallory's trichrome techniques. He reported that somatotropic (alpha) cells were the first cell-type to become differentiated in all species. Up to 13 cm crown-rump length, no individual cell-types were evidenced. Somatotropic cells were discernible at the 10.5 cm stage and cyanophils became evident at 22 cm stage. In the latter stage, Racadot (1949) also observed the presence of few carmine cells. In the chick embryo, Rahn (1939) reported that undifferentiated basophilic cells were evident on the eighth day of incubation. The first chromophil cell-type to appear was the acidophil beginning with the tenth day of incubation. Basophils with stainable secretion granules

appeared a day or two later than acidophil cells.

Romeis (1940) observed that basophil cells were the first to become differentiated in human and acidophil cells were evident in latter stages. Falin (1961) applied periodic acid-Schiff, resorcin-fuchsin, aldehyde-fuchsin and Heidenhain's azan procedures to the hypophyses of human fetuses, in his investigation of the histogenesis of adenohypophysial cells. He reported that basophil cells became evident at the eighth week of gestation and eosinophils were discernible at the ninth week. The author further reported that, to seventh month of gestation, the indifferent cells constituted a major part of the human pars distalis adenohypophysis and beyond that time, the inter-relation of cells was altered in favor of chromophil cells, particularly to that of basophils. Pavlova _et al_. (1968) obtained identical results in human fetuses by the use of histological and histochemical techniques. They reported that, after nine weeks of pregnancy, two types of mucoproteinaceous cells could be differentiated and that acidophil cells with orange G stainable granules became evident at 11-12 weeks of pregnancy. Dubois (1968) studied the histomorphology of the hypophysis from a eight-week-old human embryo with the aid of the electron microscope. At the ultrastructure level, the author could differentiate typical somatotropic cells and a second cell-type which

resembled the FSH-gonadotropic cells of the adult hypophysis. The first cell-type was far less numerous than the second type. Embryonic cells in different stages of development and synthesis of granules were also encountered.

Rumph and Smith (1926) injected hypophysial extracts from pig embryos into hypophysectomized tadpoles to determine the relationship of age and hormonal activity. They reported that hypophysial extract of a full-term (26 cm) fetus contained thyroid stimulating activity. Contopoulos and Simpson (1957) found thyroid stimulating activity in rats beginning with the 19th day of fetal life. In several species of laboratory and domestic animals, Jost (1956) also observed such activity during fetal life. Rosen and Ezrin (1966) studied 50 human hypophyses between the ages of 5 to 40 weeks of prenatal life. Periodic acid-Schiff positive basophilic cells were first evident at 9 weeks of age. By 13 weeks, the cells stained more darkly with aldehyde-thionin and could be differentiated as thyrotropic cells. At three months, it became possible to identify thyrotropin in fetal serum and in extracts of fetal pituitary gland. Pavlova *et al.* (1968) obtained positive results in thyroid stimulating activity of human fetal hypophysis at 15 weeks of prenatal life. From correlative experimental and morphological studies in chick embryos, Rahn (1939) concluded that the embryonic hypophysis exerted no influence on the thyroid

gland during early development. Using fetuses of several mammalian species, Jost (1966) proved that normal physiological function of the fetal thyroid was dependent upon hypophysial stimulation.

Presence of growth stimulating hormone in fetal hypophyses was found in the pig (Rumph and Smith, 1926; Smith and Dortzbach, 1929; Cleveland and Wolfe, 1933), in rats (Contopoulos and Simpson, 1957; Jost, 1956), in the rabbit (Jost, 1956) and in the human (Falin, 1961; Pavlova et al., 1968; Rice et al., 1968; Villee, 1969). Pavlova et al. (1968) and Villee (1969) observed that somatotropin was undoubtedly produced by fetal hypophysis in human, which could be proved by bioassay and immunoassay beginning with the ninth week of gestation. Jost (1966) pointed out that even though somatotropin was not essential for growth of fetuses in some species, e.g., rat and rabbit, it did take part in certain metabolic processes. Glycogen deposition in the liver of fetal rats and rabbits was found to be comparatively less in decapitated fetuses than in the controls.

Smith and Dortzbach (1929) implanted intramuscularly porcine fetal hypophysis into hypophysectomized rats to test the concentration of gonadotropic hormones. They obtained positive results by employing hypophyses of fetuses with a crown-rump length of 20-21 cm. Rumph and Smith (1926) observed gonad stimulating activity in hypophysial extracts

from 26 cm pig fetuses, while Cleveland and Wolfe (1933) concluded, from their transplant experiments, that hypophysis from a 17 cm pig embryo could activate the gonads of immature mouse. Hellbaum (1935) reported the presence of gonad stimulating principle in fetal hypophysis of the horse. Contopoulos and Simpson (1957) failed to gain any evidence of gonad stimulating activity in rat fetuses.

Rice *et al*. (1968) tested the hypophysis of male and female human fetuses between 6 and 7.5 months of age by the rat ventral prostate assay. They found a biologically active principle of luteinizing hormone in both sexes. Pavlova *et al*. (1968) reported sex differences in the gonadotropin content of human fetal hypophysis. In male fetuses, lutenizing hormone was found to be absent and follicle stimulating hormone was detectable at 21 weeks. In female fetuses, lutenizing hormone was first detected at 18-28 weeks and follicle stimulating hormone at 13 weeks of prenatal life. Lactotropic hormone was found to be present in the fetal hypophysis beginning with the 19th week of gestation (Pavlova *et al*., 1968).

Rahn (1939) observed that the fetal hypophysis exerted least influence on gonads in chick embryos. In rabbit, Jost (1966) proved that the fetal pituitary stimulated the testis at a maximal rate during a limited period of development, which coincided with the most important period of

somatic sexual organogenesis. Villee (1969) concluded that the fetal hypophysis was capable of producing most, if not all, of the tropic hormones regulating hormone production in the human fetus. But, as far as differentiation of the interstitial cells was concerned, morphological evidence suggested that luteinizing hormone was secreted at a latter period and thus could not possibly act as a source of gonadotropin for the purpose (Villee, 1969).

Contopoulos and Simpson (1957) found no corticotropic activity in the fetal hypophysis of rats. But Milkovic and Milkovic (1962) reported mitotic activity in adrenocortical tissue in adult rats, which contained transplants of rat fetal hypophysis. Jost (1956) proved the existence of hypophysis-adrenal feedback system in fetal rats by injecting cortisone along with adrenocorticotropin and measuring hypertrophy of the adrenal gland. In human, Pavlova et al. (1968) found corticotropin in fetal hypophysis beginning with the 9th week of prenatal life.

Blood Supply of Hypophysis

Since early part of the present century, blood supply to the hypophysis was being investigated in many species. Compared to other endocrine glands, vascular arrangements in the hypophysis were associated with distinct morphological and functional significance (Green and Harris, 1947). Not only was the existence of a portal circulation revealed in

the hypophysis (Popa and Fielding, 1930) but, as a result of its extension between the hypothalamus and hypophysis, the hypothalamohypophysial portal system was described as the pathway for hypothalamic releasing and inhibiting factors destined for the adenohypophysis (Wislocki and King, 1936; Green, 1951). The blood supply to the hypophysis of the dog was studied by Dandy and Goetsch (1911), Basir (1932), Popa and Fielding (1933), Green and Harris (1947), Jewell and Verney (1957), Torok (1954, 1960, 1964) and Szentagothai _et al_. (1968). Such detailed study of the hypophysial circulation has not been published in case of the pig.

Among other species, results of investigations have been published pertaining to the hypophysial circulation in the human (Popa and Fielding, 1930; Wislocki, 1938a; Romeis, 1940; Xuereb _et al_., 1954a, 1954b; Green, 1957; Stanfield, 1960), in the monkey (Wislocki and King, 1936; Wislocki, 1938a, 1938b; Holmes and Zuckerman, 1959), in the sheep (Daniel and Prichard, 1957a, 1957b; Baldwin, 1964), in the goat (Daniel and Prichard, 1958; Adams _et al_., 1964b), in the horse (Baldwin, 1964; Brettschneider, 1955), in cattle (Baldwin and Bell, 1960; Dellmann, 1960; Baldwin, 1964; Cummings and Habel, 1966), in the cat (Wislocki and King, 1936; Wislocki, 1937c; Green and Harris, 1947; Torok, 1960; Szentagothai _et al_., 1968), in the rabbit (Wislocki and King, 1936; Harris, 1947), in the rat (Green and Harris,

1949; Barrnett and Greep, 1951; Landsmeer, 1951; Daniel and Prichard, 1956; Glydon, 1957) and in the mouse (Worthington, 1935, 1960). Green (1951) studied the developmental trend in hypothalamohypophysial system in 44 different species including the dog and the pig. Embryological development of the hypophysial circulation has been elucidated by Wislocki (1937b), Niemineva (1950), Assenmacher (1952), Glydon (1957) and Campbell (1966). In vivo observations with regard to the direction of blood flow in the portal vessels have been reported in the rat (Green and Harris, 1949; Barrnett and Greep, 1951), in the mouse (Worthington, 1935), in the dog (Torok, 1954, 1960; Jewell, 1956; Jewell and Verney, 1957) and in the cat (Torok, 1960; Szentagothai et al., 1968).

The pattern of blood supply evident in different species was reported to differ only in minor details (Daniel, 1966). Earlier investigations revealed that the pars distalis adenohypophysis and the pars distalis neurohypophysis received separate arterial supplies (Dandy and Goetsch, 1911; Wislocki, 1937c). The rostral hypophysial arteries served as afferent vascular channels for the radix infundibuli, pars compacta infundibuli and the pars distalis adenohypophysis, while the caudal hypophysial arteries supplied the pars distalis neurohypophysis and caudal part of the pars intermedia adenohypophysis. Such an arrange-

ment has also been reported in all other species (Green, 1951; Daniel, 1966). In species where the internal carotid artery remained patent in the adult animals, it was established that the rostral hypophysial arteries took their origin from the intra-arachnoid portion of the internal carotid or from the caudal communicating artery (Basir, 1932; Torok, 1964; Stanfield, 1960). In these species, the caudal hypophysial arteries were found to be derived from the intracavernous portion of the internal carotid artery. In the ox, sheep and goat, species in which the internal carotid artery became rudimentary in the adult stage, the rostral and caudal hypophysial arteries have been demonstrated to take origin, respectively, from the arterial circle of the cerebrum and from extensions of the rostral and caudal retia present on the caudal and dorsal surfaces of the hypophysis (Daniel and Prichard, 1957a; Daniel and Prichard, 1958; Baldwin, 1964; Cummings and Habel, 1966). By the use of tracer element injection, it was determined that the pars distalis adenohypophysis was being supplied equally by each ipsilateral rostral hypophysial artery in the dog, while the pars distalis neurophypophysis was supplied solely by the internal carotid artery of one side, mostly that of the right (Jewell and Verney, 1957). No part of the vertebral arterial blood was supplied to the latter lobe. In the ruminants, it was revealed that the infundibulum and pars

distalis adenohypophysis derived their blood supply from the internal maxillary artery and the pars distalis neurohypophysis received its share from the condyloid and vertebral arteries (Cummings and Habel, 1966).

At first, existence of strictly independent arterial supply to the pars distalis adenohypophysis and pars distalis neurohypophysis was envisaged (Dandy and Goetsch, 1911; Wislocki, 1937c). But in both studies, a vascular continuity between the two parts at their rostral ends was reported as an alternate route for cases of emergency. Subsequently, by the method of experimental fat embolism in canines, it was proved that vessels crossed from the pars distalis neurohypophysis into the pars distalis adenohypophysis through the narrow zone of reflection between pars intermedia and pars distalis adenohypophysis (Morato, 1939). A functionally active anastomosis was envisaged in dogs and cats and the blood that irrigated the caudal part of the pars distalis adenohypophysis was postulated to be derived from the caudal hypophysial arteries (Morato, 1939). In later studies, such functional anastomoses were demonstrated in man (Stanfield, 1960; Adams _et al_., 1966), in sheep (Daniel and Prichard, 1957a), in the goat (Daniel and Prichard, 1958) and in cattle (Baldwin, 1964; Cummings and Habel, 1966). In all species, anastomosis between the two arterial systems was evident externally, on the dorsal surface of the

organ and also, within the parenchymal tissue.

In all species, the rostral hypophysial arteries ramified and joined the contralateral arteries in the formation of an arterial annulus around the radix infundibuli (Basir, 1932; Wislocki, 1937c; Cummings and Habel, 1966). From the annulus, branches were given to the pars infundibularis adenohypophysis, within which further branching and anastomosis resulted in the formation of a dense plexus of vessels largely of precapillary nature. This plexus was named mantel plexus by Romeis (1940) and first primary capillary bed by other authors (Stanfield, 1960; Daniel and Prichard, 1957; Cummings and Habel, 1966). A caudally directed branch also extended from each side of the annulus towards the lower infundibular stem. In human, this branch was termed artery of the trabecula (Xuereb _et al._, 1954a; Stanfield, 1960; Daniel, 1966). In other species, the arterial branch was designated as the caudoventral branch (Cummings and Habel, 1966). In the sheep and goat, occurrence of such a caudoventral branch was not observed (Daniel and Prichard, 1957a, 1958). On reaching the lower infundibular stem, the caudoventral branch anastomosed with the contralateral vessel to form a second primary capillary plexus. It had been established that branches from caudal hypophysial arteries also contributed branches to the second primary capillary plexus (Basir, 1932; Wislocki, 1937b;

Torok, 1960). In the sheep, reports indicated that the second primary capillary bed derived its blood supply solely from the caudal hypophysial arteries by way of the paired vessel which was named artery of the lower infundibular stem (Daniel and Prichard, 1957a).

The capillaries of the first primary capillary bed were found to resemble hairpin-loops with divergent shape and complexity (Green, 1948; Dellmann, 1960; Torok, 1960). These capillaries were not confined to the radix infundibuli but extended the entire length of the upper infundibular stem. They were oriented perpendicular to the infundibulum and wall of the infundibular recess. Each capillary loop possessed an afferent and an efferent limb, the former being smaller in diameter. The interposed segment between the afferent and efferent limbs occurred in the form of either a branching complex of capillary loops (arborization pattern) or an elongated helical capillary network (spike pattern) or a simple loop (simple capillary pattern) (Green, 1948; Brettschneider, 1955; Dellmann, 1960; Cummings and Habel, 1966). The spike forms pursued a dorsomedial course deep within the upper infundibular stem and extended well into the radix infundibuli (Green, 1948; Dellmann, 1960). The zona externa of the infundibulum contained mainly loops from the simple capillaries while the internal regions received loops from the arborizations and spikes. The efferent limbs

of several capillary loops joined together to form the long hypophysial portal vessels. The latter descended along the infundibulum, being embedded within the parenchyma of pars infundibularis adenohypophysis and finally, entered the pars distalis adenohypophysis. In the latter lobe, the portal vessels underwent extensive branching and constituted the secondary capillary bed.

The majority of the capillary loops did not establish connection with the long portal vessels (Szentagothai _et al_., 1968). Only those of the radix infundibuli were found to drain towards the portal vessels, while the others joined the veins of pars distalis neurohypophysis. In the rabbit, Harris (1947) reported certain peculiar morphological characters of the long portal vessels that deviated considerably from the normal mammalian pattern. The long portal vessels of the rabbit descended on the external surface of the pars infundibularis adenohypophysis and along with the latter entered the diaphragma sella through an additional foramen, separate from the one through which pars compacta infundibulum pierced the diaphragma sella.

The peculiar distribution of the short capillary loops in the zona externa of radix infundibuli and pars compacta infundibuli, and that of the long capillary loops (arborization and spike patterns) in the zona interna of the same segments, was considered as a functional necessity (Dellmann,

1960; Szentagothai *et al.*, 1968). Terminations of the axons and collateral fibers from the tuberoinfundibular tract were found in the zona externa of all species (Szentagothai *et al.*, 1968; Akmayev, 1969), while nerve endings from the supraoptico- and paraventriculohypophysial tracts were observed in the vicinity of capillary loops that penetrated into the zona externa (Dellmann, 1960; Monroe, 1967; Akmayev, 1969). Thus it was stipulated that these capillaries which contacted the terminals of tuberoinfundibular tract formed the long portal capillaries and conveyed the releasing and inhibiting factors to the adenohypophysial cells (Monroe, 1967; Szentagothai, *et al.*, 1968). In the zona interna, it was envisaged that the nerve fibers discharged their content of neurohypophysial principles into the capillaries with spike and arborization patterns, that joined the venous system directly.

A second group of capillaries, the short portal vessels, was observed to take origin from the second primary capillary bed of the lower infundibular stem. These short portal vessels were first described by Xuereb *et al.* (1954b) in human and were subsequently demonstrated in the rat (Daniel and Prichard, 1956), in the sheep (Daniel and Prichard, 1957a), in the goat (Daniel and Prichard, 1958), in the monkey (Adams *et al.*, 1963, 1966), in the dog and the cat (Torok, 1960), and in cattle (Cummings and Habel, 1966). These short portal vessels were considered important not

only because they probably carried neurohumors to certain cells of the adenohypophysis but also on account of the fact that they ensued the survival of a well defined collection of cells in the pars distalis adenohypophysis after stalk resection (Stanfield, 1960; Adams *et al.*, 1966). Daniel *et al.* (1964) placed an impermeable barrier between the cut ends of the stalk in monkeys to prevent regeneration of the long portal vessels or of the nerve fibers. The second primary capillary bed of the lower infundibular stalk was thus denervated but its afferent arterial blood supply was unimpaired by the operation. Histological examination of the area supplied by the short portal vessels revealed that the cells contained no secretion granules. Thus, it was established that the short portal vessels also carried releasing and inhibiting factors destined for the pars distalis cells.

The main supply of blood to the pars distalis adenohypophysis was provided by the long portal vessels, although the relative importance of the long and short portal vessels was reported to vary slightly among the different species. The long portal vessels supplied approximately 70-80 percent of the lobe in the rat (Adams *et al.*, 1966; Porter *et al.*, 1967), 70-90 percent in the goal (Daniel and Prichard, 1958; Adams *et al.*, 1964b), 78-90 percent in human (Adams *et al.*, 1966), 96 percent in the sheep (Daniel and Prichard, 1957b; Adams et al., 1966) and 78 percent in the

monkey (Daniel *et al*., 1964; Adams *et al*., 1966). The long portal vessels were distributed mostly in the central and ventral regions (medulla) of the pars distalis adenohypophysis, whereas the short portal vessels were distributed in the dorsolateral zones (Daniel and Prichard, 1956, 1957a, 1958; Xuereb *et al*., 1954a, 1954b; Szentagothai *et al*., 1968). It was suggested that individual portal vessels not only received blood from restricted regions of the radix infundibuli and pars compacta infundibuli, but also may deliver blood to discrete regions of the pars distalis adenohypophysis (Daniel and Prichard, 1956; Adams *et al*., 1964a, 1966).

In addition to the long and short portal vessels, a group of vessels originating from the transient zone between the infundibulum and rostral pole of the pars distalis neurohypophysis had been described in the rat (Daniel and Prichard, 1956), in the dog (Jewell, 1956) and in the monkey (Holmes and Zuckerman, 1959). These vessels were called neural lobe portal vessels. Their presence had been denied in the sheep (Daniel and Prichard, 1957a, 1957b). In species where neural lobe portal vessels were demonstrated, the destination of these capillaries were found to be the dorsal and lateral regions of the pars distalis adeno-hypophysis.

The direction of blood flow in the hypothalamohypophysial

portal system was the subject of considerable debate. The theory of a portal circulation from the pituitary to the hypothalamic region was first advanced by Popa and Fielding (1930). In the stalk of the hypophysis, certain veins were found that were assumed to collect blood from all lobes of hypophysis and convey it rostrad to the floor of the infundibular recess, where they were thought to break up into a system of fine channels forming a secondary distributing net. Housay _et al_. (1935) made the significant observation that the flow of blood in the hypophysial vessels of the toad occurred always in the downward direction, _viz_., from the hypothalamus to the pars distalis adenohypophysis. In an independent study, Wislocki and King (1936) demonstrated the same feature in the rabbit, monkey and human. A fluctuous direction of blood flow in the hypothalamohypophysial portal system depending on physiological requirements was also envisaged in man (Romeis, 1940). Confirmation as to the downward flow from the hypothalamus to the hypophysis was achieved by _in vivo_ studies in the rat (Green and Harris, 1949; Barrnett and Greep, 1951; Daniel and Prichard, 1956), in the mouse (Worthington, 1935) and, in the dog and the cat (Torok, 1960; Szentagothai _et al_., 1968). These studies also revealed that blood flow in the pars distalis adenohypophysis occurred in a rostrocaudal direction.

The pars intermedia received the least vascular supply

in all species. The rostral end of the lobe was supplied by the caudoventral branches from the arterial annulus of the hypophysis (Basir, 1932; Wislocki, 1937c). The caudal part of the lobe received branches from the caudal hypophysial arteries. Such branches were mainly confined to the junctional zone between pars intermedia and pars distalis neurohypophysis (Green, 1948; Daniel and Prichard, 1957a).

The caudal hypophysial arteries anastomosed with each other through arterial branches and formed a network on the dorsal surface of the hypophysis (Torok, 1954; Daniel and Prichard, 1957). Branches from the arterial network extended into the pars distalis neurohypophysis. Capillaries of this lobe were described as having a simple branching pattern (Basir, 1932; Daniel and Prichard, 1957a).

Green (1948) described the structure of the long portal vessels and of the tufted capillaries as being composed of a layer of collagen fibers and reticular fibers. A thick layer of postganglionic sympathetic fibers was also evident around the vessels. At the ultrastructure level, the capillaries in all regions of the hypophysis and infundibulum were found to possess fenestrated endothelium and perivascular space, delimited by a basement membrane on either side (Monroe, 1967; Szentagothai *et al*., 1968; Akmayev, 1969).

The major part of the venous drainage from the radix

infundibuli and pars compacta infundibuli was achieved by way of the long portal vessels (Torok, 1960; Szentagothai *et al.*, 1968). A fraction of the blood volume from this region, however, passed towards the subependymal capillary network of the hypothalamus. Such anastomoses between the capillaries of the median eminence and tuber cinereum had also been observed by several other workers (Daniel, 1966). The capillaries of the pars distalis adenohypophysis drained into veins which, in general, descended ventrad to terminate in the subhypophysial venous sinuses. The latter in their turn merged with the underlying cavernous sinuses (Xuereb *et al.*, 1954a; Torok, 1960). Some of the blood reaching the caudal surface of the pars distalis adenohypophysis, turned upwards and was drained over the caudal transition zone between pars distalis adenohypophysis and pars intermedia towards the vascular system of the pars distalis neurohypophysis (Basir, 1932; Szentagothai *et al.*, 1968). It was envisaged that part of the blood, draining from the pars distalis adenohypophysis towards the pars distalis neurohypophysis, passed by way of the rostrally situated loops towards the capillary system of the hypothalamus. Direction of blood flow in these vessels, in a rostral direction, was clearly revealed during *in vivo* studies in the dog (Torok, 1960; Szentagothai *et al.*, 1968).

The capillaries of the pars distalis neurohypophysis

drained into veins which opened into the subhypophysial venous sinus (Xuereb et al., 1954a; Daniel, 1966). The subhypophysial venous sinuses drained finally into the cavernous sinuses. Thus, the hormones leaving the hypophysis passed into the blood of the cavernous sinuses and thence into that of the internal jugular veins (Daniel, 1966).

Glydon (1957) and Daikoku et al. (1967) enumerated the various developmental stages through which the hypothalamohypophysial portal system attained its adult form in the rat. In its earlier stages, the entire hypophysial complex was surrounded by a plexus of meningeal vessels. The continuity of the plexus with vessels lying on the roof of the primitive pharyngeal cavity was lost, when the sphenoid bone became differentiated. Growth of the lateral or tuberal processes of the adenohypophysis led to the trapping of part of the perihypophysial plexus beneath the radix infundibuli (the supratuberal plexus). Subsequently, the vessels in the intervening area became stretched and formed the long portal vessels connecting the two consolidated capillary plexuses of the pars infundibularis and pars distalis.

In the rat, the first primary capillary plexus was observed on 3rd to 5th day of post-natal life (Glydon, 1957; Campbell, 1966). In the rabbit, the primary capillary

plexus became discernible by the 17th day of prenatal life (Campbell, 1966). In human, a well defined primary plexus was evident by the 4th month of gestation (about 16 cm crown-rump length of the fetus) (Wislocki, 1937b; Campbell, 1966).

Quantitative Changes in Hypophysis with Age

Many publications dealing with the qualitative changes that occurred in the hypophysis of domestic animals have been presented, but very few papers deal with the quantitative aspect. Latimer (1941) reported that weight of the hypophysis, as a percentage of the body weight, was significantly heavier in female dogs than in males even though absolute gland weights were not significantly different between both sexes. The hypophyses were relatively heavier in the smaller dogs and were relatively lighter in the larger animals. The weights of the hypophyses were well correlated with body weight and body length and such correlations, on the average, were higher in females than in the male dogs. These observations were confined to a sample of 155 sexually mature males and 151 mature female dogs.

Stockard (1941) subscribed that large breeds of dogs and large animals possessed heavier glands and vice versa. The relative weight of the hypophysis was heavier in smaller dogs than in larger animals. The relative weight was also found to be independent of sex influence in purebreds as

well as in breed-crosses.

White and Foust (1944) employed 203 dogs varying in age from birth to 15 years in their study of growth changes in the hypophysis of normal dogs. They reported that absolute hypophysis weight increased with age as well as with increase in body weight, while the relative hypophysis weight decreased with age. Heavier dogs were found to possess heavier hypophyses and a trend towards an increase in the actual gland weight in the older and hence larger dogs was noticed. No statistically significant sex differences were observed in this study.

Francis and Mulligan (1949) used 55 male dogs in their study on the relationship between weight of the hypophysis, body weight and age of the animal. It was observed that the hypophysis weight increased with increase in body weight. Age of the animal was found to have no significant relationship with absolute weight of the hypophysis.

Hewitt (1950) observed that the relative weight (mg/kg body weight) of the hypophysis was greater (18.12 percent) in immature dogs than in adults. In sexually mature animals, a sex difference was evident in the relative hypophysis weight, that of adult female dogs being relatively greater (17.06 percent) than adult males. Absolute weight of the hypophysis did not differ significantly in both sexes within any single weight group of adult animals. In senile animals,

the relative hypophysis weight was distinctly and significantly smaller than that of adult (sexually active) dogs.

Latimer (1954) studied the relative growth of hypophysis during prenatal and post-natal stages of dogs and reported that the hypophysis increased 30 times in weight during the fetal period, whereas the corresponding growth in postnatal period was a little over seven times.

Many reports have been presented dealing with the effects of hormones or other substances on the size of the porcine hypophysis, but very little information is available in the literature regarding the relationship of absolute weight of the hypophysis with age and body weight in pigs not subjected to experimentation. Baker et al. (1956) employed 65 Hampshire and Duroc sows, ranging in age from birth to 1400 days, in their study on the relationship between growth hormone content of the hypophysis and age of the animal. They observed that weight of the pars distalis adenohypophysis (anterior pituitary) increased rapidly until the age of 225 days after which the increase continued at a lower rate. Relative weight of the anterior pituitary decreased rapidly until the age of 300 days whence it became constant.

In the cat, Latimer (1939) found the mean hypophysis weight to be significantly different in both sexes. In male cats, it was greater than in female. But relative

weight of the hypophysis was not significantly different between both sexes. In both male and female cats, weight of the hypophysis had no significant correlation with body weight.

Weight of the hypophysis was reported to be comparatively heavier in women than in men of the same body weight (Rasmussen, 1934). This difference became evident between the two sexes beginning with teen-age, when weight of the hypophysis in females tended to become progressively heavier than that of males (Rasmussen, 1947). In both sexes, a person of large stature possessed a heavier hypophysis (Rasmussen, 1929, 1934). In men, the pars distalis adenohypophysis (anterior lobe) decreased noticeably after 50 years of age while in women, it tended to retain normality. The neural lobe increased with age in both sexes. Among the three primary lobes, the pars distalis adenohypophysis was found to be the least variable, and the pars intermedia to be the most variable part. In any age interval, the neural and intermediate lobes were consistently smaller in women than in men, but the anterior lobe was always larger in women. Similar views have also been expressed by Roessle and Roulet (1932) and Shanklin (1953).

Among the laboratory animals, relationship of hypophysis weight to age and body weight has been studied in the rat (Mixner and Turner, 1942; Bowman, 1961), in the mouse

(Blumenthal, 1955), in the guinea pig (Mixner *et al.*, 1943; Latimer, 1951) and in the rabbit (Kibler *et al.*, 1942). In male albino rats, Mixner and Turner (1942) reported the increase of hypophysis weight to be disproportionate to that of body weight. The ratio of hypophysis weight to body weight decreased as body weight increased. Bowman (1961) reported an increase in wet as well as dry weight of the hypophysis in rats with age, and with increase of body weight. The male guinea pigs possessed comparatively heavier hypophyses than females (Mixner *et al.*, 1943). In both, male and female guinea pigs, the ratio of hypophysis weight to body weight decreased as the body weight increased, but absolute hypophysis weight increased linearly with body weight (Mixner *et al.*, 1943; Latimer, 1951). Blumenthal (1955) observed that, in any age interval, female mice had heavier hypophyses than males even though body weight was consistently heavier in the latter. Weight of the hypophysis increased up to the 9-12th month in males and until the 13-16th month in female mice before declining appreciably.

In rabbits, weight of the hypophysis differed significantly in both sexes, being comparatively heavier in the female (Kibler *et al.*, 1942). In both sexes, hypophysis weight was not directly proportional to body weight. The ratio of hypophysis weight to body weight decreased as body

weight increased. Pfeiffer (1936) subjected albino and hooded rats to 18 different experimental conditions in order to determine the influence of the gonads on sex differences, in weight of the hypophysis. He concluded that the hypophysis at birth was bipotential and capable of being differentiated into either the male or the female type depending upon the gonad present. This period of undifferentiation in both sexes continued until puberty in case of castration at birth. Sex-type of the hypophysis was found to be irreversible after the age of puberty. The author concluded that sex difference in weight of the hypophysis was not a genetic factor but depended upon the presence of the differentiated gonad. Other authors also believed that weight of the hypophysis might be subject to gonadal influence (Hewitt, 1950).

Qualitative Changes in Hypophysis with Age

Age-correlated changes of the hypophysis has been considered under various topics by different authors. Many of these studies were confined to the rat, mouse and human, and very few publications dealt with other species. Among other alterations in the cellular structure of the adenohypophysis, a decrease in volume of the glandular cells was observed in the rat (Cowdry, 1952; Lowry and Hastings, 1952), in the mouse (Dawbarn, 1932; Stein _et al_., 1942; Weiss and Lansing, 1953; Blumenthal, 1955), in the golden

hamster (Spagnoli and Charipper, 1955), in the human (Lowry and Hastings, 1952; Shanklin, 1953) and in the fowl (Payne, 1946, 1952). Such a decrease in average size of the cells occurred earlier in males than in females (Blumenthal, 1955; Spagnoli and Charipper, 1955).

Changes in the mitochondria of the adenohypophysial cells with age were more evident in basophil cells than in acidophils (Payne, 1946, 1952; Andrew, 1952). The mitochondria became enlarged, swollen and vesicular. The vacuoles observed in the basophil cells of the fowl were attributed to the coalescence of such vacuolated mitochondria (Payne, 1952). In cocks, mitochondrial changes were evident at a much younger age than in hens. In the hen, vacuolated mitochondria were reported at and beyond 10 years of age (Payne, 1946). Castration was reported to hasten such mitochondrial changes in both sexes (Payne, 1952). At the ultrastructural level, Weiss and Lansing (1953) observed similar changes in the mitochondria of all cells in the adenohypophysis of the mouse.

The volume of the nucleus and that of the cytoplasm decreased with age. Payne (1946) described such changes in ageing fowl. The cytoplasm showed very little coagulated material and became watery clear. The outline of cells assumed irregular shape with angular projections. Hydration of cells with decrease of intracellular electrolytes such as

potassium, magnesium and phosphates was found in the rat and human (Lowry and Hastings, 1952). Incidence of degranulation and occurrence of pyknotic nuclei in the basophil cells with advancement of age were also observed in the golden hamster (Spagnoli and Charipper, 1955). Dawbarn (1932) reported a generalized decrease in the nuclear-cytoplasmic ratio of mice with age. The ratio increased from birth to 35 days and then decreased rapidly followed by a period of slow decrease. After 700 days of age, the ratio was less than that at birth. In cattle, Dubois and Herlant (1968) found no variation in the nuclear cytoplasmic ratio of lactotropic cells at different ages. That of thyrotropic cells, however, was significantly higher in sexually mature animals. In the liver and brain of rat and human, the nuclear-cytoplasmic ration decreased with age and a simultaneous decrease in the concentration of nucleic acids was also observed (Lowry and Hastings, 1952). Electron microscopic studies of the pars distalis adenohypophysis of aged mice revealed changes in the structure of the nuclei (Weiss and Lansing, 1953). The nucleus lost its regular spherical contour and became irregular. The double-membrane system with attached ribosomes underwent rarefaction. There was considerable increase in heterochromatin, so that the nuclear cortex was electron-dense. The nuclear changes could be correlated with changes in the nucleotides and enzymatic activities

during ageing (Weiss and Lansing, 1953).

Another change reported in the cells of the hypophysis in old age was vacuolation. In all species, vacuolation occurred in acidophil and basophil cells, but vacuolated basophils were more prevalent in males than in females (Payne, 1946; Spagnoli and Charipper, 1955). Vacuolated changes in chromophil cells were observed in the human (Cooper, 1925; Carlson, 1952; Randall, 1962; Toth and Gimes, 1963), in the rat (Wolfe, 1943), in the hamster (Spagnoli and Charipper, 1955) and in the fowl (Payne, 1946, 1949).

In some species, changes in the cell population of the ageing hypophysis have been reported. Siperstein _et al_. (1954) described cytological changes in the pars distalis adenohypophysis of the rat. According to these authors, adult patterns of cell population were established at the 17th week of post-natal life in rats. Rasmussen (1950) reported that there was a gradual increase in the percentage of eosinophils and a corresponding decrease of chromophobes in the pars distalis adenohypophysis of human beings during the first 19 years of life. During the same age interval, the percentage of basophil cells remained almost constant. In specimens from sexually mature adults (Rasmussen, 1936), the basopils were significantly greater in number in men than in women and eosinophils were significantly greater in number in women. There was a striking decrease in acidophils and an

increase in chromophobes, in both sexes beyond 50 years of age. The basophils constituted a perceptibly higher proportion in the older group of women. Rasmussen (1936) believed that the change in the proportion of basophil cells in older women might rather be related to changes in sexual function than to age.

In the male rat, Wolfe et al. (1938) found the proportion of eosinophil cells to be at a significantly lower level in sexually mature animals than in young animals. The basophils were only slightly less abundant, while the percentage of chromophobes was much higher in older animals. In the female rat Wolfe (1943) observed a progressive decrease in the relative number of eosinophils and an increase in the number of chromophobes with age (1-28 months). These changes were most pronounced during the early part of the life span.

In the golden hamster, Spagnoli and Charipper (1955) found a decrease in the percentage of acidophils in older males and an increase of basophils, particularly in older females. In old fowls (6 years and over) Payne (1946, 1949, 1952) reported a decrease in the number of functionally active basophils and also a reduction in the number of acidophils that contained secretory granules. In the dog, Francis and Mulligan (1949) observed no correlation between age and changes in the proportion of the various cell-types. The relative proportion of eosinophils, basophils and

chromophobes did not vary significantly among puppies, adolescents and adults.

Wolfe *et al*. (1938) and Wolfe (1943) reported that the number of nitoses decreased with age. Mitoses in the eosinophil cells were most abundant in immature rats, but declined rapidly in number. Mitoses were found only infrequently in rats beyond 6 months of age. In ageing mice, Blumenthal (1955) also found progressive diminution of mitotic activity with advancing age.

Decrease of the proportion of cells with advancing age was found to be accompanied by an increase in extracellular material (Lowry and Hastings, 1952). Consequently extracellular electrolytes, e.g., sodium, chloride and calcium increased and hydration of tissues, postulated to be caused by extracellular edema, was evident. In the human adenohypophysis, Cooper (1925) reported a great increase in the amount of connective tissue in advanced age. Such a change was, however, inconsistent and was not marked in the caudal and ventral aspects of the pars distalis adenohypophysis and also among the cells of pars intermedia. Romeis (1940) also failed to find a constant increase in the interstitial connective tissue in old age. He concluded that interstitial tissue elements were as strongly developed in the specimens from 30-40 year old persons as in those from 80-85 years. Shanklin (1953) reported consistent

increase of interstitial connective tissue in the human adenohypophysis. Toth and Gimes (1963) did not consider fibrosis as one of the age-correlated changes in the human.

In the young and sexually mature rats, the fibrillar material in the pars distalis adenohypophysis consisted of almost entirely reticular fibers (Lansing and Wolfe, 1942a). Such fibers were generally found only in intimate association with the wall of capillaries. An independent meshwork for the support of the parenchymal cells was not evident. In association with advancement of age, a slow but progressive increase occurred in both the number and thickness of the reticular fibrils (Lansing and Wolfe, 1942b). The fibrillar meshwork extended into the interstices of parenchymal cells from the wall of capillaries. In spite of the marked thickening of the reticular fibrils, transformation into collagen occurred very infrequently. In the pars distalis adenohypophysis of the house, a slight increase in the interstitial connective tissue was only evident with advancing age and as such, regressive connective tissue changes were considered to be insignificant in this species (Blumenthal, 1955). Spagnoli and Charipper (1955) reported that an increase, in the density of the reticular framework of the hypophysis was accompanied by disorganization with advancement of age in the hamster. In their reviews, Carlson (1952), Randall (1962), Verzar (1966) and Bourne

(1967) subscribed in support of an increase of interstitial fibrosis of the adenohypophysis with age.

Early in life, the parenchymal cells were loosely packed in the pars distalis adenohypophysis. With advancement of age, they became progressively concentrated together (Cooper, 1925; Toth and Gimes, 1963). The regular arrangement of the cell-cords was altered. In young animals and birds, the parenchyma consisted of solid cell-cords. In old age, cavities developed in the center of these cords and colloid was deposited in them (Hoser, 1941; Payne, 1952; Shanklin, 1953; Spagnoli and Charipper, 1955; Randall, 1962). Lansing and Wolfe (1942a) observed such colloid-filled follicles regularly in the pars distalis adenohypophysis and pars intermedia of rats from the 6th to 12th month of age. Wolfe (1943) reported the occurrence of such follicles as early as the 3rd month of life. Their number was found to be at the maximum level in rats which were 6-12 months old and then, the number of follicles remained approximately stationary for the remainder of the life span.

In the human, Shanklin (1953) reported the occurrence of two types of concretions in the adenohypophysis. Those associated with the follicles were termed follicular concretions and the rest, which were located in the capsule, was named mesothelial concretions. Follicular concretions reached a high incidence during the late fetal period,

underwent a rapid decrease in the early postnatal months of life and subsequently increased in number reaching a high percentage again in older age groups. The mesothelial concretions increased in number beginning at the age of 20 years and a high average in their incidence was maintained until the age of 39 years after which there was only a slight increase in their number. The incidence of ciliated cells lining the colloid-filled follicles was also found to have increased with age (Shanklin, 1953). With regard to the amount of colloid in the pars intermedia of human hypophysis, Rasmussen (1936) found no correlation with the amount of epithelium present in that lobe.

Hypophyses of old rats exhibited typical areas of hypertrophic cells which resembled those of adenomas (Wolfe, 1943). Such adenomatous changes occurred in the pars distalis adenohypophysis of 27.8 percent of the rats which were 17 months of age and above (Wolfe, 1943). Nodules were also observed in 17 percent of specimens collected from human beings of 40 years of age or older, with equal incidence in both sexes (Shanklin, 1953).

The most significant and consistent change which occurred in the pars intermedia and pars distalis neurohypophysis in the human was the invasion of these lobes by basophilic cells which migrated from the pars distalis adenohypophysis (Carlson, 1952). Rasmussen (1936) reported that both, pars

intermedia and pars distalis neurohypophysis, increased in size with age. The increase of pars intermedia was attributed to the progressive differentiation of basophilic cells which showed a tendency to grow into the pars distalis neurohypophysis, especially in older women. Carlson (1952) observed that invasion of the pars distalis neurohypophysis by the basophilic cells reflected a definite sign, if not a casual link, in the ageing process. According to Randall (1962), this occurred so commonly in aged persons that it might be considered unusual not to encounter such basophilic invasion in a person older than 30 years. In the hamster, Spagnoli and Charipper (1955) found an increase with age in the incidence of basophils within the pars distalis neurohypophysis.

Beginning at the age of 3.5 years, infiltration of the hypophysis of lymphocytes and lymphoid tissue was encountered in the human (Shanklin, 1953). The incidence of such cells increased with age and it occurred twice as frequently in the male as in the female hypophysis.

Age changes in the function of the hypophysis has been studied by several workers in the human and laboratory animals. An effect of age was not very evident in the growth hormone content of swine hypophysis (Baird _et al._, 1952; Baker _et al._, 1956). The content of growth hormone in the porcine hypophysis increased at the same rate as the dry

weight of the organ, the potency per unit of dry weight remaining constant between birth and maturity. The total hormone contained in the adenohypophysis increased throughout the life span of the animal. Amount of hormone available per kilogram of body weight was found to be high in young animals, decreased to the age of 300 days and thereafter remained constant. In the human, Gershberg (1957) demonstrated that the content of growth hormone in the hypophysis did not vary with age. Growth hormone content of the hypophysis was found to be identical in a fetus, in a 12-year-old boy and in an adult over 45 years of age. Daughaday et al. (1959) found that the sulfation activity of human serum, which probably served as an index of the activity of growth hormone, was present throughout adult life.

In the adenohypophysis of male rats, the concentration of growth hormone showed little variation with age; but the ratio of dry hypophysis weight and as such, content of growth hormone per gram of body weight decreased with age (Bowman, 1961). Contopoulos and Simpson (1957) and Solomon and Greep (1958) reported no significant change with age in the growth hormone content of the hypophysis in rats. Similar findings have been reported in the ox (Armstrong and Hansel, 1956). Decrease in body growth during adulthood was attributed to the diminution of response by body tissue to growth hormone (Everitt, 1964) and to the dilution of

available circulating growth hormone (Baker *et al.*, 1956; Bowman, 1961).

Saxton and Greene (1939) detected thyrotropic effects in the rabbit hypophysis beginning at the 10th day of post-natal life. Thyrotropic activity of hypophysial tissue from immature rabbits was found to be greater than that from mature rabbits. During senescence, the hypophysis of the female rabbit possessed less thyrotropic activity than that of males. Saxton and Loeb (1937) and Blumenthal (1954) noticed no appreciable diminution in the thyrotropin content of human hypophysis with advancing age. However, Bakke and Lawrence (1959) and Bottari (1959) reported significant decrease in the average thyrotropic hormone content of the hypophysis, as well as in the blood of human beings over 17 years of age. Severinghaus (1937) subscribed to an increase of thyrotropic hormone in the hypophysis of aged persons.

The corticotropin content of the adenohypophysis of mice showed no decline during old age (Blumenthal, 1955). Fortier (1959) and Blok (1962) also reported similar findings in rats. In the human, Blumenthal (1954) found more corticotropin (per unit weight of the hypophysis) during the first two decades of life than at later stages. But no evidence was observed for the diminution in adenohypophysial content of corticotropin with advancing age. Solomon and Shock (1950) demonstrated that exogenous

corticotropin provoked the same effect in elderly men as in the young.

In the sow, Hollandbeck *et al.* (1956) demonstrated that the amount of gonadotropin available per unit of body weight was very high in day-old animals. Afterwards, there was a rapid decrease in the hormone potency to 220 days of age, after which it remained constant. The unit potency of gonadotropic hormone decreased linearly with increasing age, while total potency increased linearly with age. Adult hypophyses contained about 5 times as much gonadotropin as infantile glands. Lauson *et al.* (1939) found no change in the gonadotropic activity of the rat hypophysis at different ages. Throughout sexual life, the activity remained at the puberal level despite continued increase in size of the hypophysis. Identical views were expressed by Solomon and Shock (1950). In female rabbits, hypophysial gonadotropin content was found to be of the same degree as young mature females. Glands of old females produced about the same degree of follicle stimulation and luteinization as those from young mature animals (Saxton and Greene, 1939).

In the human, no variation in the hypophysial content of follicle stimulating and luteinizing hormones were observed by Duncan *et al.* (1952) and Blumenthal (1954). Bahn *et al.* (1953) concluded that equal degrees of gonadotropic activity existed in adults of both sexes and

an enormous increase occurred in the follicle stimulating hormone content of postmenopausal women. Witchi (1961) showed that the follicle stimulating hormone potency per unit of dry hypophysis weight of adults was 40 times as high as that of prepubertal children. Ryan (1962) found that hypophyses of the infants contained about 1/5 of the concentration of luteinizing hormone present in the hypophyses of young women.

Increased urinary excretion of gonadotropins, especially follicle stimulating hormone, was considered as one of the characteristic age-correlated changes in women. Albert *et al.* (1956) found that gonadotropins were not detectable in the urine of children until the age of puberty. Between the ages of 10 and 50 years, there was a progressive and fourfold rise in concentration of gonadotropins in the urine. After menopause, the output of gonadotropins in the urine continued to increase to 19 years after cessation of menses, but decreased thereafter. Males manifested less of a change in the excretion of gonadotropin in urine as age progressed. Verzar (1966) concluded that increased urinary excretion of gonadotropins during menopause was the result of lack of ovarian function rather than an effect of ageing. A diminished feedback inhibition of gonadotropin production due to decrease of sex hormones was believed to be the causative factor rather than age.

Friedman *et al.* (1956) and Friedman (1957) reported a decrease in the responsiveness of the neurohypophysis during senescence in rats. The authors noticed that after large quantities of salt and water had been given to aged rats, urinary excretion resembled that seen in diabetes insipidus. The hypophysis of old rats (24 months of age) failed to respond to osmotic stimulation by overloading with 1.5 percent sodium chloride. Friedman *et al.* (1963) concluded that several changes in water and electrolyte metabolism associated with ageing might be attributed to a decrease of neurohypophysial hormone production.

MATERIALS AND METHODS

General

The dogs employed in this research consisted of purebred beagles obtained from the Beagle Colony of the Department of Veterinary Anatomy, Iowa State University. The 136 dogs involved in this study included 63 males and 73 females ranging in age from birth to 13.6 years. Table 1 provides a description of these animals.

The hogs used in this study were obtained from the Swine Farm, maintained by the Department of Veterinary Anatomy, Iowa State University. The 202 hogs employed in this study varied in age from birth to 10 years. These included 47 males and 155 females derived from 8 different purebreds and 7 breed crosses. The breeds and number of animals per sex are listed in Table 2.

Design of Program

The modern concept of ageing stipulates that the ageing process is a certain kind of change in the living system that manifests itself gradually in concurrence with the passage of time during the entire life cycle of the animal. This necessitates the histomorphological study of all tissues and organ systems of the body at all ages. It is needless to emphasize the necessity of homogenity in the sample of animals employed for such gerontological research, especially

as far as their genetic background, management and exposure to divergent environment are concerned. As early as 1942, Carlson subscribed to such a necessity of controlled study in all gerontological research (Carlson, 1952). Aware of the fact that many factors are not amenable to control, the author suggested that care should be taken to insure standardization of nutrition, overall health status, heredity and a thorough evaluation of the condition of the circulatory system and endocrine balance in the body.

Conceiving these ideas, Dr. R. Getty, Distinguished Professor, Veterinary Anatomy, Iowa State University, embarked upon a comprehensive gerontological program in 1952. A dog colony was established for the purpose of supplying specimens for research projects being carried out at that time. This colony served as an important step in evolving management conditions suitable for the present requirements of gerontological studies. In 1955, a purebred beagle colony was initiated and since then this colony has been composed of entirely purebred beagles. No outside strains are being admitted into the colony and population increase in the colony is achieved through a predetermined program of breeding among the inmates.

The dog colony is encircled by a high fence which not only minimizes possible distraction and excitement from outside sources, but also prevents direct contact of colony dogs

with stray animals. This, along with a rigid program of infectious disease prophylaxis, has kept the colony dogs free from any contagious disease. Dogs of the same age are maintained in small groups in kennels with free access to the adjacent open enclosure outside. The kennels and the yard are hosed clean every day with steaming-hot water.

All inmates of the colony receive dry commercial dog feed provided by Gaines Dog Food Division, General Foods Company, Kankakee, Illinois, throughout their stay. Feed analysis of this dry diet reveals a composition that includes 25 percent protein, 40 percent carbohydrates, 7 percent fat, essential amino-acids, vitamins and minerals, with one pound of feed providing 1500-1600 calories[1]. This self-fed diet is provided free-choice by means of self feeders along with free access to a clean fresh water supply, at all times, from an automated watering system.

In addition, the following management practices are routinely adopted to assess the general health status of the dogs. Each animal undergoes general physical check-up every three months. The physical examination is supplemented by fecal examination to determine the gastrointestinal parasitic level. Periodic administration of commercial anthelmintics is resorted to for inhibiting gastrointestinal

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[1]Feed analysis provided by the supplier, Gaines Dog Research Laboratories, Kankakee, Illinois.

parasites. Blood samples are collected from individual dogs at regular intervals and cholesterol level of each sample is determined. During the period of late pregnancy and lactation, mothers are kept isolated in maternity wards.

The hogs are procured from the Swine Farm, Animal Nutrition Department, Iowa State University and are then maintained by the Anatomy Department until the date of necropsy. The animals are selected from among those with controlled medium protein diet and known genetic background. Stress in selection is also laid on previous non-involvement in any experimentation and history of no endocrine imbalance. Management procedures adopted for maintenance of the hogs are akin to those followed in the beagle colony.

A necropsy evaluation is conducted at the time of death and specimen collection. The animal is kept off-feed for 12 hours prior to necropsy and is weighed just before euthanasia. External symptoms of endocrine malfunction, as outlined by Bloom (1962), are carefully observed. Arterial samples of blood are collected in each case and the level of cholesterol is monitored. Gross examination of heart and large vessels is undertaken for arteriosclerotic plaques before such tissues are transferred to the fixative solution. All endocrine glands and kidneys are weighed accurately to determine any quantitative deviation. Data for every animal as to date of birth, age, sex, breeding responses and weights

of all endocrine glands and kidneys are maintained on punched cards for easy reference.

Collection Procedure

A uniform necropsy procedure was adopted for every animal of both species. The animals were euthanized by means of electrocution and exsanguination was accomplished by severing axillary vessels. Decapitation followed immediately and after opening the cavum cranii, the encephalon was removed in entity. The tuber cinereum was incised in such a way that the radix infundibuli was severed at its junction with the pars parainfundibularis tuberis. Next, the diaphragma sellae was incised around its point of attachment to the sella turcica. The latter step facilitated removal of the hypophysis as a single mass due to severance of vascular branches. In older animals the sella turcica was found to be more concave than in young animals with the processus clinoideus caudalis as well as the tuberculum sellae projecting further into the cavum cranii. In these animals it was necessary to sever the arterial branches from the circulus arteriosus cerebri at a further rostral level. The entire process, from the time of euthanasia to dropping the hypophysis into the fixative, was accomplished in 15 to 20 minutes.

Histological Techniques

Immediately after removal, the hypophysis was stripped off the diaphragma sellae and was weighed to the nearest milligram. Mercury-formal was used as the general fixative. The stock solution of this fixative was composed of the following:

Sodium chloride	30.0 g
Mercuric chloride	10.2 g
Distilled water	350.0 ml

To 90 ml of the stock solution, 10 ml of 40 percent commercially available formaldehyde solution was added just before use. The hypophysis was treated in this fixative for a period of 48 hours and was then washed overnight. All tissues were embedded in paraplast after clearing in chloroform.

Hypophyses from 3 dogs and 3 hogs were also fixed in cobalt nitrate (Luna, 1968) for demonstration of Golgi apparatus. Five dog and five hog hypophyses were treated in 2.5 percent trichloroacetic acid, before fixation, for the differential solubility test (Barrnett *et al.*, 1956). A group of three dog and three hog hypophyses was also fixed in formalin-ammonium bromide solution (Luna, 1968) for silver and gold chloride techniques as well as for the demonstration of pituicytes.

In all, 64 dog and 63 hog hypophyses were employed in histomorphological study (Tables 3 and 4). All tissue

blocks were sectioned serially at five micron thickness from one extremity to another. Sectioning was carried out in a transverse plane to the longitudinal axis of the organ. In addition, 7 dog and 7 hog hypophyses were sectioned serially in a sagittal plane parallel to the longitudinal axis of the organ to facilitate orientation of parenchymal structures. Five groups of 40 sections each were collected from each hypophysis at equal intervals including the groups at either extremity of the organ. The series represented the following levels of the hypophysis:

	Dog	Hog
1-40	Pars distalis adenohypophysis Pars infundibularis adenohypophysis Radix infundibuli	Pars infundibularis adenohypophysis Radix infundibuli Pars compacta infundibuli
41-80	Pars distalis adenohypophysis Pars intermedia adenohypophysis Pars distalis neurohypophysis	Pars distalis adenohypophysis Pars intermedia adenohypophysis Pars distalis neurohypophysis
81-120	Pars distalis adenohypophysis Pars intermedia adenohypophysis Pars distalis neurohypophysis	Pars distalis adenohypophysis Pars intermedia adenohypophysis Pars distalis neurohypophysis
121-160	Pars distalis adenohypophysis Pars intermedia adenohypophysis Pars distalis neurohypophysis	Pars distalis adenohypophysis Pars intermedia adenohypophysis Pars distalis neurohypophysis
161-200	Pars distalis adenohypophysis	Pars distalis neurohypophysis

Figure 1 illustrates, schematically, the levels of sectioning across the organ in both species. The number of intermediate sections discarded, as well as the total number of serial sections obtained for each hypophysis, was recorded. Two consecutive sections were mounted on the same slide and the entire batch of 100 slides from a single organ was numbered serially. From each group of 20 slides, sections from identical locations were utilized in a particular staining procedure. In addition to these, few consecutive sections were mounted one per slide to compare the staining affinity of various cell-types.

As a routine procedure, the following staining techniques were adopted:

1. Trichrome stain of Cleveland and Wolfe (1932).

   The above technique was modified as follows:

   a. At the end of the mordanting period, the sections were dehydrated with 95 percent alcohol and were stained in an acidified solution of luxol fast blue, 0.1 percent solution in 95 percent alcohol. A staining period of 10-20 minutes was found to be adequate. Following luxol fast blue staining, the sections were rinsed in 95 percent alcohol, 70 percent alcohol and two changes of distilled water.

   b. Differentiation was achieved in 0.05 percent lithium carbonate solution, which was followed by thorough

rinsing in three changes of slightly acidified water.

2. Combined aldehyde-fuchsin and periodic acid-Schiff method (Elftman, 1959b).

The following modifications were adopted:

a. Aldehyde-fuchsin stain was prepared as recommended by Gomori (1950). It was used after four days of ripening at room temperature.

b. Staining with luxol fast blue was introduced in the sequence after staining with aldehyde-fuchsin. The same luxol fast blue procedure as adopted in case of the trichrome stain (Cleveland and Wolfe, 1932) was followed.

c. Staining with Schiff reagent was followed by three changes of sulfite rinse. The latter was prepared as follows:

Sodium metabisulfite	2 g
Distilled water	500 ml
Sulphuric acid, conc.	5 ml

d. Prior to staining with orange G, nuclear stain of iron hematoxylin (Luna, 1968) was used for better elucidation of the nuclei.

3. Alcian blue, periodic acid-Schiff and orange G method, with prior oxidation in performic acid (Heath, 1965).

The modifications included the following:

a. Though Schiff reagent solution prepared by cold

method of the author gave good results, it was found to be relatively unstable and required constant refrigeration except during periods of actual use. So the hot method (Luna, 1968) was adapted.

b. Nuclear stain of iron hematoxylin (Luna, 1968) was adapted as an additional step prior to orange G stain.

4. Aldehyde-thionin, periodic acid-Schiff and orange G method (Ezrin and Murray, 1963).

The following steps were modified:

a. Luxol fast blue stain, as elaborated for the trichrome technique (Cleveland and Wolfe, 1932) was adopted prior to oxidation with periodic acid, following the staining process of Paget and Eccleston (1960) for human hypophysis.

b. Schiff reagent was prepared as per recommendations of Luna (1968).

c. Nuclear stain of iron hematoxylin was introduced between the last sulfite rinse and orange G staining.

d. pH of orange G solution was adjusted at 4.5 following the recommendation of Peterson and Weiss (1955).

5. Gomori's (1950) aldehyde-fuchsin and counterstaining with Mallory's (1900) trichrome stain as modified by Crossman (1937).

The staining procedure adopted by Purves and Griesbach (1957a) with regard to the above sequence was followed with

the exception that light green was substituted for aniline blue. The stain was employed at pH 4.5.

6. Luxol fast blue and counterstaining with Rennels' (1957) modification of Wilson and Ezrin (1954) method.

   The modifications included the following:

   a. Luxol fast blue solution was prepared as for previous methods.

   b. Nuclear stain of iron hematoxylin was adopted previous to staining with orange G.

   c. pH of the orange G solution was adjusted at 4.5 and the staining period was extended to three minutes.

7. Alcian blue and periodic acid-Schiff method, with permanganate oxidation (Herlant, 1960).

8. Tetrachrome stain (Herlant, 1960; Racadot, 1962a), with and without counterstaining with luxol fast blue (Dubois and Herlant, 1968).

   Some sections were mordanted in Helly's fluid (Luna, 1968) at 37° C for 4 hours before commencement of actual staining schedule.

9. Azocarmine G, orange G and aniline blue method (Dawson and Friedgood, 1938).

   The staining sequence was modified by increasing the staining time in azocarmine to 2.5 hours at 57° C. Differentiation was accomplished in 1 percent solution of aniline oil in 95 percent alcohol.

10. Bargmann's chrome hematoxylin method for neurosecretory substance (Bargmann, 1950; Pearse, 1968a).
11. Performic acid-alcian blue technique for histochemical demonstration of cystine (Adams and Sloper, 1956).

Nuclear fast red (Kernechtrot), 0.1 percent in 5 percent aluminum sulfate solution was used as a background stain.

12. Verhoeff's stain for connective tissue (Gurr, 1962).
13. Weigert's method for elastic fibers (Mallory, 1961).
14. Manuel's method for reticulum (Luna, 1968).
15. Mallory's phosphotungstic acid hematoxylin method (Luna, 1968).
16. Bielschowsky's method for axis cylinders and dendrites (Luna, 1968).

In addition to these, sections were also stained with the following techniques for specific purposes:

1. Palmgren's method for selective silver staining of nerve fibers and nerve endings (Palmgren, 1948).
2. Defano's method for Golgi apparatus (Atwell, 1932).
3. MacConaill's lead hematoxylin stain (Gurr, 1962).
4. Modified methyl violet method for amyloid (Pearse, 1968a).

Staining affinity of the various cell-types was established by morphology of the cells, staining of consecutive sections in two different stains and staining and subsequent differentiation with a single dye followed by destaining and

restaining with different dyes, among which comparison was sought.

Sections of the brain passing through the hypothalamic region were obtained from the same animals employed for histomorphological study. These sections were stained by the chrome alum hematoxylin method (Bargmann, 1950), performic acid-alcian blue technique (Adams and Sloper, 1956) and the alcian blue-periodic acid-Schiff-orange G technique (Heath, 1965). The sections were utilized to study the age-correlated changes in the neurosecretory material contained by the neurons of the supraoptic and paraventricular nuclei.

The dyes used in these techniques were obtained from M/S Chroma-Gesellschaft Schmid and Company, through their distributors M/S Roboz Surgical Instrument Company, Inc., Washington, D.C. The dye, acid alizarine blue used for the tetrachrome technique (Herlant, 1960; Racadot, 1962c), was procured from M/S Allied Chemical and Dye Corporation, New York.

Age Groups

Animals of both species were divided into age groups to facilitate representation of the data and to elucidate certain morphological characters. In both species three main age intervals were represented: birth to 8 weeks; 2 to 12 months; and 1 to 13.6 years. In the dog, the second age interval included 2 to 11 months, while in the hog, the

third age interval represented 1 to 10 years. These age intervals did not necessarily correspond to any significant age-associated changes in weight or morphology of the hypophysis but were arbitrary divisions as per the weaning age of 6-8 weeks and sexual maturity at 6-8 months of age in both species. The age intervals were subdivided into the following age groups:

Birth to 8 weeks:

Age group I	Birth to 4 weeks
Age group II	4 to 8 weeks

2 to 12 months:

Age group III	8 weeks to 6 months
Age group IV	6 to 12 months

1 to 13.6 years:

Age group V	12 months to 4 years
Age group VI	4 to 7 years
Age group VII	7 to 10 years
Age group VIII	10-13.6 years (in the dog only)

Such age grouping was made in order to facilitate correlation of quantitative changes with qualitative morphological changes. It was also envisaged that these age groups would reveal the peculiar patterns of hypophysis morphology during the stages of postnatal life, growth, sexual maturity, maximum productivity, decline and senescence.

Measurements and Statistical Analysis

Age-correlated changes in weight of the hypophysis were studied in both species. Data from 136 beagle dogs (Table 1) and from 202 hogs (Table 2) were employed for the statistical analyses. In addition to these, data on hypophyses of 52 dogs, drawn from miscellaneous breeds, were used for comparison with data from the beagle sample. These miscellaneous dogs were obtained from M/S General Foods Corporation, Kankakee, Illinois. The animals had been raised and utilized solely for research purposes (as control animals with medium-protein diet) and their records were well documented. The data represented six different purebreds, each composed of animals of different age and body weight. Since body size of these dogs varied greatly among breeds, data from the mixed sample were only used for comparison in the analysis of absolute hypophysis weight on body weight.

Five variables were recorded for each animal: sex, age at necropsy, live body weight, absolute weight of the hypophysis and relative weight of the hypophysis. Body weight was recorded in kilograms; absolute weight of the hypophysis was recorded in milligrams; and, relative hypophysis weight was calculated as weight of hypophysis in milligrams per kilogram of body weight. Data on body weight, absolute hypophysis weight and relative hypophysis weight, as per sex, are listed in Tables 5 and 6 for the dog

and the pig, respectively. Statistical analyses of data consisted of simple and multiple regression techniques. Sex differences were determined by comparison of the two means as per standard 't' test employing the pooled variance.

In statistical analysis of the data employed to study age-correlated changes in the relationship of the hypophysis with other endocrine glands, weights of the hypophysis, thyroid, adrenal and gonads were used. The number of animals employed in each category and for each species was furnished in Tables 1 and 2. To compare with the results obtained from beagles, data from 188 dogs selected at random from different breeds was also used. Number of these animals as per breed and sex, has been summarized in Table 1. These animals were not raised on a controlled diet, but were of known ages and well documented life histories. In these data, weight of the paired organs, e.g., thyroid (except the pig), adrenal, testis and ovary, represented the average weight of the two organs. A preliminary survey showed that the differences between the mean unilateral weights of these organs were so small that it was not possible to attribute the differences to inherent character on the part of the organ eliminating the error factor due to chance. In this procedure, the standard 't' test for correlated observations was used. The mean unilateral weights of the various organs were presented in Table 7.

For the regression technique, the animals were classified according to age at necropsy, sex and body weight. Calculations were made of the effects of these three independent factors on each of the following dependent variables: hypophysis weight; thyroid weight; adrenal weight; and, gonad weight. In all three samples, there was variation in the number of observations for each category of organ weights. These variations had been caused by damage inflicted on the organs during necropsy, which made them unsuitable for accurate measurement. Hence, the least square method of fitting constants for multiple classifications with disproportionate subclass numbers (Harvey, 1968) was employed to obtain unbiased estimates of the effects of age, sex and body weight. The mathematical model used in the least square analysis of the data was as follows:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + (ab)_{ij} + (ac)_{ik} + e_{ijkl}$$

$i = 1$ to 2

$j = 1$ to 3

$k = 1$ to 2

Y_{ijkl} = the lth observation of the $(ijk)^{th}$ treatment combination

μ = the overall mean with equal subclass numbers

a_i = the true effect of the ith level of factor a (sex)

b_j = the true effect of the jth level of factor b (age)

c_k = the true effect of the kth level of factor c (body weight)

$(ab)_{ij}$ = effect of sex and age on the ij^{th} subclass after the average effects of sex and age had been removed

$(ac)_{ik}$ = effect of sex and body weight on the ik^{th} subclass after the average effects of sex and body weight had been removed

(These were the individual interaction effects expressed as a deviation from the mean μ.)

e_{ijkl} = random errors

Statistical analyses of the data were conducted on IBM 360/50 Computer, Computation Center, Iowa State University. A level of significance of 0.05 was used in all cases for any difference to be statistically significant.

Cytometry

Age-correlated changes were studied in the following factors:

1. Percentage of the cell-types of the pars distalis adenohypophysis, including the chromophobes.
2. Relative proportion of the cytoplasm and nucleus of five cell-types, excluding the adrenocorticotropic cells and chromophobes.
3. Relationship between the volume of the gross subdivisions of the hypophysis.

The percentage of cells was counted on sections obtained from the three middle series and stained with the luxol fast

blue-trichrome technique (Cleveland and Wolfe, 1932). Counting was carried out as per the volumetric method of Rasmussen and Herrick (1922). To demarcate the boundary of each field, a disc 21.15 mm in diameter with a net reticle 5 mm square (divided into 100 small squares) was inserted into the 10X eyepiece of the microscope. The 100X oil-immersion lens was used to explore the field in all measurements. Starting at one end, the cells of each type in every fifth field of each fifth row were counted. Among cells in contact with margins of the square, only those touching the upper and right-hand margins were included in the count for that particular field. In this systematic manner, the entire area of the pars distalis adenohypophysis was counted. Figure 2 illustrates a schematic view of the fields used for differential counting.

To record rapidly the number of cells during the process of counting, multiple-tally denominator counters were used. Each of these counters incorporated two different channels with independent manipulations and separate designation tapes atop of each channel. Each channel could handle counts to 9999 and different colored push buttons and designation tapes with name of cell-type printed on them facilitated the counting process. The total number of cells that were counted and the number of each cell-type were recorded according to sex, from which the percentage of different cell-types was calculated.

The relative volume of the cytoplasm and the nucleus of five cell-types of the pars distalis adenohypophysis was obtained by the hit method of Chalkley (1943). Instead of 5 hairs attached to the eyepiece, as was originally used by the author, the net reticle used for other cytometric procedures was employed, in combination with the 10X eyepiece and 100X objective. The cells coming in contact with the upper margin and right-hand margin of the square were taken into account. This gave a total of 100 cross-hairs in each field. The number of cross-hairs hitting the nuclei of one cell-type, under focus at the plane of the image, was recorded on a multiple-tally counter and the number of cross-hairs hitting the cytoplasm of same cells was recorded on the second channel of the counter. Fifty cells of each category, beginning with the first hit, were measured in each of the three sections on which cell counting was also carried out. The cytoplasm-nucleus ratio of each cell-type was calculated directly by the ratio of total number of hits on cytoplasm over total number of hits on nuclei of one cell-type. The final cytoplasm-nucleus ratio of each cell-type within each age group was expressed as the mean for each measurement in all animals of the same sex. These data have been recorded in Table 16.

Volumes of the pars distalis adenohypophysis, pars intermedia adenohypophysis and the pars distalis

neurohypophysis were obtained by the number of hits that covered the entire extent of each lobe. The net reticle was employed for these measurements. The volume of each lobe, as furnished in Table 15, represented the mean of the measurements taken on all animals belonging to one particular age group. The mean volume was expressed as percentage of total hits covering each of the three lobes to that of all the three lobes together.

Statistical analysis of these cytometric data was carried out by using the chi square test at a level of significance of 0.05.

OBSERVATIONS

Quantitative Changes in Weights of Hypophysis

Dog: beagle

Summaries of the regression analyses of the weight of the hypophysis and body weight with increase in age are given in Graphs 1 through 5. Regression data concerning weight of the hypophysis on the body weight has been summarized in Graph 6. Statistics pertaining to the regressions are recorded in each graph.

Absolute weight of the hypophysis increased with age in all three age intervals and no significant sex difference was observed in any interval. From birth to 8 weeks, absolute weight increased linearly (Graph 1). At 4 weeks the net increase in absolute weight amounted to 107 percent over that at birth, and between 4 and 8 weeks the increase was 54 percent. Absolute hypophysis weight increased 219 percent between birth and 8 weeks. During the latter interval, approximately 48 percent of the variation of absolute hypophysis weight could be attributed to the relationship with age whereas 57 percent of the same was accounted for by the relationship with body weight. Increase of one week in age contributed to an increase of 2.94 milligrams in weight of the hypophysis.

Between 2 to 11 months the net increase in absolute hypophysis weight amounted to 68 percent with periodic

increases of 30 percent between 2 to 6 months and 29 percent between 6 to 11 months (Graph 2). During the interval 2 to 11 months the percentage of variations of absolute hypophysis weight that were accounted for by the relationships with age and body weight were, respectively, 24 and 27. During the same interval one unit increase in age resulted in an increase of 3.15 units in absolute hypophysis weight. During the intervals 1 to 4, 4 to 7, 7 to 10 and 10 to 13.6 years the increase in absolute hypophysis weight was, respectively, 15, 13, 11 and 12 percent. During the latter period, there was an increase of 3.19 milligrams in weight of the hypophysis corresponding to an increase of one year in age. Approximately, 27 percent of the variation of absolute hypophysis weight could be attributed to differences in age and 31 percent of the same was accounted for by the relationship with body weight.

The relationship of absolute hypophysis weight and body weight, independent of age, was a curvilinear regression without any statistically significant sex difference in the means (Graph 6). Weight of the hypophysis increased with increase in body weight but at a decreasing rate, being 98 percent during the interval 1 to 5 kilograms, 52 percent during the interval 5 to 10 kilograms and 34 percent during the interval 10 to 16.5 kilograms body weight. The mean hypophysis weight constituted 0.000809 percent of the mean

body weight in the beagle.

Relative weight of the hypophysis was comparatively higher in young animals (Table 3; Graphs 1 and 3). In the first age group, relative weight decreased linearly from birth to 8 weeks (Graph 1). During the intervals birth to 4 weeks and 4 to 8 weeks the decrease in relative weight was, respectively, 22 and 30 percent. The net decrease during the interval birth to 8 weeks amounted to 45 percent. For every unit increase in age relative weight decreased by 1.91 milligrams per kilogram of body weight. Approximately, 15 percent of variations of relative weight could be attributed to differences in age and 21 percent of the same was accounted for by its relationship with body weight.

In the second age interval the relative weight decreased curvilinearly (Graph 3). The decrease continued to 11 months being 56 percent for the period 2 to 10 months. Between 10 to 11 months there was an increase of 3 percent over that at 10 months and the net decrease in relative weight between 2 and 11 months amounted to 56 percent. The decrease in relative weight during the intervals 2 to 6 and 6 to 11 months amounted to 44 and 22 percent, respectively. In the third age interval relative weight decreased linearly with age (Graph 5). During the intervals 1 to 4, 4 to 7, 7 to 10 and 10 to 13.6 years, the decrease in relative weight was 5, 6, 6 and 8 percent with a net decrease of 22 percent for

the entire interval 1 to 13.6 years. An increase of one year in age corresponded to a decrease of 0.15 milligrams per kilogram of body weight in weight of the hypophysis. Five percent of the variation of relative weight was accounted for by the relationship with age and 26 percent of the same was on account of the relationship with body weight. No significant sex difference in the relative hypophysis weight was observed in this study.

The analysis of body weight on age was a linear regression in all age intervals. The difference in mean body weight of the male and female beagles was not statistically significant in any interval. During the period from birth to 4 weeks body weight increased 192 percent over that at birth and from 4 to 8 weeks the increase in body weight was 68 percent, amounting to a 393 percent increase over the weight at birth (Graph 1). An increase of one week in age, during the interval birth to 8 weeks, contributed to an increase of 0.15 kilograms in body weight. Approximately, 72 percent of the variation of body weight was on account of the relationship with age.

Between 2 and 6, and 6 and 11 months the increase in body weight was 129 and 70 percent, respectively. The net increase for the period 2 to 11 months was 290 percent. During the latter period an increase in age by one month resulted in an increase of 3.15 kilograms in the body weight.

About 56 percent of the variation of body weight could be attributed to differences in age. In the third age interval, the increase in body weight occurred at a decreasing rate being 26 percent during the interval 1 to 4 years, 21 percent during the interval 4 to 7 years, 17 percent during the interval 7 to 10 years and 17 percent during the interval 10 to 13.6 years (Graph 4). The overall increase in body weight constituted 108 percent. Increase in one year in age corresponded to an increase of 0.69 kilograms in body weight. Approximately, 42 percent of the variation of body weight, during this interval, was accounted for by the relationship with age.

Dog: mixed breeds

Analysis of absolute hypophysis weight on body weight, independent of age, was a linear regression in the mixed breeds exclusive of the beagles (Graph 6). Mean absolute hypophysis weight of the male and female showed a sex difference in these breeds. The mean absolute hypophysis weight was comparatively larger in the female than that of the male. For a particular body weight, female dogs possessed heavier hypophyses than the males and this difference increased with the increase in body weight (Graph 6).

Pig

Summaries of the regression analyses of hypophysis weight and body weight with increase in age are given in

Graphs 7 through 11. The relationship of absolute hypophysis weight to body weight increase has been recorded in Graph 12. Statistics pertaining to the regressions are recorded in each graph.

Absolute weight of the hypophysis increased with age in all three age intervals and no significant sex difference was observed in any age interval. The relationship between absolute hypophysis weight and age was observed to be linear in the young and adult animals (Graphs 7, 8, 9). At 4 weeks the increase in absolute weight was 120 percent over that at birth and between 4 and 8 weeks the increase was 62 percent. The overall increase from birth to 8 weeks was 256 percent (Graph 7). During the entire interval, an increase of one week in age contributed to an increase of 9.82 milligrams in weight of the hypophysis. About 72 percent of the variation of absolute hypophysis weight was accounted for by the relationship with age and 84 percent of the variation of absolute hypophysis weight could be attributed to the relationship with body weight.

In the age interval 2 to 12 months, the absolute hypophysis weight increased 413 percent with periodic increments of 165 and 93 percent during the intervals 2 to 6, and 6 to 12 months, respectively (Graph 8). In this interval absolute hypophysis weight of one animal was comparatively higher (Table 4; Graph 8) which resulted in a

high standard error. One unit increase in age resulted in an increase of 16.1 units in weight of the hypophysis. Approximately, 22 percent of the variation of hypophysis weight could be attributed to the relationship with age and only 18 percent to that with body weight. In the third age interval, 1 to 10 years, the increase in absolute weight during the intervals 1 to 4, 4 to 7 and 7 to 10 years were 33, 25 and 20 percent, respectively (Graph 9). The net increase in absolute weight during the interval 1 to 10 years amounted to 100 percent. Absolute hypophysis weight increased at the rate of 6.19 units for every unit increase in age. About 43 percent of the variation of absolute weight was accounted for by the relationship with age and only 5 percent of the variation of absolute hypophysis weight could be attributed to differences in body weight.

Absolute hypophysis weight when analyzed in relation to body weight, independent of age, showed a curvilinear regression (Graph 12). The male and female animals did not differ significantly in this regression. From 1 to 300 kilograms of body weight absolute weight continued to increase at a decreasing rate and then declined. The increase in absolute hypophysis weight in the intervals 1 to 100, 100-200 and 200-300 kilograms of body weight were 1030, 56 and 13 percent, respectively. Mean weight of the hypophysis constituted 0.000324 percent of the body weight.

Relative weight of the hypophysis was comparatively higher in young animals (Table 4; Graphs 7 and 8). In the first age interval the relative weight decreased curvilinearly (Graph 7) while in the second interval it declined linearly with age (Graph 8). From birth to 4 weeks the decrease in mean relative weight was 51 percent and from 4 to 8 weeks it was 28 percent. The net decrease between birth to 8 weeks amounted to 64 percent (Graph 7). In the intervals 2 to 6 and 6 to 12 months the decrease in relative weight was 33 and 74 percent, respectively. During the interval 2 to 12 months an increase of one month in age contributed to a decrease of 0.53 milligrams per kilogram of body weight in weight of the hypophysis. Approximately, 56 percent of the variation of relative weight was accounted for by the relationship with age and 60 percent to that with body weight. In the adult group the relative hypophysis weight was almost constant in the male from 1.5 to 7.5 years of age (Graph 11). The relative hypophysis weight of sows increased linearly from 1 to 10 years (Graph 11). However, the two regressions were not significantly different.

Body weight increased linearly up to one year of age without any significant sex difference (Graphs 7 and 8). In the interval from birth to 4 weeks body weight increased 600 percent over that at birth and, from 4 to 8 weeks the increase in body weight was 99 percent, amounting to 1400 percent

increase over the weight at birth. About 88 percent of the variation of body weight was accounted for by the relationship with age. An increase of one week in age corresponded to an increase of 1.85 kilograms in body weight. Between 2 and 6 and 6 and 8 months, the corresponding increase in body weight was 405 and 120 percent, respectively (Graph 8). During the interval 2 to 12 months the net increase in body weight was 1013 percent. Corresponding to an increase of one month in age, body weight increased at the rate of 16.05 kilograms. Approximately, 93 percent of the variation of body weight was accounted for by the relationship with age.

In the adult group, the mean body weights of male and female animals differed significantly, with the relationship of body weight and age a linear regression in both sexes (Graph 10). Mean body weight was comparatively higher in male pigs than in females. Between 1.5 and 7.5 years the body weight of male pigs showed an increase of 51 percent with periodic increase of 21 percent between 1.5 and 4 years and 25 percent between 4 and 7.5 years. The increase in body weight of sows during the intervals 1 to 4, 4 to 7 and 7 to 10 years amounted to 8, 7 and 7 percent, respectively (Graph 10). The net increase during the interval 1 to 10 years was 23 percent. A unit increase in age contributed to an increase of 20.25 and 4.97 kilograms, respectively, in body weight of the male and female animals. In the male 36

percent of the variation of body weight was accounted for by the relationship with age whereas in the female it was only 6 percent.

Age-correlated Changes in the Relationship of the Hypophysis with other Endocrine Glands

<u>Dog</u>: <u>beagles</u>

Mean squares for the effects considered in the mathematical model are presented in Table 8. The partial regression of age on absolute weight of the hypophysis was significant, indicating that the older dogs had larger hypophyses. Weight of hypophysis as percentage of body weight was significantly heavier in the young animals. Identical relationship of the thyroid weight and adrenal weight was observed with age of the animal. Both absolute and relative gonad weights were independent of any age-correlated influence.

The female of the species possessed heavier adrenal glands than the male. Adrenal weight as percentage of the body weight was also heavier in the former. The significant differences between absolute and relative weights of the testis and the ovary were obvious. Effect of sex was not pronounced on the weight of either the hypophysis or the thyroid in any form. Heavier dogs possessed larger thyroid and adrenal glands. However, percent weight of both glands was not subject to significant alterations due to variations in the body weight. Body weight showed no influence on

weight of the hypophysis or the gonad. The significant interactions were observed only in case of the gonads. With the advancement of age, weight of the testis continued to increase whereas that of the ovary remained almost constant. Increase of body weight influenced the weight of the testis to a higher degree as compared to that of the ovary.

Correlation coefficients between weight of the endocrine glands, age and body weight of dogs have been presented in Table 11. Hypophysis weight was significantly correlated with age, body weight and all other endocrine glands. Older animals possessed heavier hypophyses as were animals with larger body weights (Graphs 13 and 16). Dogs with larger hypophyses had larger thyroids, adrenals and gonads. High correlation of the weight of the hypophysis as percentage of the body weight with that of thyroid, adrenal and gonads indicated age as the prime influencing factor. Increase of body weight caused a decrease in the relative hypophysis weight, suggestive of a slower rate of growth of the latter as compared to that of the former. Female animals who had higher percent weight of the hypophysis also possessed heavier ovaries, but the opposite effect was evident in the males. Dogs with larger body weights also had heavier thyroids. The animals with heavier thyroids also had larger sized adrenals and gonads. The size of the adrenal was associated with the size and unit weight of the gonads

in both sexes. Growth of the testis occurred at a much rapid pace with body weight than that of the ovaries. Male dogs who had larger body weights possessed higher percentage of testis weight whereas heavier females possessed smaller ovary weights as percent body weight. On the contrary, female animals whose adrenal weight on percent basis was high, also had heavier ovaries.

To study the variations in these relationships at different age intervals, the data were analyzed in three groups as per the age intervals mentioned earlier. The correlation between age and body weight was high in each group and thus the data were subjected to less variations on account of body weight. Between birth to 8 weeks of age, size of the hypophysis was significantly associated with the size of thyroid, testis, ovary and the adrenal gland of female animals (Graph 13). For an increase of one milligram in the weight of the hypophysis, the respective increases in the weight of the thyroid (both sexes), adrenal (female), testis and ovary were 0.004 g, 0.004 g, 0.007 g and 0.004 g. Hypophysis weight increased with increase of age as well as body weight (Graph 13), whereas relative hypophysis weight decreased with age and with increase in body weight. In both sexes, animals with larger thyroid glands also had larger adrenals and gonads. Size of the adrenal gland was associated with the size of the ovary to a much higher degree than with

that of the testis. Growth of the testis and the ovary was at almost an equal pace with advancement of age during this period.

Between 2 to 11 months, male animals with larger hypophysis also had larger adrenals and testis (Graph 14). In both sexes, size of the hypophysis was associated with the size of the thyroid gland. A unit increase in hypophysis weight was associated with an increase of 0.004 units in the thyroid weight, 0.008 units in the weight of the adrenal gland of the male and 0.006 units in the weight of the adrenal gland of the female. Hypophysis weight as percentage of the body weight was directly associated with all other glands. Female animals which possessed larger thyroid weights, also had larger weights of the adrenal and ovary. Growth of the adrenal gland was at a more rapid pace in the female than in the male and that of the testis was much more rapid with age than that of the ovary.

Between 1 to 14 years of age, the relationship between weight of the hypophysis and the other endocrine glands was not significant except with that of the adrenal gland (Graph 15). Weight of the adrenal increased at a rate of 0.006 units in the male and 0.005 units in the female corresponding to an increase of one unit in the weight of the hypophysis. Relative hypophysis weight continued to decrease with age and increase in body weight, but the rate of decrease was

not significantly related with age. This indicated the fact that growth of the hypophysis during this period was at a much lower rate than that of the body weight. The female animals who had larger weights of the hypophysis per kilogram of body weight also had higher adrenal and ovary weights on the same basis. There was no significant relationship between the weight of the adrenal gland and that of the ovary. Relative weight of the testis was not significantly related with age or body weight during this period, whereas that of the ovary decreased in direct proportion to increase in body weight and age.

The partial correlations of male and female dogs, having the effect of body weight held constant, have been presented in Table 13A and Table 13B. Most of the partial correlations were lower than those reported in Table 11A. In the male, weight of the hypophysis was associated significantly with the weight of the thyroid and the testis. Relative hypophysis weight had significant relationships with that of thyroid and adrenal. In female animals, weight of the hypophysis had significant relationship with the weight of the ovary, and relative hypophysis weight was significantly related with the absolute weight of the thyroid, relative weight of the thyroid and relative weight of the ovary. Weight of the thyroid had one significant relationship in both sexes, that with the adrenal. In the male, relationship

between weight of the adrenal and the gonad was significant, but not so in the female.

Dog: mixed breeds

Mean squares and the F ratios from the analysis of variance are presented in Table 9. The results that were obtained from the sample of mixed breeds were identical to those of the beagle sample. Older animals had lower weights of the hypophysis in terms of percent body weight. Similar results were obtained in case of the thyroid and adrenal glands, too. The partial regression of body weight on weight of the hypophysis, thyroid and adrenal was significant, indicating that heavier animals possessed larger glands.

Pig

Mean squares for the effects considered in the mathematical model are shown in Table 10. Male animals had significantly heavier thyroid glands than the female animals. All gland weights were significantly higher in older animals as compared to the young animals (Fraphs 17, 18, 19). There was, however, no pronounced influence of age on the weight of the gonads. Heavier animals possessed glands of larger size. The partial regression of body weight on the weight of the gonads was not significant. The interaction of sex and age was significant in the regression on weight of the gonads. Older boars had consistently higher weights of testis whereas

old sows possessed heavier gonad weight limited to certain age only (Graph 19). Within the sexes, older boars had higher weight of the testis in comparison with similar trend of gonad weight in the older sows.

Correlation coefficients between weight of endocrine glands, age and body weight have been summarized in Table 12A. Correlation coefficients of pigs grouped into the three age intervals were presented in Tables 12B through 12D. In the pig sample, weight of the hypophysis was significantly related with thyroid, adrenal, gonads, age and body weight of the animals. Animals which had larger hypophyses, not only had larger endocrine glands but also more units on gland tissue per kilogram of body weight. Relationship of all endocrine glands as per cent body weight was also significant. Relative hypophysis weight decreased with age and with increase in body weight, indicating slow growth rate of the hypophysis as compared to that of the body weight. Animals who had larger thyroids also had larger adrenals and gonads. Male animals possessed heavier thyroid weight than females. Size of the adrenal gland was associated with the size of the gonads in both sexes. Relative weights of both thyroid and adrenal glands decreased with age. Testis and ovary increased in size with the advancement of age and increase in body weight. Relative weight of both organs increased with increase in body weight.

During the age interval from birth to 8 weeks, animals with larger hypophysis also had larger thyroid, adrenals and ovaries. Relationship between the relative hypophysis weight and the relative weight of the adrenal and the ovary was significant. The corresponding rates of increase in unit weight per unit increase in the weight of the hypophysis were 0.021 (male) and 0.018 (female) for the thyroid, 0.006 for the adrenal and 0.005 for the ovary. Size of the thyroid gland was associated with the size of adrenal and gonads. Weight of the thyroid increased at a much faster rate in the male than in the female. Animals having larger weight of the adrenal gland also had larger weights of the gonads. There was no significant change in the relationship of the different glands between 2 to 12 months, as different from the previous interval. However, during the age interval 2 to 12 months, an increase in the weight of the hypophysis of one milligram brought about an increase of 0.016 g (male) and 0.040 g (female) in thyroid weight, 0.011 g in adrenal weight, 0.055 g in testis weight and 0.026 g in weight of the ovary.

During the age interval 1 to 10 years, relationship between the weight of the hypophysis and weight of the thyroid, adrenal and ovaries was significant. Growth of the hypophysis was better correlated with age than with body weight during this period. Actually, relative

hypophysis weight increased with increase in the weight of the hypophysis, indicating gain of weight that could be attributed to growth of the gland. Unlike the previous intervals, relative hypophysis weight increased with age. During this interval, a unit increase in hypophysis weight contributed to an increase of 0.022 (male) and 0.016 (female) units in the weight of the thyroid, 0.007 units in the weight of the adrenal and 0.006 units in the weight of the ovary. Size of the thyroid gland was only associated with the sizes of the adrenal and gonad in sows. All correlations with body weight were low. The relative weight of the hypophysis, adrenal and ovary decreased with increase in the body weight, while that of the thyroid was at a constant level.

The partial correlations reported in Tables 13C and 13D have the effects of body weight being held constant, so that the influence of this factor has been removed. As in case of the dog, most of the partial correlations were lower than those reported in Table 12A. In both sexes weight of the hypophysis was related significantly with that of the thyroid, and the relative hypophysis weight was related with that of the thyroid and the adrenal. In the female, a significant relationship existed between hypophysis weight and weight of the adrenal and the ovary. In case of sows, weight of the thyroid gland possessed significant relationships with

the weight of the adrenal and the ovary. In the male animals, weight of the adrenal was significantly related with the weight of the testis.

Histomorphology of the Canine Hypophysis

Adenohypophysis

Pars distalis adenohypophysis The pars distalis adenohypophysis circumscribed the pars intermedia and the pars distalis neurohypophysis. Its dorsal extent varied with age being more extensively in specimens from aged animals. The rostroventral part was the widest of all. The cavum hypophysis separated the pars distalis adenohypophysis and the pars intermedia adenohypophysis throughout their extension (Figure 62). The cavum was lined externally with a single layer of cells composed of all cell-types of the pars distalis adenohypophysis. Majority of the population of cells lining this margin was formed by the thyrotrope cells and FSH gonadotrope cells. The cavum hypophysis of neither the young nor the aged dogs contained colloid in any part. Rostrodorsally, the pars distalis adenohypophysis merged with the pars paraneuralis and pars infundibularis adenohypophysis without any sharp demarcation (Figure 41). The average volume of the pars distalis adenohypophysis, relative to that of the pars intermedia and pars distalis neurohypophysis, was 4.23:1:2.31.

The parenchyma of the pars distalis adenohypophysis was

composed of chromophobes and chromophil cells arranged in the form of irregular cell-cords (Figure 8). Portal capillaries and bands of collagen fibers intervened between the cell-cords. Elastic fibers were not evident among the collagen fibers. Fibroblasts were comparatively fewer in number and were less numerous in the specimens from aged dogs. The collagen fiber bundles were never encountered circumscribing the cell-cords either partially or completely. The external layer of the portal vessels was formed exclusively by collagen fibers which adopted to the contour of cells present in the vicinity of the vessels (Figure 12). Reticular fibers in the form of dense bundles were observed among the collagen fibers and along the portal vessels. Recticular fibers never penetrated in between the individual cells. Bundles of nerve fibers, probably autonomic in nature, formed a dense feltwork around the capillaries (Figure 11). Branches of such nerve fibers were not observed to be associated with the cells in any form. Seven different cell-types, including the chromophobes, were observed in the pars distalis adenohypophysis of the dog. The tinctorial affinities of various cell-types have been summarized in Table 14.

<u>Somatotrope cells</u> These cells formed the most numerous cell-type in the beagle. Their average proportion was approximately 46 percent in adult animals. Somatotropes occurred throughout the lateral zones of the pars distalis

adenohypophysis and were especially numerous in the dorsolateral zones. They were scarce in the rostroventral part, especially in its central zone. An area in the immediate vicinity of the central part of the cavum hypophysis also contained very few somatotropes. The cells occurred singly and always formed the external layer of the cell-cords. They were the smallest in diameter among the chromophil cells and possessed distinct outlines (Figure 8). Their usual forms were round and oval, with a distinct nucleus located in the central part of each cell. The nucleus was round to oval in outline and contained many fine chromatin granules among which the single nucleolus was evident distinctly (Figure 10). The cytoplasm was densely filled with very fine granules which were not discernible individually. Uniform coloration of the cytoplasm was characteristic in all the staining techniques employed.

The granules of somatotrope cells stained bluish green with luxol fast blue which served as a specific and useful stain to distinguish this cell-type from others. The granules stained yellow with orange G and showed no affinity for periodic acid-Schiff. They showed specific affinity for luxol fast blue over orange G and for orange G instead of erythrosin, in all the techniques where the stains were employed in conjunction with each other (Figure 18). The granules stained orange in the azocarmine, orange G and

aniline blue procedure of Dawson and Friedgood (1938) which enabled the cells to be identified from azocarmine positive lactotropes (Figure 8). Close to the vicinity of the nucleus, the cytoplasm contained a rounded granule-free area depicting a negative image of the Golgi body (Figure 18).

Lactotrope cells Lactotrope cells were comparatively larger in size than the somatotropes. They predominated the rostrocentral area of the pars distalis adenohypophysis and were encountered in the periphery of the lateral zones of the lobe. The lactotropes were frequently encountered in zones where the predominated cell-type was the FSH gonadotrope cell. The cells usually occurred in groups of two to four, and two or three groups of cells occurred in a single region of the lobe (Figure 13). The cells had varigated shapes which ranged from round or oval to polygonal. Occasionally the central part of the cell appeared truncated with bulbous extremities simulating a drawn out appearance. The cell borders were poorly defined. Lactotropes constituted 9 percent of the total cell population.

The cytoplasm of the lactotrope cell was not stained in any technique. The intracytoplasmic granules were large, coarse and individually discernible. They showed a tendency to accumulate towards the central part of the cell in the vicinity of the nucleus and the Golgi body (Figure 18). The granules stained intensely with azocarmine, erythrosin and

acid fuchsin (Figures 8 and 18); where orange G was used as the only stain for acidophil cells, the lactotropes stained orange. In periodic acid-Schiff procedures, the granules were light magenta, showing affinity for leuco fuchsin. However, in the techniques where Schiff reagent was employed in conjunction with aldehyde-fuchsin and orange G or with alcian blue and orange G, the cells were colored dull orange.

The nuclei of the cells were quite varied in outline; round, oval and occasionally elongate varities were the frequent shapes observed. Large numbers of diffuse chromatin granules were distributed throughout the nucleoplasm in addition to the heterochromatin masses which were clearly evident. The nucleus usually contained a single nucleolus towards one extremity. A prominent negative image of the Golgi body was evident near the nucleus.

Thyrotrope cells These cells were dispersed throughout the extent of the pars distalis adenohypophysis. They were less numerous in the rostroventral zone where FSH gonadotrope cells and lactotropes constituted the major part of the cell population. The outline of the cell was clearly defined. The cells were less variable in shape being either angular or polygonal in outline. The sides of the cell were flattened or somewhat indented by contact with adjacent cells. The usual location of the cell was the inner parts of the cell-cords, away from the wall of

the capillaries (Figure 14). Thyrotrope cells formed approximately 6 percent of the cell population of the pars distalis adenohypophysis.

The cytoplasm of this type of cell was unstained. The intracytoplasmic granules of the cells occurred either in the form of small granules or in the form of rather large clumped masses; both types were observed with equal frequency in the specimens examined. When the granules occurred in the form of clumped masses, their usual location was near the periphery of the cell along the margins (Figure 9). A usual manner of their distribution was in the form of two crescentic masses, one on either side of the nucleus, extending towards the opposite extremity of the cell and becoming progressively wider. Some of the thyrotrope cells contained a mixture of granules and vesicles. Some distance away from the nucleus, a large hyaline vacuole, filled with granular material in its central part, was evident in between the two crescent-shaped vesicles (Figure 14). The staining of both forms were the same; the vesicles usually manifested a deeper hue. The granules stained intense blue with aniline blue (Figure 18), red with periodic acid-Schiff, purplish black with aldehyde-fuchsin, distinct blue with alcian blue after prior oxidation in performic acid or acidified potassium permanganate (Figure 12) and blue black with aldehyde-thionin after prior oxidation in acidified

potassium permanganate (Figure 19). The granules stained pale blue in aldehyde-fuchsin and Crossman's trichrome procedure (Figure 14).

The nuclei of the thyrotrope cells were located eccentrically at one extremity. Their usual shapes varied being irregular, indented or oval (Figure 14). Usually more than one nucleolus was encountered in the cells. The chromatin occurred in the form of coarse clumps distributed throughout the nucleoplasm.

<u>FSH gonadotrope cells</u> These cells belonged to the largest cell-type among the glycoproteinaceous cells. Their outlines were poorly defined and the shapes were usually spherical. However, when they lined the wall of the cell-cords, their shapes tended to be polygonal with indentations for adaptation to the contour of adjacent cells. The cells were frequently encountered in the rostroventral area of the pars distalis adenohypophysis, especially in the central zone. They also occurred in small groups in many parts of the lateral zones of the lobe. Like the somatotrope cells, FSH gonadotropes were encountered frequently in association with the portal capillaries and the cells were less numerous in the least vascular regions. They lined the cavum hypophysis in large numbers. They constituted 8 percent of the total cell population.

The nucleus of the cell was distinctly oval in outline

with fine chromatin granules and a single nucleolus. Heterochromatin masses usually furnished a cart-wheel appearance to the nucleus. Occasionally a negative image of the Golgi body was observed in the cytoplasm some distance away from the nucleus; the central part of this was usually filled with granules. The cytoplasm usually remained unstained in all the techniques. The intracytoplasmic granules were very fine and were very thinly distributed throughout the cytoplasm being somewhat numerous near the cell margin. Due to the fineness of the granules and their poor density as compared to the volume of the cytoplasm, the FSH gonadotrope cells usually appeared empty and vacuolated.

The granules stained pale blue with aniline blue, red with periodic acid-Schiff procedure and purple with aldehyde-fuchsin (Figure 16). When periodic acid-Schiff procedure was applied in conjunction with orange G, the granules retained their original coloration (Figure 13), while they stained light violet when periodic acid-Schiff procedure was preceded by alcian blue staining (Figure 35). The granules stained purple with Crossman's trichrome procedure and with the aldehyde-fuchsin and Crossman's trichrome procedure (Figure 16) they appeared brownish purple. They were colored blue black with aldehyde-thionin after permanganate oxidation; when aldehyde-thionin was employed in association with periodic acid-Schiff and orange G, the granules stained

brownish purple or violet (Figure 19). Methyl blue colored the granules, specifically, purplish blue. No other cell-type in the pars distalis adenohypophysis was stained with methyl blue.

ICSH gonadotropic cells These cells predominated the rostromedian region of the pars distalis adenohypophysis. At times their distribution extended into the latter zones of the lobe. The cells appeared compact, distinctly polygonal in outline and possessed well evident margins. Their size was smaller in comparison to that of the FSH cells. They occurred singly or in groups of several cells in one locality. The cells were invariably located some distance away from the capillary wall. They constituted 1-2 percent of the total cell population.

The secretion granules of the cell were very fine and were densely distributed throughout the cytoplasm so as to make the cytoplasmic mass completely nondiscernible. Individual granules were not evident in any form of the cell (Figure 35). The granules stained violet in all techniques where aniline blue was employed in conjunction with orange G, depicting clear affinity for both stains (Figure 17). Similar coloration was also observed in aldehyde-fuchsin and Crossman's trichrome procedure where light green was substituted for aniline blue. The granules stained red with periodic acid-Schiff technique; but, due

to their affinity for orange G, the final coloration was changed to rose red whenever orange G was employed in conjunction with the Schiff reagent (Figure 12). The granules showed no affinity for aldehyde-fuchsin, alcian blue, methyl blue or aldehyde-thionin. Affinity of the cells for orange G was reduced considerably when some of the latter were employed as counterstain, especially after the use of alcian blue. The granules were selectively stained with lead hematoxylin. In the azocarmine, orange G and aniline blue procedure, the cells were colored violet.

The nucleus of the ICSH cell was large, oval in outline and was vesicular. Fine chromatin granules were dispersed throughout the nucleoplasm. The nucleus usually contained several (to 3 or 4 in number) nucleoli. A negative image of the Golgi body was never encountered in this cell-type.

<u>Adrenocorticotrope cells</u> These were represented by very large spherical cells with irregular outlines. They occurred singly along the wall of the portal capillaries; rarely did they occur in small groups. The outline of the cell was well defined. This type of cell was distributed throughout the pars distalis adenohypophysis and was less numerous in the vicinity of the peripheral margin or along the cavum hypophysis. They constituted approximately 1 percent of the total cell population.

The cytoplasm of the cell was never stained with any of

the techniques employed. It appeared empty and vacuolated (Figure 16). The entire cell manifested a foamy appearance. Very fine granules were evident lining the margins of the cell. Their number was so small as to give a faint coloration to the cell boundary. The granules stained intensely with erythrosin, weakly with periodic acid-Schiff and aldehyde-fuchsin. They were intensely stained and were revealed in increased numbers when the sections were postchromated on the slide. The nucleus of the cell was excessively large and vesicular. No nucleolus was usually observed. The nucleoplasm contained fine chromatin granules, rather densely distributed throughout its extent.

Chromophobe cells In the pars distalis adenohypophysis of the beagle, chromophobes constituted the second major proportion. They usually occurred among the collagen fiber bundles that intervened between the cell-cords, adjacent to the wall of the portal vessels. In the central part of the cell-cords few chromophobic cells were also observed. The peripheral area of the pars distalis adenohypophysis was especially rich in these cells. The cells were represented by large oval vesicular nuclei with scanty cytoplasm or none at all. The nucleoplasm contained large floccular chromatin material in discrete masses. Some of the nuclei manifested indentations and were often notched. A distinct nucleolus was rarely observed. The cells stained

weak purple or none.

<u>Cupping of cells</u> In the pars distalis adenohypophysis, cells of all types were found in close association with each other. In many instances, the entire outline of a cell was embedded within the associated cell (Figure 10). A characteristic feature of this phenomenon was the association of functionally active cells only, involving one member from acidophil class and the other from the basophil class (Figure 10). A chromophobe or a degranulated cell with an empty cytoplasm was never observed to take part in cupping. Though all types of associations were evident, that of the somatotrope cell cupped by the FSH gonadotrope cell was the most frequent type. Cupping of somatotropes and lactotropes with adrenocorticotrope cells and that of FSH gonadotrope cells and lactotropes was observed in many regions of the lobe. The thyrotrope cell was involved with both somatotrope and lactotrope cells in the cupping phenomenon. The ICSH gonadotrope cell was rarely encountered in a cupped association with other cell-types.

<u>Pars paraneuralis</u> The pars paraneuralis was very extensive in the beagle. It formed the zone of contact between pars distalis and pars intermedia adenohypophysis, and the pars infundibularis adenohypophysis. No sharp demarcation was evident between the lobes in any region, the lobes merging imperceptibly with each other (Figure 43).

The parenchymatous cells of pars paraneuralis were arranged in irregular strands or sheets extending from the pars infundibularis towards the caudal extremity of the organ. The strands were separated by the portal vessels which coursed in the same direction as the cellular strands (Figure 45). Collagen fiber bundles also intervened between the latter. Reticular fibers and a dense feltwork of nerve fibers were observed in conjunction with the portal vessels. The entire pars paraneuralis was broken up into segments due to permeation by ramifications of the cavum hypophysis in such a manner that the cellular columns and strands appeared to hang from the pars cava infundibuli.

Two types of cells that formed the parenchyma of the region were observed. The type of cells constituting the major proportion resembled in shape, size and appearance with the cells of pars intermedia and to some extent with the chromophobe cells of the pars distalis adenohypophysis. These cells revealed no staining affinity (Figure 42). The other type was composed of large cuboidal or polygonal cells with spherical nuclei and faintly stained cytoplasm. The granules of these cells were very fine and fewer in number. In their staining character, the cells were akin to the basophil cells of the pars distalis adenohypophysis (Figure 42). Such cells were always encountered along the wall of the portal vessels.

Pars infundibularis adenohypophysis This lobe was the smallest in proportion. It formed an external covering for the pars proximalis neurohypophysis and encircled the latter throughout its extent. The parenchyma was formed by large number of blood vessels, loosely packed collagen fibers, reticular fibers, and cells arranged in the form of single layered acini. Two types of cells were observed; chromophobic cells were the numerous type, while polygonal basophilic cells formed the minority group. The former type of cells composed solely the dorsal half of the lobe. Each type resembled the respective cell-type of the pars paraneuralis. A dense network of nerve fibers was present along the ramifications of portal vessels. Branches from these nerve bundles were found to penetrate the strands of collagen fibers and terminate in the vicinity of the capillary walls like tufts.

Pars intermedia adenohypophysis The pars intermedia was variable in its thickness at different regions of the same section as well as at different levels of the organ. It surrounded the pars distalis neurohypophysis and merged imperceptibly with the pars paraneuralis. At the line of junction with the pars distalis neurohypophysis, a well developed vascular plexus, lined with reticular and collagen fibers, was present. Strands of connective tissue fibers and branches from the capillary vessels penetrated into the parenchyma of the lobe dividing it into several lobular

masses. Some of these capillary branches extened to the cavum hypophysis. Except for the septal vessels, the pars intermedia was devoid of blood vessels and connective tissue.

The parenchymal cells of the lobe possessed distinct outlines and were arranged compactly. Their shapes were either oval or columnar. The nuclei were spherical or spindle-shaped in outline and contained distinct chromatin granules. Characteristically a single nucleolus was evident in each cell. The cells which constituted a major part of the population occurred in closely packed groups. Their cytoplasm was coarse and contained fine granules that stained pale blue with aniline blue, light magenta with periodic acid-Schiff procedure (Figure 49), and possessed slight affinity for alcian blue, aldehyde-fuchsin and aldehyde-thionin, so that when the latter were used in conjunction with periodic acid-Schiff a purple shade resulted due to superimposition of two different colors. The second category of cells contained large granules and their cytoplasm was densely populated with the granules as a result of which staining of these cells appeared much deeper. The granules stained red with periodic acid-Schiff technique, intense pink with acid fuchsin and pink with aldehyde-fuchsin (Figure 51). They showed no affinity for alcian blue, aldehyde-thionin and methyl blue. In the aldehyde-fuchsin and Crossman's trichrome technique, they were

colored intense pink.

Large colloid-filled follicles occurred in the pars intermedia along its junctional zone with the neurohypophysis. At many places fiber bundles from the latter came in close approximation with the parenchymal cells of the pars intermedia and Herring bodies were observed in close proximity of the cells. Such union of neurohypophysial fibers and cells of the pars intermedia was especially evident in the vicinity of the colloid follicles. The colloid stained intense magenta with the periodic acid-Schiff technique.

In the sections stained with Bielschowsky's technique for axis cylinder and dendrite, a well developed network of nerve fibers was observed around the capillaries. Bundles of such nerve fibers also coursed along the interlobular septa. That these fibers descended from the pars distalis neurohypophysis was clearly evident in the sections. These fibers did not contain neurosecretory material either during their course through the neurohypophysis or within the pars intermedia as was evidenced by Gomori's chrome alum hematoxylin procedure and Sloper's performic acid alcian blue technique. Branches from these fibers extended into the lobules along with the capillary vessels.

Neurohypophysis

Pars proximalis neurohypophysis   The pars proximalis neurohypophysis was composed of two separate parts. The radix infundibuli was comparatively very short in its extent. It formed the zone of attachment between the hypothalamus and the pars cava infundibuli. It served mainly as the pathway for the hypothalamohypophysial tract. The pars cava infundibuli was very long and served as the main part of the stalk of the hypophysis.

Radix infundibuli   The radix infundibuli was composed of a single layer of ependymal cells and two distinct zones of nerve fibers which were clearly discernible due to opposing direction of their composing fibers (Figure 54). The ependymal cells lined the inner margin of the radix infundibuli that formed the boundary of the infundibular recess. They were cuboidal or oval cells whose cytoplasm was not elucidated in the routine staining techniques. The nucleus of the ependymal cell was oval in outline, contained diffuse granular masses of chromatin and a single nucleolus. A basement membrane was not associated with the ependymal layer. Nerve fibers from the inner zone penetrated in between the ependymal cells, and Herring bodies were observed along the course of these fibers.

The nerve fibers of the inner zone coursed in a parallel direction to that of the longitudinal axis of the

hypophysis (Figure 57). They were comparatively larger and formed small bundles. Large numbers of neuroglia cells were interposed between the fibers. Two types of such cells were observed--a large and a small. Gomori's chrome alum hematoxylin technique as well as that of Adam and Sloper for cystine, revealed the presence of neurosecretory material throughout the course of infundibular fibers (Figure 60). Herring bodies were abundant throughout this zone (Figure 60). Aldehyde-fuchsin, aldehyde-thionin and alcian blue were equally useful in demonstrating the neurosecretory material contained within the nerve fibers as well as within the Herring bodies. Herring bodies occurred in the form of spherical masses of variable diameter. Large bodies usually assumed an irregular form while the small ones were distinctly spherical. The bodies were composed of spherical granules of neurosecretory material each of which was discernible against an empty background (Figure 45). Capillaries were fewer in number in this zone.

The outer zone was made up of fibers which were more delicate and of smaller diameter than those of the inner zone. These fibers coursed through the inner zone in the form of distinct bundles before spreading out in the outer zone. The direction of these fibers was distinctly perpendicular (Figure 54). The zone contained neither neuroglia cells nor capillaries. Stainable neurosecretory material

was not observed in the fibers of this zone (Figure 60).

On its external side, the outer zone was flanked by the mantel plexus. Two types of capillaries were encountered in this plexus (Figure 59). One type possessed a comparatively spherical form and three distinct layers--endothelium, tunica media and tunica externa. The tunica media was formed by a single layer of smooth muscle fibers, while the tunica externa was formed by loose collagen and reticular fibers. A feltwork of nerve fibers was evident in this tunic. This type was suggestive of the arterial end of the portal capillaries. The other variety of capillary vessels resembled venules. Their walls were composed of a layer of endothelium and a very thin layer of tunica externa. Pericapillary cells were usually associated with this type of vessel. Such vessels were observed in close proximity of each other in groups of 2 to 6, suggestive of extensive looping of the parent capillary vessel.

Pars cava infundibuli   This part was very much elongated in the beagle due to deep penetration of the infundibular recess. It intervened between the pars distalis neurohypophysis and the radix infundibuli (Figure 57). In structure, it resembled the former. However, the outer zone was comparatively less extensive and terminated some distance from the junction of both lateral counterparts of the infundibulum. Fibers of the inner zone of the

infundibulum continued as those of the pars distalis neurohypophysis. Caudal to the region of the stalk where both lateral halves united with each other, nerve fibers containing neurosecretory material were found to terminate along the portal capillaries. Herring bodies were equally numerous in the region of the pars cava infundibuli as in the radix infundibuli (Figure 60).

Pars distalis neurohypophysis The pars distalis neurohypophysis was faintly lobular in outline and was composed of irregularly distributed and extensively ramifying nerve fibers (Figure 66). The fibers were direct continuation of those that composed the zona interna of radix infundibuli. Ramification of these nerve fibers was especially extensive in the junctional zone with pars intermedia where they terminated in association with capillaries (Figure 63). Such associations were evident throughout the pars distalis neurohypophysis.

Two types of nerve fibers were evident in this part. Majority of the fibers was fine and stained vividly with luxol fast blue and aldehyde-thionin. Neurosecretory material was observed throughout the course of these fibers; but, its density was excessive in the terminal portions of the fibers adjacent to the capillaries (Figure 64). Herring bodies were numerous and were usually located some distance away from the capillaries (Figure 65). The second type of

nerve fibers was revealed only in Bielschowsky's technique. They occurred in the form of discrete bundles and formed dense feltwork around the capillaries. Stainable neurosecretory material was not evident in these fibers and they extended into the pars intermedia. These were probably postganglionic autonomic fibers.

Two types of cells were observed among the nerve fibers of the pars distalis neurohypophysis. Pituicytes were stellate cells with long irregular processes. The latter were better elucidated by the silver stains. The common neuroglia cells were represented by smaller cells with spherical or oval nuclei. Pituicytes usually constituted the pericapillary cells in this lobe. Blood vessels were numerous throughout the lobe. Transition from medium sized arteries to capillaries was evident as the caudal hypophysial artery ramified into numerous branches within the lobe (Figure 63). The capillaries were associated with collagen fibers, reticulum and a distinct basement membrane. Such fibers were, however, not present among the nerve fibers of the lobe. Each of the arterial branches was usually accompanied by satellite veins and a distinct unilateral distribution of the vessels was evident.

Histomorphology of the Porcine Hypophysis

Adenohypophysis

Pars distalis adenohypophysis   The pars distalis adenohypophysis of the pig occupied the rostroventral part of the hypophysis and extended to the dorsal border of the organ in its rostral half only (Figure 75). The dorsal border of the organ was formed entirely by the neurohypophysis. The pars distalis was very extensive on the ventral half and was comparatively wider on the right lateral half than on the left (Figure 77). The disproportionality increased from rostral to the caudal extremity of the organ. Rostrally, the pars distalis merged imperceptibly with the pars paraneuralis all around the pars compacta infundibuli of the neurohypophysis. Caudally, it terminated in a blunt point without quite approaching the caudal extremity of the hypophysis which was formed by the pars distalis neurohypophysis (Figures 74 and 75). The average volume of the pars distalis adenohypophysis, relative to that of the pars intermedia adenohypophysis and the pars distalis neurohypophysis, was 11.70:1:1.83.

The cavum hypophysis was S-shaped and was comparatively wider caudally than in its rostral end (Figure 75). It extended the entire length of the pars distalis and pars intermedia adenohypophysis. Externally the cavum was lined by a single layer of cells composed of all cell-types of

the pars distalis adenohypophysis. A single layer of pars intermedia cells lined its inner margin. Presence of colloid in the cavum hypophysis was observed in specimens collected from aged animals (Figure 125). The histological structure of the parenchyma of the pars distalis adenohypophysis of the pig appeared identical to that of the dog. The cells were arranged in distinct zones which was even evident by gross examination of the stained sections (Figure 77). Chromophobes and six different types of chromophil cells were observed.

Somatotrope cells Like the canine hypophysis, somatotropes constituted the single major cell-types (33 percent of the total cell population). They were the most predominant type in the dorsolateral area of the lateral zones of the lobe (Figure 77). In addition, they occurred in groups of several cells in specific areas of the organ well mapped out in the ventrolateral areas of both lateral zones, especially, towards the caudal extremity of the organ. The somatotrope cells were rarely encountered in the central part of the rostroventral zone. The zonal distribution of somatotropes was very well evident in the porcine hypophysis than it was in the canine. In the cell-cords, somatotrope cells usually occupied the periphery and their extremities always remained in close contact with the capillary wall.

The cells possessed distinct outlines and were usually oval or polygonal in shape. The well distinct nucleus occurred eccentrically. It was spherical or oval in outline and contained many chromatin granules distributed throughout the nucleoplasm. Usually one or two nucleoli were observed in each nucleus (Figure 86). The cytoplasm contained fine granules densely packed throughout its extent. The dense accumulation of granules made resolution of the individual granules difficult and staining on the cells always appeared in a deeper hue. The staining affinity of the granules was identical to that of homologous cells in the canine pars distalis adenohypophysis (Figure 84). A well evident negative image of the Golgi body was invariably discernible in the perinuclear region of the cytoplasm.

Lactotrope cells The lactotrope cells were comparatively larger than the somatotropes (Figure 99). Like the latter, they also possessed distinct margins. They were usually oval or spherical in outline. Their sides were compressed in adoption to the contour of adjacent cells, so that in many locations they were angular in outline. The cells occupied the periphery of the cell-cords and invariably remained in contact with the walls of the capillaries. They occurred as individual cells, several cells occurring in the same cord and in the same locality (Figure 99). Majority of these cells occupied the vicinity of the acidophil zone

in the dorsolateral region. They were evenly distributed in the central part of the lateral zones of pars distalis adenohypophysis and were comparatively rare in the midcentral zone, and in the vicinity of the cavum hypophysis. They constituted 10 percent of the entire cell population of the pars distalis adenohypophysis.

The nucleus of the lactotrope cell occurred towards one end of the cytoplasm and contained large floccular masses of chromatin. In cells with small diameter, the large mass of chromatin obscured the picture of nuclear outline and made it appear dense. The single nucleolus occurred at one end, close to the nuclear membrane. A distinct hollow sphere, close to the nucleus, represented the negative image of the Golgi body. The intracytoplasmic granules were coarse and much larger in size than those of somatotrope cells. Their staining affinity was identical to that of the lactotrope cells in the canine hypophysis (Figures 117 and 118).

Thyrotrope cells The thyrotrope cells were the smallest and widely distributed basophil cells, forming approximately 14 percent of the cell population. They occurred throughout the pars distalis adenohypophysis except the central part of the rostroventral area. They usually occurred as single cells. The cells were either oval or angular in outline and their borders were distinct. The usual location of the cell was in the deeper part of

the cell-cords, away from the capillary wall. The nucleus was spherical or oval in outline and occurred eccentrically towards one extremity (Figure 87). The large amount of chromatin material obscured the outline of the nucleus. A single nucleolus was discernible in those which possessed less chromatin material. The negative image of the Golgi body was present towards the periphery of the cell in the form of a hollow sphere with a dense centrum (Figure 116). The intracytoplasmic granules of the thyrotrope cells usually occurred in the form of two crescentic masses of deep staining floccular material, which remained separated by the nucleus and the Golgi body. Their condensation towards the peripheral margins of the cell was very conspicuous (Figures 116 and 118). Like other cell-types, staining affinity of the thyrotrope cell was identical in the canine and porcine species.

FSH gonadotrope cells   This cell-type was found in a well demarcated area extending over the central region of the rostroventral surface of the pars distalis adenohypophysis (Figure 75). This area constituted the basophil zone in contrast to the dorsolaterally located acidophil zone (Figure 76). The FSH cells always occurred in groups of a large number of cells. Such groups were also observed in the central part of the lateral zones at frequent intervals. A bilateral area, flanking the

midcentral part in the vicinity of the cavum hypophysis, was also rich in FSH cells. In all these areas, the other common cell-type present was the lactotrope cell. The relative percentage of FSH gonadotrope cells was 24.

The FSH gonadotrope cells were very variable in size. The same section often contained cells of 3 or 4 different sizes. The cells were usually polygonal in outline and their margins remained on contact with the capillary walls (Figure 94). The nucleus was spherical in outline and contained characteristically a single prominent nucleolus and few chromatin granules (Figure 94). A negative image of the Golgi body was not discernible in these cells. The intracytoplasmic granules were fewer in number and were widely distributed in the cytoplasm (Figure 94). This gave a foamy appearance to the cell. The staining affinity of the granules was akin to that described for similar cell-type of the pars distalis adenohypophysis of the dog (Figures 94 and 116).

ICSH gonadotrope cells These cells were smaller than the FSH cells and were distinctly oval in outline. They occurred throughout the lateral zones of the pars distalis adenohypophysis except the dorsal and ventral extremities. They were more numerous towards the lateral margins and were usually present as single cells in the deeper parts of the cell-cords. They constituted the least

numerous chromophil cell-type in the pars distalis adenohypophysis (approximately 3 percent of the cell population). The nucleus of this cell-type was irregular in outline and contained a dense mass of chromatin material which masked other features of the nucleus. In some cells, however, two or three nucleoli and floccular masses of chromatin material were present. The intracytoplasmic granules were small and were densely distributed throughout the cytoplasm (Figures 94 and 112). No difference was observed in the staining affinity of these granules between the pig and the dog (Figure 90).

Adrenocorticotrope cells The adrenocorticotrope cells were mainly located in the lateral zones on either side of the central midline. They were numerous in the vicinity of the cavum hypophysis. They were comparatively more numerous in the dorsolateral region than in the ventrolateral. They usually occurred in groups of small stellate-shaped cells with prominent nuclei but scanty cytoplasm. Sometimes, the entire population of the cell-cords was composed of these cells only. The cytoplasm of this cell was pale, so that the fine erythrophilic granules were distinctly discernible in it. Their affinity for erythrosin was especially enhanced when the sections were treated with potassium dichromate before actual staining. Apart from their affinity for erythrosin, the granules of the corticotrope cells remained

unstained in all other techniques. In the pig, adrenocorticotropic cells constituted 9 percent of the total cell population of the pars distalis adenohypophysis. These cells could be easily distinguished from the lactotrope cells which were also stained with erythrosin due to their irregular shape, fine granulation and lack of affinity for azocarmine.

Chromophobe cells Chromophobe cells were comparatively fewer in number in the pig than in the dog and formed only 7 percent of the cell population. They occurred in the form of one or two cells in the central part of the cell-cords. No cytoplasm was observed in these cells. The nucleus was oval in outline and contained few chromatin granules, but no nucleolus.

Cupping of cells In the pars distalis adenohypophysis of the pig, embedment of cells within the cytoplasm of another cell-type was observed in all parts of the lobe. Cells that took part in such close association represented functionally active cells with different tinctorial affinities. The common types were the association of lactotrope cells with thyrotrope cells and that of somatotrope with thyrotrope cells (Figure 116). Association of FSH gonadotrope cells with lactotrope cells was more frequently observed than that with somatotrope cells. ICSH gonadotrope cells were involved in the cupping phenomenon

primarily with lactotrope cells.

Pars paraneuralis The pars paraneuralis was equally well developed in the pig as in the dog. Unlike the latter, it formed a compact cellular mass of the junctional zone between the pars distalis adenohypophysis, pars intermedia and pars infundibularis adenohypophysis (Figure 75). The area contained many large capillary vessels and thick bands of collagen and reticulum. Around the vessels, a dense feltwork of nerve fibers, probably of autonomic nature, was present (Figure 120). Apart from the presence of large vessels, the area was comparatively less vascular than the pars distalis adenohypophysis.

The cells which constituted the parenchyma of this lobe were arranged in the form of distinct strands and acini which were separated by bands of collagen and reticular fibers. The cells possessed ill-defined outlines (Figure 119). Two types of cells were present in this part. Majority of the cells belonged to chromophobic cells. They showed no affinity for any type of stain. Their nuclei were irregular in outlines and were usually dense with chromatin material. The second cell-type was much larger in size and possessed a distinct spherical nucleus with a single nucleolus (Figure 119). In some of the latter cells, compact nuclei were also observed. The cytoplasm of these cells contained very fine granulation which stained magenta with periodic acid-

Schiff technique and pink with aldehyde-fuchsin and periodic acid-Schiff procedure. Their tinctorial affinity resembled that of the FSH gonadotrope cells in many respects.

Pars infundibularis adenohypophysis The pars infundibularis of the pig was the direct rostral continuation of the pars paraneuralis from which it could only be demarcated by locating the mantel plexus. It formed a cellular mass around the pars compacta infundibuli and the radix infundibuli (Figure 74). The pars infundibularis adenohypophysis was comparatively better developed at the level of the radix infundibuli, being about twice as wide as that in the region of the pars compacta infundibuli (Figure 74).

The cells of this part were identical to those of the pars paraneuralis except the fact that they were comparatively larger in size and stained intensely with the periodic acid-Schiff technique (Figure 122). The cells manifesting tinctorial affinity akin to that of the FSH gonadotrope cells were comparatively more numerous in this zone. The cells were mostly arranged on the form of single layered acini. The entire region was highly vascular.

Pars intermedia adenohypophysis The pars intermedia adenohypophysis of the pig varied in thickness at different regions of the organ and at different levels of the same section. It was especially extensive at the midventral

plane. Caudally, it became reflected over the caudal extremity of the pars distalis adenohypophysis, both on the right and the left lateral halves of the latter (Figure 74). Rostrally it was continuous with the pars paraneuralis without any demarcation.

The pars intermedia of the pig was distinctly divided into lobules by connective tissue septa. The latter were composed of loose collagen and reticular fibers. Capillaries and venules of different sizes coursed along these septa. The pars intermedia contained some large veins which extended to the exterior (Figure 124). Each lobule was made up of a single acinus formed by a layer of large polygonal cells. The central part of the acinus was usually hollow and contained colloid which stained strongly with periodic acid-Schiff technique (Figure 126).

The cells of the pars intermedia belonged to two different categories in the pig. The most numerous type consisted of large polygonal cells with indistinct margins. The cytoplasm of these cells appeared homogeneous without any discernible intracytoplasmic granules (Figure 125). The nuclei were dense and irregular in outline. The cells were colored magenta with periodic acid-Schiff technique. These cells manifested affinity for no other stain. Cells of the second category were fewer in number and possessed distinct oval and spherical outlines. The nuclei of these

cells were large, vesicular and contained a single nucleolus. A few cells of this type possessed compact nuclei akin to the first category of cells. The cells stained red with periodic acid-Schiff and intense pink with aldehyde-fuchsin. They showed no affinity for alcian blue, aldehyde-thionin, methyl blue and lead hematoxylin. Many of these cells contained no intracytoplasmic granules and their cytoplasm presented a foamy appearance. Such cells were not colored in any staining technique and resembled degranulated cells.

Neurohypophysis

Pars proximalis neurohypophysis In the pig, the infundibular recess did not extend beyond the radix infundibuli, so that the entire stalk was formed by the pars compacta infundibuli (Figure 74). The radix infundibuli was the intervening part between the former and the hypothalamus.

Radix infundibuli The radix infundibuli of the pig was similar in structure to the dog, but was much wider on its ventral aspect than on the dorsal (Figure 74). The ependymal layer was formed by a single layer of cells lining the infundibular recess. They were small oval cells without any discernible cytoplasm. Their nuclei contained few chromatin granules and no nucleoli.

The inner zone of the radix infundibuli was formed by longitudinally running coarse nerve fibers. In the subependymal region, the fibers were arranged loosely while

in the outer region they were densely packed. Neurosecretory material, in the form of small granular masses, was present along the course of these fibers (Figure 133). The material showed equal affinity for Gomori's chrome alum hematoxylin, alcian blue and aldehyde-thionin or aldehyde-fuchsin (following oxidation with acidified permanganate). Their accumulation in the form of Herring bodies was rarely observed. Neuroglia cells were few in number but many arterioles and capillaries were present in this zone.

The outer zone was composed of very fine nerve fibers which intercrossed with those of the inner zone to assume their perpendicular direction. All these fibers terminated in the vicinity of capillaries of the mantel plexus, which intervened between this zone and the pars infundibularis adenohypophysis (Figure 133). Neither neuroglia cells nor blood vessels were present in this layer. Neurosecretory material was not demonstrable in the fibers of this zone.

The mantel plexus consisted of large number of thin walled vessels loosely arranged between the pars infundibularis adenohypophysis and the inner zones of the radix infundibuli and pars compacta infundibuli (Figure 134). The arterioles of this plexus contained a layer of smooth muscle in between the endothelium and tunica externa, while the walls of the capillaries were composed of an endothelial layer and a layer of collagen and reticular fibers. A wide

basement membrane was evident in all techniques that employed periodic acid-Schiff staining. A dense feltwork of nerve fibers surrounded the vessels. These fibers were discernible after application of Bielschowsky's technique. Nerve fibers of the outer zone of the radix infundibuli terminated in close vicinity of these capillaries. Beginning with the caudal end of the radix infundibuli, fibers of the inner zone also terminated in close association with the capillary vessels of the mantel plexus. Branches from the vessels located in the mantel plexus often penetrated into the radix infundibuli as well as into the pars compacta infundibuli. Such vessels were highly tortuous (Figure 128).

Pars compacta infundibuli In the pig, the pars compacta infundibuli was very long and stout. It was circumscribed by the pars infundibularis adenohypophysis and the extension of the mantel plexus. In cross-sectional view, it presented a distinct lobular contour. It contained large number of blood vessels within its parenchyma (Figure 133).

The pars compacta infundibuli was mainly composed of a dense feltwork of nerve fibers. Its central part was formed by continuation of fibers from the inner zone of the radix infundibuli. These fibers contained neurosecretory material which was stainable with chrome alum hematoxylin, alcian

blue, aldehyde-fuchsin and aldehyde-thionin as was used in the usual techniques (Figure 133). The fibers terminated in the vicinity of capillaries that coursed within the substance of the pars compacta infundibuli and also in the vicinity of capillaries of the mantel plexus. In the former location Herring bodies were evident. The latter were fewer in number and were rarely encountered along the course of the fibers. The amount of neurosecretory material was comparatively less in the radix infundibuli and pars compacta infundibuli than in case of the pars distalis neurohypophysis.

The outer zone of the pars compacta infundibuli was not as well developed as in the radix infundibuli. In this zone, there was considerable admixture of fibers that stained positive for neurosecretory material than those that were negative. Throughout its extent, fibers of the former type terminated along the course of blood vessels, a fact which was not observed in the canine neurohypophysis.

Pars distalis neurohypophysis The pars distalis neurohypophysis was a massive bulbous structure in the pig. It increased in diameter, progressively, in the rostrocaudal direction and the bulbous caudal end of this region formed the caudal tip of the hypophysis (Figure 74). It was mainly formed by continuation of the fibers from the central core of the pars compacta infundibuli. The fibers were primarily oriented, in the central part of the lobe. In the peripheral

region of the lobe, there was irregular organization of fibers due to excessive branching. Fibers were sectioned in different planes in a single section. Unlike the dog, a distinct lobular arrangement was observed in the pars distalis neurohypophysis of the pig. The central part of each lobule contained a single large vessel around which neurosecretion bearing fibers terminated in random fashion, furnishing a rosette (Figure 143). Collagen and reticular fibers were only limited to the vicinity of the blood vessels. The pars distalis neurohypophysis was not clearly demarcated from the pars intermedia.

Two types of nerve fibers were observed in this lobe. One type of fibers was of small diameter, showed much branching and stained blue with luxol fast blue. These fibers carried neurosecretory material that could be stained with chrome alum hexatoxylin, alcian blue and aldehyde-thionin. The neurosecretory material occurred in the form of discrete granules all along the course of fibers and in the form of accumulated masses or Herring bodies. The former were especially dense in the vicinity of blood vessels. Herring bodies were far less numerous as compared to that of the dog, but individual fibers carried much more neurosecretory material than in case of the dog (Figure 137). The second type of nerve fibers was identified after silver staining. They occurred in bundles and formed a network

around each blood vessel. The same fibers continued into the pars intermedia. These were probably the postganglionic autonomic fibers.

Pituicytes and neuroglia cells were both present in the pars distalis adenohypophysis of the pig. Both types of cells were comparatively less numerous than that of the dog, but were identical in morphology to the homologue cell-types of the latter. The blood vessels that coursed through the pars distalis neurohypophysis of the pig were mostly thin walled vessels which resembled venules (Figure 137). Medium sized arterial branches were not observed in this lobe. The vessels were lined with loose collagen and reticular fibers. A periodic acid-Schiff positive basement membrane was evident around them.

Age-correlated Changes in the Canine Hypophysis

Adenohypophysis

Pars distalis adenohypophysis

Group I: birth to 4 weeks In this group, the earliest age at which specimens were available was 8 hours postnatal. The pars distalis adenohypophysis was comparatively more extensive on its ventral surface than on the lateral zones. It circumscribed the pars intermedia and the pars distalis neurohypophysis except for a variable zone along the dorsal border of the organ. The cavum hypophysis was wide and lined by a single row of undifferentiated cells on

its external margin. The parenchymal cells of the pars intermedia did not form a regular lining along its dorsal margin. Rostrally, the cavum hypophysis ramified into numerous channel-like extensions which penetrated into the pars paraneuralis and subdivided the latter into strands of cells that remained projected into the cavity.

The pars distalis adenohypophysis was composed of large clusters of cells separated from each other by thin strands of collagen fibers (Figure 4). A fine network of reticular fibers was also evident in the stroma. Bands of argyrophilic fibers were evident around the blood vessels. These fibers also formed a component part of the stroma and extended between the vessels. Many capillaries were present in the parenchyma of the lobe. The wall of these vessels was formed only by a thin layer of endothelium. Fibroblasts were present in the perivascular region as well as along the septa.

The parenchymal cells of the lobe were loosely arranged in the cell-clusters; but, they were compactly packed together along the peripheral margin as well as in the vicinity of the cavum hypophysis. In addition to the chromophobe cells, three other cell-types were evident at this age. The somatotrope cells were mostly present along the peripheral margins of the lobe. They possessed distinct outlines and were oval in shape. The cytoplasm contained

many small granules which stained yellow with orange G and blue green with luxol fast blue (Figure 5). The nucleus of this cell was oval in outline and was often bilobed.

The thyrotrope cells were mainly distributed in the central region of the lobe and were less numerous towards the dorsal part of the lateral zones. They were larger in size than the somatotrope cells. The intracytoplasmic granules were fewer in number as compared to the volume of the cytoplasm. The granules were mainly located along the peripheral margins of the cell. The nucleus of the cell was distinctly oval and contained 2-3 nucleoli in each cell. Bilobed nuclei were also present in some cells. Large amount of chromatin granules was discernible in the nucleoplasm. A perinuclear hollow was characteristically present in these cells. The cells stained bluish green with aniline blue and light blue with alcian blue (Figure 5). Aldehyde-thionin stained the cells bluish black (Figure 4).

The FSH gonadotrope cells were so less numerous as not to form a measurable portion of the cell population. The cells were located in the central area of the rostroventral surface, mainly towards the peripheral margins. The FSH cell was oval or polygonal in outline and contained very few intracytoplasmic granules. The nucleus of the cell was typically spherical in outline and contained a single large nucleolus (Figure 4). The cells stained weakly with

periodic acid-Schiff and light blue with aniline blue. Aldehyde-thionin employed in conjunction with periodic acid-Schiff stained the cells purple. Alcian blue in conjunction with periodic acid-Schiff and orange G also stained with cells purple.

The chromophobe cells were very numerous in the parenchyma of the pars distalis adenohypophysis. They occurred in groups of several cells densely packed together. Very few of them contained a cytoplasmic mass. The nucleus of the chromophobe cell was quite variable in shape and size. Cells with bilobed nuclei were frequently encountered. Some of the cells contained a single nucleolus while others contained as many as three per cell. Many of them showed condensed chromatin material and no nuclear outlines at all. Various stages of mitotic division were observed in the chromophobe cells (Figure 6).

Both somatotrope and thyrotrope cells showed degranulation at this age. The latter type of cells was more affected than the former. The degranulated cells were comparatively larger in size and contained very few stainable granules near their peripheral margin. The central part of the cell appeared vacuolated (Figure 7). In many instances no granules were observed in the cytoplasm of the degranulated somatotrope cells. There was no appreciable difference in the histological structure of the pars distalis between the

male and the female animals. The relative proportion of the different cell-types was as follows:

	Female (percent)	Male (percent)
Somatotrope cells	45	50
Thyrotrope cells	14	12
Chromophobe cells	41	38

In specimens collected from 3 to 28 days old puppies, the pars distalis adenohypophysis presented little variation in its histological structure. The parenchyma became progressively denser due to increase in the cell population. The cells were not arranged in follicular or cord-like fashion, but were closely adherent to each other. Thin-walled capillaries were present at intervals throughout the extent of the lobe. There was a considerable increase in the vasculature during this period. Collagen and reticular fibers encircled the vessels and extended along their branches throughout their course. In some of the vessels, deposition of colloid was observed on the endothelial lining (Figure 7). The colloid material stained intense red with periodic acid-Schiff and was also intensely orangeophilic. Vessels of smaller caliber were more severely affected than the large calibered vessels.

No alteration in the cellular composition of the lobe was evident during this period. Majority of the thyrotrope cells was in a state of degranulation (Figure 7). Number

of somatotrope cells manifesting the same phenomenon was much less. Mitosis of the chromophobe cells was still evident to the third week of postnatal life. However, the number of mitotic figures had substantially decreased by that age.

Group II: 4 to 8 weeks The parenchyma of the lobe was densely populated with cells. The latter were arranged in the form of cords which were separated from each other by capillaries and thin strands of collagen and reticular fibers (Figure 8). The capillaries were thin-walled vessels which united together to form venules and small veins. The latter type of vessels was mostly present along the lateral margin of the lobe. Colloid infiltration of the vessel walls was predominant throughout the lobe. A distinct periodic acid-Schiff positive basement membrane was discernible in the perivascular region. The lobe was widest along the ventral surface and tapered dorsally in its extension to enclose the pars distalis neurohypophysis.

In the parenchyma, lactotrope cells were observed for the first time in specimens collected from one-month-old animals. These were small cylindrical cells with truncated middle parts and broad extremities. The nucleus of the cell was dense and contained many chromatin granules (Figure 9). It was located in the central part of the cell. The intracytoplasmic granules of the lactotrope cells were not

discernible individually at this stage. The cells were stained magenta in the periodic acid-Schiff, brick red in the alcian blue-periodic acid-Schiff-orange G technique and dull pink with erythrosin. They stained red with azocarmine (Figure 8). The cells were encountered along the dorsal and ventral borders of the lobe and were not evident in its dorsolateral regions. During this age-interval, the relative proportion of the different cell-types was as follows:

	Female (percent)	Male (percent)
Somatotrope cells	55.0	53.0
Lactotrope cells	2.0	1.1
Thyrotrope cells	17.2	17.5
FSH gonadotrope cells	1.2	1.3
Chromophobe cells	24.5	27.0

The thyrotrope cells were larger in size and were mostly degranulated (Figure 9). Majority of the chromophobe cells contained some amount of cytoplasm. Mitotic figures among these cells were not observed. Very few degranulated somatotrope cells were observed. Cupping of somatotrope and thyrotrope cells was observed in the dorsolateral regions of the lobe.

<u>Group III</u>: <u>2 to 6 months</u> During this period, the number of lactotrope and FSH gonadotrope cells showed a considerable increase in number. FSH gonadotrope cells

were predominant in the central part along the ventral surface of the lobe. Some of these cells possessed dense cytoplasm while others simulated degranulated cells. Thyrotrope cells were less numerous and were smaller in size. Very few of these showed degranulating changes. The intracytoplasmic granules occurred in the form of vesicles, so that the cells stained intensely near the peripheral margins (Figure 10). Mitosis of the chromophobe cells was not evident at this age. Majority of the chromophobe cells contained small amounts of cytoplasm which did not manifest affinity for any type of stain.

In the specimens obtained from dogs aged between 4 to 6 months, adrenocorticotrope cells and ICSH gonadotrope cells were evident in the parenchyma of the pars distalis. The former cells possessed an irregular outline and occurred as single cells, predominantly, in the midventral basophil area. They were scarce in the dorsolateral areas of the lobe. Their sizes were appreciably large. The nucleus of the cell was large and did not contain any nucleolus. The cytoplasm of the cell did not take any stain and appeared gray. A faint magenta coloration of the cell margin was evident in some of the cells that were stained with the periodic acid-Schiff procedure. The ICSH gonadotrope cells were very few in number. They were spherical in shape and were smaller in diameter as compared to the other cell-types. The differ-

ential cell count revealed the following proportion of the different cell-types:

	Female (percent)	Male (percent)
Somatotrope cells	46.3	50.0
Lactotrope cells	6.4	4.2
Thyrotrope cells	10.0	12.5
FSH gonadotrope cells	3.0	2.0
ICSH gonadotrope cells	0.5	
Adrenocorticotrope cells	0.7	1.0
Chromophobe cells	33.0	30.0

Group IV: 6 to 11 months   The structure of the parenchyma was akin to that described previously. ICSH and FSH gonadotrope cells were more numerous during this period. The former cells were mainly confined to the midventral zone predominated by other basophil cells. The ICSH gonadotrope cell was comparatively smaller in size and spherical to oval in outline. The nuclei of the cells were small and were located eccentrically. Binucleated cells were frequently observed among them. The nucleus contained a single nucleolus. The cytoplasm of the cell was uniformly dense and individual secretion granules were not discernible. The granules stained red with periodic acid-Schiff and when the latter was applied in conjunction with orange G, the cells stained rose red, indicating an affinity for the Schiff reagent as well as orange G (Figure 12). In the azocarmine,

orange G and aniline blue technique, ICSH gonadotrope cells were stained purple. The somatotrope cells were smaller in size as compared to the earlier periods. The relative proportion of cells was as follows:

	Female (percent)	Male (percent)
Somatotrope cells	42.0	46.20
Lactotrope cells	10.4	8.50
Thyrotrope cells	6.0	6.6
FSH gonadotrope cells	8.3	5.2
ICSH gonadotrope cells	1.5	1.0
Adrenocorticotrope cells	1.1	3.0
Chromophobe cells	30.0	29.0

Cupping of different cell-types was much more pronounced at this stage. Lactotrope cells were involved in great many instances as compared to other cell-types.

Group V: 1 to 4 years During this period, the pars distalis adenohypophysis was densely packed with cells. Augmentation of the cell population masked their cord-like arrangement (Figure 15). Thin strands of collagen fibers were evident in the perivascular region (Figure 15). Within the parenchyma, these septa-like strands enclosed the small vessels and extended between the venules present at intervals. The reticular stroma appeared to be heavy (Figure 20). No appreciable alteration was observed in the network formed by the argyrophilic fibers (Figure 11). Colloid infiltration

of vessels showed progressive increase. Many of the affected vessels contained a fibrin network that stained blue with Mallory's phosphotungstic acid hematoxylin stain. Few hyalinized vessels were also evident in the parenchyma of the lobe.

In specimens collected from two-year-old animals, initial stages of colloid deposition within the cell-cords were observed for the first time. Small specks of periodic acid-Schiff positive material was observed in the centrum of the affected cords (Figure 21). In the cell-cords where deposition of colloid was not particularly heavy, Mallory's phosphotungstic acid hematoxylin stain revealed a fibrin network. Fibrinoid mass, simulating cavitation of the centrum, was also evident in some of the cell-cords. The cells were dispersed to the peripheral margin of the cords simulating a follicular or cyst-like arrangement. With the advancement of age, such colloid deposits were found to have increased in size and at 3.5 years of age, large sized deposits were observed. One type of colloid material stained intense red with periodic acid-Schiff, while colloid material in few other deposits stained purplish gray. Alcian blue at pH range of 0.2 to 0.4, stained the former type of deposits intense blue (Figure 27). At one stage degenerative changes in the lining cells were observed. Colloid deposition was commonly encountered along the ventral margin and was more

prevalent in the regions where majority of the cell population was formed by basophil cells. The dorsolateral zones, which were predominated by acidophil cells, did not contain such deposits.

At three years of age and beyond, colloid deposition was also observed in the interstitial spaces (Figure 26). These deposits were initiated by autolysis of the parenchymal cells. Usually the entire population of several adjacent cell-cords were involved in such degenerative changes. The cells lost their granular contents and stained less intensely. The outlines of these cells became nondiscernible and their cytoplasm became rarified. The latter appeared to become absorbed by the ground matrix. The nucleus of the cell was the last part to degenerate. Patches of such autolyzed cells were evident at different regions of the pars distalis adenohypophysis.

In such vacuolated areas, gelatinization of collagen was evident. The hyalinized mass occurred in the form of a dense ground matrix, that stained light blue with alcian blue. In this matrix pre-collagen type of fibrils was discernible (Figure 22). The fibrils stained with reticular stains and were periodic acid-Schiff positive. Cells were not evident in this matrix. In parts of these areas, colloid deposition was evident in larger quantities and the fibrils were completely masked. This interstitial

colloid stained purplish magenta with the periodic acid-Schiff reaction instead of intense red.

There was little change in the relative proportions of the various types of cells. Cupping of the cells was very conspicuous. All cell-types were involved in this phenomenon. The number of chromophobe cells was comparatively less. Majority of them was spherical in outline and contained some cytoplasmic mass. Very few degranulated chromophil cells were observed. The relative proportion of the cell-types consisted of the following:

	Female (percent)	Male (percent)
Somatotrope cells	40.0	46.8
Lactotrope cells	11.7	8.2
Thyrotrope cells	10.0	8.0
FSH gonadotrope cells	8.0	6.3
ICSH gonadotrope cells	1.3	1.5
Adrenocorticotrope cells	2.0	2.6
Chromophobe cells	27.0	26.0

Group VI: 4 to 7 years Specimens from dogs aged 4 to 7 years, revealed no major change in the histomorphological structure as compared to that of the previous group. Colloid deposits, within the cell-cords, showed augmentation in number as well as in size. The entire parenchyma contained such deposits (Figure 26). Hyalinization of the capillaries was encountered more frequently. Increase in

the thickness of the basement membrane was also evident in the pericapillary region (Figure 24). FSH gonadotropic cells were observed in larger proportions. Pyknotic changes in the ICSH gonadotrope cells were evident. Individual members in a group of such cells lost their outlines and their cytoplasm assumed a homogeneous appearance. The staining of such cells was very intense (Figure 24). The thyrotrope cells were relatively numerous and contained few intracytoplasmic granules thinly distributed in their cytoplasm. Cells with vesicular or floccular type of granules were rarely encountered. The relative proportion of the different cell-types was as follows:

	Female (percent)	Male (percent)
Somatotrope cells	40.0	48.6
Lactotrope cells	9.0	8.5
Thyrotrope cells	12.0	10.0
FSH gonadotrope cells	8.0	5.0
ICSH gonadotrope cells	2.0	2.0
Adrenocorticotrope cells	1.5	2.7
Chromophobe cells	27.2	23.0

In this age-group, two specimens had been treated with 2.5 percent trichloroacetic acid before being fixed in the mercury-formal. In both specimens cell-types that were not affected were the somatotrope and the ICSH gonadotrope cells (Figure 25). All other categories of cells were

completely degranulated. The outlines of the cells and the nuclei were only evident. Distinct nucleoli were also not discernible. The lactotrope cells no longer showed any affinity for periodic acid-Schiff and stained weakly with orange G. In the luxol fast blue and the trichrome technique, erythrosin staining of these cells was not present. The cells stained light green in contrast to the dark blue-green coloration of the somatotrope cells. Extravascular as well as intravascular colloid was not affected due to treatment with trichloroacetic acid.

Group VII: 7 to 10 years During this interval, a significant increase in the amount of collagen was observed. Dense bands of collagen circumscribed the vessels and then extended to adjacent vessels forming distinct interlobular septa. Fibroblasts were numerous along these collagen fibers. That this connective tissue proliferation was of interstitial origin was evident from the fact that the external capsule of the hypophysis did not contribute to it and was often separable from the organ. Septa were also thicker near the margin of the pars cava infundibuli as compared to the external margin. Both reticular and argyrophilic fibers showed increase in density and intricacy.

Small colloid deposits were evident in the majority of the cell-cords; rarely did their size appear larger than a small avoid. In one specimen, two large colloidal-cysts

were encountered in the pars distalis adenohypophysis (Figure 27). In some of the cell-cords, colloid material stained intense red with periodic acid-Schiff, while in others it stained dull red in its central part only. The peripheral margins of such deposits usually stained blue with alcian blue, suggestive of sulfuration of the material due to ageing (Figure 24). Intravascular colloid, however, lost its staining intensity on ageing and instead of being sulfureted, appeared to become absorbed (Figure 27).

In many parts of the pars distalis adenohypophysis, atrophy of the vascular walls was evident (Figure 33). In these instances, infiltration of colloid into the vessel wall was not very conspicuous. But the entire endothelial lining degenerated leaving only the perivascular bands of collagen fibers. In specimens where vessels of the mantel plexus showed complete occlusion and hyalinization (Figure 56), such degenerative changes of vessels in the parenchyma of the pars distalis were very conspicuous.

Between 7 to 10 years of age, ICSH gonadotrope and lactotrope cells were rarely encountered in the specimens examined under this group. Somatotrope and thyrotrope cells manifested no appreciable change in size or morphology than those of the previous group. In many areas, groups of lactotrope cells undergoing pyknotic changes were observed. The cells were no longer identifiable as individual entities.

Their cytoplasm stained densely and appeared homogeneous. The percentage of different cell-types, during this interval, was as follows:

	Female (percent)	Male (percent)
Somatotrope cells	39.6	49.3
Lactotrope cells	5.0	6.6
Thyrotrope cells	14.3	10.2
FSH gonadotrope cells	7.2	6.1
ICSH gonadotrope cells	1.0	2.0
Adrenocorticotrope cells	1.8	2.1
Chromophobe cells	31.0	23.4

<u>Group VIII</u>: <u>10-14 years</u> Eight specimens were examined during this interval. The oldest animal employed for this study was 13.6 years. Unlike the previous intervals, some variation within the group as regards to the morphology of the pars distalis adenohypophysis, was observed. In all the specimens examined, the cavum hypophysis appeared much narrower, which was very conspicuous towards the rostral extremity of the organ. In two specimens collected from 10-year-old animals, intermittent adhesions between the pars distalis and the pars intermedia adenohypophysis were observed (Figure 29). It was usually the pars intermedia cells that migrated into the former and were found intermingled with its parenchymal cells. In four other specimens, obtained from animals aged between 11 to 13.6 years, the

cavum hypophysis was completely obliterated and the two lobes were in direct contact with each other. Intermixing of the cells between the two lobes was conspicuous (Figure 29). Capillaries often extended from one lobe to the other and in some instances were also observed to penetrate the pars intermedia and extend out into the pars distalis neurohypophysis.

In all specimens, colloid deposits in the form of small spherical masses were evident in the centrum of most of the cell-cords (Figure 34). Their sizes were not very large. In many of them, a peripheral zone that stained blue with alcian blue and was periodic acid-Schiff negative, formed a sharp contrasting picture with the centrally located intense periodic acid-Schiff positive zone. In two specimens, colloid deposits in the form of large cysts were encountered near the inner and dorsal margin of the lobe. In four of the specimens, large cysts filled with colloid material were encountered. These were of immense proportions (Figure 39). Usually a single cyst was observed in each specimen. The cysts were lined with large cuboidal cells which possessed long cilia (Figure 40). In one specimen, several layers of chromophobe cells formed the lining of the colloid-cyst (Figure 38). During this interval, appreciable increase in the interstitial connective tissue stroma was evident in all the specimens (Figure 32). Thick band of collagen

fibers traversed the parenchyma and subdivided into distinct lobules. Due to excessive development of this tissue, individual cell-cords were clearly delineated in the parenchyma.

The stroma was especially heavy along the margin of adhesion between the pars distalis and the pars intermedia adenohypophysis (Figure 52). The external capsule of the hypophysis was excessively heavy at this age, but did not contribute to the interstitial growth of connective tissue (Figure 28). The reticular framework was heaviest as compared to all other previous intervals (Figure 37). In many places, it formed dense networks around the capillaries. The network of argyrophilic fibers was also found to be comparatively much heavier. Many dense plexus-like arrangements were observed around the blood vessels.

The severity of degenerative changes, evident in the blood vessels, varied from specimen to specimen and from one region of the lobe to the other. In all specimens, the dorsolateral areas were comparatively less affected. Large number of somatotrope cells were encountered in these regions (Figure 36). The region that was most affected in almost every specimen was the midventral part predominated by the basophil cell. In this region, the blood vessels were hyalinized or completely occluded. The patent vessels showed degenerative changes in their endothelial lining

and the perivascular connective tissue fibers were no longer discernible. In its place, a thick basement membrane was evident around these vessels.

During this interval, chromophobes formed major part of the cell population. In addition to widely scattered chromophobe cells, three large aggregated masses of such cells were evident (Figure 30). The largest of these occupied the midventral region while the other two were located in the dorsolateral zones. Somatotrope cells formed the major portion of chromophil cells. The somatotrope cells showed no appreciable alteration in their size (Figure 34). There were comparatively few lactotrope cells at this period. They were mostly present in groups of 3 to 4 cells and were relatively larger in size (Figure 35). Thyrotrope and FSH gonadotrope cells were comparatively larger in size (Figure 35). Both types of cells appeared to possess few intracytoplasmic granules and stained less intensely. The former type showed a numerical increase as compared to middle age groups. ICSH gonadotrope and adrenocorticotrope cells were difficult to be revealed. Their numbers were small enough not to form a measurable part in the relative proportion of different cell-types. The average percentage of different cell-types, during this age-interval was as follows:

	Female (percent)	Male (percent)
Somatotrope cells	37.2	40.0
Lactotrope cells	2.9	3.7
Thyrotrope cells	12.9	10.8
FSH gonadotrope cells	2.7	1.4
ICSH gondadotrope cells		1.0
Chromophobe cells	44.1	43.0

In many areas of the lobe, cells in different stages of autolysis were observed (Figure 33). In some of the specimens, large areas of empty spaces were evident; these areas contained remnants of blood vessels and cell-cords (Figure 33). Vacuolated cells were rarely encountered in the parenchyma of the pars distalis adenohypophysis.

Metachromasia of the sections (stained by the modified methyl violet method (Pearse, 1968a) for amyloid revealed a positive reaction at all ages. The color of the intravascular as well as the extravascular colloid material was purple red (Figure 26), and was different from the staining of the basophil cells with crystal violet.

Statistical evaluation

Chi square test was used to evaluate data obtained from cytometry. As many of the cells contained observations whose expected frequencies were less than 5, several columns were combined to overcome this difficulty. The results

indicated that the differences observed in the percentage of various cell-types were not statistically significant. Data on the volume of the lobes and cytoplasm-nuclear ratio did not also reveal any significant difference among the various age-groups.

Pars paraneuralis The pars paraneuralis was very well developed in newborn pups. It formed the wide junctional zone between the pars intermedia and pars infundibularis adenohypophysis. The entire zone appeared segmented into columns and strands of cells due to permeation by the ramifications of the cavum hypophysis (Figure 41). The columns were attached to the pars cava infundibuli and their caudal extremities remained free in the cavum hypophysis (Figure 41).

The cells of this zone were columnar in shape. They remained densely packed together and the outlines of the cells were not discernible. The nuclei were spindle-shaped and contained dense chromatin material. Mitotic figures were observed among these cells. Cells with bilobed and double nuclei were also evident. Very few thin-walled capillaries were present in the central part of the columns. Neither collagen nor reticular fibers were observed in this zone. However, a dense network of intense argyrophilic fibers were observed. These fibers were especially intricate around the blood vessels.

In subsequent days of postnatal life, the pars paraneuralis increased in volume. Mitoses of the cells were evident in appreciable measures between 6 to 20 days of age. Thereafter, cells undergoing mitosis were rarely encountered. Mitosis of the cells was predominantly evident along the free margin of the cell columns that projected into the cavum hypophysis (Figure 58). Neither an increase in the vascularity nor in the connective tissue stroma of the zone was observed.

In specimens collected from animals aged 4 to 8 weeks, mitosis was observed in very few cells of the pars paraneuralis. The number of spindle-shaped undifferentiated cells was less while that of chromophobic cells was high. Mitosis was limited to the undifferentiated cells. Blood vessels surrounded by thin strands of collagen and reticular fibers were evident in the central part of the cell-columns. The latter were comparatively smaller in size and appeared less branched. In subsequent stages, the columns of cells decreased in size and remained close together due to decreased ramification of the cavum hypophysis.

Colloid infiltration of the vessels was observed at 6 months of age. The vessels located in the dorsal region of the zone were involved to a greater extent (Figure 43). During this period, a periodic acid-Schiff positive ground substance was also evident around the vessels. Collagen and

reticular fibers increased in quantity to form discernible connective tissue strands which extended along the blood vessels. Mitosis was still evident in the cells of the pars paraneuralis.

Between 6 to 12 months of age, a major part of the cells appeared akin to chromophobe cells of the pars distalis adenohypophysis. Spindle-shaped undifferentiated cells were less numerous and formed a cellular lining along the ramifications of the cavum hypophysis. The latter were less numerous and the cell-columns were continuous with each other (Figure 42). In addition to the chromophobe cells two other cell-types were present in the pars paraneuralis. Cells that stained blue with alcian blue, and blue black with aldehyde-thionin and aldehyde-fuchsin, were observed among the chromophobic cells of the pars paraneuralis. These cells were identical in appearance to the thyrotrope cells of the pars distalis adenohypophysis. They were comparatively larger in size and occurred as single cells (Figure 43). The second type was composed of cells that stained intense red with periodic acid-Schiff (Figure 42). These cells occurred alone or in groups of several cells. These cells were akin to the FSH gonadotropic cells of the pars distalis adenohypophysis.

Deposition of colloid in the central part of groups of such parenchymal cells was evident at six months of age

(Figure 42). Cell-clusters, entirely composed of chromophobic cells, also showed such colloid deposition.

Between 1 to 4 years of age, the thyrotrope cells showed an appreciable increase in size as well as number. Majority of the cells was oriented along the course of blood vessels and few were found among the cells that lined the cavum hypophysis (Figure 45). There was no significant increase in the number of periodic acid-Schiff positive cells. Specimens collected from male animals, contained few thyrotrope cells in the pars paraneuralis as compared to that of the female. Large areas of colloid deposits were present at different regions of the pars paraneuralis (Figure 45). In specimens obtained from dogs aged 4 to 7 years, the pars paraneuralis was much smaller in size. Fissures in its parenchyma were rarely encountered. The entire zone was directly continuous with the parenchyma of the pars distalis adenohypophysis and separated the latter from the pars cava infundibuli (Figure 46). Thyrotrope cells formed a substantial portion of the cell population of this zone. Few periodic acid-Schiff positive cells were observed at this age. The number of chromophobe cells was small. Colloid was observed in the centrum of most of the cell-columns. Parenchymal cells formed a single cellular lining around these deposits. The latter never assumed large proportions.

In the two specimens treated with 2.5 percent

trichloroacetic acid, neither the thyrotrope cells nor the periodic acid-Schiff positive cells showed any coloration. All cells appeared highly degranulated. The outlines of cells and those of the nuclei were only evident. Distinct nucleoli were not discernible in these cells. Colloid accumulations were not affected in any form.

Between 7 to 10 years of age, the pars paraneuralis was no longer evident as a separate zone. It was inconspicuously merged with the rostral end of the pars distalis adenohypophysis (Figure 46). There was an appreciable increase in the connective tissue stroma and in the number of colloid deposits. Most of the parenchymal cells appeared smaller in size and stained very weakly. Intracytoplasmic granulation was not discernible in the thyrotrope cells. The pars paraneuralis was distinctly separated from the pars cava infundibuli by a dense connective septum. The latter was mostly composed of periodic acid-Schiff negative matrix and few connective tissue fibers (Figure 46).

Out of 8 specimens examined from the 10 to 14 year age group, 3 specimens contained large colloid cysts that occupied the entire pars paraneuralis and extended into the pars distalis adenohypophysis as well. The cysts were entirely filled with colloid which stained intense red with periodic acid-Schiff. The lining of these cysts was formed

by a single layer of cuboidal ciliated cells (Figure 47). In the otherwise normal specimens, majority of the cells of this zone was chromophobic in nature. The thyrotrope cells were fewer in number and contained few intracytoplasmic granules. These cells stained very weakly (Figure 46). The entire zone was ill-defined due to merger with the pars distalis adenohypophysis and contained thick bands of interstitial connective tissue fibers.

Pars infundibularis adenohypophysis In the specimens collected from pups of 8 hours postnatal age, the pars infundibularis adenohypophysis was only confined to the radix infundibuli. It was composed of groups of undifferentiated cells arranged in the form of follicles (Figure 53). Usually several layers of cells were compactly arranged on the walls of these follicles. Large numbers of fibroblast cells were distributed among the follicles. Minute capillaries coursed through the interfollicular spaces. Neither reticular fibers nor collagen fiber bundles were observed at this stage. Argyrophilic fibers were present around the capillary vessels.

In specimens collected from puppies, 14 days of age, the pars infundibularis adenohypophysis presented an identical structure as the previous stage. The only conspicuous change was the increase in the number of cells in each follicle. There was an increase in the number

and size of the vessels also. Few arterioles were evident in this region and each of these vessels was accompanied by its satellite vein.

In specimens obtained from dogs between 4 to 8 weeks of age, few spherical cells were observed among the undifferentiated cells of this zone (Figure 59). The former contained large nuclei and small volumes of cytoplasm. They did not show any affinity for acid fuchsin, aldehyde-fuchsin of periodic acid-Schiff. Mitosis was still evident in the undifferentiated cells during this period. Many small arteries and arterioles were evident in the external region of the pars infundibularis. Structurally, these vessels resembled those of the systemic circulation. Between 2 to 6 months of age, little alteration was observed in the structure of the pars compacta infundibuli.

Between 6 to 12 months of age, connective tissue fibers increased in the perivascular regions and also between the follicles. The parenchymal cells were larger in size, but still appeared as undifferentiated cells. They resembled the chromophobe cells of the pars paraneuralis and exhibited a definite tendency to become arranged in the form of large follicles. In specimens collected from beagles between 1 to 4 years of age, undifferentiated cells were no longer evident in the follicles of the pars compacta infundibuli. The cells resembled chromophobe cells of the pars distalis

adenohypophysis and those of the pars paraneuralis. Strands of collagen and reticular fibers were evident between the follicles. Colloid deposition in these follicles was observed during this age interval and progressed with age.

There was no appreciable change in the histological structure of the pars infundibularis adenohypophysis, in specimens collected from 4 to 7 year old dogs. Increase in thickness of the basement membrane in the perivascular region and increase in the interstitial connective tissue was appreciable. In the two specimens treated with 2.5 percent trichloroacetic acid, the cells appeared as highly degranulated cells, the plasma and the nuclear membranes being the only parts discernible. Colloid was unchanged in its staining affinity. During the age interval of 7 to 10 years, the entire zone of pars compacta infundibuli exhibited degenerative changes. Majority of the interstitial vessels had been occluded due to colloid infiltration. Hyalinization and fibrosis of these vessels were extensive. The cells were no longer evident in the form of follicular masses, but appeared as groups of pyknotic cells. The nucleus was the only part of the cell that was clearly discernible in the sections. Increase in the size of the intrafollicular colloid deposits was not conspicuous. The entire zone appeared to be formed only by the interstitial stroma.

In all the specimens collected from dogs 10 to 14 years of age, the appearance of the pars infundibularis adenohypophysis simulated a thick mass of connective tissue fibers (Figure 61). All interstitial blood vessels were hyalinized and there was extensive proliferation of connective tissue fibers. The parenchymal cells were no longer evident and remnants of a few of these were present in the vicinity of the colloid deposits.

Pars intermedia adenohypophysis At birth the pars intermedia consisted of a dense mass of cells with poorly defined outlines. The width of the lobe varied from region to region. It was more extensive in the ventral half than in its doral half. The parenchymal cells possessed large spindle-shaped nuclei, which contained dense masses of chromatin granules (Figure 48). These resembled the undifferentiated cells of the pars paraneuralis and like the latter occurred in large groups. Mitosis was frequently observed in these cells (Figure 68). The cells showed no affinity for any type of stain. In addition to these undifferentiated cells, small groups of chromophobic cells were also present. This type of cells contained large vesicular nuclei and variable masses of cytoplasm. Secretion granules were not discernible in these cells and they stained comparatively lighter than the undifferentiated cells (Figure 48). Blood vessels were not present among

the parenchymal cells. Few capillaries and a fibrocellular layer were present at the junctional zone between the pars distalis neurohypophysis and the pars intermedia.

Rostrally, the pars intermedia merged imperceptibly with the pars paraneuralis and the pars distalis adenohypophysis. Ramifications of the cavum hypophysis penetrated into the parenchyma of the lobe and divided the cells into columns. Caudally it was bilaterally reflected on the caudal extremity of the pars distalis adenohypophysis.

At 14 days of postnatal life, two types of cells were discernible in the pars intermedia. The chromphobe cells occurred in groups of 2 to 6 cells. These cells had distinct outlines and possessed large vesicular nuclei that contained a single nucleolus in each. The cells stained magenta with periodic acid-Schiff and pink with aldehyde-fuchsin (Figure 49). The second category of cells represented the undifferentiated cells, and they were the parent cell-type present in the lobe. Multiplication of these cells through mitosis contributed to the increase in the cell population of the pars intermedia. Mitosis was mostly limited to cells which formed the lining of cell columns that projected into the cavum hypophysis. There was no increase in the vascularity of the lobe. The cells were densely packed together and connective tissue fibers were not observed in between the cells.

Between 4 to 8 weeks of postnatal age, the number of chromophobe cells increased progressively in the pars intermedia (Figure 49). Mitosis was still evident in the undifferentiated cells. The differentiated cells appeared in the form of sharply contrasted groups in the midst of the undifferentiated cells. Their size increased progressively and their cytoplasm stained more and more intensely with the advancement of age. Granules were not discernible in these cells.

At 2 months of age, the cell columns of the pars intermedia, that projected into the cavum hypophysis, were less conspicuous. The number of differentiated cells was much higher than those from the previous age-interval. The cells located near the junctional zone with the pars distalis neurohypophysis were comparatively larger in size and stained more intensely than the cells located towards the periphery. During this period colloid deposition was first observed in the parenchyma of the pars intermedia. At the initial phase, the centrum of the cell cluster became homogeneous and the cells were dispersed along the peripheral margin of the cluster. Subsequently, the central part became filled with colloid material. The latter stained intensely with periodic acid-Schiff (Figure 50). Some of the colloid deposits, probably of longer duration, stained purple with the periodic acid-Schiff. Colloid deposition was character-

istically encountered at the junctional zone with the pars distalis neurohypophysis. In many instances, proliferation of the pars intermedia cells located in the vicinity of the colloid follicle was observed. As a result of such proliferation, cells of the pars intermedia protruded into the pars distalis neurohypophysis (Figure 67).

In specimens collected from 6 to 12 month old dogs, colloid follicles were encountered in large numbers. The cells which formed the walls of such follicles stained magenta with periodic acid-Schiff and were larger in size than normal parenchymal cells. Columns, made up of such differentiated cells and containing colloid follicles, extended into the parenchyma of the pars distalis neurohypophysis. The entire pars intermedia was comparatively more extensive than that of the previous age-intervals.

Three types of cells were present in the parenchyma of the pars intermedia at this age. The undifferentiated cells were spindle-shaped and contained no distinct outlines. They were predominantly found in the rostral extremity and usually formed the lining of the cavum hypophysis. Mitosis was no longer observed in these cells. The chromophobic cells constituted a major part of the cell population. They were oval or spherical in shape, but had ill-defined outlines. They always occurred in groups. Their nuclei were irregular in shape and contained a single nucleolus. The

cells stained light magenta with periodic acid-Schiff (Figure 49). In procedures where the latter was employed in conjunction with alcian blue, aldehyde-thionin or aldehyde-fuchsin, the cells stained weak purple (Figure 49). Better staining of these cells was obtained in the latter combination. Among the above category of cells, large oval or polygonal cells, which stained intense red with periodic acid-Schiff, were observed (Figure 51). Combination of periodic acid-Schiff with counterstains as aldehyde-thionin and alcian blue did not alter the staining affinity of these cells. They stained intense purple with aldehyde-fuchsin. These cells occurred in a single or grouped manner.

Between 1 to 4 years of age, little change was evident in the structure of the pars intermedia adenohypophysis. The entire lobe seemed to be twice as extensive as that observed in the 2 to 6 month age interval. The parenchymal cells extended into the pars distalis neurohypophysis forming distinct strands or columns. The latter invariably contained colloid follicles (Figure 68). Cells that occupied the vicinity of these follicles were greatly hypertrophied. During the age interval of 4 to 7 years, there was no change in the histology of the pars intermedia as distinct from that observed in the previous age-group. In two specimens, large accumulations of colloid were observed in the part that had protruded into the parenchyma

of the pars distalis neurohypophysis (Figure 70). No change was observed in the staining affinity of the cells (Figure 70). Trichloroacetic acid, in the strength of 2.5 percent, caused dissolution of the cytoplasm as well as nucleoplasm of the pars intermedia cells. Colloid material, contained in the cysts, remained unaltered by this treatment.

Between 7 to 10 years of age, the parenchymal cells of the pars intermedia showed extensive proliferation and invasion of the pars distalis neurohypophysis. In some specimens as much as one-third of the volume of pars distalis neurohypophysis was encroached upon by the former cells. The latter appeared very active and hypertrophy was evident from their size and intense staining character. Cells that were located within the limits of the pars distalis neurohypophysis exhibited such tendency to a greater extent than the cells that lined the cavum hypophysis. Mitosis of the latter cells was observed in all the specimens and number of cells undergoing mitosis was appreciable. During this interval, the pars intermedia was the only part that seemed very active in its function as compared to the previous intervals.

The pars intermedia adenohypophysis was very extensive in specimens obtained from 10 to 14 year old dogs. It formed adhesion of variable extent with the pars distalis adenohypophysis and there was considerable intermixing of

parenchymal cells from both lobes (Figure 52). At places, though, a thick connective tissue septum intervened between the two lobes. Invasion of the pars distalis neurohypophysis was very pronounced during this period. There was no increase in the vascularity or connective tissue stroma of the lobe. The parenchymal cells remained densely packed together as was the condition at birth (Figure 52). Two types of cells were still present in the parenchyma of the pars intermedia. One type stained light magenta with periodic acid-Schiff and the other stained red with the same procedure. Undifferentiated cells were rarely encountered beyond 10 years of age. Colloid deposits were larger in size and there was a numerical increase, also. Some of them appeared as cysts with a single layer of lining cells (Figure 52).

Neurohypophysis

Radix infundibuli   The youngest age at which specimens were available in the first age group was 8 hours postnatal. In these specimens, the radix infundibuli was only limited to a small zone situated on the floor of the third ventricle. The infundibular recess was very wide, as a result of which the two lips of the radix were widely separated from each other. The radix infundibuli was composed of a layer of prominent ependymal cells, a zona interna and a zona externa (Figure 54). The zonal arrangement of the fibers was quite

lucid due to different direction of the fibers and due to the fact that zona interna was highly cellular while zona externa contained very few cells. Stainable neurosecretory material was not discernible in the fibers of the zona interna (Figure 53). The mantel plexus was undeveloped. It contained a small number of the fine capillaries located between the zona externa and the pars infundibularis adenohypophysis. It extended caudally between the pars paraneuralis and pars cava infundibuli, and terminated at the junction of the latter with the pars distalis neurohypophysis. Fibroblast cells were numerous in this region, but connective tissue fibers were not present. A network of intensely stained argyrophilic fibers was observed in this zone.

In specimens collected from pups after 6 days of post-natal life, neurosecretory material was evident in the fibers of the zona interna. The neurosecretory material stained purple with the chrome alum hematoxylin and bright blue with alcian blue at low pH. Though its amount was scanty, neurosecretory material was widespread throughout the zone. During this period the zona interna and the zona externa appeared fully developed. Fiber bundles of the zona externa coursed through the former to assume their perpendicular direction, which made them very conspicuous (Figure 54). The mantel plexus contained large calibered capillaries which occurred in small groups. Some of these

vessels penetrated into the zones of the radix infundibuli. Thin strands of collagen and reticular fibers were evident around these vessels.

At four weeks of age, there was no appreciable change in the structure of the radix infundibuli. The zona interna contained large number of Herring bodies, and a large amount of neurosecretory material was also evident along the fibers. The zona interna and zona externa were clearly distinguishable. The former contained many neuroglia cells and pituicytes while the latter contained very few of them. Looped capillaries projected into the zona externa from the mantel plexus. The latter showed no major change in growth at this stage (Figure 59). In the perivascular region, a distinct basement membrane that stained red with periodic acid-Schiff was discernible at this stage. It was especially distinct in the proximal part of the radix infundibuli.

In specimens collected from dogs between 1 to 4 years of age, the radix infundibuli was very extensive. The two zones were broad and contained many tortuous capillaries. Stainable neurosecretory material was conspicuous in the fibers of the zona interna and Herring bodies occurred in large numbers. Colloid infiltration of the vessels located in the mantel plexus was first noticed during this interval. Only the veins were affected and complete occlusion of vessels was not evident.

Between 4 to 7 years of age, there was some decrease in the amount of the neurosecretory material carried by the fibers of the radix infundibuli. Herring bodies were also comparatively scarce. The parenchyma of the zones appeared dense due to accumulation of ground matrix and fibers of the zona externa showed degenerative changes. There was a progressive increase in the colloid infiltration of the walls of capillaries located in the mantel plexus and in the parenchyma of the radix infundibuli. Complete occlusion of the latter vessels was also evident (Figure 56). On the contrary, branches of the rostral hypophysial artery and satellite veins were unaffected.

In specimens treated with trichloroacetic acid, the fibers of the zona interna as well as zona externa were clearly discernible as individual fibers (Figure 55). Stainable neurosecretory material was not present in these fibers. However, Herring bodies were evident in the zona interna.

In the specimens from dogs between 7 to 10 years of age, very conspicuous alterations were observed in the structure of the radix infundibuli. The fibers of the zona externa appeared to undergo progressive degeneration. Connective tissue fibers extended from the region of the mantel plexus into the interstices of the fibers. Almost all the capillaries within the zone appeared hyalinized

(Figure 56). The zona interna was very cellular and contained no stainable neurosecretory material. Few Herring bodies were only present. The mantel plexus was completely masked by excessive proliferation of the interstitial connective tissue. All the vessels showed hyalinization and fibrosis. Some of the arterial branches were relatively free of such degenerative changes.

Between 10 to 14 years of age, the radix infundibuli showed no additional changes in its zones. Herring bodies were comparatively less numerous and stained very weakly. The mantel plexus was no longer evident as a vascular zone. A dense mass of connective tissue fibers and matrix was present in the part subjacent to the zona externa (Figure 61).

Pars cava infundibuli At birth, the recessus infundibuli was very wide and extensive, due to which the two lips of the pars cava infundibuli were widely separated from each other. They united at the middle part of the organ to form the pars distalis neurohypophysis. The pars cava infundibuli consisted of an identical structure as the radix infundibuli. The zona interna was comparatively much wider in the former than in the latter.

Neurosecretory material was first observed among the fibers of the zona interna of the pars cava infundibuli in pups 6 days of age. The amount of neurosecretory material

was much less. Within the zona interna, the neuroglia cells were comparatively less numerous at this stage. Many capillaries, some of which were looped, were evident within the zona externa as well as in the zona interna. During subsequent age-intervals, the amount of neurosecretory material increased substantially in the fibers of the zona interna. There was a higher incidence of Herring bodies in the pars cava infundibuli than in the region of the radix infundibuli (Figure 59). Neurosecretion bearing fibers were found in the vicinity of the capillaries that lined the zona externa, which was especially prevalent at the junctional zone with the pars distalis neurohypophysis. Termination of such fibers was also noticed in the vicinity of the ependymal cells.

During subsequent stages, the pars cava infundibuli increased in size. The zona externa as well as the zona interna was comparatively much thicker at 10 months of age than they were beforehand. Neurosecretory material that could be revealed by staining increased in quantity. Herring bodies were discernible in large numbers (Figure 60). In the junctional zone with the pars paraneuralis, connective tissue stroma increased progressively with age. Colloid infiltration of the vessels of this zone also became evident with greater incidence as specimens from progressively aged animals were examined. Thickening of the

perivascular connective tissue was also progressive with the advancement of age.

In specimens collected from 7 to 10 year old dogs, the pars cava infundibuli manifested identical structural alterations as the radix infundibuli. The zona externa was invaded by interstitial connective tissue and contained very few neurohypophysial fibers (Figure 61). Capillaries located within the parenchyma were completely hyalinized and fibrosed. The zone was separated from the underlying pars paraneuralis by a dense zone of periodic acid-Schiff negative matrix and precollagen type of fibrils (Figure 61). The zona interna contained no stainable neurosecretory material and Herring bodies were rarely observed.

Between 10 to 14 years of age, the same structural changes described previously continued to be enhanced with age. The zona externa appeared much smaller in size and the zone of connective tissue between it and the underlying pars paraneuralis became progressively wider (Figure 61). Vessels with patent lumen were not evident in this region during this interval.

Pars distalis neurohypophysis In the specimens collected from pups 8 hours postnatal age, the pars distalis neurohypophysis formed the most conspicuous lobe. It increased in size in the rostrocaudal direction and was bulbous at its caudal extremity. It consisted of large

number of irregularly arranged unmyelinated fibers. The fibers contained neurosecretory material in small quantities so that faint coloration of the fibers was only evident in the chrome alum hematoxylin as well as Sloper's alcian blue technique (Figure 62). Large numbers of neuroglia cells and pituicytes were evident among the fibers. The latter possessed larger nuclei than the neuroglia cells. Mitosis was not observed in these cells. The entire lobe appeared much more cellular than fibrous (Figure 62).

The parenchyma of the lobe contained four small arteries and their satellite veins. The arteries were evident bilaterally at equal intervals. These represented branches of the caudal hypophysial arteries. In the rostral half of the lobe, the vessels were of smaller caliber. Few capillaries were also present in the parenchyma of the lobe at this age. The capillaries were formed by a layer of endothelium reinforced externally by a single layer of fibroblast cells. Throughout the lobe, fibroblast cells were plentiful in the perivascular zones.

During subsequent days of postnatal life, the pars distalis neurohypophysis manifested little alteration in its structure. The number of small arteries and veins decreased sharply and that of capillaries increased progressively. Looping of these capillaries was observed in the central as well as peripheral regions of the lobe. At the junctional

zone between the pars intermedia and pars distalis neurohypophysis, a well developed vascular plexus was located. Beginning with the 14th day of postnatal life, neurosecretory material became discernible in the pars distalis neurohypophysis in greater quantities. Fibers located near the junctional zone with the pars intermedia contained a denser accumulation of the neurosecretory material as compared to the fibers located in the central part of the lobe.

In specimens obtained from dogs 4 to 8 weeks of age, the pars distalis neurohypophysis contained reduced number of cells and an increased number of neurosecretion bearing fibers (Figure 63). Neurosecretory material was denser towards the terminal portions of the fibers (Figure 63). Large numbers of Herring bodies were present among the nerve fibers. They were variable in shape and size. Individual globular masses of neurosecretory material was discernible within the Herring bodies.

The number of capillaries contained in the parenchyma of the lobe showed an appreciable increase at this time. These were thin-walled vessels, reinforced externally by collagen and reticular fibers (Figure 63). Looping of these capillaries was evident in the peripheral regions of the lobe. At the rostral and caudal extremities venules and small veins were evident extending to the exterior of

the organ. Periodic acid-Schiff positive basement membrane was evident in the perivascular regions and colloid infiltration of the vessels was extensively observed. Many of the capillaries were completly occluded with colloid. Between 2 to 6 months of age, little alteration was observed in the preceding structural pattern of the pars distalis neurohypophysis.

Between 6 to 12 months of age, fibers located within the parenchyma of the pars distalis neurohypophysis contained major quantities of stainable neurosecretory material (Figure 64). Both Gomori's chrome alum hematoxylin and alcian blue at low pH elucidated the neurosecretory material contained within the fibers. In female animals, depletion of the neurosecretory material was evident in many regions of the lobe during the interval (Figure 65). Colloid infiltration and hyalinization of vessels were especially conspicuous in the peripheral zone. The parenchymal cells of the pars intermedia adenohypophysis that were located in the vicinity of such occluded vessels showed hypertrophy. They appeared to encircle these vessels and extend out into the parenchyma of the pars distalis neurohypophysis. Infiltration of parenchymal cells from the pars intermedia was observed to be progressive with age (Figure 67).

In all the specimens, Bielschowsky's technique revealed two types of nerve fibers (Figure 66). In addition to the

normal neurohypophysial fibers, coarse bundles of nerve fibers were evident in the perivascular regions. The fibers extended towards the caudal extremity of the lobe, arborizing progressively towards the periphery of the pars distalis neurohypophysis. This type of argyrophilic fibers was distributed widely throughout the lobe. Neurosecretory material was not discernible in these fibers.

Between 1 to 4 years of age, the volume of the pars distalis neurohypophysis decreased due to progressive invasion by the pars intermedia cells (Figure 68). Many Herring bodies were present in the central part of the lobe. Neurosecretory material was evident along the course of the fibers. The number of large arteries had decreased further, while capillaries were more numerous (Figure 67). Perivascular connective tissue was present in an appreciably increased quantity. Colloid infiltration of the vessels also showed augmentation with age.

In specimens treated with 2.5 percent trichloroacetic acid, no major alterations in structure were noticed. In contrast to the fibers of the radix infundibuli which lost all stainable neurosecretory material due to the treatment, neurohypophysial fibers, contained within the parenchyma of the pars distalis neurohypophysis, retained their neurosecretory material. Herring bodies, located in the parenchyma of the lobe, were also not affected by the

trichloroacetic acid treatment.

The most conspicuous change observed in the pars distalis neurohypophysis of dogs 7 to 10 years of age was a decrease in its relative volume by almost one-third to half of its original volume. The entire peripheral zone was occupied by proliferating strands of intermedia cells. The latter exerted an atrophic influence upon the neurohypophysial fibers. Masses of the latter enclosed between two adjacent invading cell-columns showed degenerative changes. The fibers lost their staining character and usually appeared as rarified masses (Figure 68). Herring bodies located in these parts usually showed pregressive depletion.

All the major vessels located within the parenchyma of the pars distalis neurohypophysis showed great increase in the perivascular basement membrane (Figure 71). The latter stained light blue with alcian blue and contained pre-collagen type of reticulum. Collagen fibers were also numerous around these vessels (Figure 72). Extension of the collagen fibers between the larger vessels and along the course of capillaries formed distinct septa-like structures (Figure 72). In the peripheral region, deposition of colloid on the wall of capillaries, contained among these fibers, simulated a distinct periodic acid-Schiff positive septum. Almost all the veins were partially or completely occluded with colloid. Arteries showed no colloid infiltra-

tion on their walls (Figure 73).

Content of neurosecretory material, relative to the volume of the pars distalis neurohypophysis, showed no significant change during this period. Neuroglia cells appeared more numerous than pituicytes at this age.

In the specimens collected from dogs with ages between 10 to 14 years, the structure of the pars distalis neurohypophysis was identical to that observed during the previous interval. There was no appreciable decrease either in the content of neurosecretory material carried by the fibers or in the number of Herring bodies. Proliferation of interstitial connective tissue was appreciable.

Age-correlated Changes in the Porcine Hypophysis

Adenohypophysis

Pars distalis adenohypophysis

Group I: birth to 4 weeks The animals employed for this age group included one female which was 14 hours old. The youngest male utilized for the study was 3 days old. The porcine hypophysis consisted of all of its adult morphological subdivisions at 14 hours of age. The pars distalis adenohypophysis was distinctly boat-shaped and was more extensive on its rostroventral than on its dorsocaudal surface. The rostral extremity was substantially more voluminous than the caudal. The lobe was also more extensive on its right lateral half than on the left. A

wide cavum hypophysis separated the pars distalis from the pars intermedia adenohypophysis. The cavity was lined on both margins by a layer of undifferentiated or chromophobic cells. This cellular lining was not distinct in many regions. Rostrally, the lumen was comparatively narrower and several ramifications from it penetrated into the pars paraneuralis, pars infundibularis and pars intermedia (Figure 119). Caudally, the lumen followed the lines of reflection of the pars intermedia into the caudodorsal margins of the pars distalis adenohypophysis. This resulted in the caudal extremity of the latter remaining free within the lumen of the cavum hypophysis.

The parenchyma of the lobe consisted of dense masses of cells closely packed together (Figure 79). The parenchymal stroma was not very evident. The entire lobe presented an avascular appearance. Very few capillaries were evident, and these vessels coursed in a radiating manner along the centroperipheral axis of the lobe. The walls of these vessels were formed by a layer of endothelial cells which resembled mesenchymal cells. Their nuclei were large and oval in outline. The cells occurred at long intervals along the lumen. On the external side, the vessels were lined by a layer of fibroblast cells. The latter were distinctly large and spindle-shaped cells, whose cytoplasm was scanty; their cytoplasmic processes seemed to be in union with

each other. Deposition of collagen was not evident in any of these specimens. The only other connective tissue fiber observed among the cells was the precollagen type of fibers revealed by the resorcin-fuchsin and Van Gieson's technique (Figure 78). Such fibers occurred as very thin strands and were only evident in some regions of the lobe. In many instances, they encircled one or two parenchymal cells only. General reticular stains failed to reveal the presence of a reticular network at this age (Figure 81). However, Bielschowsky's ammonium silver technique elucidated an extensive network of strongly argyrophilic fibers which were especially intricate around the blood vessels (Figure 104). These argyrophilic fibers were widespread and were even present in areas where no stroma was discernible after resorcin-fuchsin staining.

Three different cell-types were observed in the pars distalis adenohypophysis during this stage. The somatotropes were the most numerous cell-type. They possessed distinct oval outlines with evident margins. They predominated the rostral and caudal extremities of the lobe and were more numerous on the inner margin along the cavum hypophysis than along the peripheral margin (Figure 79). The central area of the lobe was relatively free of these cells. The staining intensity of the cells was very dense, indicating the presence of intracytoplasmic granules (Figure 79). The

cells possessed tinctorial affinity for orange G and luxol fast blue. The second cell-type that also formed an appreciable number was the thyrotrope cell. The latter cells were also fully granular and were evenly distributed throughout the lobe being more numerous in the central and peripheral regions. In some locations they occurred in groups of isolated cells (Figure 79). They stained red with periodic acid-Schiff, blue black with aldehyde-thionin and aldehyde-fuchsin (Figure80), and purple blue with alcian blue (Figure 79). They stained dark blue with aniline blue. The granules of these cells occurred in the form of vesicles which were dense near the cell borders. Accumulation of the secretory material in the granular form was not observed in this cell.

The FSH gonadotrope cells were more numerous in the rostral half of the lobe than in the caudal. They were usually encountered in the central and extreme peripheral part of the lobe along the course of the blood vessels. They occurred in groups of several cells and possessed distinct polygonal outlines. Their cytoplasm contained large granules which were rather uniformly distributed throughout the cell. They stained red with periodic acid-Schiff and were stained magenta whenever the latter was employed in conjunction with such counterstains as alcian blue, aldehyde-thionin and aldehyde-fuchsin (Figure 80). They were the only cells

stained in periodic acid-Schiff-methyl blue-orange G procedure. They stained light blue with aniline blue. Resorcin-fuchsin colored the cells light red, while the thyrotrope cells stained intensely with the same dye. Lactotrope, adrenocorticotrope and ICSH gonadotrope cells were not observed in these specimens.

The chromophobe cells appeared to be less numerous and were plentiful in the rostral half of the lobe. The caudal part of the lobe contained more chromophils than chromophobe cells. The cells contained no stainable cytoplasm. Their nuclei were much larger in size and contained a heavy mass of chromatin material. The peripheral margin of the lobe and areas adjacent to the capillaries contained many chromophobe cells.

By the third day, a thin poorly developed reticular framework was evident in the stroma of the pars distalis. The reticulum was not extensive towards the lateral circumference of the lobe. Collagen fibers were not discernible during this period and the chromophil cells appeared to be more numerous, and many cells formed small clusters in the peripheral parts of the lobe as a prelude towards cord-like or follicular arrangement. The long capillary vessels seemed to have ramified as a result of which there was a numerical increase in the vascularity of the lobe. Some of these vessels contained small plaques of colloid on their walls.

There was no change in the cellular population except for an increase in the number of cells, especially in the FSH gonadotrope category. The relative proportion of the different category of cells was as follows:

	Female (percent)	Male (percent)
Somatotrope cells	44.9	41.4
Thyrotrope cells	14.9	9.3
FSH gonadotrope cells	18.7	24.6
Chromophobe cells	21.0	24.0

In specimens collected from 7-day-old pigs, the parenchymal cells appeared to have been arranged into groups. Those at the caudal extremity and lateral margins showed definite follicular or cord-like arrangement. The stroma showed enrichment with further development of reticular framework and argyrophilic fibers. Thin bands of collagen fibers had reinforced the wall of the vessels and fibroblast cells were smaller in size and were less numerous. The vessels had become still smaller in length and the vascularity of the lobe showed appreciable increase. The vessels occurred as thin-walled capillaries with endothelial and collagenous linings.

An increase in the number of cell-types was conspicuous at this stage. The entire pars distalis appeared to contain somatotrope cells evenly distributed with groups of FSH gonadotrope cells forming well discernible islands among

them (Figure 82). Sections stained with luxol fast blue and trichrome technique revealed the presence of erythrophilic cells in the pars distalis adenohypophysis. Adjacent sections stained with azocarmine, orange G and aniline blue failed to elucidate the same cells which indicated that the erythrophilic cells were probably adrenocorticotropic cells. They were observed in the hypophysis of the male pig and their number was far less as to form a measurable part of the cell population. These cells possessed ill-defined outlines and contained erythrophilic granules very thinly distributed within the cytoplasm.

By the fourteenth day, the pars distalis adenohypophysis presented a morphological pattern of well organized parenchyma. The cells were found in the form of definite cell-cords. The stroma was predominantly collagenous with a well developed network of argyrophilic fibers. The reticular fibers were still poorly developed and were mainly confined to the central part of the lobe. The blood vessels occurred in the form of numerous minute capillaries, intervening between the cell-cords. Few medium-sized veins were evident at the rostral and caudal extremities of the lobe, while in its central part many large calibered capillaries were evident between the cell-cords. These large-sized vessels seemed to have small tributaries all around them and were interconnected with four to six adjacent vessels of

identical caliber through the tributaries. Vessels resembling arterioles were not observed. Vessels of every caliber were made up of an inner endothelial layer and an external layer of thin collagen. The latter varied in thickness depending upon the size of the vessel and continued along the fine tributary capillaries to adjacent vessels.

The large capillaries and venules showed deposition of colloid on their endothelial lining (Figure 82). The endothelium was found intact in all the vessels that presented colloid infiltration. The colloid occurred in the form of a dense homogeneous mass applied closely to the endothelial lining. It stained intense red with periodic acid-Schiff and was strongly orangeophilic. Vessel walls infiltrated to minor degrees showed accumulations in the form of crescentic masses of diagonally opposite sides (Figure 82). The union points with the finer tributaries were occluded in all cases and appeared as the foci of infiltration. At many locations, the fine tributary branches were filled with colloid and were completely occluded. These appeared as wide septa containing colloid among the collagen fibers. Colloid in the latter locations showed identical tinctorial affinity as the colloid observed within the vessels. The lumina of most of the affected vessels were narrow and in many instances appeared occluded to half of their diameters. Differential counting of the parenchymal

cells revealed the following proportion of the cell-types:

	Female (percent)	Male (percent)
Somatotrope cells	31.9	37.9
Thyrotrope cells	12.7	12.3
FSH gonadotrope cells	28.6	22.6
Chromophobe cells	26.6	26.9

At 20 days of age, the histological structure of the pars distalis adenohypophysis was not much different from that illustrated previously. The number of all cell-types was augmented appreciably as compared to that of a day-old animal. The stroma was mainly composed of thin bands of collagen fibers. The reticular framework extended throughout the lobe but was still poorly developed. Colloid infiltration into the vessels showed a progressive increase and was much pronounced near the peripheral margin of the lobe and in the vessels of larger caliber.

Group II: 4 to 8 weeks The specimens studied in this group presented an identical morphology as that observed towards the latter part of the previous group. The main features were an increase in the stroma and in the number of different cell-types. The cells were comparatively larger in size than those of the previous group. The somatotrope and FSH gonadotrope cells showed prominent negative images of the Golgi body. Cytoplasm of all cells was densely packed

with secretion granules. Some of the thyrotrope cells were hypertrophied and were completely degranulated. The shape of such cells was polygonal with indented margins. Few intracytoplasmic granules were only evident in their cytoplasm towards the plasma membrane. Nuclei of many somatotrope and FSH gonadotrope cells were large and bilobed. The number of chromophobe cells had augmented considerably. These formed a compact cellular mass along the peripheral margin of the lobe. They were undifferentiated cells without any cytoplasm. The somatotrope cells were comparatively more numerous in the female than in the male at this stage. Thyrotrope cells followed the reverse pattern.

In the pars distalis adenohypophysis of the pig, lactotrope and adrenocorticotrope cells were observed for the first time during this interval. The staining affinity of the lactotrope cells was comparatively weaker. The cells stained with azocarmine as well as erythrosin. The granules were fewer in number and the cells were small and spherical in outline. The adrenocorticotrope cells were stellate-shaped cells and contained very few erythrophilic granules. Their nuclei were the only parts stained and elucidated in the azocarmine, orange G and aniline blue technique. The number of adrenocorticotrope cells was very less. The relative proportion of the different cell-types was as follows:

	Female (percent)	Male (percent)
Somatotrope cells	37.9	37.7
Lactotrope cells	15.0	12.0
Thyrotrope cells	14.6	16.9
FSH gonadotrope cells	27.4	23.4
Adrenocorticotrope cells	0.5	0.2
Chromophobe cells	4.4	9.7

The connective tissue stroma of the lobe showed a progressive increase. The collagen fiber bands extended between the cell-cords and along the capillary walls. Reticular network was extensive and supplemented the collagen framework. The argyrophilic fibers formed a much denser network in the stroma which was especially conspicuous around the vessels with many tributary capillaries. Colloid infiltration of the vessels continued progressively. In many specimens, deposition of colloid was observed around the individual cells. The cell-type most affected by this intercellular infiltration was the thyrotrope cell. In these extravascular locations, colloid occurred in the form of dense crescentic masses around individual cells. Except for a limited area, the major part of the outline of the cell was embedded within the colloidal mass. The latter showed the same type of staining affinity as the intravascular colloid.

<u>Group III</u>: <u>2 to 6 months</u> At two months of age, the pars distalis adenohypophysis resembled a well vascularized organ. The cells were distinctly arranged in the form of cords throughout the lobe. Except for a few cell-cords where somatotropes formed the only composing cell-type, somatotropes and thyrotropes combined in the composition of the cords. The FSH gonadotropes occurred as aggregated masses of cells and were not evident in association with the cell-cords. Somatotrope cells were more numerous in the dorsolateral regions and formed the predominant cell-type in these regions. All cell-types possessed distinct negative images of the Golgi body and their cytoplasm was saturated with granules. Specimens from the male animals contained adrenocorticotrope cells in small numbers. These cells were predominantly found in areas where FSH gonadotrope cells occurred as groups. In the specimens obtained from the female animals, adrenocorticotrope cells were encountered in large numbers. Some of the FSH gonadotrope cells contained a single large vacuole towards one end of the cytoplasm. The vacuolar area did not stain in any of the techniques employed. More than one such vacuole was rarely encountered in a single cell. Vacuolated FSH cells were comparatively more numerous in the specimens from female animals than those from the male. Vacuoles in somatotrope cells were observed less frequently.

At 2 months, lactotrope cells were numerous in the specimens from female animals. In the male pig, their number was comparatively less at this age. These cells were smaller than the other cell-types and were mainly located on the periphery of the area occupied by somatotrope cells, and few were observed intermingled among the FSH gonadotrope cells. Another feature evident at this period was the cupping of cells. The cells associated with this phenomenon were the FSH gonadotrope cells and somatotrope cells (Figure 86). Thyrotrope cells were involved in cupping with the somatotrope cells in fewer instances. In the specimens collected from 3 month old pigs, ICSH gonadotrope cells were observed for the first time. These cells contained few intracytoplasmic granules which stained red with periodic acid-Schiff and their affinity for orange G was poor. The number of adrenocorticotrope cells was also augmented at this stage. Somatotrope cells were less numerous in the female as compared to that of male animals. The average relative percentage of the different cell-types were as follows:

	Female (percent)	Male (percent)
Somatotrope cells	36.0	39.3
Lactotrope cells	18.2	16.5
Thyrotrope cells	10.0	12.1
FSH gonadotrope cells	29.6	22.8
ICSH gonadotrope cells	1.0	0.5

	Female (percent)	Male (percent)
Adrenocorticotrope cells	1.5	1.0
Chromophobe cells	3.3	76.

The stroma consisted of well developed reticular and collagen fibers. Both types of fibers followed the path of the capillaries and were never observed circumscribing the cell-cords, either partially or completely. The network of argyrophilic fibers was especially dense during this period. Capillaries were numerous and many of them were of larger caliber. The latter joined with each other to form large veins which were primarily oriented towards the rostral or the caudal extremity of the lobe. At either of these extremities, a large vein was observed on each side of the pars distalis adenohypophysis. This vein was located in the mass of undifferentiated cells that formed the reflection of pars intermedia onto the pars distalis at the caudal extremity, while rostrally it occupied the angle formed by the pars distalis adenohypophysis and the pars paraneuralis. These veins extended to the exterior. The wall of these vessels consisted of a layer of endothelial cells, which were rather larger in size. The tunica adventitia was formed by a thick layer of collagen fibers. Reticular fibers were also observed among the collagen fiber bundles. In none of the sections, venous drainage from the pars distalis

adenohypophysis appeared to take place from the lateral margins of the lobe.

Within the pars distalis adenohypophysis, a majority of the large veins showed colloid infiltration (Figure 84). Their lumina were occluded to variable degrees. Occlusion of the small stromal capillaries augmented the clear follicular arrangement of the parenchymal cells (Figure 88). In the peripheral zone, some venules appeared to have been completely occluded with colloid. Some among these presented a light magenta color with periodic acid-Schiff representing the commencement of hyalinization of these vessels. Extravascular colloid was evident at this stage in the vicinity of the cells.

Between 4 and 6 months, the main changes in growth of the pars distalis adenohypophysis included no appreciable increase in the number of different cell-types or in the size of the cells. However, lactotrope and ICSH gonadotrope cells did indicate some increase in their relative proportions.

Group IV: 6 to 12 months There was no major alteration in the histomorphology of the pars distalis adenohypophysis during this interval. Presence of vacuoles in the different cell-types was rarely observed. The cells possessed dense masses of intracytoplasmic granules, and well developed negative images of the Golgi body. The parenchyma of the lobe appeared much more vascular. Many small capillaries intervened between the cell-cords, and

drained into venules. The capillaries as well as the venules showed colloid infiltration. The reticular framework was dense as was the network of argyrophilic fibers.

The somatotrope cells showed distinct zonal arrangement in the dorsolateral regions of the lobe and the FSH gonadotrope cells were primarily localized in the rostroventral part of the lobe. The thyrotrope cells were more widely distributed and showed less incidence of aggregation. Vacuolation of the FSH gonadotrope cells was evident during this period. The number of such vacuolated cells was comparatively less. The relative proportion of the different cell-types was as follows:

	Female (percent)	Male (percent)
Somatotrope cells	29.4	33.7
Lactotrope cells	16.5	13.2
Thyrotrope cells	11.0	13.1
FSH gonadotrope cells	30.2	21.8
ICSH gonadotrope cells	2.5	2.4
Adrenocorticotrope cells	4.5	6.6
Chromophobe cells	5.6	9.0

In the specimens obtained from 9-month-old female pigs, deposition of colloid was observed in the central part of the cell-cords (Figure 92). Such cell-cords were usually composed of somatotrope, thyrotrope and FSH gonadotrope cells and contained no chromophobic cells in their centrum.

The latter showed a fibrin network in all the affected cell-cords. In many other follicles, a fibrinoid mass was evident in the central part of the cell-cords (Figure 92). Colloid appeared on the fibrinoid mass and increased progressively with age. The colloidal mass stained intensely with periodic acid-Schiff. In later stages, deposits of colloid were evident in the central part of many follicles in diverse shapes and sizes. The most common was the rosette pattern. Intercellular deposition of colloid was not evident in any of the specimens studied. Occlusion of small capillaries. due to colloid infiltration was evident rhoughout the lobe.

<u>Group V: 1 to 4 years</u> At one year of age, the histological picture of the pars distalis adenohypophysis was akin to the previous age group. The stroma was much more developed at this stage and contained large bundles of collagen fibers, a dense network of reticulum, and an intricate mass of argyrophilic fibers around the vessels. Neither the collagen fibers nor the reticular fibers penetrated into the cell-cords in any part of the lobe. Colloid infiltration was extensive. Many vessels were completely occluded and some of them showed hyalinization. The hyaline mass stained poorly with periodic acid-Schiff and was non-reactive to crystal violet metachromasia. Judging from the small number of these hyalinized vessels, it was apparent that the long standing occluded vessels had been either

reabsorbed or their colloid content spread along the connective tissue strands. The large veins located on the caudal and rostral extremity of the organ also had colloid infiltration on or in their walls at this age (Figure 124). Intercellular deposition of colloid was not observed in any specimen during this period. Even though many vessels were occluded, the adjacent cells revealed no apparent cytological changes. In two of the specimens, however, the entire population of the cell-cords adjacent to an occluded vessel consisted of only chromophobe cells. Infiltration of fibroblasts, leucocytes or any other type of phagocytic cells was not evident in these areas.

Many of the FSH and ICSH gonadotrope cells showed large vacuoles in their cytoplasm. More than one single vacuole was never observed in these cells (Figure 93). Vacuoles were predominantly observed in the male than in the female. Cupping of the cells was observed to a comparatively higher degree than the previous age-groups. The relative proportion of the different cell-types, revealed by differential cell count, was as follows:

	Female (percent)	Male (percent)
Somatotrope cells	31.4	33.0
Lactotrope cells	14.0	9.9
Thyrotrope cells	11.7	14.3
FSH gonadotrope cells	28.6	23.6
ICSH gonadotrope cells	3.6	2.5

	Female (percent)	Male (percent)
Adrenocorticotrope cells	7.5	9.2
Chromophobe cells	3.0	7.2

Specimens obtained from 2-year-old animals contained colloid deposition in the cavum hypophysis. The colloidal mass did not fill the entire cavity and was comparatively of smaller volume. The initial deposition occurred along the margins formed by the cells. The amount of colloid was comparatively heavier towards the caudal extremity of the organ. Colloid deposits were also observed at this age within the parenchyma of the lobe. Deposits lined by parenchymal cells were more prevalent in the central regions of the lobe while deposits lined with collagen and reticular fibers were more common towards the periphery. The dorsolateral zone contained many such deposits.

In the pars distalis adenohypophysis of specimens collected from animals aged three years or over, autolysis of cells, in the adjacent areas of occluded vessels, was observed (Figure 95). All cells which composed the cord showed such degenerative changes and cells at various stages of the process were observed. The cells lost their outlines at the initial stage. The cytoplasm stained lightly and became progressively rarefied. During later stages, the entire mass of cytoplasm was lost and a pale stained

nucleus was the only cellular component left behind. The nucleus was the last structure to undergo autolysis. No phagocytic cells were observed in the vicinity of the cells and the cells did not become dispersed out of the cord which suggested an autolytic process rather than a process of phagocytosis. Many such large vacuolar areas, representing autolyzed cells, were evident. Their predominance was higher along the peripheral margin of the pars distalis adenohypophysis where most of the venules and veins were located.

Group VI: 4 to 7 years The pars distalis adenohypophysis showed much evidence of degenerative changes during this interval. The majority of the vessels contained heavy colloid infiltration. The infiltrated mass was not only confined to the vessels but was evident in the stroma between the cell-cords, too. This presented a picture of enhanced lobulation (Figure 94). The number of completely occluded vessels was more than in previous years. Around the occluded vessels and also those affected partially, a wide basement membrane was evident. The matrix of this did not stain with periodic acid-Schiff but stained light blue with alcian blue at pH 0.2. No collagen or reticular fibers were evident within this matrix. The broad lightly stained areas around the vessels furnished sharp contrast to the deeply stained colloid mass contained within the lumina.

The perivascular basement membrane was conspicuous around the large calibered vessels. During subsequent periods, increase in the basement membrane and involvement of a large number of vessels increased the stroma. There was an increase in the amount of collagen present in the lobe. The reticular fibers manifested a thick network in the stroma.

Many spherical colloid deposits were evident in the parenchyma of the pars distalis adenohypophysis. Every specimen studied, contained such deposits. Some of the latter possessed a cellular lining, while others were lined with stromal fibers. The outlines of such deposits were well discernible. Both lightly stained and intensely stained colloid deposits were present. A basement membrane identical to the perivascular membrane mentioned earlier was observed around larger deposits. The amount of vascular as well as extravascular colloid infiltration was comparatively less in the male than in the female. There was a conspicuous increase in the amount of colloid, deposited within the cavum hypophysis (Figure 125). The entire lumen was occluded by this material. Within this colloidal mass degenerative parenchymal cells of the pars distalis adenohypophysis were evident. In the rostral half of the organ, the lumen of the cavum hypophysis appeared narrower and the pars distalis adenohypophysis and the pars intermedia were only separated from each other by a thin band of colloid.

The pars distalis adenohypophysis contained many vacuolated areas during this interval (Figure 111). Cells in different stages of autolysis were evident throughout the lobe. The parenchymal cells presented no other significant alterations. Vacuolation was evident in a larger proportion of the FSH gonadotrope cells. ICSH gonadotrope cells were involved in the latter change to a lesser extent. A decrease in the number of FSH gonadotrope cells was apparent. Many of these cells were larger in size as compared to the previous periods (Figure 94). Throughout this age group, somatotrope and ICSH gonadotrope cells showed progressive decline in their relative proportions. The thyrotropic cells contained intracytoplasmic droplets. Such droplets were of irregular shape and size. The following average percentage of the different cell-types was obtained during this interval:

	Female (percent)	Male (percent)
Somatotrope cells	27.0	32.4
Lactotrope cells	19.5	15.5
Thyrotrope cells	15.2	17.0
FSH gonadotrope cells	22.5	21.6
ICSH gonadotrope cells	2.5	2.5
Adrenocorticotrope cells	8.5	7.2
Chromophobe cells	41.0	3.6

Group VI: 7 to 10 years Beginning with this age interval, there was pronounced decrease in the number of somatotrope and ICSH gonadotrope cells (Figure 98). The decrease in the relative proportion of these cells increased progressively from 7 to 10 years of age. Few ICSH gonadotrope cells were only observed in specimens collected from sows 10 years of age. The lactotrope cells increased in their relative proportion to a higher degree than the decrease observed in preceding cell-types (Figure 98). The cells were evident throughout the pars distalis adenohypophysis and as many as 3 to 4 cells were observed in some of the cell-cords. They were especially predominant in the central part of the lobe close to the cavum hypophysis and in comparatively avascular zones near the peripheral margin of the lobe. The cells contained dense accumulations of large intracytoplasmic granules that stained intensely with erythrosin and azocarmine. The increase in the relative proportion of the thyrotrope cells was not so conspicuous. The cells were widely distributed throughout the pars distalis adenohypophysis, and occurrence of such cells was also observed in small groups. In many instances, the cell-cord was primarily composed of thyrotrope cells. Intracytoplasmic colloid droplets were distinctly evident in some of these cells. The relative proportion of the cell-types was as follows:

	Female (percent)
Somatotrope cells	17.0
Lactotrope cells	32.4
Thyrotrope cells	15.0
FSH gonadotrope cells	21.4
ICSH gonadotrope cells	0.5
Adrenocorticotrope cells	8.0
Chromophobe cells	5.6

Increase in size of all categories of cells was evident in this age group (Figure 116). The largest increase in comparative size with those of earlier age intervals was noted in FSH gonadotrope cells (Figure 117). Some of the somatotrope cells also showed increase in their size. While somatotrope cells of small diameters were encountered at 8 to 10 years of age, all the FSH cells showed augmented proportions in the corresponding period. Between 8 to 10 years of age, the number of FSH gonadotropic cells reaching gigantic proportions was appreciable (Figure 112). The increased size in the lactotrope cells was reflected in their augmented polygonal outlines and the dense mass of large erythrophilic granules contained by them (Figure 116). All other cell-types also contained heavy accumulation of intracytoplasmic secretory granules which imparted a deeper hue in all staining techniques. Degranulated cells of any

category were not observed. Many of the heavily granulated FSH gonadotrope and lactotrope cells contained spherical vacuoles within their cytoplasm (Figure 116). Bilobed and double nuclei were especially conspicuous in the somatotrope and FSH gonadotrope cells (Figure 112), while large lobulated nuclei with dense masses of chromatin material were observed in the thyrotrope cells. Some of the somatotrope and lactotrope cells contained pyknotic nuclei. The nucleus of such cells possessed a thick nuclear membrane and the nucleoplasm occurred in fragmented form, detached from the nuclear membrane.

The entire pars distalis adenohypophysis was subdivided into lobular zones due to excessive deposition of collagen between the cell-cords. Reticulum also occurred in an increased proportion and the argyrophilic fibers were masked due to this stromal growth (Figure 114). At places, gelatinization of collagen fibers was evident. The septa-like partitions were considerably widened due to accumulation of the hyaline mass (Figure 103). In the midst of this substance, fine wavy fibrils formed an ill-defined network. These fibrils were argyrophilic and were faintly colored in the periodic acid-Schiff procedure. In the vicinity of blood vessels, a basement membrane that stained red with the latter technique was observed in specimens obtained from sows 7 and 8 years of age. In older animals, periodic

acid-Schiff positive ground substance was comparatively scanty.

Beginning with 7 years of age, small aggregations of chromophobe cells became evident in the pars distalis adenohypophysis (Figure 101). These aggregated masses were of small sizes and were not clearly defined from the adjacent chromophil cells. In specimens examined from older animals, their incidence increased progressively with age. Rarely did they show appreciable change in their size. Capillaries were not evident within such cellular masses nor were they circumscribed by connective tissue fibers.

Deposition of colloid was particularly heavy in this age group. Deposition of colloid occurred in all parts of the lobe and as much as half of the entire area of the pars distalis adenohypophysis was occupied by these deposits (Figure 109). During the latter part of this age interval, numerical growth of such colloid deposits appeared to be less conspicuous than the increase in their size. In the intercellular location, colloid masses circumscribed individual parenchymal cells to variable extents (Figure 115). The follicular colloid assumed spherical of rosette shapes and usually filled the cavity of the follicle all around its margin (Figure 118). Colloid deposits in the form of cystic measses were especially conspicuous during this period (Figure 109). Some of these were of immense

proportions and coalescence of adjacent deposits resulted in the formation of small pools of colloid material within the parenchyma of the lobe. Some of these cysts were lined with degenerative connective tissue fibers, while others possessed a lining formed by a single layer of parenchymal cells. Any type of transformation such as development of cilia or conversion into chromophobe cells was not observed in the lining cells. Transformation of colloidal masses into concretions was evident in many of the follicles. Condensation in the central part of many such follicles indicated initiation of the process in that area.

Colloid deposition within the vessels increased progressively throughout this age group (Figure 102). In all the specimens collected from animals 10 years of age, almost every vessel presented colloid infiltration. The number of portal capillaries becoming completely occluded was very high in this age group. Colloid infiltration of the vessel was so extensive that nearly three-fourths of their lumina were obliterated (Figure 107). Hyalinization of occluded vessels was evident in appreciable numbers. The hyaline mass stained less intensely with periodic acid-Schiff and was mostly circumscribed by dense ground matrix. Peripheral vacuoles were evident in all of these masses. The hyaline mass showed gradual separation from the adjacent tissue due to shrinkage. In the vicinity of such hyalinized

vessels, areas of avascular necrosis were evident (Figure 107). The degenerative change was autolytic and there was no evidence of phagocytosis. Cells in various stages of autolysis were evident in such areas.

Like that of the dog, colloid contained within the vessels and in the extravascular locations manifested positive reaction for amyloid (Figures 87 and 108). Statistical evaluation of data on percentage of different cell-types, volume of the lobes, and cytoplasm-nuclear ratio did not reveal statistically significant differences among the age groups.

Pars paraneuralis   In the specimen of the youngest animal examined in this study, the pars paraneuralis was not evident as a separate zone. Few chromophobe cells were only observed at the junctional zone between the pars distalis and the pars intermedia adenohypophysis and these cells appeared to be a part of the latter. Parenchymal cells of the pars distalis adenohypophysis extended to the junction with pars distalis neurohypophysis at the dorsolateral angles. In specimens collected from animals 2 days of age, a narrow band of chromphobe cells was evident at the dorsolateral angle and the parenchymal cells of the pars distalis were rarely present in this zone. These chromophobe cells were arranged in columns or solid clusters. Connective tissue fibers that intervened between the cell-clusters were

composed of collagen and reticular fibers. A dense network of intensely argyrophilic fibers was evident in the stroma and in the perivascular region. Infiltration of colloid was not evident in these vessels. The latter were large calibered veins which opened to the exterior of the organ. Capillaries were not observed among the cell-clusters.

Between 4 to 8 weeks of age, the pars paraneuralis was discernible as a separate zone. The cells possessed distinct oval or columnar outlines and were loosely packed in the columns. A cavity was observed in the central part of some of the columns simulating a follicle. The peripherally located cells were arranged in several layers. The nuclei were distinct and oval in outline. Nucleoli were not observed in them. The cell-columns projected into the cavum hypophysis so that the latter was subdivided into several narrow channel-like extensions (Figure 119).

During the age interval 2 to 6 months, the cells of the pars paraneuralis were comparatively more numerous but possessed small cytoplasmic volumes. Permeation of the cavum hypophysis into this zone was not evident and the cells formed a distinctly separate zone between the pars intermedia and the pars distalis adenohypophysis. The stroma was comparatively heavy and the cells were arranged in the form of distinct columns or clusters. The number of cells was almost twice that observed during the previous

groups. In subsequent periods, the cells not only showed progressive increase in number but also in size. The cells were arranged in compact masses separated by connective tissue fibers. The central cavity was not evident within the columns. Vascularity of the zone was very poor. Apart from few large venules, the pars paraneuralis contained no other vessels. Colloid infiltration was evident in these vessels at this stage.

The cells of this zone were small columnar or oval cells. The nucleus was larger than the volume of the cytoplasm. The latter appeared dense but did not stain in any technique. In periodic acid-Schiff procedure, the cells were colored light magenta indicating a slight affinity of the cells for Schiff reagent.

In specimens collected from pigs, between 6 months to 4 years of age, the pars paraneuralis was very well developed. It contained large columnar type of cells with distinct outlines. The cells possessed larger mass of cytoplasm which stained magenta with periodic acid-Schiff reagent. Among them, few cells stained intense red and their cytoplasm was comparatively denser. Both rostrally and caudally, the cells merged with the parenchymal cells of the pars distalis adenohypophysis and the pars intermedia. No distinct variation in the tinctorial affinity of the cells that constituted the pars intermedia, pars infundibularis

and pars paraneuralis was evident.

The pars paraneuralis presented a similar histological picture during 4 to 7 years of age. The cells were comparatively smaller in size and the number of intensely stained cells was small. Though thick strands of connective tissue were observed in the pars intermedia, increase of stroma was not conspicuous in the pars paraneuralis. Colloid deposits were observed in the central part of the cellular columns as well as between them. The latter were circumscribed by stromal fibers. Colloid infiltration of the large veins located within this zone was also evident.

Between 7 to 10 years of age, the cells of the pars paraneuralis showed hypertrophy. The cells were arranged in distinct columns which extended laterally towards the dorsal surface of the pars distalis neurohypophysis. Such lateral extension was very conspicuous in the caudal half of the organ. The stroma did not show any increase during this period, nor was there any conspicuous change in the vascularity of the region. The cells were large and columnar in shape. They possessed large spherical nuclei with abundant chromatin material. The cytoplasmic volume of these cells was much greater as compared to previous stages. Although the cells were hypertrophied, there was no apparent change in their staining character, nor was there any evidence of secretory granules within their

cytoplasm.

Pars infundibularis adenohypophysis In the pig, the pars infundibularis adenohypophysis formed the external zone that lined the radix infundibuli and pars compacta infundibuli. The part that lined the radix infundibuli was comparatively wider and contained a large number of cells. In the specimens collected from the day-old piglet, the pars infundibularis was constituted by a group of undifferentiated cells arranged irregularly beneath the capsule. The cells were only represented by their nuclei. The latter were dense and their intense orangeophilia suggested a heavy content of chromatin material. Collagen or reticular fibers were not evident in the parenchyma of this lobe. Very few delicate capillaries coursed through this part. These were composed of an endothelial lining only.

In specimens obtained from pigs 7 days of age, the pars infundibularis showed a definite pattern of organization. The cells, though not changed in size, showed distinct follicular arrangement. The central parts of the follicles were empty, and the cells were arranged loosely in the follicles. Thin bands of collagen and reticular fibers were observed between the follicles. Blood vessels possessing the structural pattern of small capillaries coursed through the stroma (Figure 127). Besides an endothelial lining, a lining of fibroblast cells or thin

strands of collagen fibers was evident. In the peripheral region of this zone, few small arterioles were present. These vessels consisted of a tunica intima, a tunica media composed of a single layer of smooth muscle fibers, and a thin layer of tunica adventitia. A network of intense argyrophilic fibers was evident around these vessels, and these fibers extended along the capillaries, too (Figure 120).

During the second age interval, the morphology of the zone was more or less unchanged, except for an increase of vascularity. The cells still appeared as undifferentiated cells. The follicles were smaller in size and the cells were arranged compactly in these follicles. Large numbers of arterioles and venules were observed at this age. The vessels presented the typical structure of similar systemic vessels. During later stages, the main changes observed in this part consisted of regular follicular arrangement of cells and increase in their size. The stroma also showed a progressive increase. Periodic acid-Schiff staining of the basement membrane was observed in the perivascular spaces.

At 4 months of age, the pars infundibularis assumed its typical morphology. The cells appeared identical to those of pars intermedia and pars paraneuralis but were smaller in size. They were oval in outlines and contained large vesicular nuclei. The cells showed definite follicular

arrangement with interfollicular connective tissue septa. They remained unstained in all of the staining techniques employed, except for a nonspecific magenta color imparted on them by the Schiff reagent. Among these follicles, small groups of large polygonal cells were evident. The number of such cells as compared to the chief cells was small. The cytoplasm of these cells contained secretory granules which stained intense red with periodic acid-Schiff reagent (Figure 122).

The number of large caliber vessels was less than the number of smaller arteries and veins. The vessels were mostly in groups of 3 or 4 in each location, indicating looping of their parent trunks (Figure 128). They were distributed throughout the zone. The veins were thin walled and were continuous with those of the radix infundibuli and pars compacta infundibuli.

In specimens collected from pigs 10 months of age, colloid deposition was observed in the centrum of the follicles (Figure 122). These deposits were usually spherical in shape and rarely occupied the entire centrum. The colloid material stained intense red with periodic acid-Schiff and yellow with the orange G. Presence of these deposits within the follicles did not seem to alter the lining cells in any form. The walls of the veins were infiltrated with colloid and the lumina of many vessels

were partially occluded.

In the subsequent periods, the number of periodic acid-Schiff positive cells showed a decrease. The follicles contained colloid deposits, and colloid infiltration of vessels showed progressive increase. Arterial branches were not involved in the phenomenon of colloid infiltration. Many vessels had been completely occluded and showed different stages of hyalinization. The basement membrane was clearly discernible around these vessels. Both collagen and reticular fibers increased in quantity. Argyrophilic fibers manifested no such change. The collagen fibers formed dense interwoven septa around the follicles. Connective tissue cells outnumbered the parenchymal cells at this age.

Between 7 and 10 years of age, the cells of pars infundibularis showed atrophic changes. The follicular arrangement was ill-defined. The cells were smaller in size and were much fewer in number. Various stages of autolytic changes were evident in these cells (Figure 123). Many follicles were either empty or contained one or two degenerating cells (Figure 123). Very few periodic acid-Schiff stained cells were present at this stage and these occurred as single isolated cells. The stroma showed appreciable augmentation. Wide bands of collagen fibers formed an extensive network in the parenchyma (Figure 129). The collagen fibers masked completely the reticular fibers

and made the empty follicles conspicuous. Many fibroblasts were present along these fibers. The blood vessels were fewer in number. The majority of the veins was occluded due to colloid infiltration. Increase in thickness of the basement membrane was very conspicuous.

Pars intermedia adenohypophysis In the specimens obtained from animals 14 hours after birth, the pars intermedia adenohypophysis was comparatively much smaller in size. It lined the cavum hypophysis on its dorsocaudal margin and formed its rostral and caudal extremities due to its reflection on the pars distalis adenohypophysis. It circumscribed the pars distalis neurohypophysis and was much more extensive in the rostral half of the organ than in the caudal half (Figure 135).

Its parenchyma was formed by large number of densely packed basophilic cells. The cells possessed no distinct outlines but showed an ill-defined follicular arrangement. The cytoplasmic mass of all cells present in a column, appeared as a homogeneous mass. The nuclei were large, spherical in outline and contained two to four nucleoli. Fine chromatin granules were dispersed throughout the nucleoplasm.

The parenchyma of the lobe was interrupted by many small capillaries. These were composed of an endothelial lining only. The vessels were primarily oriented along the

rostrocaudal axis and drained caudally. At the latter extremity, confluence of several veins gave rise to tributaries of the caudal hypophysial vein, which joined similar veins of the pars distalis adenohypophysis. Connective tissue fibers were not evident at this age in the interstices of the follicles. A thin reticular framework was observed along the course of the blood vessels. An intricate mass of intense argyrophilic fibers coursed along the blood vessels. The main growth changes evident between birth to 2 months of age were hyperplasia of the parenchymal cells, increase in the vascularity of the lobe and development of the stroma.

At 2 months of age, the cells were larger in size but were not identifiable due to poorly discernible outlines. The cells were distinctly arranged in the form of clusters. Each cluster was compactly packed with several layers of cells. The nuclei of the cells contained chromatin granules and a single large nucleolus. The cells were poorly stained with periodic acid-Schiff and aldehyde-fuchsin. No difference was apparent among the cells.

The stroma consisted of thin bands of collagen and reticular fibers. These were mostly confined to the course of blood vessels and did not circumscribe the cell-clusters. Large venules and veins were evident in the pars intermedia. They were composed of endothelium and a tunica adventitia

made up of collagen and reticular fibers. The large venules were located close to the cavum hypophysis. A large number of vessels was also present in the junctional zone between the pars intermedia and the pars distalis neurohypophysis. These vessels were continuous with the vessels present in the parenchyma of the latter and drained its peripheral zones. The vessels in the pars intermedia showed colloid infiltration of their walls to variable degrees. Vessels of smaller caliber were affected to a larger degree than the large venules.

At 6 months of age, the parenchymal cells of the pars intermedia possessed distinct outlines. Their cytoplasm was comparatively larger in volume and appeared homogeneous. No granules were discernible in the cytoplasmic mass. The cells stained magenta with periodic acid-Schiff and pinkish purple with aldehyde-fuchsin. The connective tissue septa located between the cell-clusters were wide and contained the large veins. Small capillary type vessels were not evident in the lobe. Colloid infiltration of vessel walls showed progressive increase. Complete occlusion of smaller vessels was evident at this stage.

In specimens collected from pigs of 1 to 4 years of age, the pars intermedia presented an identical structure as in the previous age-interval. There was great proliferation of the stromal fibers and cells, but the parenchymal cells

showed little alteration. In addition to the latter, few deep-staining cells were also observed during this period. These cells occurred singly and contained large vesicular nuclei. Their cytoplasm stained intensely with periodic acid-Schiff and aldehyde-fuchsin. In specimens collected from animals 2 years of age, colloid deposits were observed within the follicles of the parenchyma (Figure 125). These deposits varied in their size. In some of the follicles, an intense staining mass of periodic acid-Schiff positive material was observed, while in others the peripheral margins of the colloid mass was only stained. The central part of the latter type of deposits stained weakly with alcian blue. These stages, probably, represeted colloid deposits of different duration. Hyalinized vessels did not stain intensely with periodic acid-Schiff which served as a suitable differentiating guide for follicular and vascular colloid.

Between 4 to 7 years of age, the size of the pars intermedia was small. The cells were also smaller in size and did not stain as intensely as earlier stages. Many cells in various stages of degeneration were evident. Degenerative changes in the cells was especially conspicuous near the cavum hypophysis that contained colloid. Many vessels showed complete occlusion and hyalinization. In the middle part of the rostral half of the hypophysis, the pars intermedia and the pars distalis adenohypophysis attained

continuity across the cavum hypophysis due to absorption of the colloid. Parenchymal cells of the latter and blood vessels extended into the pars intermedia and were evident on either side of the union point. Intermingling of pars intermedia and pars distalis cells was conspicuous in this zone.

During the age interval of 7 to 10 years, the pars intermedia was smaller in size as compared to the previous interval. The decrease was primarily due to atrophy of the parenchymal cells. There was less significant variation in the structure of the lobe at different periods of this interval. The pars intermedia was almost completely separated from the pars distalis neurohypophysis by a septum of collagen and reticular fibers. This septum occupied the identical location of the vascular plexus observed in earlier periods. In this interval, however, very few capillaries were observed in the junctional zone between the two lobes.

In the rostral half of the hypophysis, the cavum hypophysis was intermittently occluded so that blood vessels and parenchymal cells of the pars distalis and the pars intermedia adenohypophysis were found to be intermixed in this region (Figure 125). This union between the two lobes was mainly confined to the central region. Rostrally as well as caudally the cavum hypophysis contained a heavy

mass of colloid. The latter stained red with periodic acid-Schiff and appeared identical in its affinity to the colloid observed in the pars distalis adenohypophysis and the pars intermedia. The marginal cells of both lobes showed degenerative changes and groups of such cells were evident within the colloidal mass.

In the region where continuity of pars distalis adenohypophysis and pars intermedia was evident, capillaries often extended from the pars distalis into the pars intermedia. Cellular strands from the former continued along the course of these vessels and remained intermingled with chromophobic cells of the pars intermedia, and in some instances even projected into the pars distalis neurohypophysis. Many large FSH gonadotropic cells were encountered in the pars intermedia of sows 10 years of age. In such specimens the pars intermedia appeared to have lost its individual character and delineation.

The parenchymal cells of the pars intermedia were atrophic in the specimens collected from animals 7 years of age. This condition increased progressively with age. Parenchymal cells located in the vicinity of the cavum hypophysis and occluded vessels resembled normal cells of the lobe. In the former location many of the cells contained nuclei densely packed with chromatin material. Extension of cell-columns from the pars intermedia into the pars distalis adenohypophysis was not a constant feature in the pig.

Groups of hypertrophied cells were rarely encountered in the junctional zone between the pars intermedia and the pars distalis neurohypophysis.

Colloid was found in the central cavity in the majority of the follicles (Figure 126). These deposits were of larger size and in some follicles manifested the characteristic rosette pattern. Extravascular colloid stained more intensely than the intravascular colloid, so that both were easily distinguishable. Colloid infiltration was observed in all vessels, those of small diameters having undergone complete occlusion. Hyalinization and fibrosis of some of these occluded vessels presented evidence of an early start of colloid deposition in these vessels. Many atrophied vessels were also evident in this lobe, suggestive of colloid infiltration of their parent vessels located in other regions of the organ. Such atrophied vessels contained a thin band of collagen fibers circumscribing a hollow space. The interstitial stroma showed no appreciable increase during this period.

<u>Neurohypophysis</u>

<u>Radix infundibuli</u> In the specimens obtained from pigs which were less than one day of age, the radix infundibuli occurred in the form of a wide zone. The nerve fibers coursed mainly in the longitudinal direction and a dense mass of neurosecretory material was evident in these

fibers. The neurosecretory material stained purple with Gomori's chrome alum hematoxylin and intense blue with alcian blue following performic acid oxidation. Herring bodies were not observed in any part of the radix infundibuli. Arrangement of fibers in the form of an inner zone and an outer zone was not discernible at this stage. Many neuroglia cells and pituicytes were present along the neurosecretory fibers. The former were small-sized cells represented only by their nuclei. The pituicytes appeared as large cells containing a vesicular nucleus which was relatively larger in volume than the cytoplasm.

Blood vessels were not present within the radix infundibuli. The mantel plexus was not developed at this stage. The zone located between the radix infundibuli and pars compacta infundibuli contained large number of fibroblast cells and fine capillaries (Figure 127).

In the specimens obtained from animals 7 days of age, the neurosecretory fibers were mainly confined to the subependymal layer and were comparatively less numerous towards the outer zone. Though neurosecretory material was evident in these fibers, Herring bodies were not observed among them. The mantel plexus was composed of minute capillary vessels and a large number of fibroblast cells. These vessels coursed perpendicularly towards the external layer of the radix infundibuli.

At one month of age, two distinct zones could be identified in the radix infundibuli. Fibers of the inner zone occupied the subependymal layer and were densely packed. Neurosecretory material was stainable in these fibers and Herring bodies were evident in small numbers. The fibers in the external zone coursed in a perpendicular direction and did not reveal the presence of neurosecretory material. The mantel plexus was well developed. The vessels were thin-walled capillaries and arterioles. A tunica adventitia was not evident in these vessels, but a well developed network of argyrophilic fibers was present around each vessel.

Between 2 to 6 months of age, the ependymal layer, inner zone, external zone and the mantel plexus were clearly discernible. Neurosecretory material was stainable in the fibers of the inner zone. Herring bodies were present in some of the specimens examined. Large number of neuroglia cells and pituicytes were observed in association with the fibers of the inner zone. Very few cells were evident in the external zone. The mantel plexus was very extensive and contained medium-caliber arteries and veins, in addition to a large number of capillaries. The latter occurred in groups of 2 to 6, suggestive of extensive looping of these vessels (Figure 128). Thin strands of collagen and reticular fibers were evident around the vessels.

In the subsequent age-intervals, there were less

significant changes in the structure of the radix infundibuli. The amount of stainable neurosecretory material in the zona interna decreased progressively with age. The vessels of the mantel plexus increased in size, number and tortuosity. The amount of collagen and reticulum, present among the vessels, increased progressively with age. Colloid infiltration of the vessels was first observed at one year of age. Veins were only affected in this process and partial occlusion of their lumina was observed.

In specimens collected from pigs between 4 to 7 years of age, the amount of stainable neurosecretory material was considerably less in the zona interna. The zona externa was ill-defined. The neurosecretory fibers contained in the latter zone were not discernible as individual fibers due to the presence of a homogeneous ground matrix. The latter was periodic acid-Schiff negative and stained light blue with alcian blue at pH 0.2. In the mantel plexus, many hyalinized vessels were evident. Almost each vein and capillary showed colloid infiltration on their walls. However, colloid was not evident in the extravascular location. A large number of collagen fibers formed interlacing bundles around the vessels (Figure 129). The number of fibroblast cells was appreciably enhanced.

The radix infundibuli showed many significant structural changes between 7 to 10 years of age. It lacked the char-

acteristic zonal arrangement of fibers. Neurosecretory material was rarely encountered in the fibers of the zona interna which was very well evident in older specimens (Figure 132). The vessels of this zone possessed distinct thickening of their walls due to accumulation of a ground matrix and collagen fibers. The former showed affinity for alcian blue at pH 0.2 (Figure 130). In the midst of this ground substance argyrophilic fibers were evident. The tunica adventitia of the vessels was very thick as was the interstitial connective tissue. All vessels manifested colloid infiltration of their wall and the majority of them was hyalinized. A fibroid type of hyaline mass was more commonly observed in the vessels of the radix infundibuli than a colloid mass.

The vessels of the mantel plexus manifested identical changes as those of the radix infundibuli. All small-caliber vessels were completely occluded due to colloid deposition, and hyalinization of these vessels was evident.

<u>Pars compacta infundibuli</u> The structure of the pars compacta infundibuli resembled that of the radix infundibuli at all ages. At birth, the infundibular recess was very extensive and permeated the entire length of the pars compacta infundibuli as well as the pars distalis neurohypophysis. With the advancement of age, it became occluded and the layer of ependymal cells forming its inner

margins degenerated. At 2 months of age, the part of the recessus infundibuli, contained within the pars compacta infundibuli, had become completely obliterated.

In its growth pattern, the pars compacta infundibuli followed that of the radix infundibuli. In the former, however, the zona externa contained an admixture of fibers some of which stained with Gomori's chrome alum hematoxylin while others did not. Tèrmination of the former type of fibers was observed in the vicinity of the blood vessels of the mantel plexus and also around the vessels that were present within the zone (Figure 133). In addition to the neurosecretory material carried in the fibers, aggregated masses of the same in the form of Herring bodies were frequently encountered in the pars compacta infundibuli. Their incidence was very common in specimens from animals 4 months to 2 years of age. Beyond 4 years of age, Herring bodies were rarely encountered.

In specimens, obtained from animals 6 to 10 years of age, the number of neurosecretory fibers was considerably decreased. A dense ground matrix was evident among the fibers. Degeneration of the neurosecretion-bearing fibers was observed in some of the specimens. In such areas, a dense mass of replacement fibrosis was evident. This fibrous mass consisted of a ground matrix that stained light blue with alcian blue at low pH and contained thin individual

fibrils, interlaced with each other in the form of a network. The fibrils were periodic acid-Schiff positive and were argyrophilic. A large number of fibroblast cells was associated with these fibers.

Pars distalis neurohypophysis The pars distalis neurohypophysis was comparatively less extensive in development at birth. In specimens collected from piglets at 14 hours of age, the pars distalis neurohypophysis occupied the entire length of the pars distalis adenohypophysis. The infundibular recess extended into its parenchyma. The histological structure of the lobe was typical of growing nervous tissue. The entire parenchyma consisted of a large number of neuroglia cells (Figure 135). Pituicytes were larger in size than the neuroglia cells and were comparatively less numerous.

The nerve fibers were irregularly distributed within the parenchyma. In the peripheral margins, a lobular arrangement was evident. The nerve fibers stained deeply with chrome alum hematoxylin and with alcian blue at low pH (Figure 135). Accumulation of neurosecretory material in the vicinity of capillaries was not observed at this age. There was no discernible connective tissue stroma. Few fibroblast cells were only present in the vicinity of the blood vessels. Branches of the caudal hypophysial artery entered the lobe at its dorsocaudal and caudal extremities. A satellite vein

accompanied each branch of the artery. Within the parenchyma branches of the vessels were evident as small arteries and veins. Capillaries were absent at this stage. The blood vessels were circumscribed by a feltwork of intense argyrophilic fibers.

At one month of age, there was an appreciable change in the structure of the parenchyma. The number of neuroglia cells was comparatively less. The presence of a large number of arteries and satellite veins suggested enhanced ramification of the caudal hypophysial artery (Figure 136). The vessels were circumscribed by thin strands of collagen and reticular fibers at this stage. Herring bodies were observed throughout the parenchyma of the lobe. These were composed of a large number of spherical masses of neurosecretory material. Their shape and size varied extensively. The ground matrix of Herring bodies did not reveal any staining affinity. At this age, the recessus infundibuli showed signs of occlusion at the rostral end of the pars distalis neurohypophysis. The cavity became obliterated and the ependymal cells of both of its margins appeared in close contact with each other. The caudal extremity of the recess remained patent until later stages.

Between 2 to 6 months of age, the parenchyma was composed of very few cells, and was predominantly fibrous. The fibers contained neurosecretory material that was stainable

with chrome alum hematoxylin as well as alcian blue (Figure 136). Condensation of neurosecretory material was very conspicuous near the terminal ends of the fibers and adjacent to the wall of blood vessels (Figure 136). The neurosecretion-bearing fibers showed less branching in the central part of the lobe. Towards the peripheral margins they showed extensive ramifications which were arranged in the form of tufts around the capillaries. Pituicytes were observed in close association with these fibers. Herring bodies were present in large numbers among the fibers. They were more commonly encountered in the central part of the lobe than in the peripheral region. The remnant of the infundibular recess was present as a dilated cavity in the central part of the rostral half of the lobe. The recess was lined with well developed ependymal cells. Neurohypophysial fibers terminated in close proximity of the ependymal cells and were evident between them (Figure 136). Herring bodies and neurosecretory material was evident all round the recess. In later stages, a hyaline cast developed in the cavity and the latter became progressively obliterated with age.

At two months, the parenchyma of the pars distalis neurohypophysis contained many capillaries and few small arterioles and veins. The capillaries were oriented in a radiating manner along the centroperipheral axis of the lobe (Figure 136). Extensive looping and ramifications of

these capillaries were apparent. Thin bands of collagen and reticular fibers formed a connective tissue sheath along a series of such capillaries. The veins from the rostral region of the lobe drained into the pars intermedia, while those from the caudal one-third of the lobe drained directly into the caudal hypophysial vein.

In the subsequent age-groups, very little alteration was observed in the structure of the parenchyma. The remnant of the recessus infundibuli became progressively obliterated due to a hyaline cast formed in its centrum. Complete obliteration was not achieved until 11 months of age and in few specimens until 2 years of age. Looping of the capillaries showed great increase in all regions of the lobe. Neurosecretory material and Herring bodies were found in abundance throughout the lobe.

In specimens collected from one-year-old pigs, colloid infiltration of the capillaries was evident in the pars distalis neurohypophysis. The staining affinity of the intravascular colloid material was identical to that present in the pars distalis adenohypophysis. Beginning with this age, proliferation of connective tissue was evident. This was, however, limited to the perivascular region.

In specimens collected from pigs 2.5 years of age and over, the neurchypophysial fibers became progressively reduced in number. The amount of neurosecretory material also

decreased and was evident only in small quantities in sections taken from the rostral and middle portions of the lobe. However, in the caudal extremity of the lobe, the amount of neurosecretory material appeared to be unaltered. Concurrent with the reduction in the number of neurohypophysial fibers, replacement fibrosis became evident (Figure 138). This fibrous mass was of an identical structure as that of the tissue observed in the pars compacta infundibuli. The ground matrix of this tissue mass was periodic acid-Schiff negative. In this matrix fine interwoven fibers were present (Figure 140). These fibers stained magenta with periodic acid-Schiff and were intensely argyrophilic (Figures 139 and 142). Routine collagen stains failed to reveal these fibers.

Masses of such fibrous tissue contained minute capillaries and a large number of fibroblast cells. The latter occupied the enclosed spaces between the interwoven fibers and appeared to have been entrapped by them. The fibrous tissue occurred in the form of cones with their narrow ends pointing towards the centrum of the lobe and the broad ends towards the periphery (Figure 138).

During this and subsequent age-intervals, there was a progressive increase in the amount of connective tissue fibers that circumscribed the blood vessels. The latter showed progressive ramification while some of them became obliterated due to colloid infiltration. Incidence of

hyalinization of capillaries showed an increase with the advancement of age. Content of neurosecretory material showed a progressive decrease in quantity with age.

Between 7 and 10 years of age, the lobular arrangement of the pars distalis neurohypophysis, in its contour as well as in its structure, was less evident. In the rostral part of the pars distalis, no stainable neurosecretory material was present in any of the specimens studied. The amount of neurosecretory material present in the vicinity of the vessels was scanty. In the caudal half of the lobe, the fibers also contained decreased amounts of neurosecretory material. The latter was evident only in the vicinity of the vessels (Figure 143). Herring bodies were not encountered during this age interval.

In the pars distalis neurohypophysis of the specimens studied in this age-group, replacement fibrosis was very conspicuous. The number of typical neurohypophysial fibers was greatly reduced. Colloid infiltration of the vessels was conspicuous. By 10 years of age, almost all the small vessels were hyalinized and medium and large vessels were affected to variable degrees. Connective tissue fibers formed septa-like strands that extended throughout the pars distalis neurohypophysis. Colloid-filled capillaries within these septa made them conspicuous (Figure 142).

Invasion of the pars distalis neurohypophysis by cellular

columns from the pars intermedia adenohypophysis occurred in all the specimens (Figure 142). These columns were mostly confined to the rostral two-thirds of the lobe. Extension of such cellular columns often followed the course of occluded blood vessels. It was characteristic that these penetrating columns were composed of hypertrophied cells which appeared as functionally active cells. Invasion of the pars distalis neurohypophysis by the parenchymal cells of the pars intermedia was not so extensive as that observed in the dog.

Age-correlated Changes in the Hypothalamus

The present study was confined to the investigation of neurosecretory material contained in the paraventricular and supraoptic nuclei of the dog and the pig. In the beagle, the paraventricular nucleus was located on either side of the ependymal lining of the third ventricle. The neurons, constituting this nucleus, were distributed over a wide area, some distance away from the ependymal wall and dorsomedial to the anterior columns of the fornix. This area contained a large number of capillaries and few neuroglia cells.

The neurons of the paraventricular nucleus were very large cells. The nucleus of the neuron was vesicular and was characteristically spherical in outline with a single nucleus located towards one end (Figure 146). The nucleus itself was eccentrically located. The cytoplasm was compara-

tively larger in volume and stained uniformly purple with chrome alum hematoxylin and blue with alcian blue. An area in the immediate neighborhood of the nucleus did not reveal any staining with the above dyes. The cells possessed large numbers of dendrites. The large unmyelinated axon usually originated with a dendrite and manifested the same staining character as the cytoplasm. The axon was not uniform in diameter, but it contained spherical enlargements throughout its course that could be followed in the sections. Collateral branching was also evident in these axons.

Though many such large neurons were evident in the nucleus, all of them did not contain neurosecretory material. A large proportion of them appeared as functionally inactive cells. Their cytoplasm showed no affinity for the stains. Such neurons contained a large number of Nissl bodies in their cytoplasm. The Nissl bodies were located near the peripheral margin of the perikaryon and were mostly concentrated on the opposite end of the nucleus and at the point of origin of the processes. In addition to these, many neurons of comparatively smaller size were present among the larger neurons. Some of the former showed typical staining of the neurosecretory material while others did not reveal any affinity for such stains.

The individual axons pierced through the fibers of the anterior column of the fornix and united into discrete

bundles on the ventrolateral surface of the latter. A large compact bundle was not evident during the downward course of the axons to the supraoptic nucleus. The small bundles crossed each other during their course, which could be followed easily throughout its extent due to the characteristic staining of their neurosecretory material (Figure 147). Along the course of the paraventriculohypophysial tract, many small neurosecretory neurons were present. These were small cylindrical cells with ill-defined processes. Their neurosecretory content was evident from the characteristic staining of their cytoplasm by chrom alum hematoxylin and alcian blue.

The supraoptic nucleus occurred in two separate parts. The dorsolateral group (pars suprachiasmatica) of neurons was located in a limited area beneath the pia-glial membrane that straddle the optic chiasma. Their location was rostral and ventral to that of the paraventricular neurons further lateral to the infundibular recess of the third ventricle. The neurons that composed this nucleus were comparatively larger than the paraventricular neurons and were of uniform size. In structure, they resembled the large neurons of the latter nucleus. As stated earlier, many small neurosecretory neurons were evident along the paraventriculohypophysial tract and extended between the two nuclei. These constituted the accessory paraventricular

nucleus (nucleus paraventricularis pars accessoria). The neurons of the supraoptic nucleus contained few processes and their axons united with those of the paraventricular to form a prominent hypothalamohypophysial tract (Figure 145). The latter could be easily delineated in sections stained with chrome alum hematoxylin. Considerable intricate patterns were evident by crisscrossing of neurosecretion-bearing fibers from both nuclei in the region of the supraoptic nucleus (Figure 167).

The second part of the supraoptic nucleus (pars postchiasmatica) consisted of a small group of neurons located on the ventrolateral surface of the optic chiasma (Figure 149). Their usual site of location was bounded between the radix infundibuli and the optic tract. The perikarya located in this nucleus were comparatively smaller in size than the dorsolateral group and were of uniform size. Their structure was akin to the latter neurons, too. Axons of these neurons did not form a distinct fasciculus before uniting with the hypothalamohypophysial tract in the radix infundibuli.

Stainable neurosecretory material could be demonstrated in specimens obtained from pups 6 days of age. Perikaryon of both nuclei revealed the presence of neurosecretory material at this age, but their number was higher in the supraoptic nucleus. In hypothalamic sections collected from

puppies 3 days and 8 hours of age, neurosecretory material could not be revealed either by Bargmann's chrome alum hematoxylin technique or Adam and Sloper's alcian blue technique (Figure 144). In the supraoptic nucleus, the neurons contained small cell bodies without many processes and discernible Nissl bodies. Neither the paraventriculo-hypophysial tract nor the hypothalamohypophysial tract was discernible. Very few fibers and neuroglia cells were evident in both the nuclei. Whereas the paraventricular nucleus contained very few large neurons with eccentrically located nuclei, the supraoptic nucleus contained a fairly large number of them.

Beginning with the age of 6 days, incidence of stained perikarya increased in both nuclei. The axons that formed the paraventriculohypophysial tract were discernible at an early age whereas an appreciable number of cell bodies were not noticed in either nuclei until 2 months of age. Numbers of collateral branches that originated from the axons also increased progressively with age, and intricacy of fibers of the hypothalamohypophysial tract progressed with age. The ventrolateral part of the supraoptic nucleus became clearly evident in specimens collected from pigs 2 months of age. The smooth homogenous staining of the perikaryon was progressively changed to globe-like masses of stained material with the advancement of age.

Apart from the preceding, very little significant variation was noticed between different age intervals up to 7 years of age. Beyond that period degenerative changes became apparent in both the perikarya as well as the axons. There was considerable variation in the severity of these changes between specimens of the same age group. Usually the degenerative changes were confined to a limited number of cell bodies, while some neurons appeared as normal neurons and others as inactive cells.

Three different types of alterations in the morphology of the cells were evident. In some specimens, the cytoplasmic mass contained a gray stained area in the opposite direction of the nucleus. This area was devoid of Nissl bodies and appeared rarified. The neurons was not stained by any of the techniques. Such changes resembled typical chromatolysis of the cell bodies in the central nervous system. Incidence of neuronophagia increased progressively with age. Between 10 and 14 years of age, there was appreciable reduction in the number of cell bodies. Vacuoles as well as various stages of degenerating neurons were evident in these specimens. Both the preceding types of structural alteration were commonly observed in the neurons of the supraoptic than in those of the paraventricular nuclei.

Vacuolation of the perikaryon was frequently encountered in the paraventricular nuclei (Figure 159). Only a small

percentage of the neurons presented such an affection. A small vacuole was evident in the main cytoplasmic mass of perikarya in certain cells while in others the entire perikaryon was composed of a signet ring-like mass of stained neurosecretory material (Figure 160). Tigrolysis occurred in both the nuclei with equal incidence. Tigrolysis was commonly encountered only in specimens of the older age groups (between 11 to 14 years of age). Large globes of intensely stained bodies were evident along the peripheral margin of the perikaryon while the main mass stained gray. In contrast to this, many cell bodies contained a dense mass of intensely stained neurosecretory material without a discernible nucleus (Figure 149). Dendrites were not evident in such cells. The entire perikaryon appeared as a cell with an excess accumulation of neurosecretory material. In fact, it simulated a Herring body with a long outward process.

Degenerative changes also involved a decrease in the size of the neurons. The largest size of neurons was observed between 2 month to 4 years of age, after which there was a gradual decreasing trend in the average size of the neurons. Neurons of specimens obtained from beagles 12 to 14 years of age contained perikarya of appreciably reduced dimensions. Concurrent with the reduction in the size of perikarya, the number of dendrites became less evident as a result of which very few stellate-shaped cells were evident in the nuclei.

Beyond 7 years of age, the fibers of the hypothalamo-hypophysial tract also decreased in number. The intricate network of the fasciculi was no longer evident in the vicinity of the supraoptic nucleus. The entire area appeared to have been encroached upon by glial cells and non-neurosecretion-bearing fibers. Herring bodies, evident among the fibers of the paraventriculohypophysial tract, showed an increase in number with age. But in old subjects they stained weakly. The incidence of the preceding type of degenerative changes was higher in the hypothalamus of beagles whose pars distalis neurohypophysis was affected by invasion of pars intermedia cells. In older subjects, in which the pars distalis neurohypophysis was considerably reduced in volume, changes such as tigrolysis and chromatolysis were evident with equal severity.

The morphology of the supraoptic and paraventricular nuclei of the pig was identical to their homologue counterparts in the beagle. The neurons were considerably larger in size which was especially noticed in the case of the supraoptic nucleus. In the pig, stainable neurosecretory material was evident by the third day of postnatal age. The changes evident in the nuclei of the dog were also observed in the pig. Vacuolation of neurons and chromatolysis were much more common in the pig than in the dog. Accumulation of neurosecretory material in the form of cysts was

observed in the pig. Such cysts were usually located along the course of the hypothalamohypophysial tracts at its point of union with the radix infundibuli (Figure 156). Severe degenerative changes were evident in the hypothalamic nuclei of animals that contained fibrous masses in their pars distalis neurohypophysis. In specimens obtained from 8 to 10 year old sows, the majority of the supraoptic neurons showed chromatolysis and neuronophagia (Figure 161). The small capillaries appeared as occluded vessels with periodic acid-Schiff-positive material (Figure 161).

DISCUSSION

Comparative Study on Quantitative Changes in Weight

Comparative study of age-correlated changes in weight of the hypophysis revealed identical patterns in the dog and pig. Absolute weight of the hypophysis increased from birth to 13.6 years in the dog and from birth to 10 years in the pig. In both species, percentage of variation of the hypophysis weight that could be attributed to the relationship with either age or body weight decreased considerably with increase of both variables. Between birth and 8 weeks of age, the period during which the hypophysis manifested the highest rate of growth in both species, a significant proportion of the variation of the absolute hypophysis weight was on account of the relationship with age or body weight whereas in the third interval, during which the rate of growth was the lowest of the three age intervals, the relationship with age and/or body weight was appreciably low, especially in the pig. In the latter, only five percent of the variation of absolute hypophysis weight could be attributed to the relationship with body weight (Graph 9). This was also reflected in the regression analysis of absolute hypophysis weight on body weight of the pig (Graph 12). The increase in absolute weight during the interval 1-100 kilograms body weight was 1030 percent whereas during the intervals 100-200 and 200-300 kilograms body weight it

reduced to 56 and 13 percent, respectively. These facts suggested that age and body weight of the animal influenced growth rate of the hypophysis to a much higher degree in the sexually immature animal than in the sexually mature animal.

Relative growth of the hypophysis was considerably higher in animals of both species between birth and 8 weeks of age and was the lowest in the adult group (Tables 3 and 4). There was an increase in absolute hypophysis weight in all age intervals where body weight also manifested an increase. However, the percentage increase in absolute hypophysis weight and body weight occurred at different rates with age. In the second age interval of the dog, the relationship between relative hypophysis weight and age was a curvilinear regression indicating a disproportionate increase in absolute hypophysis weight and body weight. In the 9 to 11 month interval the percentage gain in absolute hypophysis weight was greater than that of body weight (Graph 3). This period approximated the time of sexual maturity in beagles. In the pig, analysis of relative weight on age was a curvilinear regression in the first age interval, birth to 8 weeks, which resulted primarily from a higher growth rate of the hypophysis between 6-8 weeks of age (Graph 7).

In the third age interval of pigs the difference in mean relative weight of the male and female animals was

mainly due to differences in the body weight (Graph 11). Body weight in the adult males was significantly larger than that of the females (Graph 10) and absolute hypophysis weight increased with age from 1 to 10 years without any sex difference (Graph 9). This caused the relative hypophysis weight to remain constant in the male from 1.5 to 7.5 years while that in the female increased linearly. In the sow, the high positive linear regression on age occurred as a result of few large relative weights between 8 to 10 years of age.

In mixed breeds of dogs other than beagles, regression analysis of absolute hypophysis weight on body weight differed in the male and the female (Graph 7). This might have been caused by significant variations between samples of the two sexes (Table 1). The number of male dogs was appreciably low and did not include representations from large breeds, viz., Fox Terrier, German Shepherd etc., all of which constituted a significant proportion of the female sample. However, a sex difference in absolute hypophysis weight was observed by Latimer (1941) and White and Foust (1944) in samples of mixed breeds of dogs, which was not however, statistically significant. Hewitt (1950) reported no significant sex difference in the hypophysis weight within any weight group of adult dogs. However, it should be noted that the sample used by Hewitt (1950) included 52 animals

out of a total of 91, whose body weights ranged from 5 to 9 kilograms. In the present study the sex difference in the ratio of hypophysis weight to body weight was more evident in the higher ranges of body weight than in the lower ranges (Graph 6); no sex difference was evident in the beagle sample where the range of body weight was 0.2 to 16.5 kilograms (Table 3). The present finding of a higher ratio of absolute hypophysis weight to body weight in females than in males of the mixed sample is in conformity with similar observations of Latimer (1941) and Hewitt (1950) in samples of mixed breeds. Thus, the validity of a true sex difference could not be firmly established without the employment of a large sample size equally weighted at either end of the body weight range.

The present study revealed that absolute weight of the canine hypophysis was influenced by age and body weight. Absolute hypophysis weight increased with age as body weight also increased with age. White and Foust (1944) reported similar findings in their study on the dog. Francis and Mulligan (1949) observed that the individual body weight of the dog is an accurate indication of the weight of the hypophysis whereas age of the animal is not constantly related to either of them. In the present study influence of both age and body weight was evident in all age intervals of the dog though in decreasing proportions. Latimer (1941),

White and Foust (1944) and Hewitt (1950) reported a decrease of relative weight of the hypophysis in the dog from birth to senescence, akin to observations in the present study. Employing mixed breeds of dogs Latimer (1941) and Hewitt (1950) found the relative hypophysis weight to be significantly higher in the female than in the male. The latter author further substantiated that such a difference between both sexes was only evident in sexually mature animals. However, Latimer (1941) observed no significant sex difference in absolute hypophysis weight and Hewitt (1950) found no difference in weight of the hypophysis within any single weight group of adult animals. In the present study, where the sample consisted of beagles only, sex difference in relative hypophysis weight was not observed in puppies, growing animals or adult animals. Considering the fact that weight of the hypophysis was affected by body weight, a great variation in body size of the different breeds of dogs could have affected the data in samples employed by Latimer (1941) and Hewitt (1950).

Baker _et al_. (1956) subscribed that weight of the anterior lobe of the hypophysis increases at a rapid rate up to the age of 225 days in the sow after which the increase continues at a lower rate. They also stated that the ratio of anterior pituitary weight to body weight decreases rapidly until the age of 300 days when it becomes constant.

These observations by Baker *et al.* (1956) appeared identical to the present observations even though the entire hypophysis was used in the present study. In the male animals relative hypophysis weight was found to be constant beyond the age of one year whereas in sows there was a slight increase which was probably caused by few higher weights between 8 to 10 years of age. Cupps *et al.* (1969) reported that sows attain puberty at 6 to 8 months of age and body weight of the animals averages 70 to 80 kilograms at the time of the onset of puberty. In the present study, body weight of the pigs, at 6 months of age was found to be 80 kilograms and it was interesting that absolute hypophysis weight increased 165 percent during the interval 2 to 6 months, which was the highest observed among all age groups.

The hypophysis of the female has been reported to be heavier in the mouse (Blumenthal, 1955), rat (Pfeiffer, 1936; Verzar, 1966) and rabbit (Kibler *et al.*, 1942). In the cat (Latimer, 1939) and guinea pig (Mixner *et al.*, 1943) weight of the hypophysis has been demonstrated to be heavier in the male of these species. In the human, Rasmussen (1947) observed that women possess heavier hypophyses than men and this sex difference becomes significant only after puberty. The present study revealed no significant sex-linked difference in absolute hypophysis weight or in the relative hypophysis weight of either the

dog or the pig. These variations may be a reflection of species differences in weight of the hypophysis.

Pfeiffer (1936) subscribed that sex difference of the hypophysis is not a genetic factor and that testes of newborn animals secrete a small amount of androgen which acts on the hypophysis to make the pattern of hypophysial secretion the male pattern. Hewitt (1950) concluded that weight of the hypophysis is subject to gonadal influence being increased in the female by estrus, pregnancy and the administration of chorionic gonadotropin, theelin and theelol. On the contrary, the hypophysis of the male is not influenced by the administration of testosterone. Ganong and Kragt (1969) observed that sex-pattern of the hypophysis is not fixed and it is the hypothalamus that differentiates according to the presence of testosterone. Hypophysis is dependent on the sex-pattern of the brain under which it is located. Both endogenous and exogenous testosterone, administered to newborn rats, has been found to be capable of typical feminine rhythmic periodicity of hypothalamic activity whereas castration of male rats at birth leads to differentiation of feminine type of hypothalamus. In view of the fact that species differences exist in ex-linked differences of the hypophysis weight it is not possible to attribute the sex difference in weight of the hypophysis either to genetic factors or gonadal

factors without further elucidation.

The two variables that seem to affect the absolute hypophysis weight in the pig and beagle samples are age and body weight. The influence of body weight is much more pronounced in the young than in the adult animals. Compared to the dog, absolute hypophysis weight increases appreciably at an early age in the pig which corresponds to the occurrence of puberty at a comparatively earlier age in the sow than in the bitch. Relative hypophysis weight is consistently higher in young animals than in sexually immature and adult animals.

The present study revealed high correlations between the endocrine glands in both species. Subdividing the data into three specific age intervals revealed the fact that correlation of all the organs with age of the animal decreased with advancement of age, denoting a diminution in their growth rates. The only exception was the adrenal gland of the beagle which manifested an increased rate of growth in the sexually mature animal. The relationship of body weight and the other endocrines was significant during the entire span as well as during the different age intervals. This indicated that both age and body weight had marked influence on the weight of all endocrine organs.

The interrelationship among the endocrine organs carried high correlation coefficients in both species, which

showed a general tendency of decrease with age. When partial correlations were computed holding body weight constant, the correlations were reduced in most instances. This indicated that body weight had a marked effect on most of the interrelationships.

The available literature indicated diversified results among the different species. Stockard (1941) stated that a relatively large amount of thyroid tissue is not regularly associated with either a high amount or a low amount of hypophysis in the dog. White and Foust (1944) also reported similar findings in a sample composed of mixed breeds. The present study indicated that a given weight of the hypophysis is always related to a particular amount of thyroid tissue. Even after computing the partial correlations, holding body weight as a constant, the above relationship was statistically significant in the dog as well as in case of the pig. The latter results are in agreement with that of Baird *et al.* (1952) in case of pigs. The latter authors have also reported that the hypophysis and thyroid weights of rapid and slow growing genetically different lines do not differ significantly. Thus a difference due to variety or breed is negligible.

In both species, the pattern of growth of the thyroid is identical with the reported results by Haensly *et al.* (1964) and Haensly and Getty (1970). In the guinea pig,

Mixner *et al*. (1943) found a significant difference in the weight of the thyroid gland between both sexes as is evident in case of the pig sample in the present study. The authors have concluded that the thyroid gland of mature females is heavier than the male, as female thyroid grows at a faster rate with increase in body weight. The present study revealed that the weight of the thyroid gland of the boar, but not the sow, increased at a faster rate with increase in body weight and hypophysis weight while that of the sow increased at a faster rate with age (Graphs 19 and 20). In the adult cat, Latimer (1939) found no significant correlations between weight of hypophysis, thyroid, adrenal and testis. Like the present observations on the canine sample, a sex difference in the weight of the thyroid gland has not been reported in the cat (Latimer, 1939) and the rabbit (Kibler *et al*., 1943).

In both the dog and the pig, a significant relationship was evident between the weight of the hypophysis and the adrenal gland. The same relationship was also significant when weights of the organs were considered as percent body weight. In both species, weight of the thyroid was also significantly related with the weight of the adrenal, on the basis of absolute as well as relative weights. This indicated that a given weight of the adrenal tissue is associated with a particular weight of all other glands. A sex difference in the weight of the adrenal gland was only

evident in case of the canine species. Between birth to 1 year of age, there was very little difference between the two sexes. Beyond the period of sexual maturity, the adrenal gland of the male increased sharply followed by a decline, while in the female, it increased consistently with age (Graph 15). A sex difference in the growth of the adrenal gland has not been reported in the rabbit (Kibler _et al_. (1943), in the guinea pig (Mixner _et al_., 1943; Latimer, 1951) or in the cat (Latimer, 1939). Haensly and Getty (1965) and Haensly and Getty (1968) have reported observations on samples composed of mixed breeds of dogs and pigs, which are similar to present findings.

The significant relationship between adrenal and gonads was an interesting observation. Body weight showed the least influence in this relationship in case of the male animals of both species. Weight of the testis was related to age, body weight and also with the weight of the hypophysis and the thyroid. The relationship of the ovary differed from the former in the respect that after sexual maturity there was very little growth of the ovary. Identical results have also been reported in swine (Bal _et al_., 1969) and in a sample of mixed canine breeds (Stott, 1970).

In the dog, weight of the testis increased at a much faster rate than the body weight while that of the ovary decreased with increase in body weight. Maximum growth of

the testis occurred between 2 to 11 months of age after which there was no significant increase in its weight (Graph 16). Similar results have been observed by Mixner et al. (1943) in the guinea pig and Kibler et al. (1943) in case of rabbits. Bal et al. (1969) reported that ovary weights of swine increased at a decreasing rate with increase in body weight up to 180 kg. At body weights exceeding 180 kg ovary weight was found to be relatively stable. An identical relationship of the body weight and weight of the ovary was also evident in the present study (Graph 20).

The present study indicated that age has a profound influence on the growth of the hypophysis, thyroid, adrenal and the gonads, and age affected their interrelationships. Body weight of the animals also influenced the correlations between these glands. Since age and body weight are closely related with each other, effect of one cannot be evaluated without considering the other.

Morphology

Adenohypophysis

In both species, six different types of chromophil cells were observed in the present study. There was neither a difference in the tinctorial affinity of the various cell-types between the species nor was there any difference within the species at different ages. Two minor deviations were only evident in the present study. The FSH gonadotrope

cell of the newborn puppy stained magenta with periodic acid-Schiff whenever the latter was employed in conjunction with alcian blue, aldehyde-thionin or aldehyde-fuchsin. Affinity of the cell for the latter dyes increased in later periods. Similarly the intracytoplasmic granules of the ICSH gonadotrope cell showed enhanced affinity for orange G as the age of puberty was approached.

The acidophil cells could be easily differentiated by the use of dye combinations such as luxol fast blue and orange G, orange G and azocarmine, orange G and acid fuchsin or orange G and erythrosin. The latter two combinations were not as useful as the first two procedures. In the presence of erythrosin or acid fuchsin, the granules of the somatotrope cells did not manifest the typical yellow coloration with orange G. Achievement of correct degree of differentiation was found to be critical in both combinations. Increasing the time of azocarmine staining as suggested by Hartmann _et al_. (1946) brought about clear differentiation between the somatotrope and lactotrope cells. Sections from very young animals required a slightly longer period of staining in orange G and aniline blue mixture without which the former cells were colored dull orange and the basophil cells were stained in shades of greenish blue.

Luxol fast blue served as a dye of choice to differentiate the somatotrope cells (Figure 18). No other cell-

type of the pars distalis adenohypophysis manifested any affinity for this dye as had been reported by Paget and Eccleston (1960) in case of the rat and human. In the present study, luxol fast blue was employed in conjunction with Schiff reagent, aldehyde-fuchsin, aldehyde-thionin, methyl blue, orange G and erythrosin. Though luxol fast blue was used at the initial phase of the staining sequence (except for aldehyde-fuchsin and aldehyde-thionin) it did not alter the coloration of cells by the latter stains. The differentiation of the dye in the sections was easy to control. Combination of luxol fast blue and erythrosin served as a very useful sequence. The intracytoplasmic granules of somatotrope cells and lactotrope cells were selectively stained by the respective dyes, and in addition, erythrosin served as a nuclear stain for suitable delineation of the margins of cellular components. There was no effect of postchromation or oxidization of sections with iodine on the staining character of luxol fast blue. On the other hand postchromation of sections enhanced the staining of lactotrope cells.

The staining character of the somatotrope and lactotrope cells of the canine hypophysis, observed in the present study, is identical to the results obtained by Wolfe and Cleveland (1932), Hartmann _et al_. (1946), Goldberg and Chaikoff (1952a), Purves and Griesbach (1957a), Wolfe (1959)

and Dawson (1963). Mikami and Ono (1956) have reported that the somatotrope cells of the canine hypophysis are stained by the azocarmine dye while the lactotrope cells stain with orange G. This appeared as the opposite case of present observations as had also been concluded by other authors. In both species, the lactotrope cells manifested affinity for the Schiff reagent. Earlier, Purves (1961, 1966) and Heath (1965) had reported that the lactotrope cells were periodic acid-Schiff positive in the mammalian species including the dog and the pig. Thus, the present observations confirm the earlier reports.

The basophil cells were easily differentiated by the use of dyes, such as alcian blue, aldehyde-thionin and aldehyde-fuchsin when employed in conjunction with periodic acid-Schiff. Alcian blue furnished a better coloration of the cells following oxidation with performic acid than with acidified potassium permanganate. It also stained the neurosecretory material contained by the neurohypophysial fibers and by the perikarya of the supraoptic and paraventricular nuclei. In order to stain entire sections of the hypophysis alcian blue can be utilized with a greater advantage than aldehyde-fuchsin. The disadvantage of the dye was due to tendency of the sections to become detached during performic acid treatment. In the present study, formaldehyde fumigation served as a suitable means to over-

come this difficulty without impairing the staining of the tissue components.

While prior oxidation with acidified potassium permanganate was not found essential for aldehyde-fuchsin staining, it was found necessary for aldehyde-thionin. However, the latter dye was easy to prepare and it gave uniform results. Aldehyde-thionin also stained the neurosecretory material in all locations with equal lucidity as alcian blue at low pH. Coloration of the thyrotrope cells with aldehyde-thionin was much more vivid than that by aldehyde-fuchsin. But the former dye showed a general tendency to impart a light hue over every tissue element in the section. This was especially conspicuous in the dull greenish blue coloration of the somatotrope cells following luxol fast blue staining. In both species, the thyrotope cells were selectively stained by all of the three dyes; the FSH gonadotrope cells possessed affinity for all three stains as well as for the Schiff reagent due to which they were stained purple; and the ICSH gonadotrope cells presented no affinity for any of the stains and were, thus, strongly colored by the periodic acid-Schiff reaction (Figure 100). These staining characteristics of the basophil cells are identical to the earlier observations of Bugnon (1963b) and Heath (1965) in case of the pig, and Paget and Eccleston (1960), Heath (1965), Purves (1966) and Carlon (1967) in case of the dog.

Comparing with other species, identical results have been reported in human (Ezrin and Murray, 1963; Pearse and Van Noordan, 1963; Conklin, 1968), in the rat (Purves and Griesbach, 1951b, 1954; Halmi, 1952a), in the bat and the mole (Herlant, 1964), in the cat (Racadot and Herlant, 1958; Racadot, 1961, 1962) and in the sheep (Racadot, 1962b). The gonadotrope cells of the dog and the pig differed from those of cattle in their staining affinity. The FSH gonadotrope cell of the latter species had little affinity for alcian blue or aldehyde-thionin (Dubois and Herlant, 1968), whereas that of the dog and the pig manifested clear staining by the above dyes.

In differentiating the gondaotrope cells, methyl blue and lead hematoxylin were found very useful. FSH gonadotrope cells manifested specific affinity for the former dye while ICSH gonadotrope cells were selectively stained by the latter. These results are in agreement with similar observations by Rennels (1957) in the rat.

The adrenocorticotrope cell differed in its morphology in the two species. In the dog, it is a large cell with great amounts of cytoplasm. It is better elucidated in the luxol fast blue-tichrome technique and in the aldehyde-fuchsin and Crossman's trichrome technique. In the former it appeared as a degranulated cell with faint erythrophilic granules. In the latter procedure, it was evident as a large gray cell

without any granules (Figure 16). The latter type was akin to the zeta cell of Mikami (1956) who established the functional identity of this cell by adrenalectomy of the dogs. Purves and Griesbach (1957a) described the cell as the pale blue cell and believed it to be a type of gonadotrope. This was later corrected by Purves (1966) as being the neutrophil concerned with the secretion of adrenocorticotropin. Carlon (1967) has observed similar type of adrenocorticotropin cells in the canine adenohypophysis after tetrachrome staining. In the present study, Mallory's phosphotungstic acid hematoxylin stain clearly outlined these cells along the margin of the portal vessels in case of the dog. Many portal capillaries were found to be associated with one or two such cells.

In the pig, the adrenocorticotrope cells were found as small stellate-shaped chromophobic cells that contained a small volume of cytoplasm. Similar morphological characteristics of these cells have also been reported by Bugnon (1963a) and Bugnon and Racadot (1963). Bugnon (1963b) has confirmed the functional significance of these cells by studying the hypophysis of animals suffering from various types of stress.

The trichrome technique of Cleveland and Wolfe (1932) served as a very suitable procedure for staining all the cell-types in a single section of the adenohypophysis. A

great advantage of the sequence is that there is no critical step for differentiation of any of the three stains employed, *viz.*, erythosin, orange G and aniline blue. The procedure necessitated postchromation of the sections on the slide which facilitated the staining affinity of lactotrope cells to a minor extent but revealed the staining of adrenocorticotrope cells to a great extent. Two other advantages of the staining sequence are the clear declineation of the nuclear and cytoplasmic outlines of all cells without the application of an additional nuclear stain, and clear cut differentiation of all cell-types. It was found preferable to Herlant's (1960) tetrachrome method due to the fact that the latter required critical steps of differentiation and there was poor differentiation between the FSH gonadotrope cell and the thyrotrope cell, both of which stained in different shades of blue. In order to reveal the ICSH gonadotrope cell, the tetrachrome method utilizes a fourth acid stain, alizarine acid blue. The latter not only requires a specific composition but an adjustment of its pH in the solution and two critical steps of differentiation in alcoholic phosphomolybdic acid (Racadot, 1962c). Moreover, this technique required Helly's or Bouin-Hollande fixation of the tissue and did not give good differentiation of cells following mercury-formal fixation, whereas Cleveland and Wolfe's (1932) technique gave the same type of reaction after

mercury-formal, formalin-ammonium bromide and buffered neutral formalin fixation of tissues.

In the present study, luxol fast blue was employed in conjunction with Cleveland and Wolfe's trichrom technique for the first time. Application of this sequence has not been reported in any other species. Addition of luxol fast blue differentiated the somatotrope cells selectively and masked the bluish hue of aniline blue (Figure 83). The lactotrope cells stained brick red by erythrosin and the thyrotrope cells stained clear blue (Figure 18). The two types of gonadotrope cells could be differentiated early by the gray blue coloration of FSH cells and vivid violet coloration of the ICSH gonadotrope cells (Figure 116). The dye orange G could not be eliminated from the sequence as it imparted the violet coloration to the ICSH cells in association with erythrosin, and thus facilitated their clear differentiation from the lactotrope cells.

Two other staining procedures employed in the present study have not been reported in the earlier literature as having been employed for the hypophysis. Bielschowsky's ammonium silver technique revealed the postganglionic autonomic fibers in all the subdivisions of the hypophysis. The minute branches given off to the vessel walls were also evident (Figures 11 and 104). Another advantage of this technique was that even the smallest capillaries were revealed in the

sections due to staining of the perivascular nerve fibers. Results of this technique have been discussed in greater detail at a later stage.

Mallory's phosphotungstic acid hematoxylin stain served as a useful stain for its general applicability. All types of connective tissue fibers were stained in the same section and the outlines of the cells were vividly delineated. Among the fibers, collagen stained red brown, reticulin was colored red with blue lining; fibrin stained blue and fibrinoid masses were stained brown (Figure 92). The stain was especially useful in the study of age-correlated changes because it elucidated the deposition of fibrin in the centrum of the cell-cords and in the vessels as a prelude to the deposition of colloid. The hyperplastic chromophobic nodules were more vividly elucidated in this technique even at their early phases than in the other techniques.

In both species, the shape and size of the basophil cell-types were found to be beneficial guidelines to supplement the differentiation of cell-types on the basis of their staining affinity. The thyrotrope cell in either species was angular in outline and possessed medium proportions. The FSH gonadotrope cell was variable in its contour and was the largest cell-type of the adenohypophysis in both species. The ICSH gonadotrope cell was the least numerous. It was equal or slightly smaller in diameter than the thyrotrope

cell and possessed distinct polygonal to spherical outline. Such a supplementary guideline was not evident in case of the acidophil cells. The only useful criterion was the large size of the intracytoplasmic granules in the lactotrope cells when erythrosin was employed as the staining medium.

Two features of the staining technique employed in the present study were considered worthy of elucidation. Regardless of the species studied, sections ought to be procured from different levels of the organ and a set of sections obtained from one particular level should be stained with different techniques. Although distinct zonal arrangement of the cell-types was not evident in some species, e.g., the canine, certain types of cells were usually more predominant in some locations while in other locations they were distributed as isolated cells. This had a direct bearing on the cytometry of the cells. When cells were found in groups, they usually assumed a cylindrical or truncated outline for accommodation within the cell-cords, whereas they were distinctly spherical or polygonal with comparatively larger dimensions in locations where they occurred as single cells. Few average-sized somatotrope and lactotrope cells were observed in the rostral one-third of the gland whereas the reverse was apparent in case of the FSH gonadotrope cells.

In any type of study, a useful guideline is the tinctorial affinity of the intracytoplasmic granules of the different

types of adenohypophysial cells. In the present study, where morphological classification of the cell-types was the only criterion utilized to achieve differentiation of the cells, employment of different types of staining sequences to consecutive serial sections served as a very valuable practice. Ambiguity in the differential type of cells could be avoided by staining consecutive sections in two types of differentiating stains. The latter was especially evident in case of adrenocorticotrope and lactotrope cells. The possibility of misrepresentation of the latter cell could be avoided by the use of azocarmine technique. Similarly the lactotrope cells could be easily differentiated from the ICSH gonadotrope cells in periodic acid-Schiff procedures by the use of erythrosin on the following or the preceding section.

A characteristic difference observed in the morphology of the canine and the porcine adenohypophysis was the more vivid zonal preponderance of the various cell-types in the latter species. As reported earlier by Giroud and Desclaux (1947a, 1947b) and Racadot (1955), localization of the FSH gonadotrope cells was found to be mainly confined to an area of the rostroventral surface (Figure 75). The somatotrope cells were mostly confined to the dorsolateral region (Figure 77). It was interesting to observe that the FSH zone was continuous with the pars paraneuralis as well as with the

pars infundibularis adenohypophysis without any conspicuous demarcation (Figure 75). Continuity of blood vessels between these regions was suggestive of a functional relationship between these cells.

A second characteristic difference between the two species was mitosis of the parenchymal cells. In the canine hypophysis, mitosis was evident in all the subdivisions of the adenohypophysis. In the pars distalis, the chromophobe cells were the only ones that were involved in mitotic division. In none of the subdivisions of the porcine hypophysis, mitosis of the parenchymal cells was observed. A feature worth mentioning in this connection is that Weigert's resorcin-fuchsin and Mallory's phosphotungstic acid methods revealed very fine collagen fibers that circumscribed a small group of cells and in certain instances only one or two cells (Figure 78). In later stages, each cell-cord was found to contain at least six to eight cells. If it has to be believed that the same cord-like arrangement of the newborn piglet continues and there is no intermediate reorganization of cells (as it is more likely to be the case, since there is no sign of degenerative changes in the collagen fibers at such an early age), the cells contained in these cords would have to multiply in some form. Further elucidation of this problem would be beneficial. A suitable source for such study will be to utilize fetuses at different age

intervals and observe the pattern of growth in the adenohypophysis.

Mitosis of the adenohypophysial cells and their diminution with age have been reported in the mouse (Yamada et al., 1960) and in the rat (Wolfe, 1943). Sommers (1959) stated that mitosis of parenchymal cells was not evident in the entire series of specimens from human material. Yamada et al. (1960) observed mitosis in somatotrope and chromophobe cells. In the adenohypophysis of the dog, however, mitoses were limited to chromophobe cells and undifferentiated cells of the pars paraneuralis and pars intermedia.

The nomenclature adapted in the present study was in conformity with the staining affinity of the cell and did not necessarily establish the elaboration of any particular hormone. However, correlative studies indicated that the same nomenclature can be extended to the functional criteria also. Racadot (1955) used specimens from pregnant and castrated pigs to establish the fact that the cells located in the centromedian region of the porcine adenohypophysis are FSH gonadotrope cells. Giroud and Martinet (1948a, 1948b) proved by transplantation experiments that adenohypophysial cells from different regions of the lobe evoke diverse growth responses in immature rats. Responses akin to the administration of follicle stimulating hormone, growth hormone and thyroid stimulating hormone were obtained

by implanting cells from the central, dorsolateral and rostromedian regions, respectively. Bugnon (1963b) utilized specimens collected from sows in different stages of reproductive cycle and from animals after castration. There was a definite relationship between the tinctorial affinity of the cell-types and the reactive changes evident in the cells during gestation, lactation and following castration. Marshall (1951) used immunofluorescent technique to establish the adrenocorticotropic hormone in the porcine adenohypophysis. The locations described by the author are identical to the local distribution of adrenocorticotrope cells in the present study. Corte and Biondi (1964) found a positive immunofluorescent reaction with FSH antibodies in the (beta) cells of the centromedian region. This area contained primarily, the FSH gonadotrope cells in the present study and the staining affinity of the cells, as described by the authors are similar to the present observations.

In the dog, Goldberg and Chaikoff (1952b) used ^{131}I to destroy the thyroid gland and observed hypertrophy of the aldehyde-fuchsin positive beta cells (thyrotrope cells). Purves and Griesbach (1957a) administered thyroid extract and estrogen to dogs to establish the functional criteria of various cell-types. The thyrotrope cells (blue cells of Purves and Griesbach, 1957a) showed hypertrophy. Similarly, the lactotrope (red acidophils) cells were hypertrophied in

male and female dogs following estrogen treatment. Carlon (1967) experimented with castration and treatment with metopirone (SU-4885) and reserpine to establish the functional criteria of various cell-types in the dog. The present observations are in full agreement with the staining characters reported by Carlon (1967). As identical staining affinity and structure of the different cell-types in the porcine and canine hypophysis are observed between the preceding studies and the present work, the nomenclature adopted here, probably, corresponds to the functional criteria of the cell-types, too.

In both species studied in this work, elastic fibers were not observed in the stroma. Three different staining techniques, viz., Verhoeff's elastic tissue stain, Weigert's iron hematoxylin-resocin-fuchsin stain and Mallory's phosphotungstic acid hematoxylin stain were employed to study collagen, elastic fibers and fibrin in the pars distalis adenohypophysis. The stroma was mainly composed of collagen and reticular fibers confined to the perivascular region. Bloom and Fawcett (1968) and Pearse (1968a) subscribed that elastic fibers are concerned with reestablishment of the volume of the organ when the latter undergo changes in its dimensions, while collagen fibers and reticulin are concerned with maintenance of rigidity in the organs and holding the various tissue elements in respective positions. Since the

adenohypophysis is not involved in alterations of its volume to a significant degree like some other organs, absence of elastic fibers in the former can be visualized.

In the present study, the intricate mass of argyrophilic fibers observed in the perivascular region (Figure 11) was the postganglionic autonomic fibers. These fibers were revealed by the ammoniacal silver technique of Bielschowsky which stains selectively the axis cylinders and dendrites. Ample proof of this fact was obtained by the staining of unmyelinated neurohypophysial fibers in the pars distalis neurohypophysis (Figure 66). Two other facts contributed towards this interpretation. In specimens of day-old animals reticulum was poorly developed, and in the pig was not evident at all in many regions (Figure 81). The reticular network increased in thickness with age. On the other hand, the mass of argyrophilic fibers was evident with equal lucidity at all ages. Secondly, the fibers formed an intricate mass by numerous fine branches in the perivascular region of each lobe of the hypophysis, which was especially conspicuous in the region of the pars infundibularis adenohypophysis (Figure 120). Reticular fibers were found to be poorly developed in the infundibularis adenohypophysis and in the pars distalis neurohypophysis. Presence of postganglionic autonomic fibers in the perivascular region of the adenohypophysis has been reported earlier by Harris

(1948) and Green (1948, 1951).

In his *in vivo* studies on the mouse adenohypophysis, Worthington (1935) observed that the portal capillaries are capable of changing their calibers to a marked degree by active contraction or dilatation. In certain circumstances, a small group of capillaries disappeared completely from the field. Local application of epinephrine brought about an identical result. The present morphological evidence of a well distinct mass of intricate postganglionic autonomic fibers around each portal vessel lends ample support to the earlier work.

In this connection, it is worth mentioning that, in both species, the adrenocorticotrope cells were closely associated with the wall of blood vessels and formed pericapillary cells. In aldehyde-fuchsin and Crossman's trichrome technique as well as in Mallory's phosphotungstic acid hematoxylin stain, outline of the cells was clearly revealed in apposition close to the vascular wall. Coupled with the fact that there existed a distinct network of postganglionic autonomic fibers in the perivascular areas, the portal capillaries of the adenohypophysis appeared, on morphological grounds, to be under active systemic control. It is a temptation to quote the remarks made by Worthington (1935, p. 350) and appreciate their validity: "If specific areas in the median eminence or stalk are secreting, or specific groups of capillaries

are receiving different substances which are delivered to the pars distalis, controls of the rates of flow through such specific areas under different physiologic conditions would have an effect on the rates of arrival of these substances in the pars distalis, and the relative proportion of such substances which arrive there. Thus flow rates in a given group could determine the capacity of the region to deliver the substance at a maximal rate. This could be extended to include the rates at which the target organ hormones could be brought to the sites of production or release of these hypothetical substances." With regard to the first sentence in the author's statement, it would not be out of place to mention here that between 1935 and the present date, the releasing and inhibiting factors for all adenohypophysial hormones have been established and in some cases, the releasing factors have been isolated in pure form (Ganong and Kragt, 1969; McCann and Porter, 1969).

Two other factors also appeared to be correlated with the above conclusions: cellular distribution in the pars distalis adenohypophysis and cupping of cells. Studies on the vascular supply of the pars distalis adenohypophysis have revealed an apparent tendency for a certain type of zonal distribution of the portal vessels (Daniel and Prichard, 1956, 1957b; Adams *et al.*, 1963; Daniel, 1966). The median or central area of the lobe is supplied by the portal vessels

that originate from the primary capillary bed of the radix infundibuli; the lateral rostral areas are supplied by the stalk portal vessels that take their origin from the pars cava (or pars compacta) infundibuli; neural lobe portal vessels are mainly distributed in the posterolateral and posterior areas; and, short portal capillaries that originate from the second primary capillary bed of the lower infundibular stem are located in the deep median part of the lobe. This description is in conformity with the basic pattern of cellular distribution observed in the present study and also, reported earlier by several authors (Racadot, 1962a; Herlant, 1964; Holmes, 1964). The somatotrope cells are predominant in the dorsolateral region; the lactotrope cells show predominance in the rostral and ventral parts of the lobe; the FSH gonadotrope cells are dominant in the rostromedian area; the ICSH gonadotrope cells are predominant in the posterolateral area and the thyrotrope cells are dispersed widely in the central region of the lobe with rostral predominance. Thus interrelationship with specific groups of hypothalamic nuclei and groups of adenohypophysial cells may be visualized.

Cupping of the cells in the adenohypophysis was evident in both species. Cupping of the cells has also been observed in the rat (Nakane, 1970). As functionally active cells are only involved in the cupping phenomenon, it may be conceived

that such embeddment of cells within the cytoplasm of a second cell may hinder the diffusion of releasing and inhibiting factors from the blood to the concerned cell. Nakane (1970) attributed the inverse secretion of gonadotropins during suckling in rats to the cupping of gonadotrope cells by the lactotrope cells and thus isolating them from the releasing factors.

In both species, colloid was associated with the portal vessels from a very early age. There is no available literature on the occurrence of intravascular colloid in the dog or the pig and the present study is the first report of its kind. Extravascular colloid has been observed in various other species by Puech et al. (1953), Siperstein et al. (1954), Spagnolli and Charipper (1955), Charipper et al. (1961) and Lopez and Piazza (1962). In the present study, both intra- and extravascular colloid was strongly periodic acid-Schiff positive, indicating a high content of carbohydrates. In some instances, colloid in both locations stained light magenta. The latter type probably represented deposits of longer duration. In Mallory's phosphotungstic acid hematoxylin stain, a fibrin network was evident in the initial stages and fibrinoid was observed in the centrum of cell-cords prior to colloid deposition.

Pearse (1968a) subscribed that hypophysial colloid is a carbohydrate substance rich in the amino acid, tyrosine.

As such it may be conceived as a tissue product of local origin. The fact that colloid appeared earlier at the venous end of the capillaries and a set of capillaries in the pars distalis, and arteries in the pars infundibularis adenohypophysis and pars distalis neurohypophysis remained relatively free of colloid infiltration suggests that colloid is being synthesized by tissue elements within the hypophysis. The colloid contained in the portal capillaries and tissue spaces showed positive reaction for amyloid. Unlike the latter, colloid did not show a "spill over" in the tissue spaces. The occluded vessels appeared in reduced numbers during later periods of the life span which indicated that colloid is being reabsorbed from them. Occurrence of vacuoles along the peripheral margins of occluded vessels (Figure 89) also lends support to the above fact.

The pars paraneuralis consisted of undifferentiated cells in the beagle and the pig. The morphology of the lobe was observed to be identical in both species during earlier stages (Figures 41 and 119). In both species, the parenchymal cells seemed to be intimately associated with the cavum hypophysis. In the dog, mitosis and cell-differentiation were only evident among the marginal cells and changes in the pars paraneuralis were not evident until the cavum hypophysis became obliterated. The embryonic structure of cell-columns separated by ramifications of the

cavum hypophysis was retained until a very late age in the dog, whereas in the pig, the identical embryonic structure was modified at a comparatively earlier age. It will be of academic interest to determine whether the tissue fluid of the cavum hypophysis carries any tropic substance. The fact that colloid was deposited in the central part of the cell-columns and a certain portion of the parenchymal cells was transformed into thyrotrope cells (Figure 45) indicates that the parenchymal cells of this lobe are active cells. Colloid deposition in the follicles was found not only confined to those that contained thyrotrope cells but also was evident in those which were primarily composed of cells that did not show affinity for any type of stains. There is no available report on the function of these cells in any species.

In the dog, the pars infundibularis adenohypophysis is only composed of chromophobe cells whereas that of the pig contains a large number of highly granular periodic acid-Schiff positive cells in addition to chromophobe cells. The present study is the first report on the occurrence of such cells in the pig. There is some amount of similarity between these cells and the FSH gonadotrope cells of the pars distalis adenohypophysis. However, the former did not show any affinity for alcian blue, aldehyde-thionin and aldehyde-fuchsin, and contained a large number of

intracytoplasmic granules. Dawson (1948) and Green (1948) have postulated that the parenchymal cells of the pars infundibularis adenohypophysis are functionally related with the vessels of the mantel plexus.

The pars intermedia of the two species manifested certain characteristic differences. In both species, two types of cells were observed: a chromophobe type of cell whose cytoplasm stained light magenta with periodic acid-Schiff and a granular cell whose cytoplasm stained red with periodic acid-Schiff. In the dog, undifferentiated cells were evident at all ages, and proliferation of the pars intermedia occurred through mitosis of these cells. The latter process was not observed in the pig nor undifferentiated cells were evident at any age. Purves and Bassett (1963) have subscribed that the pars intermedia of the domestic animals is composed of a single cell-type. Wingstrand (1966b) was of the opinion that there are two types of cells in the pars intermedia of the mammals. The light cells of Wingstrand (1966b) correspond to the light magenta cells of the present study in their distribution and morphology, while the dark cells are akin to the granular cells. The present study definitely showed the presence of two cell-types in the pars intermedia of the dog and the pig. The two types of cells differed in their morphology, staining affinity and relative proportions. As functional significance of the pars intermedia has not been

elucidated in mammals it is not possible to evaluate the function of the two types of cells. Pearse (1968b) believed that the pars intermedia cells of mammals share many structural similarities with the light cells of thyroid, adrenocorticotrope cells of the adenohypophysis and "c" cells of the ultimobranchial bodies. Except for the volume of the cytoplasm, the light magenta cells of the dog do appear identical to the adrenocorticotrope cells of the canine hypophysis (Figure 49).

A distinguishing feature between the two species was the vascularity of the intermediate lobe. In the dog, blood vessels were not evident. Only Bielschowsky's ammonium silver technique revealed the presence of slender capillary vessels between the cell masses. In the pig, however, the pars intermedia was highly vascular and contained many large veins. On either side, a large tributory of the caudal hypophysial vein traversed the entire length of the pars intermedia and joined similar veins from the caudal extremity of the pars distalis adenohypophysis (Figure 124). The majority of the smaller veins located in the junctional zone between the pars intermedia and the pars distalis neurohypophysis joined the former vessel. Coupled with the fact that there were very few major venous channels located in the pars distalis neurohypophysis of the pig and that the capillaries of this lobe followed its radial axis, the pars intermedia of the

pig appeared to serve as the main outlet for venous drainage of the pars distalis neurohypophysis. In the dog, the latter lobe contained many large sized arteries and veins, which showed a bilateral arrangement within this lobe. This indicated that the pars distalis neurohypophysis of the dog served as its own outlet.

This difference, probably, has a bearing on the diverse pattern of age-correlated changes observed in the two species. In the pig, all major changes, _viz_., colloid deposition, colloid infiltration of vessels and degeneration of parenchymal cells occurred in the pars intermedia. On the other hand, the parenchymal cells of the dog appeared as functionally active cells throughout the age period of the animal. Considering the fact that the hypothalamic factor is inhibitory to the pars intermedia cells, it would be expected that the cells of the more vascular lobe are less active. That is what has been observed in the present study. Hyperplasia of the canine intermedia was so extensive that it enchroached upon the pars distalis neurohypophysis (Figure 68).

Neurohypophysis

In both species, the radix infundibuli was composed of the same zones as reported in case of the rat (Monroe, 1967; Kobayashi _et al_., 1966), in the monkey (Bodian, 1966), in the mouse (Oota, 1963) and in human (Lederis, 1965). The

pars compacta infundibuli of the pig and the pars cava infundibuli of the dog contained terminations of axon colaterals in the vicinity of the blood vessels which indicated a possible site for the exchange of neurosecretory substance between the neurohypophysial fibers and the blood vascular system. In the dog, a large number of the above fibers terminated among the ependymal cells that formed the marginal layer of the infundibular recess. Herring bodies were also evident in the vicinity of these lining cells. This was more marked in young animals than in the aged. Though a functional relationship has not been established between the neurosecretory fibers and the cerebrospinal fluid, the morphological association of the two lends enough speculative conceptions in this regard. Monroe (1967) and Akmayev (1969) have subscribed to the fact that the pars proximalis neurohypophysis is the main site of exchange for neurohypophysial hormones while pars distalis neurohypophysis comes into play during emergency situations. The long pars compacta infundibuli of the pig can serve as a suitable site for such an exchange, while in the dog, the shortness in the length of the pars cava infundibuli may necessitate a supplementary route. The widely dilated recessus infundibuli of the dog may thus be conceived as a route for the above purpose. The preceding fact may also be a reflection on the low content of neurosecretory material in the pars distalis

neurohypophysis of the pig as compared to that of the dog.

In this study, staining of the neurosecretory material was achieved with equal success by Gomori's chrome alum hematoxylin, Adam and Sloper's alcian blue procedure with prior oxidation in performic acid, aldehyde-fuchsin with or without prior oxidation and aldehyde-thionin following oxidation with acidified potassium permanganate solution. Kernechtrot was used in conjunction with alcian blue. The latter dye did not hinder the histochemical reaction for cystine, but made the image of tissue sections more vivid. Heath's (1965) performic acid-alcian blue-periodic acid-Schiff-orange G sequence furnished excellent results in the staining of the neurosecretory material in both species (Figures 70 and 136).

Herring bodies were more numerous in the neurohypophysis of the dog than in the pig. Electron microscopic studies have established the fact that Herring bodies are contained within the neurohypophysial fibers and undergo depletion like the latter (Monroe and Scott, 1966). In the present study, a definite age-correlated change in the pattern of their incidence and growth was not observed.

In the pars distalis neurohypophysis of the pig, the caudal end of the embryonic infundibular recess was found to have been retained until a late age. It was interesting to note that neurohypophysial fibers and Herring bodies were

present in close association with the ependymal linking of this cavity. This is ample proof of the fact that neurohypophysial fibers are associated with the cerebrospinal fluid in the embryo. The hyaline cast that developed in the lumen of this embryonic vestige was reabsorbed and did not leave any trace in specimens obtained from aged animals. Since blood vessels were not associated with this structure, the only conceivable substance that could have contributed to the formation of the hyaline cast was either the neurosecretory material or some product of the ependymal cells.

An interesting feature of the present study is the fact that neurosecretory material was discernible in the fibers of the pars distalis neurohypophysis of the pig as well as that of the dog on the first day of postnatal life (Figure 135) even though sections of the hypothalamus of the same animals did not reveal any discernible neurosecretory material in the neurons of the supraoptic or the paraventricular nuclei (Figures 144 and 152). This can be attributed to either one or both of the following concepts: the neurohypophysial principles are synthesized in the neurons, but firm conjugation with neurophysin is not established until they reach the pars distalis neurohypophysis or site of exchange. The second conceivable factor can be that the consistent axonal flow carries down the conjugated protein-peptide complex at a much faster rate than that of synthesis

by the neurons. It is an established fact that the Golgi complex is poorly developed in the cells of newborn animals and both endoplasmic reticulum and Golgi complex become enriched with age in developing animals. Coupled with this fact, it was also interesting to note that in the specimens treated with 2.5 percent trichloroacetic acid, stainable neurosecretory material was only present in the fibers of the pars distalis neurohypophysis, even though Herring bodies were evident in all regions. Trichloroacetic acid has been employed to dissociate the hormones from the protein complex in sodium chloride treated crude extracts of the neurohypophysis (Bargmann, 1966; Gabe, 1966). In view of this and the fact that staining of neurosecretory material is due to conjugation with neurophysin (Bargmann, 1966; Pearse, 1968a), the first concept appeared to be more appropriate than the second. In the human infant, Raiba and Hjelt (1957) reported a definite correlation between the onset of neurosecretory activity and development of the hypophysial portal system. In the present study, an ill-defined mantel plexus was evident in both species at birth and there seemed to be no relationship between the neurosecretory activity and growth of the portal system.

The morphology of the neurohypophysis, as observed in the present study, cor esponds to that described by Bargmann (1950, 1966) and Sloper (1955) in case of the dog. Available

literature on the morphology of the porcine neurohypophysis is scant and the present study is the first report in many aspects.

Age-correlated Changes

In both species, a uniform pattern of structural changes, associated with the age of the animals, was observed. There was no difference between the two species in the different types of changes. The main structural changes observed in this study may be classified into two separate categories: primary and secondary. The primary age-correlated changes include the following: adhesion of lobes; variaton in the relative proportion of cells of the pars distalis adenohypophysis; alterations in the cytoplasm-nuclear ratio; gelatinization or denaturation of collagen; interstitial colloid infiltration; occurrence of chromophobic nodules and degeneration of the neurohypophysial fibers. The secondary changes consist of the following: deposition of colloid in the follicles; degeneration of portal capillaries; degranulation and autolysis of parenchymal cells; occurrence of vacuoles in the cells; and, hyperplasia of the pars intermedia adenohypophysis.

Some of these age-correlated changes have also been reported in the adenohypophysis of the rat, mouse, golden hamster and human. These have been discussed in the chapter on Review of Literature. There are very few publications on

the age-correlated morphological changes of the pars intermedia and the pars distalis neurohypophysis in case of domestic animals or laboratory animals.

In the beagle, adhesion of the pars distalis adenohypophysis and the pars intermedia was observed at 10 years of age and that in the pig was evident at 7 years of age. In the porcine hypophysis, no appreciable change was noticed which could be attributed to have been caused by such adhesion of the lobes. In the dog, however, the undifferentiated cells did not undergo mitotic divisions after the adhesion and their number was progressively reduced with age. As specific function of the cavum hypophysis or its colloid content has not been elucidated, the cause and effect of its occlusion are difficult to interpret.

From birth to senility, a conspicuous change is evident in the relative proportion of the cell-types. Immediately after birth, the somatotrope and thyrotrope cells increased in size as well as relative proportions. This increase was conspicuous to the end of the third age group. A relative stability was maintained afterwards except during the terminal group where a decrease in the proportion of somatotrope cells was evident. The increase in the percentage of thyrotrope cells during the last two age intervals was primarily caused by transformation of the parenchymal cells of paraneuralis into thyrotrope cells (Figure 46). The

latter was especially conspicuous in the beagle. It was interesting to note that the weight of the thyroid gland also showed an increase during this late period of life (Graph 16).

In their study on the growth hormone content of the porcine hypophysis, Baker et al. (1956) reported that potency of the hormone per unit of dry pituitary weight remains constant between birth and maturity, and the content of growth hormone increases at the same rate as the dry weight of the organ. This would indicate that the relative proportion of the somatotrope cells would be expected to increase with increase in the weight of the hypophysis, which has been observed in this study. Pecile et al. (1965) found a significant decrease in the concentration of the growth hormone releasing factor in rats beyond one year of age. If this holds true in other species also, the decrease in the relative proportion and cytoplasm-nuclear ratio of the somatotrope cells is obvious. McCann and Porter (1968) subscribed to a decrease with age in the responsiveness of the somatotrope cells to the releasing factor elaborated by the hypothalamus. All these studies lend definite support towards diminished activity of the somatotrope cells with age.

Daughaday (1968) and McCann and Porter (1969) observed that the dog and the pig contained higher amounts of thyroid

stimulating hormone, second only to the rat and the mouse. The present study also indicated a higher relative percentage of the thyrotrope cells in both species. It is a proven fact that young animals possess actively growing thyroid glands and in the puppies many of the tracts of the central nervous system do not become myelinated until a long period after birth. Since thyroxin helps in the maturation of the nervous system, it would be expected that a higher requirement of the thyroid stimulating hormone ought to be released during this period. This is probably reflected in the large number of degranulated thyrotrope cells that were evident in the pars distalis adenohypophysis of puppies. These cells resembled the typical "thyroidectomy cells" reported to occur in the adenohypophysis of the adult dog (Goldberg and Chaikoff, 1952b) and of the rat (Halmi, 1950; Purves and Griesbach, 1956).

In the pig, an increase in the relative proportion of the lactotrope cells during old age was probably a secondary change due to a sharp decrease in the vascularity of the organ. Occlusion of the portal vessels was much more pronounced in this species, and the occlusion was supplemented by the great increase in the thickness of the basement membrane and perivascular collagen. Thus, the diffusion of material to and from the blood is likely to be decreased. Since the hypothalamic factor is inhibitory to

the lactotrope cells (Ganong and Kragt, 1969), an increase in their size and proportion would not be expected.

The FSH gonadotrope cells manifested two types of changes. There was a definite increase in their size and relative proportion between birth and 4 years of age. During the interval of 6 to 12 months of age, the maximum in the percentage of these cells had been reached. This period corresponds to the age of puberty in both species (Cupps _et al_., 1969). Beyond that age, the relative proportion of these cells tended to decrease while the average diameter of the cells increased. During late periods of the age span, large-sized FSH gonadotrope cells were evident in the pars distalis adenohypophysis of both species. The available literature indicates a definite increase in the gonadotropin output during the later part of life which is especially pronounced in aged women (Heller and Shipley, 1951; Albert _et al_., 1956). Witchi (1961) reported that the follicle stimulating hormone potency per unit of dry hypophysis weight of adult human is 40 times higher than prepubertal children, indicating a great increase in the diameter and percentage of the hormone secreting cells. This is what has been observed in the beagle and also in the pig.

The increase in the urinary output of gonadotropins with age has been looked upon as a function of age (Randall, 1962) and/or that of impaired pituitary gonad feedback system

(Verzar, 1966). The concluding remarks of Heller and Shipley (1951, p. 961) may be more appropriate than either of the above: "In late life, two opposite influences may act upon the pituitary-primary gonadal failure which tends to cause an increased output, and senile change which promotes a decreased output." The increased size of the gonadotrope cells definitely points to a less responsive gonad-pituitary axis, while the decreased proportion of gonadotrope cells appeared to be the function of age changes of the hypophysis itself. It was an interesting observation that correlation between weight of the gonads and that of the hypophysis was significantly lower in the third age interval (1 to 14 years) than the other two age intervals (Table 12D). Although it is not authentically known as to what degree the correlation between weight of endocrine glands is reflected on their function and vice versa, a positive relationship seemed likely from the results obtained in this study.

In spite of several atrophic changes in the adenohypophysis of the two species, *viz.*, degenerative changes in collagen, intrafollicular colloid formation development of chromophobe nodules and decreased vascularity, the percentage of different cell-types was not significantly different. The differential affluence to different regions of the lobe may have been the corrective factor in this regard.

The cytoplasm-nuclear ratio indicates a general

functional capability of the cell (Dawbarn, 1932). In the present study, a significant difference was not found in the ratio at different ages. The only appreciable difference was evident in case of the FSH gonadotrope cells in both species. This increase was probably due to decreased feedback inhibition from the gonads as has been subscribed by Verzar (1966). Thus, it may be concluded that there is no appreciable diminution in the functional capacity of the individual cells of the pars distalis adenohypophysis as long as they are intact. Pyknotic changes in the nuclei were only evident in a low percentage of cells which further indicate that the adenohypophysial cells retain their functional capacity until a very old age.

A conspicuous primary change in the histomorphology of the pars distalis adenohypophysis, pars infundibularis adenohypophysis and mantel plexus of both species was an increase in the connective tissue stroma. Reticular tissue increased with age from birth to old age at a very slow rate. The adenohypophysis of older pigs and dogs contained a thick reticular framework (Figure 37). The poor development of reticular fibers in the piglets and in the younger animals in general may be attributed to the organized nature of the parenchyma during the said period. Collagen fibers manifested a definite increase between birth to 7 years of age. Beyond that age, there was little proliferative change but more of

a degenerative change. Fibrin was not evident in the parenchyma of the adenohypophysis. It occurred during the interval 6 months to 4 years of age as a prelude to intra-follicular colloid deposition. Thus development of fibrin seemed to be a function of age.

Lansing and Wolfe (1942a) observed that the fibrillar material in the pars distalis adenohypophysis is entirely composed of reticular fibers and do not change to collagen at any age. However, in the dog and the pig, both reticular and collagen fibers were observed. In the human adenohypophysis, Cooper (1925) and Shanklin (1953) reported significant increase in the connective tissue stroma, while Romeis (1940) did not consider it as significant. But the adenohypophysis of the dog and the pig revealed conspicuous increases in the connective tissue stroma with age.

In the other species, denaturation of collagen has not been reported. In the present study, gelatinization or denaturation changes in the collagen were evident as early as 4 years of age. These changes were much more severe in the canine adenohypophysis than in the porcine. The collagen fibers lost their individual contours and were broken into small segments. These masses then developed into large hyalinized areas between the cell-cords (Figure 22). Verzar (1964) has attributed gelatinization of collagen to a phase transition in its threshold melting point.

According to him denaturation of collagen would occur with age only when shrinkage temperature or tissue collagen becomes lower than the body temperature. The author has explained that the lowering of shrinkage temperature of tissue collagen can be caused by the dissolving out of the mucoprotein from the collagen fibers. The exact cause of such changes in the adenohypophysis requires elucidation. A similar mechanism may also be functioning in the hypophysis. The decreased vascularity of the region may be envisaged to cause an increased depletion of mucroprotein from the stroma to meet the demand of parenchymal cells. Adrenocorticotropic hormone of the adenohypophysis is also known to bring about a decrease in the protein mucopolysaccharide content of the ground substance (Bloom and Fawcett, 1968). The decreased vascularity of the lobe may bring about increased concentration of adrenocorticotropic hormone leading to the connective tissue changes.

Another conspicuous change was the increase of ground substance. This was evident in the perivascular region of all the lobes, in the mantel plexus and in the radix infundibuli. In the young animals, the ground substance manifested a positive reaction with periodic acid-Schiff. In older animals, a pale blue color was manifested when the sections were stained with alcian blue at low pH. This is indicative of the presence of acid mucopolysaccharides and

decreased permeability (Pearse, 1968a). Bloom and Fawcett (1968) subscribed that a change in the ground matrix can hamper the diffusion of substances through it since the exchange of materials is influenced by the viscous properties of the ground substance. Electron microscopic studies of Farquhar (1961) and Herlant (1963, 1964) have revealed the fact that the discharged granules from adenohypophysial cells become dissolved in the perivascular basement membrane. Intact granules have never been observed in the lumen of the capillaries. Since adenohypophysial cells are actively involved in the exchange of substances, to and fro, with the portal capillaries, impairment in the structure of the ground substance is likely to cause severe setbacks in their function. It may be possible that alterations in the structure of the ground matrix (either due to decreased vascularity or increased concentration of gonadotropin) is the cause of extravascular colloid deposition.

Out of the two types, the extravascular colloid, especially follicular colloid, was also one of the primary changes correlated with age. Formation of fibrin and fibrinoid was found to be the primary change and colloid deposition occurred secondarily (Figure 92). Payne (1952), Shanklin (1953) and Rynearson (1962) described the initial changes as cavitation of the centrum of the cell-cords. On the contrary, the present study clearly revealed the fact

that development of fibrinoid is the initial stage. As has been reported by Wolfe (1943) in rats, incidence of intrafollicular colloid was found to be very high during the period of maximum reproduction capability of the animal.

Hyperplastic chromophobic nodules were observed in the pars distalis adenohypophysis of the dog and the pig. In the former, they occurred mainly as large aggregated masses often occupying a substantial volume of the lobe (Figure 39). On the other hand, they always occurred as small nodular masses distributed throughout the lobe in case of the pig (Figure 101). Location of these chromophobic patches near occluded vessels suggested an avascular reactive phenomenon of the degranulated chromophil cells. It is very likely that in the areas where collateral circulation can be established after occlusion of the primary vessel, the degranulated chromophil cells become the chromophobe cells of these hyperplastic nodules while in all other locations they undergo autolysis. Among other species such hyperplastic nodular transformation of the adenohypophysial cells has been observed in the rat (Wolfe _et al._, 1938; Wolfe, 1943) and in human (Shanklin, 1953; Sommers, 1959).

Several experimental studies have proved the fact that estrogen administration brings about a reduction in the number of chromophil cells and a concurrent increase in the chromophobes (Tuchmann-Duplessis, 1952). Whether degranula-

tion of cells and development of hyperplastic nodules in the ageing hypophysis is related with an alteration in the estrogen feedback mechanism on the hypothalamus or hypophysis is not well known. In their recent study, Yoshimura et al. (1969) reported success in transplanting adenohypophysial chromophobic cells into the hypothalamus. These cells differentiated either into the acidophil or the basophil type depending upon the region from which the cells were obtained. It will be of academic interest to study the effect of such transplantations on the hyperplastic chromophobe cells of the senile hypophysis.

A conspicuous age-correlated change of the neurohypophysis was the axonal degeneration of neurohyophysial fibers. This was evident in the pig as well as in the dog. The number of fibers in the pars distalis neurohypophysis and the pars compacta (or pars cava) infundibuli decreased progressively with age. In the pig, replacement fibrosis was evident in a very severe form, while in the dog loss of neurohypophysial fibers was followed by proliferation of the adjacent pars intermedia cells to occupy their space. That the pars distalis neurohypophysis is the primary site of such degenerative changes is derived from the study of hypothalamic sections of these animals. Axonal degeneration and their subsequent replacement by fibrosis has not been reported earlier in the pig or in any other species. A

possible cause of such degenerative changes may be the excess accumulation of neurosecretory material in the fibers due to decreased vascularity and blood circulation. As colloid infiltration of veins located in the pars distalis neurohypophysis is evident in a much more severe form than that of the dog, the severity of degenerative changes in the porcine neurohypophysis can be visualized.

The different type of reaction in the dog can be conceived as a secondary change due to impairment in the inhibitory control of the pars intermedia cells. As discussed earlier, the venous drainage of the pars distalis neurohypophysis and the pars intermedia occurs by way of the former. Once that the capillary loops are occluded in the junctional zone, inhibitory control of the hypothalamus on the pars intermedia cells is likely to be either reduced or inhibited completely. Thus, proliferation of the pars intermedia cells encroaches upon the parenchyma of the pars distalis neurohypophysis. Invasion of the latter by basophil cells has been considered as one of the primary age-correlated changes in the pars intermedia and the pars distalis neurohypophysis in case of the human (Carlson, 1952; Randall, 1962).

In the pars distalis neurohypophysis, significant changes in the distribution pattern of the blood vessels were observed in this study. In the day-old animal, the vessels consisted

of very few large arteries and veins. These vessels manifested a definite pattern of looping and "in series" arrangement with the advancement of age. A somewhat similar pattern of growth has been reported by Xuereb (1954) in the human neurohypophysis. Such changes in the vascular pattern of the pars distalis neurohypophysis may be envisaged as a secondary controlling factor in the volumetric transfer of neurohypophysial principles from this lobe.

During old age, hyalinization of capillaries coupled with degeneration of neurohypophysial fibers indicated appreciable atrophic changes at the morphological level. Though much evidence is not available regarding impairment of function in the domestic animals, Friedman (1957) and Friedman _et al_. (1963) observed a decrease in the responsiveness of the neurohypophysis to administration of large quantities of salt and water during senescence in rats. The latter authors believed that several changes in the fluid and electrolyte metabolism associated with ageing is caused by decreased availability of neurohypophysial hormones. The severity of changes observed in the present study lends support to these postulations.

The secondary changes were evident in progressive incidence with age but their primary cause appeared to be colloid infiltration of the vessels. The latter was evident from birth to senility and hence cannot be associated

with ageing of the hypophysis. However, its severity increased with age and probably brought about these secondary changes. One such change was the higher incidence of intracellular colloid droplets and intercellular accumulation of colloid. Such changes were predominant in specimens obtained from aged animals and in the same section were conspicuous in the least vascular regions. Degranulation of the cells during advanced age is probably caused by decreased nutrition due to diminished vascularity of the lobe.

The present study revealed that colloid infiltration of the vessels was initiated in the smaller capillaries and involved the veins. Late appearance of colloid in the vessels of the pars infundibularis adenohypophysis and in those of the mantel plexus lends support to this view. Degenerative changes in the portal capillaries located in the pars distalis adenohypophysis were not evident until vessels in the latter region were affected. Colloid infiltration of the vessels in the pars distalis neurohypophysis can be attributed to the venous return from the rostrodorsal part of the pars distalis adenohypophysis by way of the former lobe (Basir, 1932; Szentagothai et al., 1968). Autolysis of the cells is probably another degree of such vascular necrosis. Herlant (1963) and Smith and Farquhar (1966) have demonstrated a rich lysosome system in the adenohypophysial cells. Impairment of nutrition is one of the potent causes which

activates the lysosome system in cells. As such, decreased vascularity of the pars distalis adenohypophysis may be the primary cause of such autolytic changes.

Vacuolation of the parenchymal cells in the pars distalis adenohypophysis was evident in both species and involved all cell-types. In the sexually immature animals, it was mainly confined to few somatotrope cells in female animals, while it was predominantly encountered in the FSH gonadotrope cells after the age of puberty. Moreover, vacuolated cells were more preponderant in the male than in the female. Between 7 and 10 years of age, the adenohypophysis of the pig also contained many vacuolated lactotrope cells. These facts indicated that appearance of vacuoles in the cytoplasm is related more with the functional state of the cell than age. However, incidence of vacuolation increased with age in the same cell-type.

Smith and Farquhar (1966) explained that granulophagy of the adenohypophysial cells occurs in two forms. The immature granules combine with multivesicular lytic bodies and the mature granules become united with dense lytic bodies. In subsequent steps, the material entering the lysosomal system (myelinated body) through the two pathways is progressively degraded to yield a common residual body, the vacuolated dense body. The latter is composed of a vacuole formed by a lipid droplet. The latter progressively

protrudes out and eventually becomes separated from the dense part of the "body". These lipid droplets merge with each other to form larger cytoplasmic lipid droplets. This mechanism not only serves to prove the existence of an alternative pathway for the turnover of secretory protein but also the existence of an intracellular disposal mechanism. It may be very likely that the intracytoplasmic vacuoles observed in functionally active cells are end products of granulophagy by the lysosomal system of the cell, granulophagy manifesting higher incidence during advanced ages due to decreased vascularity of the lobe.

The changes observed in the perikarya of the supraoptic and paraventricular nuclei cannot be attributed to age-correlated changes only. In all probability degeneration of the neurohypophysial fibers is the main cause, and the changes, viz., tigrolysis, accumulation of neurosecretory material in the cell body and vacuolar degeneration are probably secondary changes. The only age-correlated change may be the increase in the amount of neurosecretory material with age. The large number of inactive neurons that are evident in the hypothalamic nuclei, probably, represents a state of cyclic activity in these cells. Scharrer (1954) has contributed towards such an hypothesis. Sloper (1962) considered excessive accumulation of the neurosecretory material in the cytons as a sign of degenerative change of

the hypothalamic neurosecretory cells. The present observations lend support to this view.

Correlative Studies on Age-correlated Changes in Other Endocrine Glands

It is a well established fact that function of the hypophysis is dependent on the hypothalamus, and in its turn regulates the function of other endocrine glands of the body and is also influenced by structural changes manifested in those glands (Ganong and Kragt, 1969). Though a large number of studies have been made in this field, the full concept has not yet been clearly elucidated. Concerning correlative ageing of other endocrine glands, Spagnoli and Charipper (1955) observed extensive morphological alterations in the thyroid and the testis of the golden hamster concurrent with age-associated changes in the hypophysis. Blumenthal (1955) also observed identical structural alterations in the endocrine glands along with that of the hypophysis in several strains of mice. Similar reports are available in the literature regarding many other species of animals (Charipper _et al_., 1961; Bourne, 1967). These studies have established the fact that age changes of the hypophysis are reflected in the other endocrine glands.

The changes in the thyroid consisted of typical changes similar to those of hypothyroidism. The follicular cells decreased in their dimensions; the colloid of adjacent follicles coalesced giving rise to large cavitations.

Individual follicles decreased in size and many of them became devoid of colloid (Charipper *et al.*, 1961). The present study revealed that there is some amount of hyperplasia of the thyrotrope cells in the adenohypophysis during old age. The transformation of undifferentiated cells of the pars paraneuralis into thyrotrope cells may be looked upon as a defensive mechanism by the body to counter senile hypothyroidism.

Hullinger (1966, 1968) observed significant changes during growth of the canine adrenal. At birth, the cortical zones are not distinctly delineated. The zona glomerulosa and the zona fasciculata become discernible on the 4th day of postnatal life, while the zona reticularis becomes evident as a separate zone on the 14th day of life. Corresponding to these changes, neither the present study nor the available literature indicated an appreciable change in the morphology of the corticotrope cells. In this study, the latter cells were not evident close to the period of sexual maturity. One thing can be pointed out in this connection. The adrenal gland is more of a self-adjusting organ than most of the other endocrines (Hullinger, 1966). The nodules in the capsule during earlier days of life and the cortical nodules during the later part of life are definitely capable of compensating for any lack of tropic hormones. Basing this conclusions on these findings,

Hullinger (1966) has expressed the view that the adrenal gland, probably plays a secondary role in the ageing process.

Contrary to these, the influence of the adrenal gland on the hypophysis seems to be profound (Tuchmann-Duplessis, 1952). Adrenalectomy brings about a reduction in the number of acidophil and basophil cells and also in the general hormonal content of the gland (Tuchmann-Duplessis, 1952). However, it is not yet clear whether utilization of corticosteriods by the tissues is the main factor in the regulation of adrenocorticotropin production by the adenohypophysis.

Atrophic changes have been observed in the testis of senile rats and mice. Such changes include a decrease in the height of germinal cells, disorganization of seminiferous tubules and rarefaction of their wall, and a great increase in the connective tissue stroma (Blumenthal, 1955; Bourne, 1967). A decrease in the number of interstitial cells is evident in all species. Collaborative changes of the preceding are evident in the hypophysis during old age (Charipper _et al._, 1961). The present study also indicated that the gonadotrope cells decrease in proportion and the FSH gonadotrope cells manifest hypertrophy. Corresponding to these, the zona reticularis of the adrenal cortex also undergoes hypertrophy (Hullinger, 1966, 1968). While the zona glomerulos and zona fasciculata decrease in their

respective thickness, the zona reticularis of the dog increases with age and is widest in senile animals.

In the ovary of the sow, Bal (1966) observed the appearance of mature graffian follicles at 2 months of age and corpora lutea and corpora albicana at 4 months of age. These age periods correspond to the presence of the gonadotrope and lactotrope cells in the pars distalis adenohypophysis of the pig. Many of the cyclic ovarian changes are also reflected in the morphology of the adenohypophysis (Cleveland and Wolfe, 1933). Ovulation in the sow was found to be accompanied with a slight decrease in the percentage of acidophil and basophil cells, and an increase in the number of chromophobe cells. McEntee and Jubb (1957) reported that the gonadotrope cells of the adenohypophysis of cows with cystic corpora lutea degranulate at a later stage and follicular cysts cause retention of granules by the gonadotrope cells to varying degrees. Identical results in the ovary-adenohypophysis relationship have also been observed in the canine species (Stott,1970; Wolfe _et al_., 1933). In case of the ovary, all feedback mechanisms have been envisaged to act via the hypothalamic centers (Szentagothai _et al_., 1968; Ganong and Kragt, 1969). Hence it will be difficult to evaluate the reciprocal influence of gonadal changes on the hypophysis. But the positive influence of the adenohypophysis on the ageing

ovary is an established fact (Jones and Krohn, 1959).

The present study revealed a definite influence of age on the quantitative measurements as well as histomorphological structure of the hypophysis in the dog and in the pig. Many of these structural alterations indicate a process of dysfunction and are well correlated with diminution in the function of the organ. The available literature also indicates structural changes in other endocrine glands that can be linked with ageing changes of the hypophysis and vice versa. Thus, the hypophysis may be envisaged to have a significant contribution towards the general ageing process of the body and to play a primary role in this ageing process.

SUMMARY

Age-correlated changes in the quantitative measurements and histomorphology of the hypophysis were studied in the canine and porcine species. The former consisted of purebred beagles and the latter included eight purebreds and four breed-crosses. All the animals employed in this study had been raised by the Department of Veterinary Anatomy solely for research purposes with proper documentation of their dietary and breeding records. The dog sample consisted of 157 beagles ranging in age from 8 hours to 14 years and the pig sample was composed of 215 animals within the range of 14 hours to 10 years of age.

In the study of quantitative measurements, the effect of age and of body weight on the absolute weight and weight of the hypophysis per kilogram of body weight (relative weight) was determined in both species. The effect of age and body weight on the interrelationship between the weight of the hypophysis, thyroid, adrenal, testis and ovary was also studied. Animals of both species were grouped into three age intervals: birth to 8 weeks; 2 to 12 months; and, 1 to 14 years. Statistical analysis of the data consisted of simple and multiple regressions, and analysis of variance. The least square method of fitting constants for multiple classifications with disproportionate subclass numbers was used to obtain unbiased estimates of the effects

of age, body weight and sex. Partial correlations were computed holding body weight as a constant to determine the influence of the latter upon the factors investigated.

In both species, absolute hypophysis weight increased with age and with increase in body weight. Hypophysis weight was not significantly different between the male and female animals of both species. Weight of the hypophysis as percent body weight was heaviest in the pre-weaned animals and decreased consistently with age. In both sexes, the ratio of hypophysis weight to body weight decreased as body weight increased indicating a lower rate of growth than that of the latter.

In the beagle, weight of the thyroid increased with age and with increase in the body weight. There was no significant difference between the two sexes. The ratio of thyroid weight to body weight decreased with age and with increase in body weight. In the pig there was no difference in the growth of the thyroid between the two sexes to 4 weeks of age. Beyond that, the thyroid in the female increased at a faster rate than it did in the male. Males with larger body weights than females had larger thyroid glands on weight basis. There was no difference in the ratio of gland weight to body weight between the two sexes.

The pig displayed no difference in the adrenal weight due to the sex of the animals. There was a marked increase

in the rate of adrenal growth between birth to 1 year of age as compared to animals over 1 year of age. During the latter interval, correlation between body weight and adrenal weight was also low. The ratio of adrenal weight to body weight increased with age in the sexually mature animals, but decreased consistently with increase in body weight. In the beagle, a significant difference due to sex was evident. In both sexes, weight of the adrenal gland increased at an equal rate to the age of 1 year. In sexually mature animals, it increased at a much rapid pace in the male than in the female. However, beyond 10 years of age, adrenal weight decreased in the male with the advancement of age; in the female, it continued to increase consistently. In both sexes, ratio of gland weight to body weight increased with age in the sexually mature animals.

In the beagle, weight of the testis increased with age and with increase in body weight. A sharp increase was observed between 2 to 11 months of age. During the interval birth to 8 weeks, ratio of testis weight to body weight decreased with age as well as with increase in body weight. In later periods, it increased with increase in both of the independent variables. In the pig, absolute weight of the testis increased with age and with increase in body weight, while relative weight of the testis decreased with age and increase in body weight.

Weight of the ovary increased in the beagle to 1 year of age. Beyond that age, the ovary weight remained constant. In relation to body weight, weight of the ovary manifested an increase throughout the entire span. The ratio of ovary weight to body weight decreased with age and with increase in body weight. In the pig, the growth pattern of the ovary was identical to that of the dog except that there was a slight increase in weight between 1 to 10 years of age. The ovary of swine increased at a faster rate as compared to age and body weight during the interval of 2 to 12 months of age.

In both species, animals with larger hypophysis also had larger thyroid, adrenal, testis and ovary. Size of the thyroid was associated positively with that of adrenal, testis and ovary. Likewise animals who possessed larger adrenals also had larger gonads. Subdividing the data into three specific age intervals showed a decrease in the quantitative relationship of the endocrines. The only gland that showed a significant increase in its relationship with the hypophysis and gonads was the adrenal gland. These correlations were also reduced in most instances when partial correlations were calculated holding body weight as the constant factor which indicated the influence of body weight upon the factors investigated.

For histomorphological study, hypophyses from 62 animals of either species were employed. Several cytological

and connective tissue stains were employed in this study. Luxol fast blue was applied in conjunction with the trichrome stain of Cleveland and Wolfe (1932) for the first time. Other special techniques included Bielschowsky's method for axis cylinders and dendrites (Luna, 1968) and modified methyl violet method for amyloid (Pearse, 1968a). Cytometry of the tissue included volumetric measurements on the lobes and cytoplasm-nuclear ratio of the cells at different ages. Differential counting of the cell-types at different age intervals was also carried out.

There was no difference in the staining affinity of the various cell-types at different ages or between the two species. Six different types of chromophil cells were observed and the nomenclature of Herlant (1965) and Carlon (1967) was adopted to name the various cell-types. Nomenclature recommended by the International Committee on Veterinary Anatomical Nomenclature (1968) was adopted for morphological descriptions.

In the day-old beagle, somatotrope, thyrotrope and chromophobe cells constituted the parenchyma of the pars distalis adenohypophysis. In swine, FSH gonadotrope cells were also present at birth. In both species, the cells occurred in irregular masses, and the stroma was composed of an ill-defined reticular framework. The cells became organized into cords, with age, and collagen fibers became

evident in the perivascular region. Somatotrope and thyrotrope cells increased in size and relative proportions to 4 years of age. Beyond that period the former decreased in both aspects but the latter showed an increase in percentage. The lactotrope and ICSH gonadotrope cells showed no appreciable decline in their proportions until the later part of life. In swine, lactotrope cells increased in size and proportion beyond 7 years of age. The FSH gonadotrope cells increased in proportion to the age of puberty followed by a period of stability that extended to 4 years of age. Beyond that age, there was a decrease in the relative proportion of this cell-type accompanied with appreciable increase in their size. Adrenocorticotrope cells showed the least variation with age.

Mitosis of the chromophobe cells was observed in the adenohypophysis of the dog. Mitosis was evident until 4 to 7 years of age in the pars paraneuralis and pars intermedia, but it was no more evident among the cells of pars distalis adenohypophysis beyond 1 month of age. Mitosis was not observed in the porcine adenohypophysis. Incidence of degranulated cells of all categories increased with age. Intracellular colloid droplets were evident in the thyrotrope cells with a higher incidence beyond 7 years of age. Deposition of colloid in the interstices between individual cells was also evident beyond 7 years of age. Autolysis of

cells was observed in the vicinity of portal capillaries that had become occluded due to colloid infiltration.

In the pars distalis adenohypophysis, reticulin and collagen increased with age. Beyond 4 years of age, there was very little proliferative growth in the collagen fibers. Gelatinization or denaturation of collagen was evident during this period. The collagen fibers became fragmented and hyalinized, which was very conspicuous in the canine species. Postganglionic autonomic fibers were associated with the blood vessels of all the lobes from birth to senility and showed no change with age. The perivascular basement membrane increased in its thickness with the advancement of age.

Hyperplastic chromophobic nodules occurred in the canine adenohypophysis between 4 to 7 years of age while in the porcine hypophysis such chromophobic nodules were evident beyond 7 years of age. The nodular masses did not contain any capillaries and were not circumscribed by connective tissue fibers.

Colloid infiltration into the portal capillaries was observed in the day-old animal and its incidence increased progressively with age. Occlusion of vessels and reabsorption of their colloid contents were also evident. Specimens obtained from animals between 7 to 10 years of age contained many atrophic portal vessels. Colloid infiltration into the vessels of the pars intermedia and pars distalis

neurohypophysis was also observed with increasing incidence with age. Deposition of colloid in the central part of the cell-cords was evident at 9 months of age and increased progressively with age. In the pig, coalescence of adjacent follicles resulted in the formation of large follicular masses. In the beagle, follicular colloid was not so conspicuous; but, beyond 10 years of age, cysts were encountered with increasing predominance. Usually a single cyst occupied the entire pars paraneuralis and a substantial portion of the pars distalis adenohypophysis. Such cysts were either lined by chromophobe cells or ciliated cells. Follicular colloid was also observed in the pars intermedia, pars paraneuralis and pars infundibularis adenohypophysis. Both intra- and extravascular colloid stained positively for amyloid. Evidence that follicular colloid deposition is mediated through fibrin and fibrinoid has been presented.

The pars paraneuralis was composed of undifferentiated cells and chromophobe cells during the interval from birth to 4 years of age. Beyond that age, the fissures of the cavum hypophysis became obliterated and many thyrotrope cells were evident in the parenchyma of the lobe. In swine, the embryonic structure of the lobe was altered at a much earlier age than the beagle.

The pars infundibularis adenohypophysis was ill-defined at birth. It became progressively differentiated with age.

The parenchyma of the lobe was composed of chromophobic cells arranged in the form of distinct follicles. In case of the pig, a large number of periodic acid-Schiff positive cells was observed in this zone. The latter cells were of very large proportions and contained a large number of periodic acid-Schiff positive granules in their cytoplasm. Beyond 7 years of age, the entire zone became fibrosed.

The pars intermedia of the pig was a very vascular zone, while in the dog, it was the least vascular region. In the latter species, a large number of undifferentiated cells was present to 7 years of age. These cells showed mitotic division throughout their existence. In both species, chromophobic cells and periodic acid-Schiff positive cells were observed in this lobe. In the pig, the parenchymal cells showed progressive atrophy and degeneration due to excessive colloid deposition, while in the beagle, there was hyperplasia of the cells leading to invasion of the neural lobe.

The cavum hypophysis became progressively obliterated beginning with the fourth year of life. Beyond the eighth year, the pars distalis adenohypophysis and the pars intermedia remained in apposition with each other and considerable intermingling of the parenchymal cells was evident. In the pig, adhesion of the two lobes was preceded by colloid deposition in the cavum hypophysis and its

subsequent reabsorption.

Neurosecretory material was observed in the fibers of the neurohypophysis beginning with the first day of postnatal life. Its incidence was not high until after the fourteenth day. Herring bodies showed increased predominance with age. In the pars distalis neurohypophysis of the pig, the vestige of the infundibular recess was retained to variable ages in different animals. But in the region of the stalk, it became obliterated by the second week of life. Progressive arborization of blood vessels with age was evident in this lobe. Beginning with the third year, degeneration of neurohypophysial fibers was evident. In swine, fibrosis replaced the degenerating fibers, while in the beagle, the invading cell-columns from the pars intermedia replaced them.

The neurons of the supraoptic and paraventricular nuclei contained stainable neurosecretory material beginning with the third to sixth day of postnatal life. Its incidence increased with age. Herring bodies were observed among the fibers of the hypothalamohypophysial tract. Changes in the cytons of the neurosecretory neurons consisted of axonal degeneration, accumulation of neurosecretory material and vacuolation. Cysts containing neurosecretory material were observed along the course of the hypothalamohypophysial tract of the porcine.

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Quantitative and histomorphological studies on age-correlated changes in canine and porcine hypophysis

by

Lakshminarayana Das

Volume 2 of 2

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APPENDIX A. TABLES

Table 1. Breeds of dogs and numbers studied

Age and breed	Male	Female	Total
Birth to 8 weeks			
Beagle	13	14	27
Dalmatian	2	0	2
2 to 11 months			
Beagle	20	23	43
Dachshund	4	2	6
Dalmatian	1	2	3
1 to 16.4 years			
Basenji	1	0	1
Beagle	29	37	66
Black Laborador	0	1	1
Cocker Spaniel	1	2	3
Dachshund	1	2	3
Fox Terrier	0	3	3
German Shepherd	0	3	3
German Short Haired Pointer	0	2	2
Golden Retriever	2	4	6
Irish Setter	1	2	3
Laborador Retriever	1	2	3
Siberian Husky	0	1	1
Welch Corgi	1	2	3
W. H. Terrier	2	1	3
Yellow Laborador	1	0	1
Totals	81	107	188

Table 2. Breeds of swine and numbers studied

Age and breed	Male	Female	Total
Birth to 8 weeks			
Chester White	1	3	4
Duroc	0	2	2
Florida Wild	3	0	3
Hampshire	1	0	1
Poland China	0	2	2
Yorkshire	3	8	11
Chester White-Duroc cross	0	1	1
Duroc-Poland China-Landrace cross	1	5	6
Yorkshire-Hampshire cross	2	2	4
Yorkshire-Landrace cross	2	0	2
2 to 12 months			
Chester White	1	2	3
Duroc	3	3	6
Hampshire	0	1	1
Yorkshire	2	6	8
Duroc-Poland China-Landrace cross	0	2	2
Landrace-Poland China cross	0	1	1
Yorkshire-Landrace cross	0	5	5
Yorkshire-Landrace-Poland china cross	0	12	12
1 to 10 years			
Chester White	0	7	7
Duroc	5	7	12
Farmer's Hybrid	1	4	5
Florida Wild	1	2	3
Hampshire	6	3	9
Landrace	1	15	16
Poland China	12	0	12
Yorkshire	2	6	8
Yorkshire-Chester White cross	0	1	1
Yorkshire-Landrace cross	0	45	45
Yorkshire-Landrace-Poland China cross	0	10	10
Totals	47	155	202

Table 3. Hypophyses of 64 beagle dogs employed for histomorphological study

Age group	Interval	ID number	Sex	Age at necropsy Yr	Mo	Day
I	Birth to 4 weeks	B 9	F	0	0	1
		B 7	M	0	0	1
		B 110	F	0	0	6
		B 111	M	0	0	6
		B 50	M	0	0	14
		E 76	F	0	0	14
		E 75	F	0	0	20
		B 113	M	0	0	21
II	4 to 8 weeks	B 45	F	0	1	0
		E 85	M	0	0	27
		B 28	M	0	1	5
		B 29	F	0	1	5
		B 97	F	0	1	10
		B 98	M	0	1	10
		B 99	F	0	1	19
		B 47	M	0	1	28
III	2 to 6 months	068	M	0	2	15
		072	F	0	2	18
		B 100	F	0	3	0
		B 26	M	0	3	5
		E 27	M	0	4	0
		B 69	F	0	4	0
		B 106	F	0	5	0
		B 107	M	0	5	0
IV	6 to 11 months	B 115	F	0	6	2
		C 35	M	0	6	0
		B 60	M	0	6	23
		B 61	F	0	6	24
		B 49	F	0	8	4
		B 71	M	0	8	3
		B 124	M	0	9	1
		A 46	F	0	9	9
		B 86	F	0	10	1
		B 125	M	0	10	0
		C 17	F	0	10	25
		B 126	M	0	11	0
		C 8	F	0	11	0

Table 3. (Continued)

Age group	Interval	ID number	Sex	Age at necropsy Yr	Mo	Day
V	1 to 4 years	B 128	M	1	0	0
		B 62	F	1	0	0
		B 80	M	1	8	6
		B 25	F	2	3	4
		B 88	M	3	1	2
		B 75	F	3	2	12
		B 82	F	3	3	7
VI	4 to 7 years	B 95	M	4	0	0
		B 51	F	4	1	20
		071	F	5	4	0
		047	M	5	6	0
		003	F	6	0	0
		002	M	6	0	0
		B 18	F	6	0	0
VII	7 to 10 years	B 30	F	7	6	0
		B 44	F	7	9	0
		B 42	F	8	6	28
		B 33	F	9	1	0
		B 63	M	9	2	6
VIII	10 to 13.6 years	B 64	F	10	0	13
		B 15	F	10	4	19
		B 73	F	11	10	13
		B 123	F	11	8	19
		B 131	F	12	8	18
		B 31	F	12	4	14
		B 32	M	13	6	21
		B 14	F	13	7	20

Table 4. Hypophyses of 62 pigs employed for histomorphological study

Age group	Interval	ID number	Sex	Age of necropsy Yr	Mo	Day
I	Birth to 4 weeks	4	F	0	0	1
		1448	M	0	0	3
		1262	M	0	0	7
		1263	F	0	0	7
		1320	M	0	0	14
		1322	F	0	0	14
		3184	F	0	0	20
		3181	M	0	0	20
II	4 to 8 weeks	3180	M	0	1	3
		1653	F	0	1	6
		999	M	0	1	11
		518	F	0	1	11
		3051	M	0	1	19
		3105	F	0	1	18
		93	M	0	1	27
		68	F	0	1	27
III	2 to 6 months	1650	M	0	2	9
		1651	F	0	2	9
		W-708	F	0	2	22
		W-671	M	0	2	23
		5250	F	0	3	17
		72-B	M	0	3	21
		9713	F	0	4	7
		2250	F	0	4	7
IV	6 to 12 months	9310	F	0	6	1
		6022	F	0	8	0
		1285	M	0	8	0
		5093	F	0	9	3
		2512	M	0	10	0
		3923	F	0	10	12
		DU-4	M	0	10	0
		9442	F	0	11	5

Table 4. (Continued)

Age group	Interval	ID nu ber	Sex	Age of necropsy Yr	o	Day
V	1 to 4 years	L-914	M	1	2	0
		3430	F	1	2	3
		AEC	M	1	6	0
		4471	F	1	5	16
		6333	F	1	6	17
		3091	M	2	3	28
		2653	F	2	3	16
		1424	F	2	4	17
		3203	F	2	7	23
		1361	F	3	0	17
		29-3633	M	3	0	17
		5132	F	3	9	9
		4631	M	3	9	15
VI	4 to 7 years	203	M	4	2	6
		BB-2	F	4	6	0
		4734	M	5	0	28
		BB-5	F	5	6	0
		22-160	M	5	10	26
		29-260	M	6	0	4
		1350	F	6	1	15
		312	F	6	9	21
VII	7 to 10 years	3093	F	7	0	10
		FH-4	M	7	6	0
		ET-4	F	7	9	0
		N.F.	F	8	0	0
		1561	F	8	6	0
		9090	F	9	1	10
		254	F	9	2	0
		Merrick	F	10	0	0
		37-258	F	10	0	0

Table 5A. Age, body weight, absolute hypophysis weight and relative hypophysis weight of 27 beagle dogs ranging in age from birth to 8 weeks

Sequence number	ID number	Sex	Age (weeks)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
1	B 105	F	0.142	0.210	10.000	47.619
2	B 110	F	0.857	0.320	10.000	31.250
3	B 111	M	0.857	0.340	10.000	29.411
4	B 103	F	1.142	0.397	20.000	50.377
5	B 104	M	1.142	0.422	20.000	47.393
6	B 114	F	1.857	0.567	10.000	17.636
7	B 34	M	1.857	0.680	10.000	14.705
8	E 83	M	2.000	0.396	10.000	25.252
9	E 76	F	2.000	0.530	20.000	37.735
10	B 35	F	2.000	0.567	15.000	26.455
11	B 50	M	2.000	0.499	10.000	20.040
12	B 109	M	2.285	0.680	15.000	22.058
13	E 75	F	2.857	0.680	20.000	29.411
14	E 89	M	2.857	0.816	10.000	12.254
15	B 116	F	2.857	0.794	20.000	25.188
16	B 113	M	3.000	0.862	15.000	17.401
17	E 85	M	3.857	1.020	25.000	24.509
18	B 121	F	4.000	0.952	20.000	21.008
19	B 45	M	4.285	1.406	30.000	21.337
20	B 46	F	4.285	1.043	40.000	38.350
21	B 122	F	4.857	1.588	30.000	18.891
22	B 28	M	5.000	0.907	30.000	33.076

Table 5A. (Continued)

Sequence number	ID number	Sex	Age (weeks)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
23	B 29	F	5.000	1.000	30.000	30.000
24	B 97	F	5.714	0.907	20.000	22.050
25	B 98	M	5.714	1.134	30.000	26.455
26	B 99	F	7.000	0.907	20.000	22.050
27	B 47	M	8.285	1.565	30.000	19.169

Table 5B. Age, body weight, absolute hypophysis weight and relative hypophysis weight of 43 beagle dogs ranging in age from 2 to 11 months

Sequence number	ID number	Sex	Age (months)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
28	E 26	M	2.033	1.701	20.000	11.757
29	068	M	2.500	2.722	50.000	18.368
30	072	F	2.600	2.549	30.000	11.769
31	B 100	F	3.000	2.268	60.000	26.455
32	B 101	M	3.000	2.268	50.000	22.045
33	B 26	M	3.166	4.083	45.000	11.021
34	B 27	M	3.266	2.631	44.000	16.723
35	E 27	M	4.000	3.289	50.000	15.202
36	B 69	F	4.000	3.176	40.000	12.594
37	B 108	F	4.000	3.062	40.000	13.063
38	B 112	F	4.000	4.083	40.000	9.796
39	B 106	F	5.000	4.537	60.000	13.224
40	B 107	M	5.000	4.083	50.000	12.245
41	B 102	F	5.000	5.217	60.000	11.500
42	049	F	5.366	6.946	30.000	4.319
43	B 76	M	5.866	5.671	50.000	8.816
44	C 35	M	6.000	10.435	70.000	6.708
45	B 40	F	6.433	7.332	60.000	8.183
46	B 66	F	6.466	6.805	50.000	7.347
47	B 59	F	6.766	5.807	60.000	10.332
48	B 60	M	6.766	9.210	60.000	6.514
49	B 61	F	6.800	4.764	50.000	10.495

Table 5B. (Continued)

Sequence number	ID number	Sex	Age (months)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
50	A 41	M	7.633	7.259	80.000	11.020
51	B 48	F	7.866	8.847	60.000	6.781
52	B 72	M	7.866	8.166	70.000	8.572
53	B 79	F	8.000	6.578	60.000	9.121
54	B 70	M	8.060	8.393	75.000	8.936
55	B 71	M	8.100	7.372	50.000	6.782
56	B 49	F	8.133	12.704	80.000	6.297
57	C 34	M	8.633	5.898	70.000	11.868
58	B 124	M	9.033	11.343	90.000	7.934
59	B 74	F	9.233	6.352	65.000	10.232
60	A 46	F	9.300	7.259	50.000	6.888
61	B 67	F	9.333	6.098	40.000	6.559
62	B 53	M	9.933	11.615	120.000	10.331
63	B 125	M	10.000	7.259	60.000	8.265
64	B 86	F	10.000	4.537	50.000	11.020
65	B 73	F	10.433	9.754	60.000	6.151
66	C 17	F	10.833	6.352	60.000	9.445
67	B 55	M	11.000	13.520	90.000	6.656
68	B 126	M	11.000	9.528	60.000	6.297
69	C 8	F	11.000	10.435	40.000	3.833
70	C 10	F	11.000	6.805	50.000	7.347

Table 5C. Age, body weight, absolute hypophysis weight and relative hypophysis weight of 66 beagle dogs ranging in age from 1 to 13.6 years

Sequence number	ID number	Sex	Age (years)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
71	B 54	M	1.000	9.255	30.000	3.241
72	B 56	M	1.000	11.932	80.000	6.704
73	B 57	M	1.000	9.936	50.000	5.032
74	B 58	M	1.000	15.199	100.000	6.579
75	B 62	F	1.000	7.259	80.000	11.020
76	2BC	M	1.005	6.125	60.000	9.795
77	5FF	F	1.008	8.121	60.000	7.388
78	5FD	M	1.010	5.172	40.000	7.733
79	2BB	M	1.013	6.669	70.000	10.496
80	5FC	M	1.019	7.168	80.000	11.160
81	B 83	F	1.021	6.352	60.000	9.445
82	2BD	F	1.076	5.580	40.000	7.168
83	B 132	M	1.106	9.074	60.000	6.612
84	B 133	M	1.107	8.620	70.000	8.120
85	B 37	M	1.194	7.032	70.000	9.954
86	710	M	1.249	9.074	60.000	6.612
87	494	M	1.268	7.713	80.000	10.372
88	B 90	F	1.331	7.713	70.000	9.075
89	B 96	F	1.410	7.259	50.000	6.888
90	A 50	M	1.493	7.259	50.000	6.888
91	B 77	M	1.542	8.393	60.000	7.148
92	B 81	M	1.545	9.074	80.000	8.816

Table 5C. (Continued)

Sequence number	ID number	Sex	Age (years)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
93	B 129	F	1.583	7.032	75.000	10.665
94	B 80	M	1.673	9.528	60.000	6.297
95	B 41	F	1.701	4.650	70.000	15.053
96	B 128	M	2.000	9.528	80.000	8.396
97	B 127	F	2.019	6.805	70.000	10.286
98	B 24	F	2.254	7.940	40.000	5.037
99	B 25	F	2.257	7.940	40.000	5.037
100	B 68	F	2.673	6.352	80.000	12.594
101	B 135	M	2.723	12.250	100.000	8.163
102	B 91	F	2.923	9.074	60.000	6.612
103	B 92	F	2.923	9.528	100.000	10.495
104	B 93	F	2.923	8.166	70.000	8.572
105	B 94	F	2.923	10.435	100.000	9.583
106	046	F	3.000	9.074	70.000	7.714
107	B 87	F	3.087	8.166	60.000	7.347
108	B 88	M	3.087	10.435	60.000	5.749
109	B 75	F	3.197	7.486	70.000	9.350
110	B 82	F	3.265	8.166	70.000	8.572
111	B 16	M	3.320	13.611	80.000	5.880
112	B 89	M	3.323	12.704	80.000	6.297
113	B 36	M	3.400	11.456	100.00	8.729
114	B 65	F	3.564	14.745	70.000	4.747

Table 5C. (Continued)

Sequence number	ID number	Sex	Age (years)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
115	B 95	M	4.000	11.343	60.000	5.289
116	B 130	F	4.000	9.981	70.000	7.013
117	B 51	F	4.136	10.208	90.000	8.816
118	071	F	5.328	10.889	80.000	7.346
119	047	M	5.500	12.250	90.000	7.346
120	003	F	6.000	12.704	30.000	2.361
121	002	M	6.000	11.343	80.000	7.052
122	B 134	M	6.723	13.157	45.000	3.420
123	B 119	F	6.868	11.343	75.000	6.612
124	B 117	F	6.915	14.292	70.000	4.897
125	B 30	F	7.500	11.796	100.000	8.477
126	B 43	F	7.567	10.662	80.000	7.503
127	B 44	F	7.739	11.116	60.000	5.397
128	B 42	F	8.569	10.889	120.000	11.020
129	B 136	M	9.000	16.243	100.000	6.156
130	B 137	F	9.000	16.515	115.000	6.963
131	B 33	F	9.082	14.972	120.000	8.014
132	B 63	M	9.180	12.023	70.000	5.822
133	B 64	F	10.035	11.297	90.000	7.966
134	B 31	F	12.367	12.250	80.000	6.530
135	B 131	F	12.706	8.620	90.000	10.465
136	B 32	M	13.550	13.611	100.000	7.346

Table 6A. Age, body weight, absolute hypophysis weight and relative hypophysis weight of 36 pigs ranging in age from birth to 8 weeks

Sequence number	ID number	Sex	Age (weeks)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
1	1	M	0.142	1.142	33.000	25.984
2	2	F	0.142	1.134	18.000	15.873
3	3	F	0.142	1.179	25.000	21.204
4	4	F	0.142	1.270	25.000	19.685
5	1448 B	M	0.428	1.043	30.000	28.763
6	1449 B	M	0.428	1.134	20.000	17.636
7	1243	F	1.000	1.950	24.000	12.307
8	1260	F	1.000	1.633	34.000	20.820
9	1262	M	1.000	2.450	42.000	17.142
10	1263	F	1.000	1.950	35.000	17.948
11	1322	F	2.000	2.949	41.000	13.903
12	1320 M	M	2.000	4.537	56.000	12.342
13	1320 F	F	2.000	4.310	50.000	11.600
14	1329	F	2.000	3.629	61.000	16.809
15	FA-2	M	2.000	2.903	40.000	13.778
16	3181	M	2.857	4.990	46.000	9.218
17	3183	F	2.857	3.629	24.000	6.613
18	3184	F	2.857	4.083	49.000	12.000
19	FA-3	M	4.285	4.582	70.000	15.277
20	3180	M	4.714	7.713	57.000	7.390
21	1652	F	5.142	6.352	64.000	10.075
22	1653	F	5.142	9.074	69.000	7.604

Table 6A. (Continued)

Sequence number	ID number	Sex	Age (weeks)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
23	1662	F	5.142	10.435	106.000	10.158
24	999	M	5.857	11.343	72.000	6.347
25	518	F	5.857	11.796	71.000	6.018
26	527	F	5.857	11.796	92.000	7.799
27	480	F	6.000	14.065	92.000	6.541
28	3105	F	6.857	12.250	90.000	7.346
29	3083	F	6.857	11.796	90.000	7.629
30	3051	M	7.000	16.333	112.000	6.857
31	1244	F	7.142	9.981	108.000	10.820
32	68	F	8.142	19.509	138.000	7.073
33	69	F	8.142	12.250	52.000	4.244
34	92	F	8.142	18.148	133.000	7.328
35	93	M	8.142	17.695	110.000	6.216
36	FA-4	M	8.142	10.889	60.000	5.510

Table 6B. Age, body weight, absolute hypophysis weight and relative hypophysis weight of 38 pigs ranging in age from 2 to 12 months

Sequence number	ID number	Sex	Age (months)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
37	1171	F	2.066	22.232	121.000	5.442
38	1178	F	2.066	19.056	120.000	6.297
39	1187	M	2.066	12.704	98.000	7.716
40	1188	F	2.066	14.065	130.000	9.242
41	592	F	2.166	16.333	120.000	7.347
42	627	F	2.166	14.519	60.000	4.132
43	1650	M	2.300	19.509	110.000	5.638
44	1651	F	2.300	20.417	145.000	7.101
45	1680	F	2.333	23.139	130.000	5.618
46	1682	F	2.333	14.065	100.000	7.109
47	5330	F	2.400	18.148	90.000	4.959
48	1264	M	2.533	18.602	120.000	6.450
49	1272	F	2.533	35.390	200.000	5.651
50	1237	F	2.566	22.686	135.000	5.950
51	1234	F	2.566	19.963	120.000	6.011
52	5353	F	2.700	20.871	120.000	5.749
53	W-716	F	2.733	24.500	165.000	6.734
54	W-708	F	2.733	24.500	130.000	5.306
55	Y-650	F	2.766	30.852	210.000	6.806
56	W-671	M	2.766	29.945	145.000	4.842
57	5250	F	3.566	24.954	190.000	7.614
58	72 B	M	3.700	50.816	550.000	10.823

Table 6B. (Continued)

Sequence number	ID number	Sex	Age (months)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
59	5262	F	3.766	42.196	190.000	4.502
60	9753	F	4.166	61.252	240.000	3.918
61	9713	F	4.233	52.177	210.000	4.024
62	2250	F	4.233	56.715	210.000	3.702
63	9310	F	6.033	104.355	200.000	1.916
64	1292	F	6.166	92.558	260.000	2.809
65	6022	F	8.000	111.161	250.000	2.248
66	634	F	8.133	121.597	260.000	2.138
67	2210	F	8.200	125.680	260.00	2.068
68	3920	F	8.633	125.226	360.000	2.874
69	5093	F	9.100	115.698	220.000	1.901
70	DU-4	M	10.00	83.030	350.000	4.215
71	2211	F	10.033	179.673	440.000	2.448
72	3923	F	10.400	175.136	420.000	2.398
73	9442	F	11.166	156.533	370.000	2.363
74	7973	F	11.866	163.339	340.000	2.081

Table 6C. Age, body weight, absolute hypophysis weight and relative hypophysis weight of 128 pigs ranging in age from 1 to 10 years

Sequence number	ID number	Sex	Age (years)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
75	FS-1	F	1.082	83.938	230.000	2.740
76	3439	F	1.172	173.774	400.000	2.301
77	2944	F	1.235	204.174	420.000	2.057
78	2360	F	1.306	176.497	460.000	2.606
79	5930	F	1.260	186.025	440.000	2.365
80	2021	F	1.378	200.544	560.000	2.792
81	4461	F	1.449	196.007	400.000	2.040
82	4471	F	1.454	178.312	490.000	2.747
83	4460	F	1.454	153.357	420.000	2.738
84	4475	F	1.460	187.840	510.000	2.715
85	FS-Boar	M	1.500	127.041	410.000	3.227
86	6333	F	1.539	158.802	480.000	3.022
87	FA	F	1.657	68.058	500.000	7.346
88	3521	F	1.687	156.533	510.000	3.258
89	3204	F	1.701	192.831	500.000	2.592
90	9000	M	1.739	263.157	600.000	2.280
91	990	M	1.821	134.119	530.000	2.263
92	50	M	1.824	224.137	740.000	3.301
93	4491	F	1.931	229.582	490.000	2.134
94	4496	F	1.950	222.323	590.000	2.653
95	4513	F	1.967	166.515	420.000	2.522
96	4512	F	2.005	185.117	550.000	2.971

Table 6C. (Continued)

Sequence number	ID number	Sex	Age (years)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
97	6154	F	2.027	166.969	670.000	4.012
98	6153	F	2.109	167.876	620.000	3.693
99	5912	F	2.117	176.950	590.000	3.334
100	3420	F	2.123	252.268	810.000	3.210
101	3523	F	2.126	260.435	900.000	3.455
102	5931	F	2.167	190.562	750.000	3.935
103	2363	F	2.183	242.740	720.000	2.966
104	5913	F	2.194	199.637	560.000	2.805
105	2362	F	2.208	224.591	630.000	2.805
106	5933	F	2.241	190.562	640.000	3.358
107	5911	F	2.216	195.099	650.000	3.331
108	2443	F	2.221	195.099	520.000	2.665
109	2442	F	2.224	220.508	580.000	2.630
110	2943	F	2.227	246.823	760.000	3.079
111	2440	F	2.235	205.989	700.000	3.398
112	6020	F	2.235	192.831	670.000	3.474
113	2903	M	2.246	328.493	600.000	1.826
114	6024	F	2.249	188.294	680.000	3.611
115	5910	F	2.257	190.562	690.000	3.620
116	4640	F	2.260	238.656	470.000	1.969
117	4641	F	2.260	238.656	470.000	1.969
118	4740	F	2.265	208.711	500.000	2.395

Table 6C. (Continued)

Sequence number	ID number	Sex	Age (years)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
119	2941	F	2.268	198.729	740.000	3.729
120	2654	F	2.271	202.359	730.000	3.607
121	2651	F	2.273	190.562	670.000	3.515
122	2480	M	2.279	295.372	760.000	2.573
123	2653	F	2.317	228.675	790.000	3.454
124	3091	M	2.323	261.796	560.000	2.139
125	4741	F	2.347	254.083	490.000	1.928
126	1470	F	2.361	158.802	730.000	4.596
127	1790	F	2.364	229.582	850.000	3.702
128	1472	F	2.375	209.618	800.000	3.816
129	1424	F	2.375	158.802	830.000	5.226
130	1610	F	2.383	215.517	710.000	3.294
131	1573	F	2.405	189.655	600.000	3.163
132	3202	F	2.564	175.136	660.000	3.768
133	632	F	2.575	161.070	650.000	4.035
134	ISU-951	F	2.575	235.934	550.000	2.331
135	3203	F	2.638	176.950	670.000	3.786
136	3195	F	2.750	208.711	850.000	4.072
137	3196	F	2.756	188.294	720.000	3.823
138	1040	F	2.778	208.711	870.000	4.168
139	7122	F	2.895	229.128	800.000	3.491
140	1362	F	2.895	185.571	760.000	4.095

Table 6C. (Continued)

Sequence number	ID number	Sex	Age (years)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
141	29-3633	M	3.046	235.934	620.000	2.627
142	1361	F	3.046	167.876	690.000	4.110
143	4383	M	3.342	290.381	710.000	2.445
144	4870	M	3.361	290.381	740.000	2.548
145	4876	F	3.500	240.471	610.000	2.536
146	3652	M	3.638	254.083	680.000	2.676
147	2324-58	M	3.698	349.364	300.000	0.858
148	5132	F	3.764	170.145	750.000	4.408
149	4631	M	3.780	281.306	930.000	3.306
150	2260	F	3.786	207.803	740.000	3.561
151	4966	F	3.969	176.950	580.000	3.277
152	6308	F	4.000	254.083	610.000	2.400
153	4915	F	4.076	190.562	600.000	3.148
154	4919	F	4.082	217.785	520.000	2.387
155	4192	M	4.084	251.814	890.000	3.534
156	203	M	4.180	294.918	610.000	2.068
157	1271	F	4.219	240.471	660.000	2.744
158	622	M	4.263	358.439	750.000	2.092
159	BB-1	F	4.500	326.792	650.000	1.989
160	BB-2	F	4.500	326.792	810.000	2.479
161	BB-3	F	4.500	326.678	570.000	1.744
162	190-10	F	4.501	249.546	1040.000	4.167

Table 6C. (Continued)

Sequence number	ID number	Sex	Age (years)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
163	7710	F	4.536	254.083	700.000	2.755
164	4110	M	4.558	274.500	830.000	3.023
165	5335	F	4.602	197.368	550.000	2.786
166	392	F	4.624	210.980	670.000	3.175
167	5898	F	4.854	238.203	980.000	4.114
168	6015	F	4.857	201.905	780.000	3.863
169	3651	M	4.901	276.769	790.000	2.854
170	966	M	5.027	326.678	790.000	2.418
171	4734	M	5.076	330.308	980.000	2.966
172	3654	M	5.164	276.769	880.000	3.179
173	24	M	5.202	335.753	670.000	1.995
174	BB-5	F	5.500	356.170	850.000	2.386
175	PC-25	M	5.852	272.232	850.000	3.122
176	22-160	M	5.893	260.435	590.000	2.265
177	6043	F	6.000	220.054	610.000	2.772
178	5895	F	6.000	251.814	770.000	3.057
179	29-260	M	6.002	276.769	690.000	2.493
180	4583	F	6.076	240.471	720.000	2.994
181	323-160	F	6.098	255.444	890.000	3.484
182	1350	F	6.123	174.682	700.000	4.007
183	930-259	M	6.304	390.199	690.000	1.768
184	19-259	M	6.312	366.606	1010.000	2.755

Table 6C. (Continued)

Sequence number	ID number	Sex	Age (years)	Body weight (kg)	Absolute weight (mg)	Relative weight mg/kg body wt
185	221	F	6.427	267.695	1080.000	4.034
186	312	F	6.797	228.675	720.000	3.148
187	9014-259	F	7.000	334.845	1040.000	3.105
188	26-258	F	7.241	286.751	790.000	2.755
189	3093-259	F	7.027	281.306	1040.000	3.697
190	FH-4	M	7.500	372.050	860.000	2.311
191	ET-13	F	7.739	173.774	730.000	4.200
192	ET-1	F	7.739	200.998	720.000	3.582
193	ET-11	F	7.739	178.765	700.000	3.915
194	1816-260	F	7.822	186.025	1460.000	7.848
195	13-258	F	8.000	231.397	810.000	3.500
196	LJF-1	F	8.000	204.174	840.000	4.114
197	54-1175	F	8.246	199.601	1130.000	5.661
198	N.F.	F	8.000	158.802	850.000	5.352
199	1287-260	F	8.276	206.442	1350.000	6.539
200	D-5	F	8.821	131.578	1380.000	10.488
201	9090	F	9.109	197.822	870.000	4.397
202	37-258	F	10.000	190.562	960.000	5.037

Table 7. Mean of the unilateral weights of endocrine glands in different species of animals

Species	Number of observations	Thyroid (g) Right	Thyroid (g) Left	Number of observations	Adrenal (g) Right	Adrenal (g) Left
Dog: Beagles	156	0.400	0.401	156	0.672	0.646
Dog: Mixed breed	111	0.579	0.570	119	1.132	1.122
Pig	213			214	5.175	4.971

Table 7. (Continued)

Species	Number of observations	Testis (g) Right	Left	Number of observations	Ovary (g) Right	Left
Dog: Beagles	66	6.614	6.632	74	0.468	0.470
Dog: Mixed breed	29	8.633	8.609	43	1.090	1.121
Pig	44	358.805	364.824	160	7.977	8.411

Table 8. Mean squares and F ratios (in parentheses) from analysis of variance: beagle data

Source	d.f.	Hypophysis weight	Relative hypophysis weight	Thyroid weight
Sex	1	80.989 (0.150)	17.730 (0.439)	0.052 (2.505)
Age	2	6467.541 (11.959)**	1384.021 (34.241)**	0.209 (9.997)**
Body weight	1	74.020 (0.137)	148.331 (3.670)	0.184 (8.821)**
Sex x age	2	849.737 (1.571)	43.789 (1.083)	0.019 (0.894)
Sex x body weight	1	1390.796 (2.572)	0.844 (0.021)	0.009 (0.409)
Error	149	540.811	40.421	0.021

*P < 0.05
**P < 0.01

Relative thyroid weight	Adrenal weight	Relative adrenal weight	Gonad weight	Relative gonad weight
0.0018 (0.100)	0.439 (8.429)**	0.01005 (5.094)*	1499.396 (195.998)**	13.541 (205.067)**
0.09907 (56.534)**	1.337 (25.684)**	0.01471 (7.461)**	0.412 (0.054)	0.035 (0.529)
0.00052 (0.299)	0.567 (10.899)**	0.00377 (1.913)	0.048 (0.006)	0.004 (0.068)
0.00409 (2.331)	0.042 (0.799)	0.00216 (1.094)	41.228 (5.389)**	0.723 (10.944)
0.00628 (3.585)	0.000 (0.000)	0.00050 (0.253)	71.087 (9.292)**	0.052 (0.783)
0.00175	0.052	0.00197	7.650	0.066

Table 9. Mean squares and F ratios (in parentheses) from analysis of variance: miscellaneous dog data

Source	d.f.	Hypophysis weight	Relative hypophysis weight	Thyroid weight
Sex	1	3404.425 (1.722)	6.654 (0.215)	0.121 (0.941)
Age	2	2487.318 (1.258)	935.586 (30.249)**	0.223 (1.743)
Body weight	1	16829.815 (8.510)**	81.907 (2.648)**	1.157 (9.029)**
Sex x age	2	1511.703 (0.764)	2.337 (0.076)	0.081 (0.633)
Sex x body weight	1	2892.469 (1.463)	71.607 (2.315)	0.038 (0.294)
Error	186	1977.642	30.930	0.128

*P < 0.05
**P < 0.01

Relative thyroid weight	Adrenal weight	Relative adrenal weight	Gonad weight	Relative gonad weight
0.00023 (0.250)	1.097 (4.067)*	0.00258 (1.815)	1105.277 (76.030)**	3.426 (58.566)**
0.00696 (7.561)**	5.244 (19.434)**	0.00847 (5.969)*	1.210 (0.083)	0.012 (0.200)
0.00082 (0.885)	3.000 (11.120)**	0.00662 (4.663)*	2.099 (0.144)	0.000 (0.000)
0.00022 (0.241)	0.061 (0.227)	0.00026 0.185	107.929 (7.424)**	0.369 (6.303)**
0.00017 (0.184)	0.587 (2.175)	0.00179 (1.263)	9.153 (0.630)	0.012 (0.200)
0.00092	0.270	0.00142	14.537	0.058

Table 10. Mean squares and F ratios (in parentheses) from analysis of variance: pig data

Source	d.f.	Hypophysis weight	Relative hypophysis weight	Thyroid weight
Sex	1	24,723.446 (0.572)	13.749 (1.499)	1045.936 (28.607)**
Age	2	1,960,652.880 (45.396)**	440.769 (48.045)**	916.638 (25.070)**
Body weight	1	322,260.048 (7.461)**	5.942 (0.648)	535.626 (14.650)**
Sex x age	2	11,383.011 (0.264)	2.605 (0.284)	11.619 (0.318)
Sex x body weight	1	18,317.134 (0.424)	0.140 (0.015)	120.441 (3.294)
Error	207	43,190.143	9.174	36.563

*$P < 0.05$
**$P < 0.01$

Relative thyroid weight	Adrenal weight	Relative adrenal weight	Gonad weight	Relative gonad weight
0.00109 (0.870)	1.243 (0.279)	0.00067 (2.188)	5,689,248.149 (440.194)**	72.818 (384.600)**
0.02433 (19.499)**	217.854 (48.901)**	0.01529 (50.045)**	640.831 (0.050)	0.014 (0.074)
0.000 (0.000)	46.450 (10.426)**	0.00035 (1.144)	131.190 (0.010)	0.000 (0.000)
0.00066 (0.525)	1.811 (0.407)	0.00027 (0.883)	56,795.915 (4.395)**	2.518 (13.299)**
0.00022 (0.176)	5.171 (1.161)	0.000 (0.000)	23,031.151 (1.782)	0.913 (4.824)*
0.00125	4.455	0.00031	12,924.416	0.189

Table 11A. Correlations between weight of endocrine glands of beagles from birth to 14 years

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight	Relative thyroid weight
Hypophysis weight		-0.59**	0.62**	-0.71**
Relative hypophysis weight			-0.64**	0.84**
Thyroid weight				-0.39**
Relative thyroid weight				
Adrenal weight				
Relative adrenal weight				
Gonad weight				
Relative gonad weight				
Body weight				

*P < 0.05
**P < 0.01

Adrenal weight	Relative adrenal weight	Gonad weight	Relative gonad weight	Body weight
0.75M** 0.77F**	-0.53M** -0.31F**	0.76M** 0.65F**	0.68M** -0.51F**	0.84**
-0.67M** -0.66F**	0.63M** 0.56F**	-0.67M** -0.55F**	-0.68M** 0.73F**	-0.75**
0.71M** 0.67F**	-0.32M* -0.34F**	0.62M** 0.62F**	-0.53M** -0.46F**	0.72**
-0.60M** -0.67F**	0.76M** 0.57F**	-0.68M** -0.55F**	-0.69M** 0.74F**	-0.74**
	-0.25M -0.05F	0.85M** 0.73F**	0.75M** -0.46F**	0.85M** 0.85F**
		-0.45M** -0.14F	-0.45M** 0.63F**	-0.55M** -0.44F**
			-0.89M** -0.08F	0.92M** 0.72F**
				0.77M** -0.60F**

Table 11B. Correlations between weight of endocrine glands of beagles during the age interval birth to 8 weeks

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight	Relative thyroid weight
Hypophysis weight		0.04	0.68**	-0.40*
Relative hypophysis weight			-0.30	0.68**
Thyroid weight				-0.15
Relative thyroid weight				
Adrenal weight				
Relative adrenal weight				
Gonad weight				
Relative gonad weight				
Age				
Body weight				

*P < 0.05
**P < 0.01

Adrenal weight	Relative adrenal weight	Gonad weight	Relative gonad weight	Age	Body weight
0.50M 0.79F**	-0.42M -0.24F	0.73M** 0.74F**	-0.15M -0.18F	0.71**	0.77**
-0.41M -0.29F	0.42M 0.48F	-0.56M** -0.28F	-0.19M 0.47F	-0.44*	-0.52*
0.74M** 0.83F**	-0.13M -0.28F	0.76M** 0.80F**	0.25M -0.24F	0.60*	0.70**
-0.15M -0.49F	0.71M** 0.67F**	-0.54M** -0.49F	0.23M 0.50F	-0.65**	-0.70**
	0.15M 0.06F	0.70M** 0.90F**	-0.04M 0.01F	0.61M* 0.83F**	0.74M** 0.73F**
		-0.49M -0.07F	-0.18M 0.65F**	-0.44M -0.28F	-0.47M -0.53F*
			0.23M 0.23F	0.76M** 0.79F**	0.93M** 0.63F*
				-0.27M -0.24F	-0.11M -0.55F*
					0.86**

Table 11C. Correlations between weight of endocrine glands of beagles during the age interval 2 to 11 months

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight	Relative thyroid weight
Hypophysis weight		-0.16	0.34*	-0.32
Relative hypophysis weight			-0.35*	0.41**
Thyroid weight				0.50**
Relative thyroid weight				
Adrenal weight				
Relative adrenal weight				
Gonad weight				
Relative gonad weight				
Age				
Body weight				

*P < 0.05
**P < 0.01

Adrenal weight	Relative adrenal weight	Gonad weight	Relative gonad weight	Age	Body weight
0.64M** 0.28F	-0.49M* 0.06F	0.85M** 0.22F	0.68M** 0.08F	0.50**	0.69**
-0.52M* -0.51F*	0.63M** 0.31F	-0.76M** -0.21F	-0.62M** 0.22F	-0.66**	-0.74**
0.35M 0.73F**	0.04M 0.01F	0.17M 0.45F*	0.03M 0.03F	0.28	0.46**
-0.23M -0.09F	0.66M** 0.37F	-0.66M** 0.11F	-0.59M** 0.31F	-0.48**	-0.49**
	0.10M 0.47F*	0.61M** 0.57F**	0.59M** 0.14F	0.57M* 0.66F**	0.61M** 0.71F**
		-0.60M** 0.36F	-0.39M 0.44F*	-0.60M** -0.02F	-0.67M** -0.27F**
			0.87M** 0.84F**	0.91M** 0.64F**	0.92M** 0.31F*
				0.86M** -0.23F	0.65M** -0.22F
					0.73**

Table 11D. Correlations between weight of endocrine glands of beagles during the age interval 1 to 14 years

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight	Relative thyroid weight
Hypophysis weight		0.42**	0.14	-0.32**
Relative hypophysis weight			-0.36**	0.01
Thyroid weight				0.65**
Relative thyroid weight				
Adrenal weight				
Relative adrenal weight				
Gonad weight				
Relative gonad weight				
Age				
Body weight				

*P < 0.05
**P < 0.01

Adrenal weight	Relative adrenal weight	Gonad weight	Relative gonad weight	Age	Body weight
0.35M* 0.37F*	-0.01M -0.09F	0.33M 0.20F	-0.13M -0.22F	0.47**	0.53**
-0.33M -0.17F	0.05M 0.40F*	-0.43M* -0.12F	-0.08M 0.32F*	-0.16	-0.50**
0.55M** 0.18F	0.33M -0.17F	0.44M* 0.27F	0.00M -0.03F	0.08	0.43**
0.05M -0.17F	0.51M** 0.15F	-0.24M 0.00F	-0.02M 0.26F	-0.36*	-0.36*
	0.68M** 0.55F**	0.68M** 0.29F	0.24M -0.09F	0.73M** 0.76F**	0.66M** 0.50F**
		0.13M 0.04F	0.22M 0.39F*	0.27M 0.22F	-0.06M -0.42F**
			0.63M** 0.66F**	0.65M** 0.03F	0.77M** 0.34F**
				0.23M -0.40F*	0.02M -0.41F*
					0.60**

Table 12A. Correlations between weight of endocrine glands of pigs from birth to 10 years of age

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight	Relative thyroid weight
Hypophysis weight		-0.47**	0.89M** 0.82F**	-0.37**
Relative hypophysis weight			-0.63M -0.52F**	0.49**
Thyroid weight				-0.25M** -0.14F*
Relative thyroid weight				
Adrenal weight				
Relative adrenal weight				
Gonad weight				
Relative gonad weight				
Body weight				

*P < 0.05
**P < 0.01

Adrenal weight	Relative adrenal weight	Gonad weight	Relative gonad weight	Body weight
0.87**	-0.41**	0.93M** 0.82F**	0.80M** 0.67F**	0.85**
-0.49**	0.85**	-0.63M** -0.55F**	-0.54M** -0.46F**	-0.66**
0.85M** 0.85F**	-0.67M** -0.40F**	0.85M** 0.77F**	0.69M** 0.59F**	0.93M** 0.82F**
-0.31**	0.51**	-0.50M** -0.45F**	-0.48M** -0.47F**	-0.53**
	-0.27**	0.89M* 0.77F**	0.72M** 0.60F**	0.83**
		-0.68M** -0.49F**	-0.59M** -0.40F**	-0.59**
			0.90M** 0.86F**	0.96M** 0.88F**
				0.78M** 0.65F**

Table 12B. Correlations between weight of endocrine glands of pigs during the age interval birth to 8 weeks

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight	Relative thyroid weight
Hypophysis weight		-0.63**	0.88M** 0.74F**	-0.20
Relative hypophysis weight			-0.76M** -0.74F**	0.04
Thyroid weight				0.01M 0.35F
Relative thyroid weight				
Adrenal weight				
Relative adrenal weight				
Gonad weight				
Relative gonad weight				
Age				
Body weight				

*P < 0.05
**P < 0.01

Adrenal weight	Relative adrenal weight	Gonad weight	Relative gonad weight	Age	Body weight
0.85**	-0.75**	0.75M** 0.69F**	-0.14M -0.64F**	0.86**	0.92**
-0.78**	0.81**	-0.72M** -0.45F*	0.05M 0.74F**	-0.83**	-0.77**
0.96M** 0.85F**	0.72M** 0.71F**	-0.70M** 0.51F*	-0.22M -0.67F**	0.89M** 0.90F**	0.96M** 0.89F**
0.02	0.27	-0.32M -0.22F	0.00M 0.01F	-0.04	-0.07
	-0.70**	0.71M** 0.61F**	-0.20M -0.70F**	0.91**	0.93**
		-0.72M** -0.51F*	0.05M 0.78F**	-0.85**	-0.82**
			0.35M -0.11F	0.91M** 0.61F**	0.78M** 0.64F**
				-0.01M -0.79F**	-0.19M -0.73F**
					0.94**

Table 12C. Correlations between weight of endocrine glands of pigs during the age interval 2-12 months

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight	Relative thyroid weight
Hypophysis weight		-0.69**	0.78M 0.86F**	-0.43*
Relative hypophysis weight			-0.91M** -0.74F**	0.40*
Thyroid weight				-0.04M -0.12F
Relative thyroid weight				
Adrenal weight				
Relative adrenal weight				
Gonad weight				
Relative gonad weight				
Age				
Body weight				

*P < 0.05
**P < 0.01

Adrenal weight	Relative adrenal weight	Gonad weight	Relative gonad weight	Age	Body weight
0.89**	-0.64**	0.67M 0.82F**	0.57M 0.50F**	0.89**	0.93**
-0.79**	0.64**	-0.73M -0.65F**	0.49M -0.26F	-0.83**	-0.85**
0.63M 0.82F**	-0.51M -0.61F**	0.81M 0.81F*	-0.39M 0.36F*	0.79M 0.93F**	0.87M 0.92F**
-0.45	0.21	-0.24M -0.32F	0.34M -0.45F**	-0.39*	-0.40*
	-0.44**	0.77M 0.79F**	-0.35M 0.48F**	0.90**	0.93*
		-0.08M -0.49F**	0.33M -0.09F	-0.61**	-0.66**
			0.07M 0.72F**	0.71M 0.86F**	0.74M 0.83F**
				-0.57M 0.47F	-0.55M 0.41F
					0.98**

Table 12D. Correlations between weight of endocrine glands of pigs during the age interval 1 to 10 years

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight	Relative thyroid weight
Hypophysis weight		0.62**	0.34M 0.50F**	0.36**
Relative hypophysis weight			-0.29M 0.13F	0.29**
Thyroid weight				0.87M** 0.85F**
Relative thyroid weight				
Adrenal weight				
Relative adrenal weight				
Gonad weight				
Relative gonad weight				
Age				
Body weight				

*P < 0.05
**P < 0.01

Adrenal weight	Relative adrenal weight	Gonad weight	Relative gonad weight	Age	Body weight
0.55**	0.34**	0.35M 0.37F**	-0.10M 0.13F	0.72**	0.35**
0.18	0.54**	-0.11M -0.03F*	0.33M 0.28F**	0.45**	-0.44**
0.39M 0.62F**	0.12M 0.32F**	0.11M 0.33F**	-0.36M 0.02F	0.19M 0.55F**	0.61M** 0.43F**
0.42**	0.42**	-0.05M 0.10F	-0.17M 0.15F	0.41**	0.02
	0.72**	0.51M* 0.26F**	-0.03M -0.03F	0.71**	0.43**
		0.18M -0.07F	0.16M 0.08F	0.58**	-0.24*
			0.66M** 0.72F**	0.28M 0.21F*	0.43M* 0.51F**
				-0.23M -0.02F	-0.38M -0.20F*
					0.57M** 0.31F**

Table 13A. Partial correlations between weights of endocrine glands of 157 beagles: male (body weight held constant)

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight
Hypophysis weight		0.34**	0.18*
Relative hypophysis weight			-0.09
Thyroid weight			
Relative thyroid weight			
Adrenal weight			
Relative adrenal weight			
Testis weight			
Relative testis weight			

*P < 0.05
**P < 0.01

Relative thyroid weight	Adrenal weight	Relative adrenal weight	Testis weight	Relative testis weight
-0.06	0.15	0.03	0.37**	0.44**
0.32**	-0.06	0.46	0.13	0.04
0.32**	0.27**	0.15	-0.06	-0.01
	0.03	0.35**	-0.03	-0.21
		0.47**	0.31**	0.30**
			0.09	0.03
				0.89**

Table 13B. Partial correlations between weights of endocrine glands of 157 beagles: female (body weight held constant

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight
Hypophysis weight		0.18*	0.04
Relative hypophysis weight			-0.16*
Thyroid weight			
Relative thyroid weight			
Adrenal weight			
Relative adrenal weight			
Ovary weight			
Relative ovary weight			

*P < 0.05
**P < 0.01

Relative thyroid weight	Adrenal weight	Relative adrenal weight	Testis weight	Relative testis weight
-0.28**	0.07	-0.08	0.51**	0.15
0.50**	-0.08	-0.01	0.06	0.58**
0.11	0.17*	-0.03	0.12	0.00
	-0.07	0.50**	-0.09	0.34**
		0.55**	0.09	0.02
			0.02	0.04
				0.64**

Table 13C. Partial correlations between weights of endocrine glands of 215 pigs: male (body weight held constant)

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight
Hypophysis weight		0.07	0.14*
Relative hypophysis weight			-0.02
Thyroid weight			
Relative thyroid weight			
Adrenal weight			
Relative adrenal weight			
Testis weight			
Relative testis weight			

*P < 0.05
**P < 0.01

Relative thyroid weight	Adrenal weight	Relative adrenal weight	Testis weight	Relative testis weight
0.11	-0.08	-0.09	0.01	0.01
0.17*	0.00	0.84**	-0.08	-0.07
0.73**	-0.08	-0.08	-0.01	-0.04
	-0.02	0.18**	-0.11	-0.14
		0.27**	0.30**	0.20**
			-0.04	-0.06
				0.92**

Table 13D. Partial correlations between weights of endocrine glands of 215 pigs: female (body weight held constant)

Traits	Hypophysis weight	Relative hypophysis weight	Thyroid weight
Hypophysis weight		0.30**	0.42**
Relative hypophysis weight			0.07
Thyroid weight			
Relative thyroid weight			
Adrenal weight			
Relative adrenal weight			
Ovary weight			
Relative ovary weight			

*P < 0.05
**P < 0.01

Relative thyroid weight	Adrenal weight	Relative adrenal weight	Testis weight	Relative testis weight
0.22**	0.41**	0.16*	0.21**	0.24**
0.22**	0.11	0.73**	0.06	-0.05
0.62**	0.46**	0.15*	0.21**	0.18
	0.24*	0.28**	0.08	-0.13
		0.49**	0.12	0.13
			0.04	0.04
				0.85**

Table 14. Tinctorial affinity of different cell-types of the canine and porcine pars distalis adenohypophysis

Technique	STH cell	LTH cell	TSH cell	Gonadotrope FSH cell	Gonadotrope ICSH cell	ACTH cell
Luxol fast blue and trichrome stain of Cleveland and Wolfe (1932)	blue-green	brick red	dark blue	light blue	violet	erythrophilic
Tetrachrome (Herlant, 1960; Racadot, 1962)	yellow	brick red	dark blue	light blue	violet	erythrophilic
Aldehyde-fuchsin and Crossman's trichrome (Purvis and Griesbach, 1957)	orange	intense pink	light green	purple	purple to violet	gray
Azocarmine, orange G and aniline blue (Dawson and Friedgood, 1938)	orange	red	dark	light	bluish	chromophobic
Performic acid, alcian blue, PAS and orange G (Heath, 1965)	yellow	magenta	blue	purple	rose red	

Table 14. (Continued)

Technique	STH cell	LTH cell	TSH cell	Gonadotrope FSH cell	Gonadotrope ICSH cell	ACTH cell
Aldehyde-thionin PAS and orange G (Ezrin and Murray, 1963)	yellow	light magenta	blue-black	purple	rose red	
Aldehyde-fuchsin, PAS and orange G (Elftman, 1959b)	yellow	magenta	purple-blue	purple	rose red	chromophobic
Potassium permanganate, alcian blue and PAS (Herlant, 1960)			blue	purple	red	chromophobic
Luxol fast blue, PAS, orange G and methyl blue (Rennels, 1957)	blue-green	magenta	red	dull purple	red	
Lead hematoxylin (MacConaill, 1947)					intense purple	

Table 15. Volumetric proportion (in percent) of the pars distalis adenohypophysis (PDA), pars intermedia (PI) and the pars distalis neurohypophysis (PDN) during different age intervals in the dog and the pig

Age interval	Dog			Pig		
	PDA	PI	PDN	PDA	PI	PDN
Birth - 4 weeks	53.0	9.0	38.0	65.0	9.3	25.7
4 - 8 weeks	54.2	10.8	35.0	72.4	8.0	19.6
2 - 6 months	54.8	12.0	34.2	75.0	8.0	17.0
6 months - 4 years	56.1	13.3	30.6	80.5	6.9	12.6
4 - 7 years	55.7	14.5	29.8	82.2	7.2	10.6
7 - 10 years	55.4	16.0	28.6	81.3	7.7	11.0
10 - 14 years	55.8	15.8	29.4			

Table 16. Cytoplasm-nuclear ratio of different cell-types of the pars distalis denohypophysis in the dog and the pig

Cell type	Birth - 4 weeks		4 - 8 weeks		2 - 6 months	
	Dog	Pig	Dog	Pig	Dog	Pig
STH cell	2.50	3.25	4.00	2.50	4.00	4.50
LTH cell			2.50	2.25	3.67	3.75
TSH cell	3.50	2.44	3.00	2.00	4.00	2.50
FSH cell		2.40	3.50	2.59	3.50	2.66
ICSH cell					2.00	1.50

6 - 12 months		1 - 4 years		4 - 7 years		7 - 14 years	
Dog	Pig	Dog	Pig	Dog	Pig	Dog	Pig
4.00	4.00	4.00	4.00	4.00	3.00	3.50	2.50
3.33	3.00	3.00	4.50	4.00	4.25	4.33	4.25
3.00	2.50	2.20	3.00	3.50	2.67	4.40	2.67
4.00	3.00	3.00	4.00	3.33	4.25	4.00	7.33
2.67	2.00	2.67	3.33	3.50	2.00	2.00	1.75

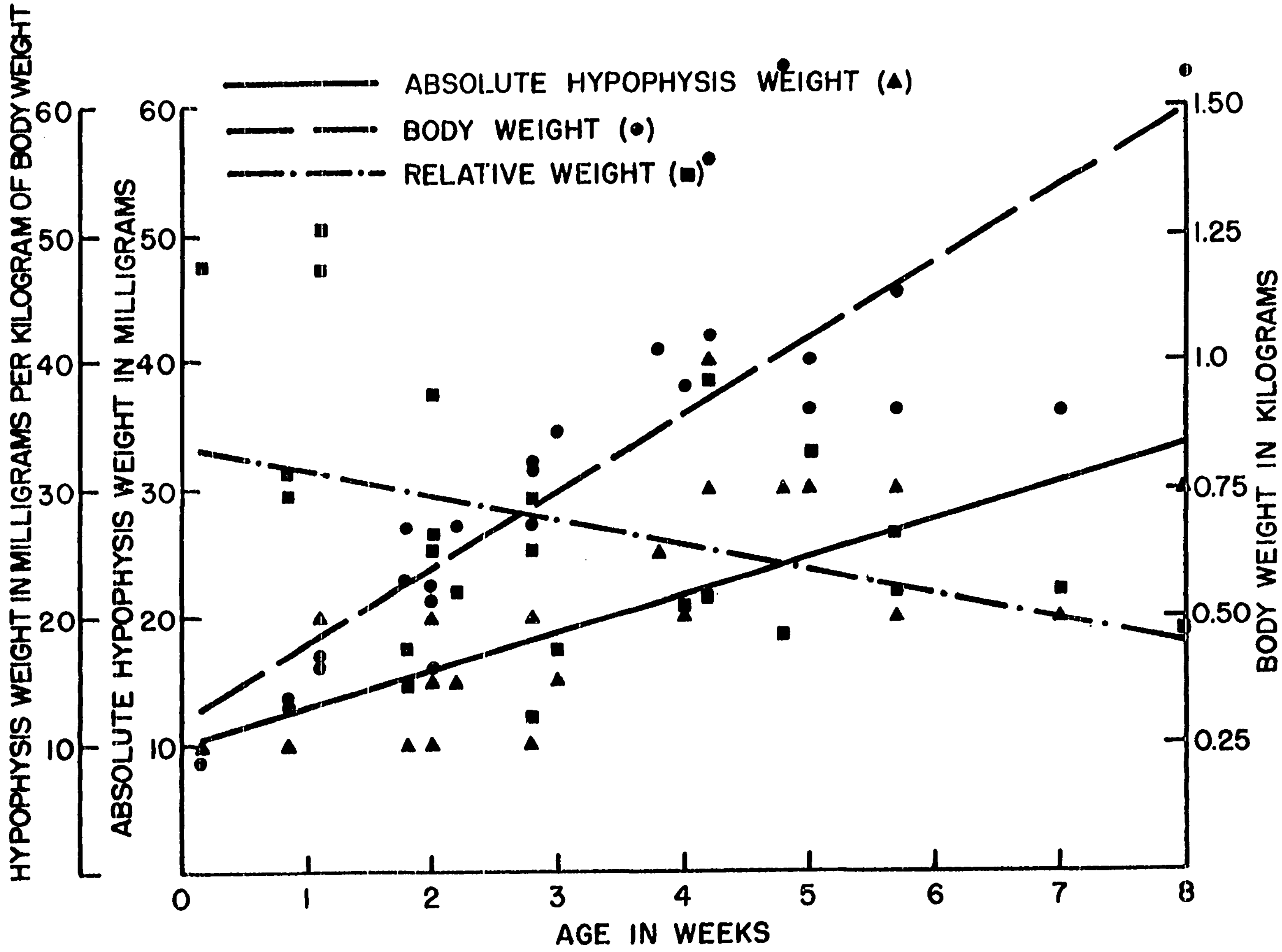
APPENDIX B. GRAPHS

Graph 1. Absolute hypophysis weight, relative hypophysis weight and body weight of dogs from birth to 8 weeks

Absolute weight of the hypophysis in mg versus age in weeks. Linear regression data: $N = 27$; $Y = 10.088 + 2.937\ X, \pm 6.296$ mg; $b = 2.937, \pm 0.615, P < 0.01$; $\bar{y} = 19.630, \pm 1.212, P < 0.01$; $r = 0.691, P < 0.01$

Relative weight of hypophysis in mg/kg body weight versus age in weeks. Linear regression data: $N = 27$; $Y = 33.269 - 1.905\ X$, ± 9.388 mg/kg body weight; $b = - 1.905, \pm 0.916, P < 0.05$; $\bar{y} = 27.08$, $\pm 1.807, < 0.01$; $r = 0.384, P < 0.05$

Body weight in kg versus age in weeks. Linear regression data: $N = 27$; $Y = 0.285 + 0.154\ X, \pm 0.198$ kg.; $b = 0.154, \pm 0.019$, $P < 0.01$; $\bar{y} = 0.785, \pm 0.037, P < 0.01$; $r = 0.847, P < 0.01$



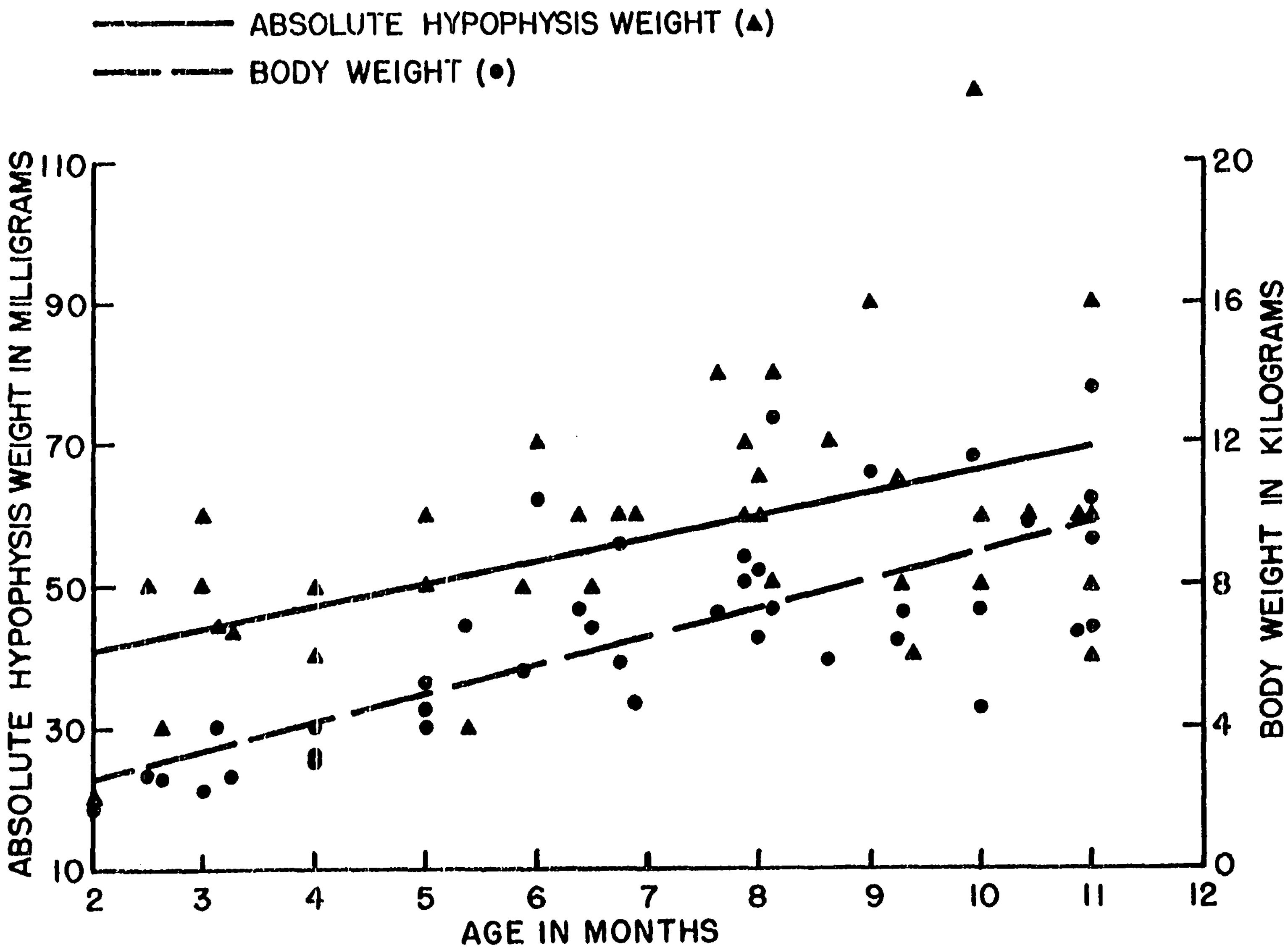
ABSOLUTE HYPOPHYSIS WEIGHT (▲)
BODY WEIGHT (●)
RELATIVE WEIGHT (■)
HYPOPHYSIS WEIGHT IN MILLIGRAMS PER KILOGRAM OF BODY WEIGHT
ABSOLUTE HYPOPHYSIS WEIGHT IN MILLIGRAMS
10
20
30
40
50
60
0
1
2
3
4
5
6
7
8
AGE IN WEEKS
0.25
0.50
0.75
1.0
1.25
1.50
BODY WEIGHT IN KILOGRAMS

Graph 2. Absolute hypophysis weight and body weight of dogs from 2 to 11 months

Absolute weight of the hypophysis in mg versus age in months.

Linear regression data: $N = 43$, $Y = 35.137 + 3.154\ X$, $\pm$ 15.698 mg; $b = 3.154$, $\pm$ 0.872, $P < 0.01$; $\bar{y} = 56.953$, $\pm$ 2.394, $P < 0.01$; $r = 0.492$, $P < 0.01$

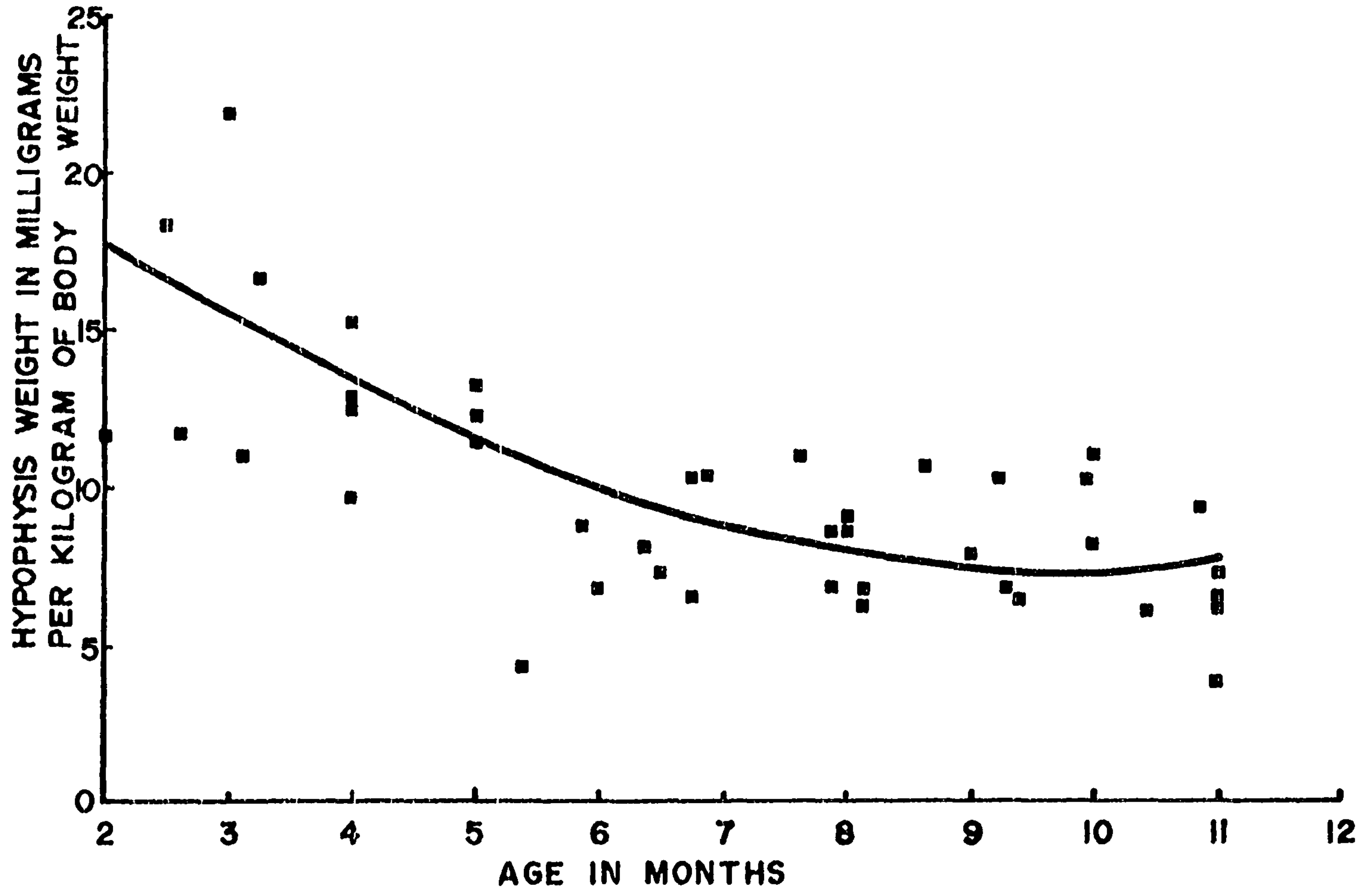
Body weight in kg versus age in months. Linear regression data: $N = 43$; $Y = 0.891 + 0.808\ X$, $\pm$ 2.021 kg; $b = 0.808$, $\pm$ 0.112, $P < 0.01$; $\bar{y} = 6.482$, $\pm$ 0.308, $P < 0.01$; $r = 0.747$, $P < 0.01$



ABSOLUTE HYPOPHYSIS WEIGHT (▲)
BODY WEIGHT (●)
ABSOLUTE HYPOPHYSIS WEIGHT IN MILLIGRAMS
10
30
50
70
90
110
2
3
4
5
6
7
8
9
10
11
12
AGE IN MONTHS
BODY WEIGHT IN KILOGRAMS
0
4
8
12
16
20

Graph 3. Relative hypophysis weight of dogs from 2 to 11 months

Relative weight of hypophysis in mg/kg body weight versus age in months. Curvilinear regression data: $N = 43$, $Y = 23.764 - 3.315 X_1 + 0.169 X_2$, ± 3.257 mg/kg body weight; $b_{y1.2} = - 3.315, \pm 1.027$, $P < 0.01$; $b_{y2.1} = 0.169, \pm 0.075$, $P < 0.05$; $\bar{y} = 10.205, \pm 0.497$, $P < 0.01$



HYPOPHYSIS WEIGHT IN MILLIGRAMS
PER KILOGRAM OF BODY WEIGHT
0
5
10
15
20
25
2
3
4
5
6
7
8
9
10
11
12
AGE IN MONTHS

Graph 4. Absolute hypophysis weight and body weight of dogs from 1 to 13.6 years

Absolute weight of the hypophysis in mg versus age in years.

Linear regression date: $N = 66$; $Y = 60.919 + 3.186\ X$, 18.413 mg;

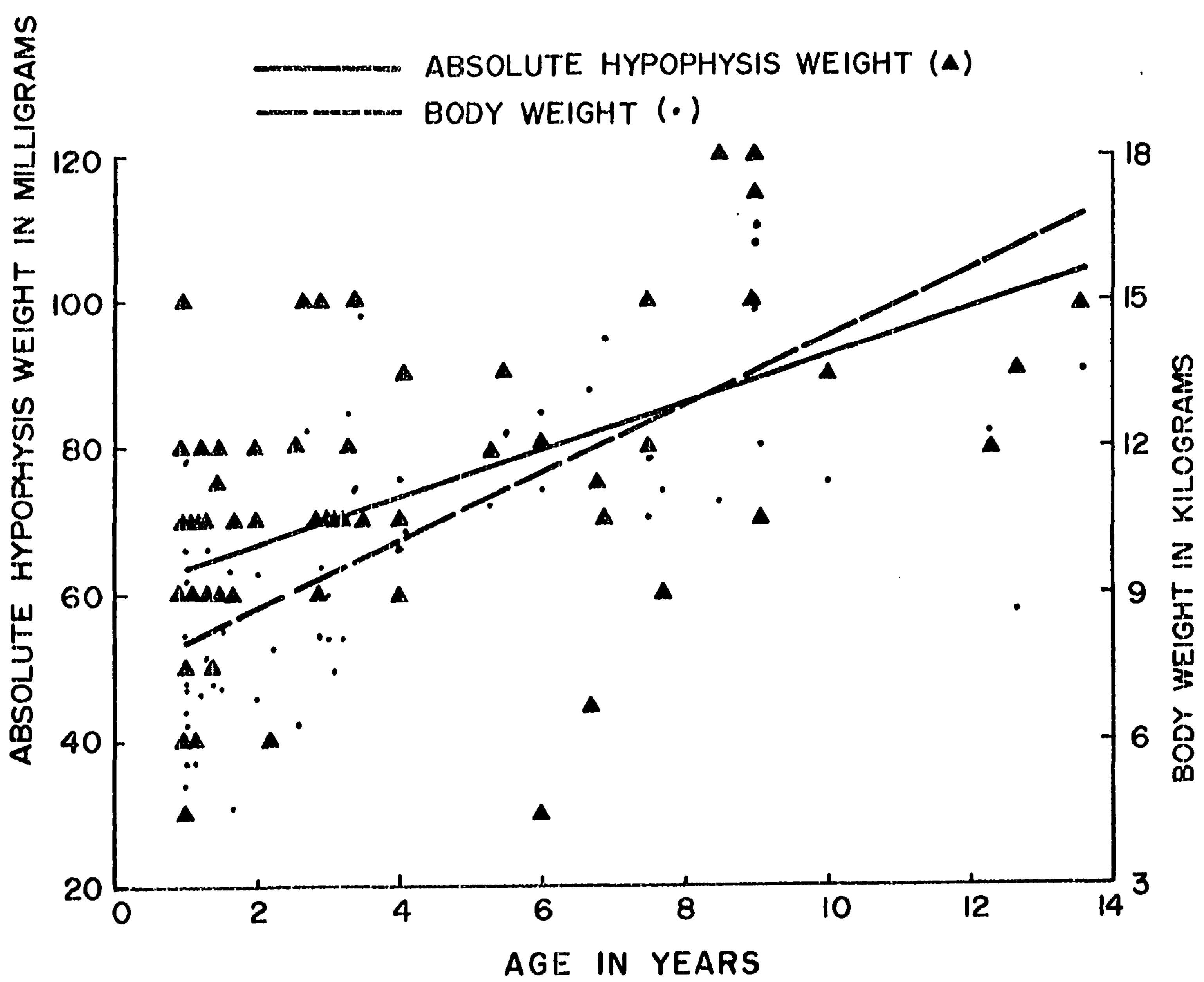
$b = 3.186$, $\pm$ 0.662, $P < 0.01$; $\bar{y} = 73.788$, $\pm$ 2.267, $P < 0.01$;

$r = 0.516$, $P < 0.01$

Body weight in kg versus age in years. Linear regression data:

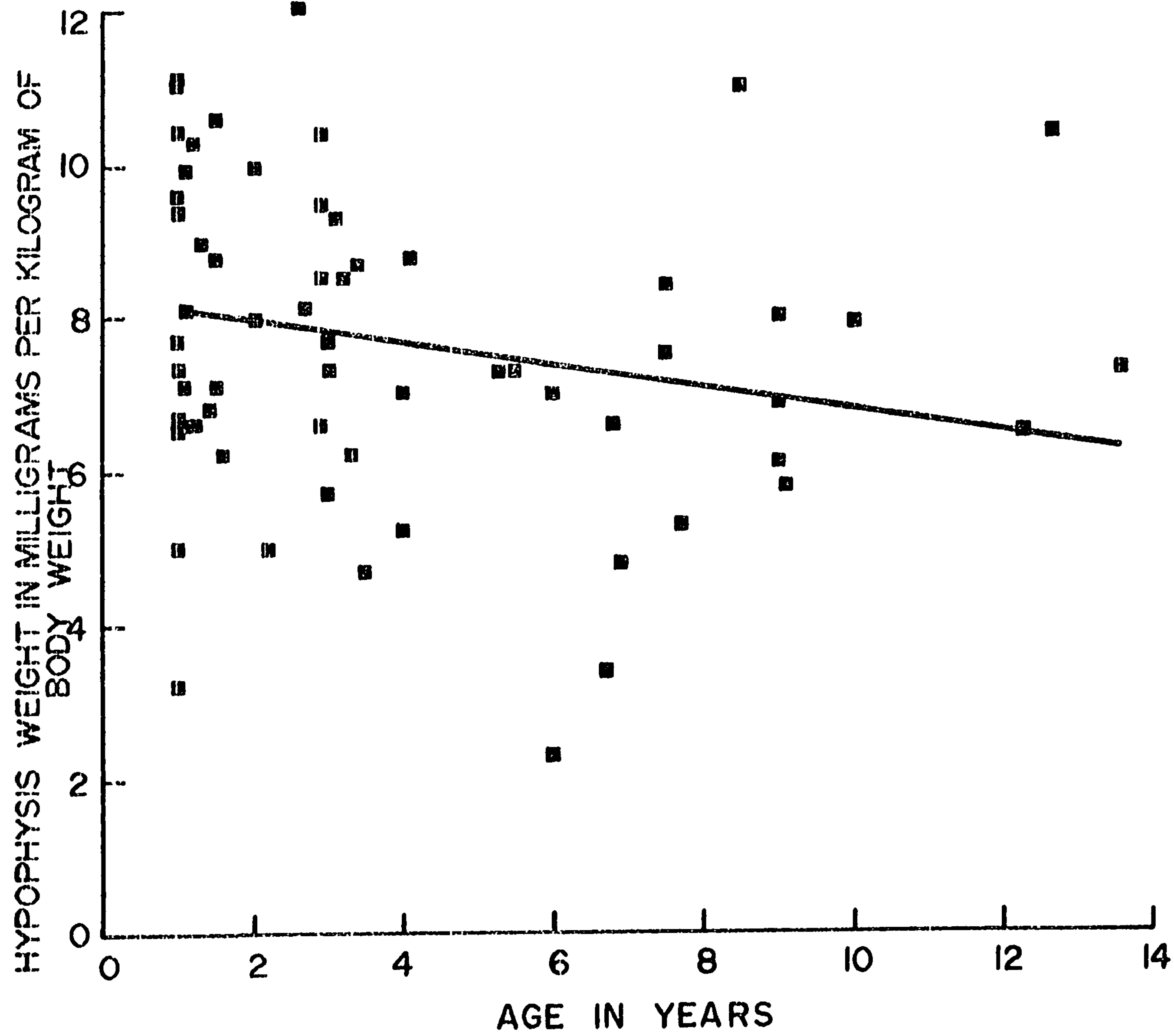
$N = 66$; $Y = 7.344 + 0.691\ X$, $\pm$ 2.827 kg; $b = 0.691$, $\pm$ 0.102,

$P < 0.01$; $\bar{y} = 10.135$, $\pm$ 0.348, $P < 0.01$; $r = 0.648$, $P < 0.01$


ABSOLUTE HYPOPHYSIS WEIGHT (▲)
BODY WEIGHT (•)
ABSOLUTE HYPOPHYSIS WEIGHT IN MILLIGRAMS
120
100
80
60
40
20
0
2
4
6
8
10
12
14
AGE IN YEARS
18
15
12
9
6
3
BODY WEIGHT IN KILOGRAMS

Graph 5. Relative hypophysis weight of dogs from 1 to 13.6 years

Relative weight of hypophysis in mg/kg body weight versus age in years. Linear regression data: $N = 66$; $Y = 8.276 - 0.145\ X$, $\pm\ 2.243$ mg/kg body weight; $b = -\ 0.145$, $\pm\ 0.081$, $P < 0.05$; $\bar{y} = 7.692$, $\pm\ 0.276$, $P < 0.01$; $r = 0.219$, $P < 0.05$

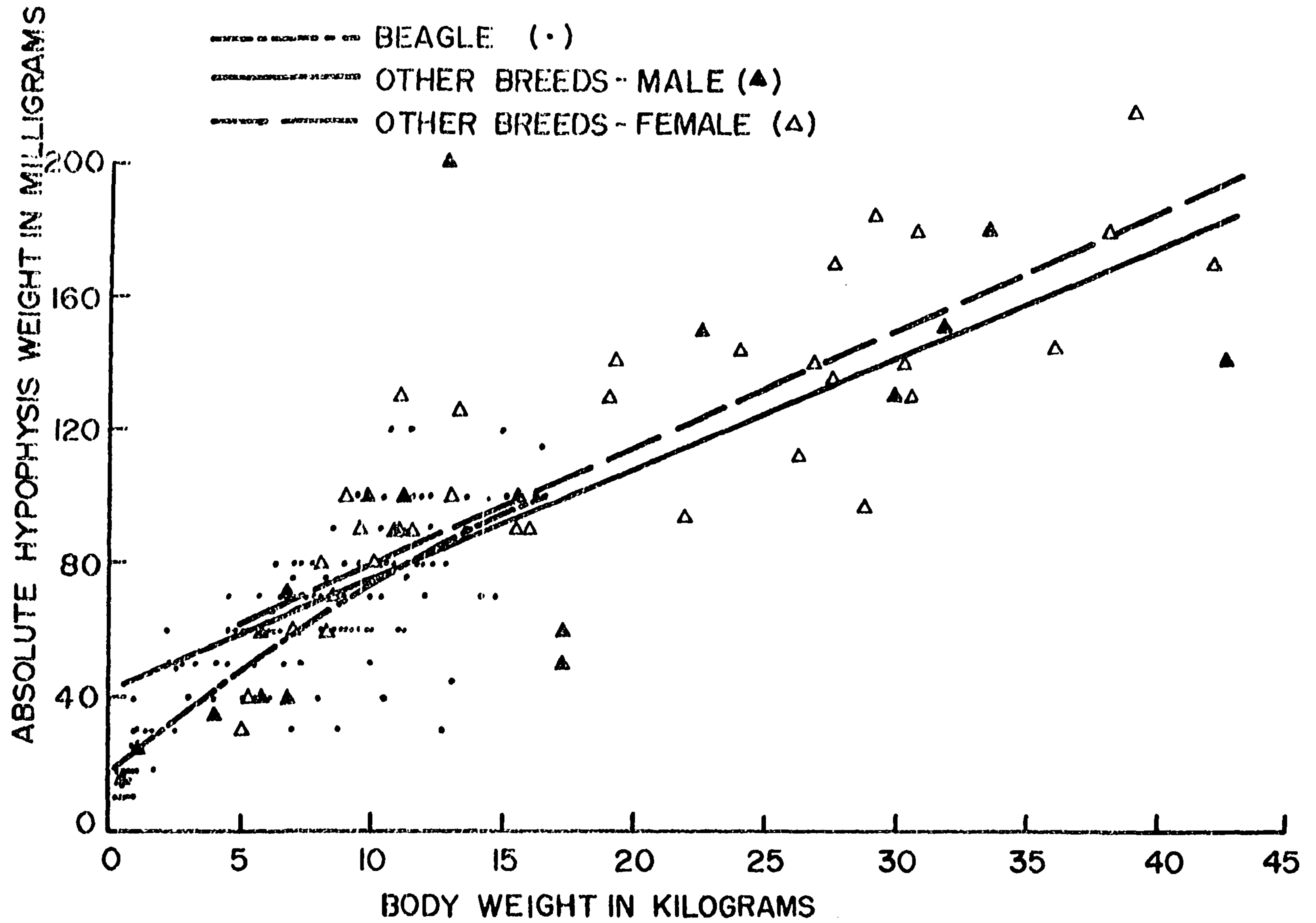

HYPOPHYSIS WEIGHT IN MILLIGRAMS PER KILOGRAM OF BODY WEIGHT
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
AGE IN YEARS

Graph 6. Absolute hypophysis weight of dogs ranging in body weight from 0.210 to 13.611 kg

Absolute weight of the hypophysis in mg versus body weight in kg. Beagles. Curvilinear regression data: $N = 136$; $Y = 17.926 + 6.627 X_1 - 0.103 X_2$, $\pm$ 15.044 mg; $b_{y1.2} = 6.627$, $\pm$ 0.590, $P < 0.01$; $b_{y2.1} = - 0.013$, $\pm$ 0.029, $P < 0.01$; $\bar{y} = 57.676$, $\pm$ 1.210, $P < 0.01$

Absolute weight of the hypophysis in mg versus body weight in kg. Male of breeds other than beagle. Linear regression data: $N = 17$; $Y = 43.429 + 3.293 X$, $\pm$ 40.541 mg; $b = 3.293$, $\pm$ 0.860, $P < 0.01$; $\bar{y} = 92.353$, $\pm$ 9.833, $P < 0.01$; $r = 0.703$, $P < 0.01$

Absolute weight of the hypophysis in mg versus body weight in kg. Female of breeds other than beagle. Linear regression data: $N = 35$; $Y = 44.721 + 3.522 X$, $\pm$ 23.308 mg; $b = 3.522$, $\pm$ 0.358, $P < 0.01$; $\bar{y} = 112.400$, $\pm$ 3.940, $P < 0.01$; $r = 0.864$, $P < 0.01$

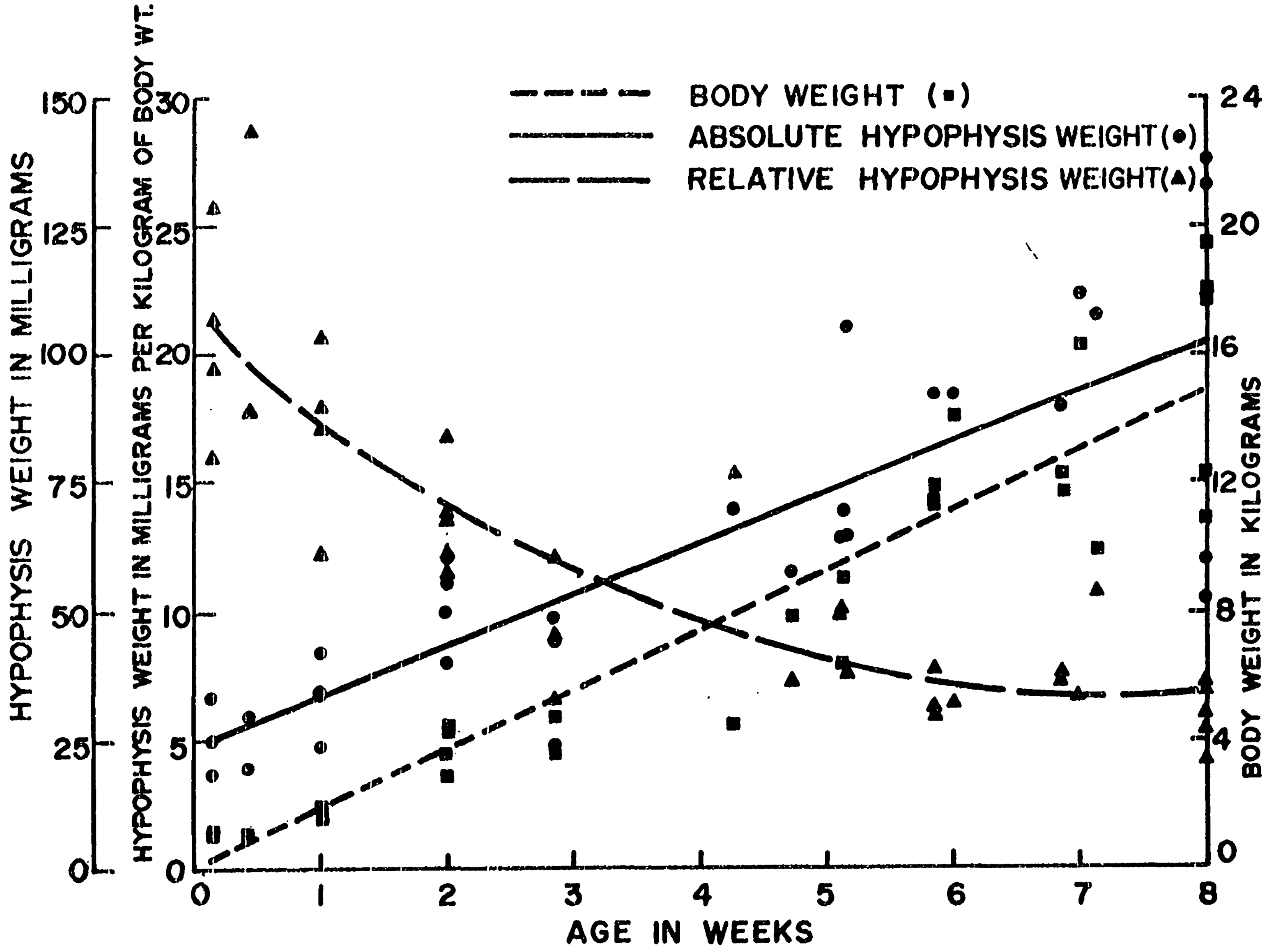

BEAGLE (•)
OTHER BREEDS - MALE (▲)
OTHER BREEDS - FEMALE (△)
ABSOLUTE HYPOPHYSIS WEIGHT IN MILLIGRAMS
0
40
80
120
160
200
0
5
10
15
20
25
30
35
40
45
BODY WEIGHT IN KILOGRAMS

Graph 7. Absolute hypophysis weight, relative hypophysis weight and body weight of pigs from birth to 8 weeks

Absolute weight of the hypophysis in mg versus age in weeks. Linear regression data: $N = 36$; $Y = 23.841 + 9.823\ X, \pm 17.699$ mg; $b = 9.823, \pm 1.049$, $P < 0.01$; $\bar{y} = 62.194, \pm 2.950$, $P < 0.01$; $r = 0.849$, $P < 0.01$

Relative weight of hypophysis in mg/kg body weight versus age in weeks. Curvilinear regression data: $N = 36$; $Y = 21.415 - 4.096\ X_1 + 0.286\ X_2, \pm 3.083$ mg/kg body weight; $b_{y1.2} = -4.096, \pm 0.745$, $P < 0.01$; $b_{y2.1} = 0.286, \pm 0.089$, $P < 0.01$; $\bar{y} = 12.052, \pm 0.514$, $P < 0.01$

Body weight in kg versus age in weeks. Linear regression data: $N = 36$; $Y = 0.047 + 1.852\ X, \pm 1.950$ kg; $b = 1.852, \pm 0.116$, $P < 0.01$; $\bar{y} = 7.279, \pm 0.325$, $P < 0.05$; $r = 0.940$, $P < 0.01$

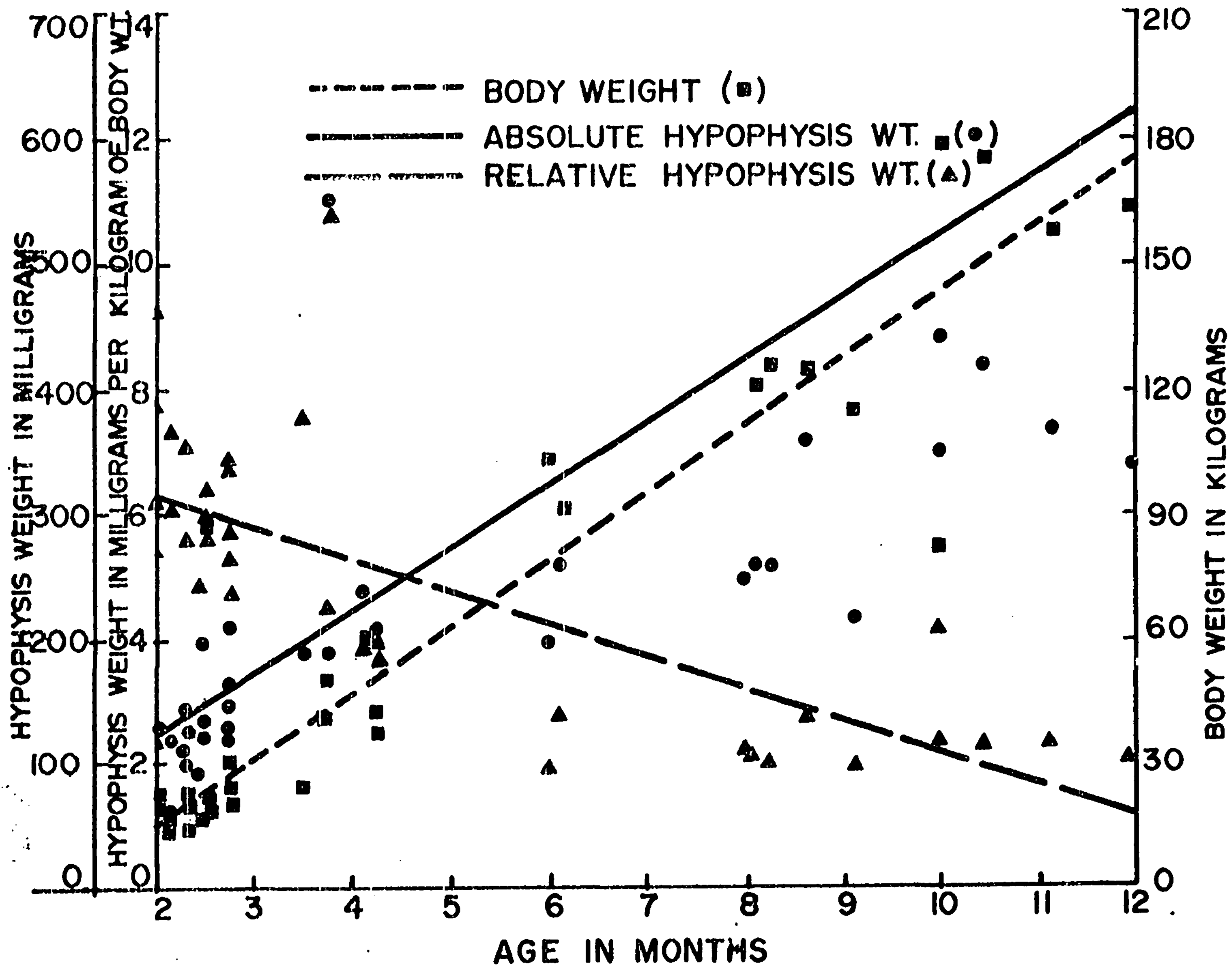

BODY WEIGHT (■)
ABSOLUTE HYPOPHYSIS WEIGHT (●)
RELATIVE HYPOPHYSIS WEIGHT (▲)
HYPOPHYSIS WEIGHT IN MILLIGRAMS
HYPOPHYSIS WEIGHT IN MILLIGRAMS PER KILOGRAM OF BODY WT.
BODY WEIGHT IN KILOGRAMS
AGE IN WEEKS
0
25
50
75
100
125
150
0
5
10
15
20
25
30
0
4
8
12
16
20
24
0
1
2
3
4
5
6
7
8

Graph 8. Absolute hypophysis weight, relative hypophysis weight and body weight of pigs from 2 to 12 months

Absolute weight of the hypophysis in mg versus age in months. Linear regression data: $N = 38$; $Y = 21.140 + 50.491\ X$, ± 307.160 mg; $b = 50.491$, ± 16.055, $P < 0.01$; $\bar{y} = 259.711$, ± 49.820, $P < 0.01$; $r = 0.464$, $P < 0.01$

Relative weight of hypophysis in mg/kg body weight versus age in months. Linear regression data: $N = 38$; $Y = 7.439 - 0.527\ X$, ± 1.491 mg/kg body weight; $b = -0.527$, ± 0.078, $P < 0.01$; $\bar{y} = 4.951$, ± 0.242, $P < 0.05$; $r = 0.748$, $P < 0.01$

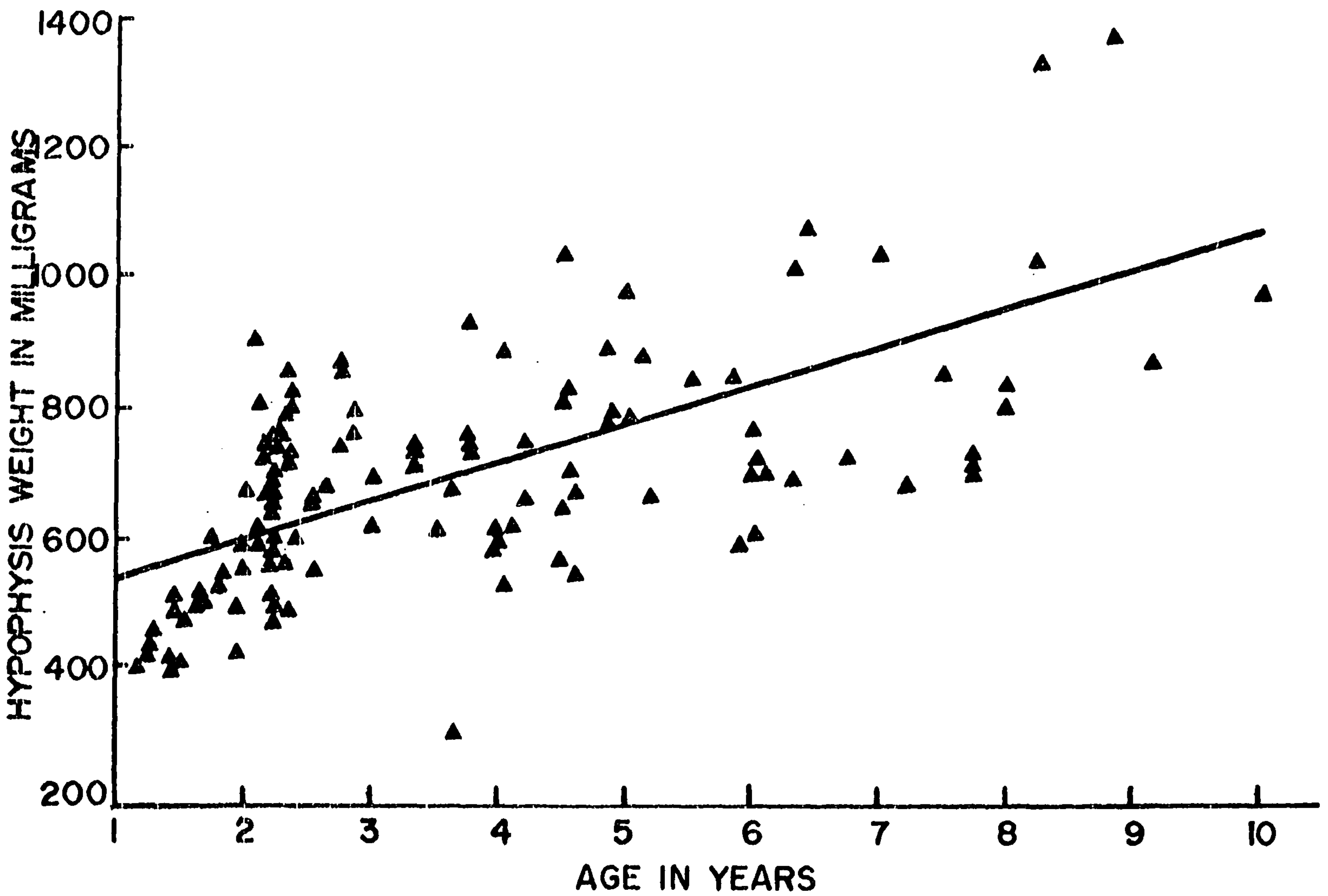
Body weight in kg versus age in months. Linear regression data: $N = 38$; $Y = -16.257 + 16.048\ X$, ± 14.547 kg; $b = 16.048$, ± 0.760, $P < 0.01$; $\bar{y} = 59.568$, ± 2.360, $P < 0.01$; $r = 0.962$, $P < 0.01$


BODY WEIGHT (■)
ABSOLUTE HYPOPHYSIS WT. (●)
RELATIVE HYPOPHYSIS WT. (▲)
HYPOPHYSIS WEIGHT IN MILLIGRAMS
HYPOPHYSIS WEIGHT IN MILLIGRAMS PER KILOGRAM OF BODY WT.
BODY WEIGHT IN KILOGRAMS
AGE IN MONTHS
700
600
500
400
300
200
100
0
14
12
10
8
6
4
2
0
210
180
150
120
90
60
30
0
2
3
4
5
6
7
8
9
10
11
12

Graph 9. Absolute hypophysis weight of pigs from 1 to 10 years

Absolute weight of the hypophysis in mg versus age in years.

Linear regression data: $N = 128$; $Y = 476.924 + 59.747\,X$, $\pm\ 150.017$ mg; $b = 59.747$, $\pm\ 6.191$, $P < 0.01$; $\bar{y} = 703.125$, $\pm\ 13.260$, $P < 0.01$; $r = 0.652$, $P < 0.01$

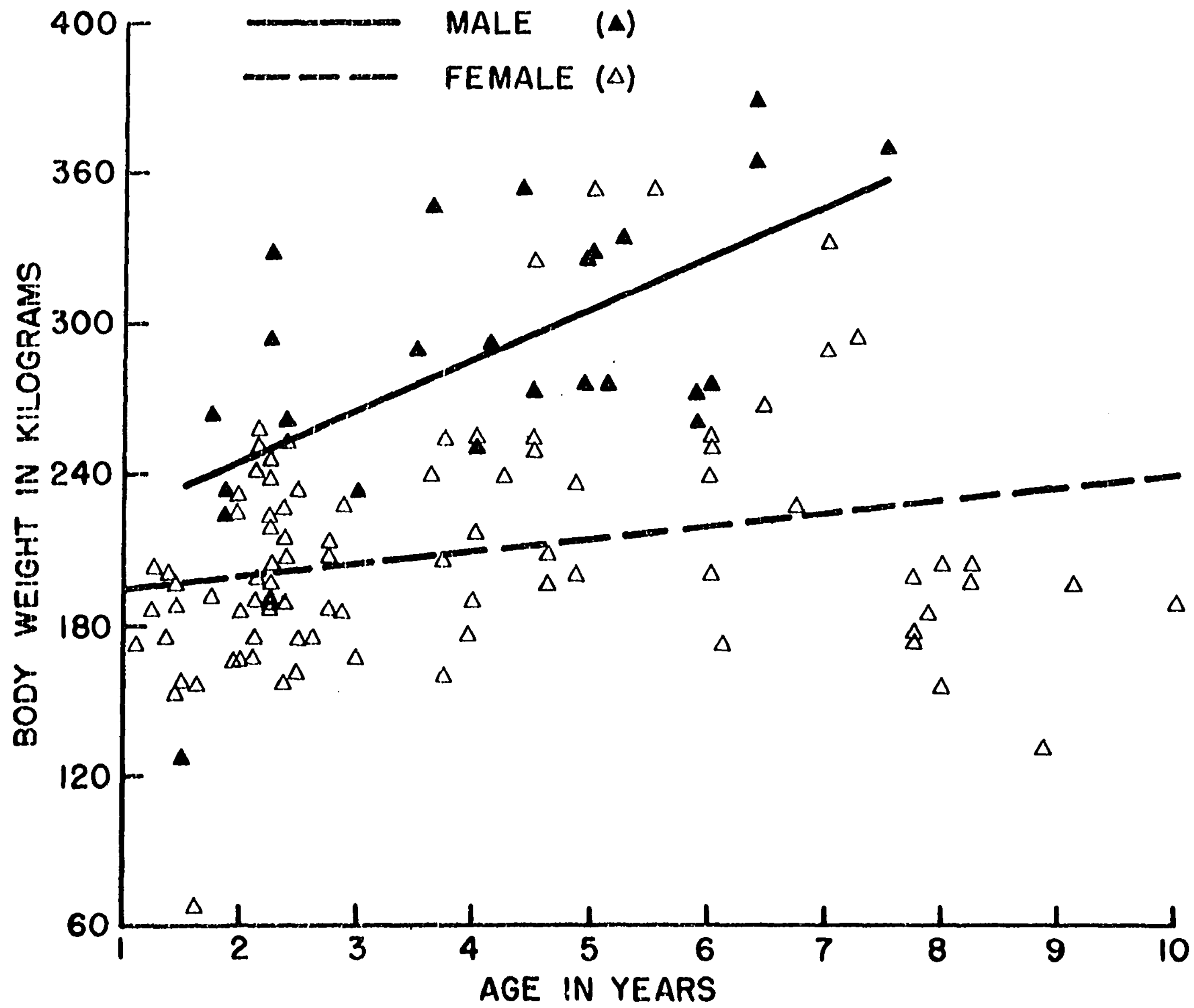


HYPOPHYSIS WEIGHT IN MILLIGRAMS
1400
1200
1000
800
600
400
200
AGE IN YEARS
1
2
3
4
5
6
7
8
9
10

Graph 10. Body weight of pigs from 1 to 10 years

Body weight in kg versus age in years. Males. Linear regression data: $N = 28$; $Y = 206.182 + 20.247\,X$, $\pm$ 44.429 kg; $b = 20.247$, $\pm$ 5.251, $P < 0.01$; $\bar{y} = 289.279$, $\pm$ 8.396, $P < 0.01$; $r = 0.603$, $P < 0.01$

Body weight in kg versus age in years. Females. Linear regression data: $N = 100$; $Y = 190.416 + 4.968\,X$, $\pm$ 44.768 kg; $b = 4.968$, $\pm$ 1.979, $P < 0.01$; $\bar{y} = 208.783$, $\pm$ 4.477, $P < 0.01$; $r = 0.246$, $P < 0.05$

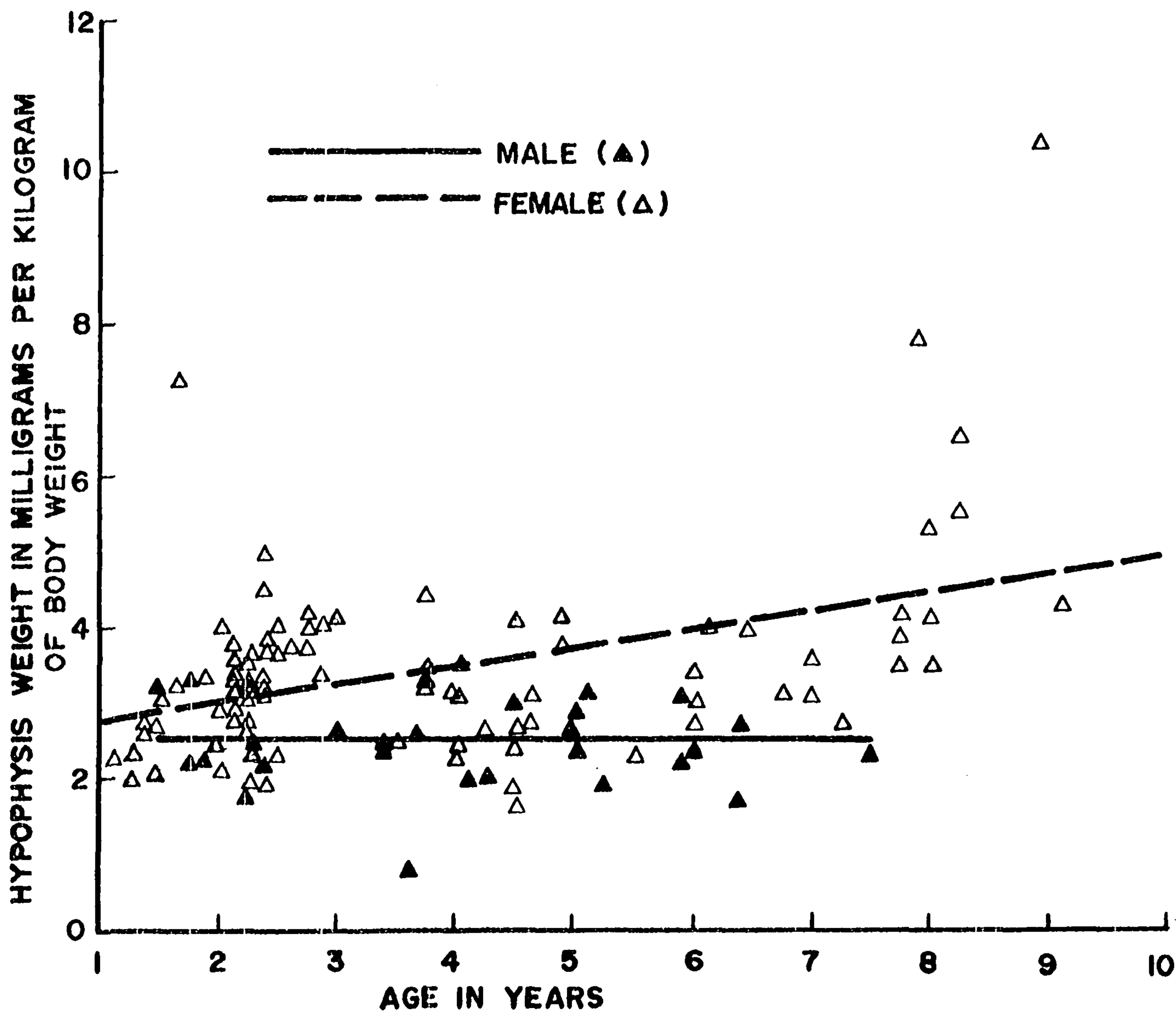


MALE (▲)
FEMALE (△)
BODY WEIGHT IN KILOGRAMS
400
360
300
240
180
120
60
1
2
3
4
5
6
7
8
9
10
AGE IN YEARS

Graph 11. Relative hypophysis weight of pigs from 1 to 10 years

Relative weight of hypophysis in mg/kg body weight versus age in years. Males. Linear regression data: $N = 28$; $Y = 2.578 - 0.011\ X$, $\pm\ 0.590$ mg/kg body weight; $b = -0.011$, $\pm\ 0.070$, $P < 0.05$; $\bar{y} = 2.533$, $\pm\ 0.114$, $P < 0.05$; $r = 0.031$, $P < 0.05$

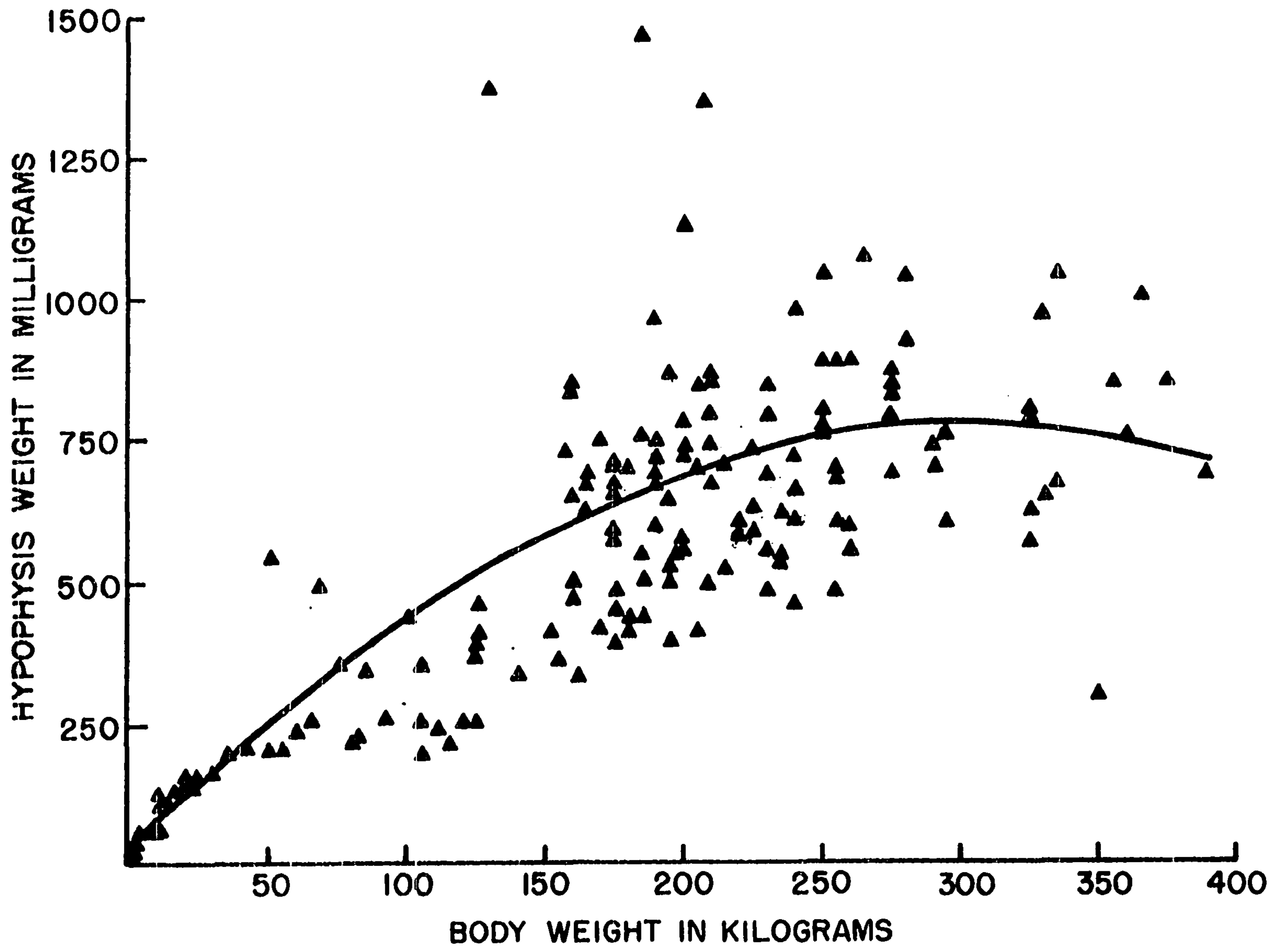
Relative weight of hypophysis in mg/kg body weight versus age in years. Females. Linear regression data: $N = 100$; $Y = 2.542 + 0.250\ X$, $\pm\ 1.127$ mg/kg body weight; $b = 0.250$, $\pm\ 0.050$, $P < 0.01$; $\bar{y} = 3.466$, $\pm\ 0.113$, $P < 0.05$; $r = 0.452$, $P < 0.01$



MALE (▲)
FEMALE (△)
HYPOPHYSIS WEIGHT IN MILLIGRAMS PER KILOGRAM
OF BODY WEIGHT
0
2
4
6
8
10
12
1
2
3
4
5
6
7
8
9
10
AGE IN YEARS

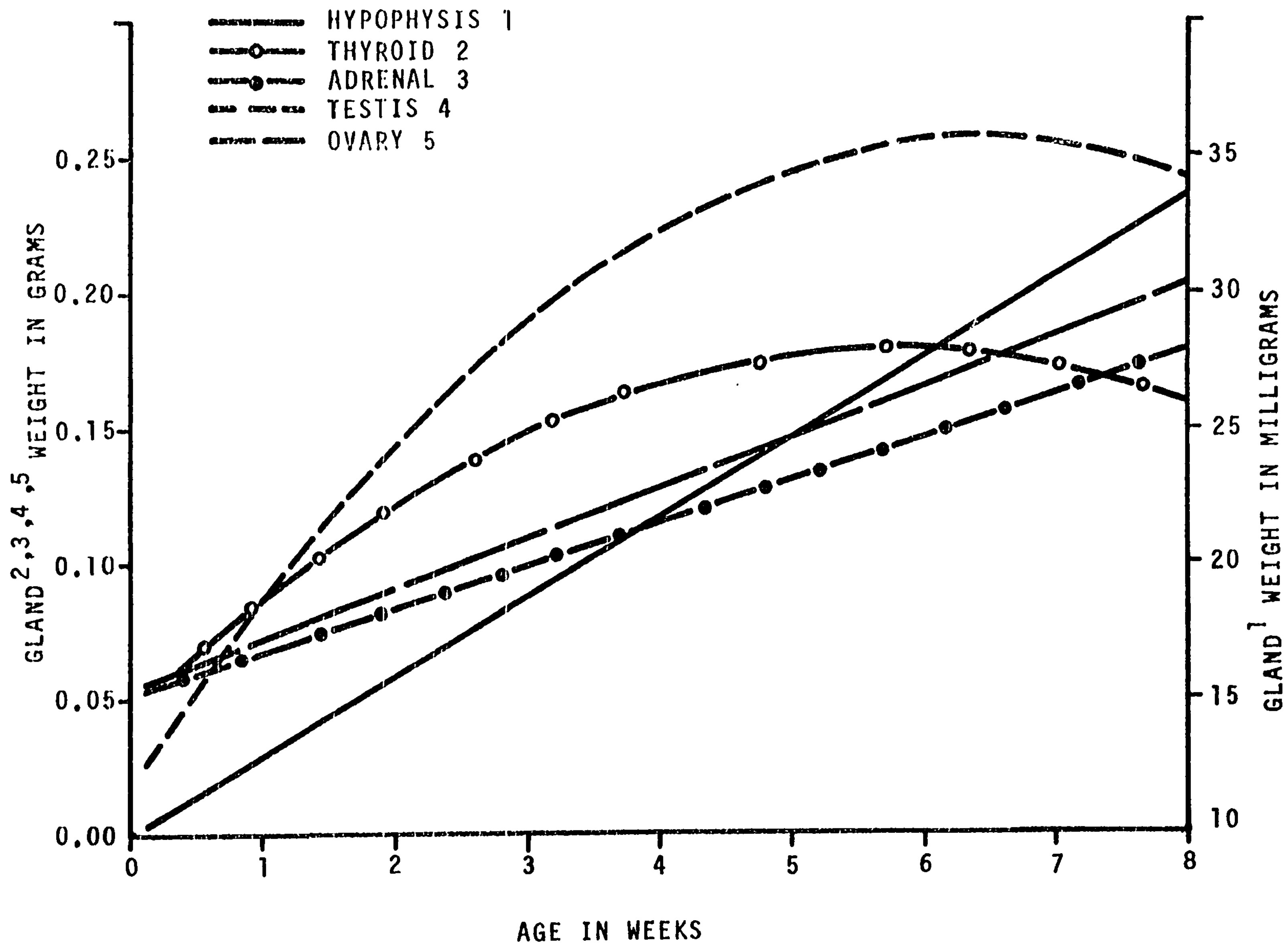
Graph 12. Absolute hypophysis weight of pigs ranging in body weight from 1.04 to 390.20 kg

Absolute weight of the hypophysis in mg versus body weight in kg. Curvilinear regression data: $N = 202$; $Y = 34.302 + 4.864\ X_1 - 0.008\ X_2$, $\pm$ 203.221 mg; $b_{y1.2} = 4.864$, $\pm$ 0.419, $P < 0.01$; $b_{y2.1} = -0.008$, $\pm$ 0.001, $P < 0.01$; $\bar{y} = 505.485$, $\pm$ 14.298, $P < 0.01$

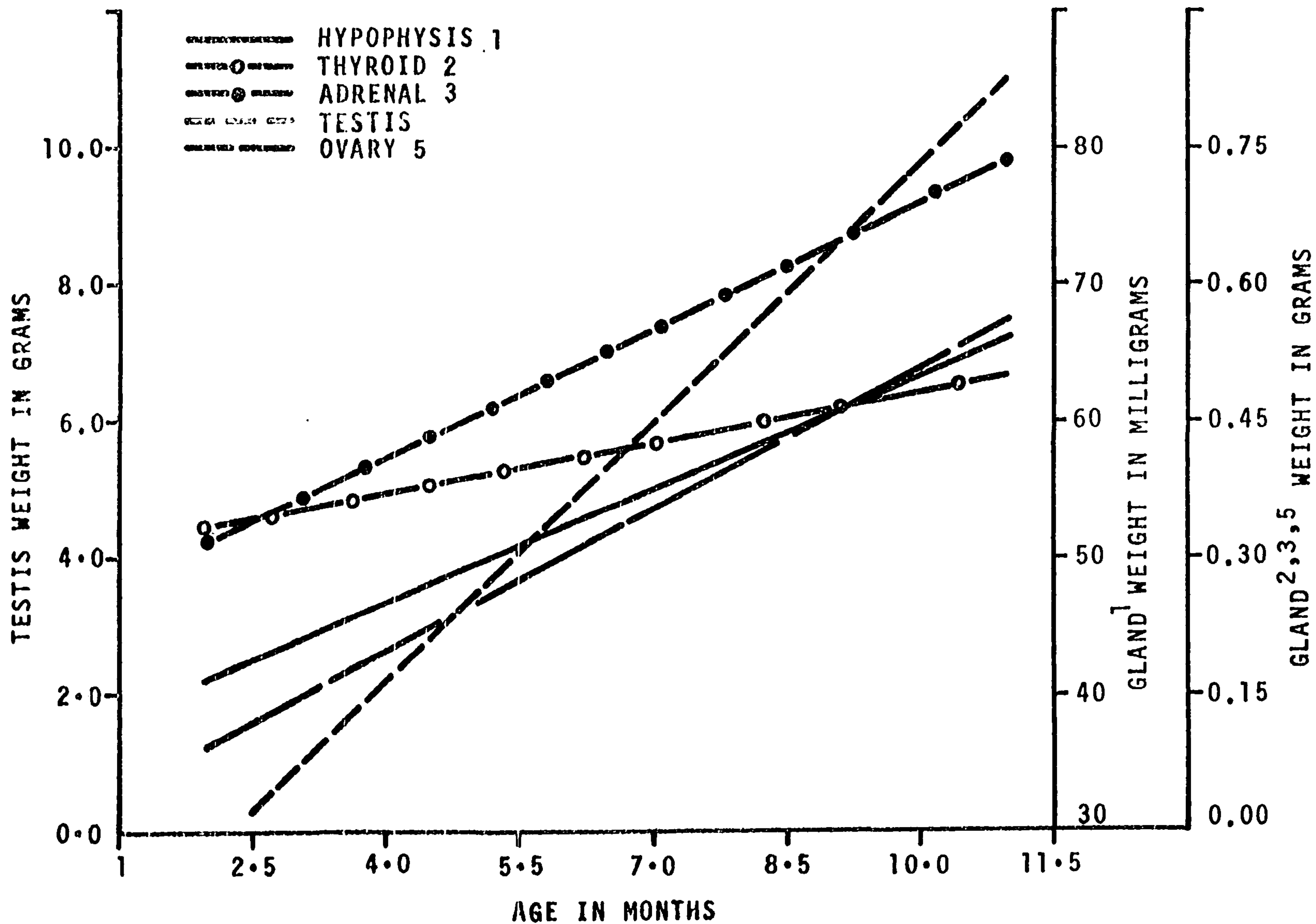


HYPOPHYSIS WEIGHT IN MILLIGRAMS
1500
1250
1000
750
500
250
50
100
150
200
250
300
350
400
BODY WEIGHT IN KILOGRAMS

Graph 13. Absolute weights of hypophysis, thyroid, adrenal, testis and ovary from beagles versus age in weeks


HYPOPHYSIS 1
THYROID 2
ADRENAL 3
TESTIS 4
OVARY 5
GLAND 2,3,4,5 WEIGHT IN GRAMS
0.00
0.05
0.10
0.15
0.20
0.25
GLAND 1 WEIGHT IN MILLIGRAMS
10
15
20
25
30
35
0
1
2
3
4
5
6
7
8
AGE IN WEEKS

Graph 14. Absolute weights of hypophysis, thyroid, adrenal, testis and ovary from beagles versus age in months


HYPOPHYSIS 1
THYROID 2
ADRENAL 3
TESTIS
OVARY 5
TESTIS WEIGHT IN GRAMS
10.0
8.0
6.0
4.0
2.0
0.0
1
2.5
4.0
5.5
7.0
8.5
10.0
11.5
AGE IN MONTHS
80
70
60
50
40
30
GLAND1 WEIGHT IN MILLIGRAMS
0.75
0.60
0.45
0.30
0.15
0.00
GLAND2,3,5 WEIGHT IN GRAMS

Graph 15. Absolute weights of hypophysis, thyroid, adrenal, testis and ovary from beagles versus age in years

HYPOPHYSIS 1
THYROID 2
ADRENAL 3
TESTIS
OVARY 5

♂
♀

TESTIS WEIGHT IN GRAMS

18.0
16.0
14.0
12.0
10.0
8.0
6.0

GLAND[1] WEIGHT IN MILLIGRAMS

110
100
90
80
70
60
50

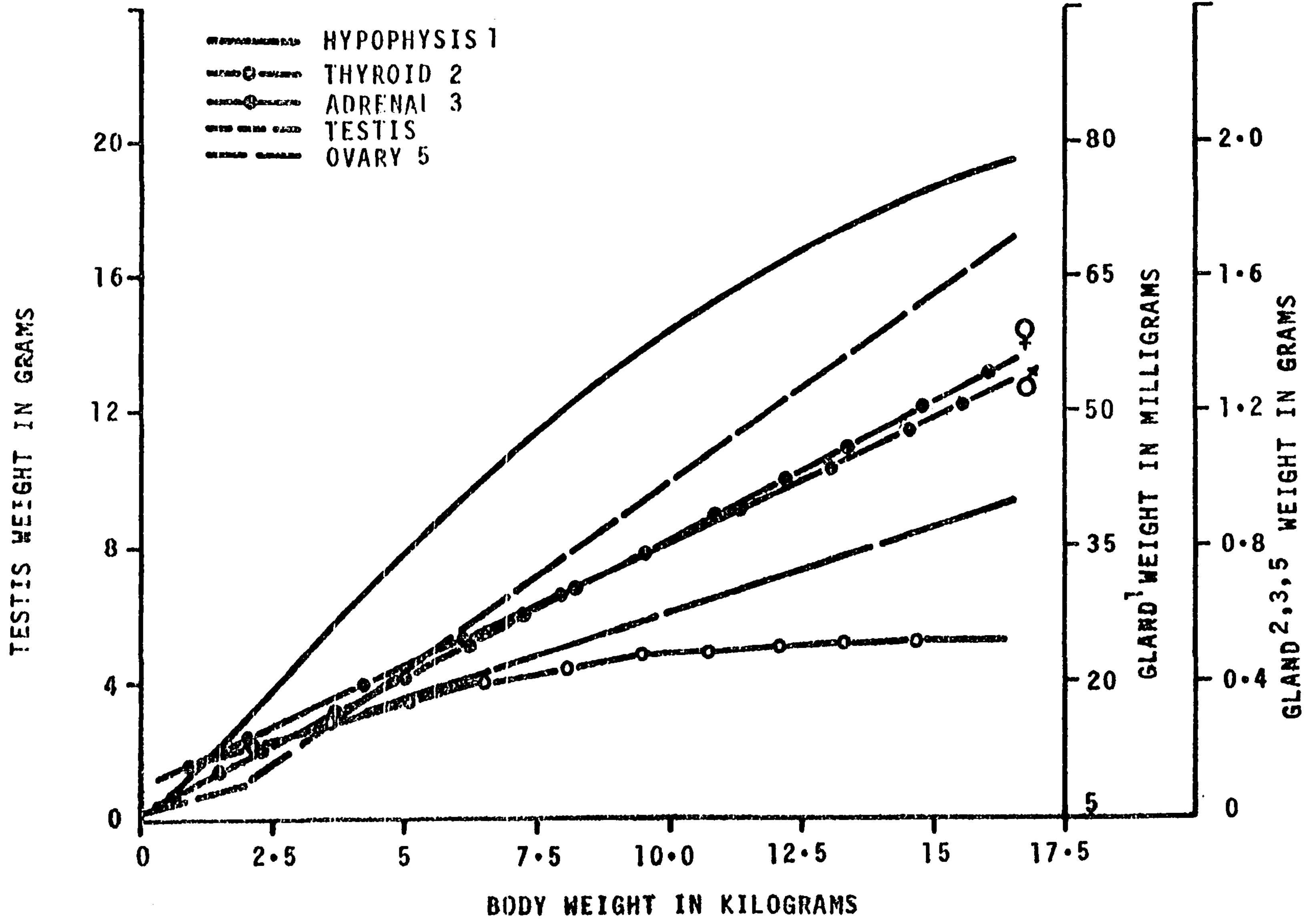
GLAND 2,3,5 WEIGHT IN GRAMS

1.4
1.2
1.0
0.8
0.6
0.4
0.2

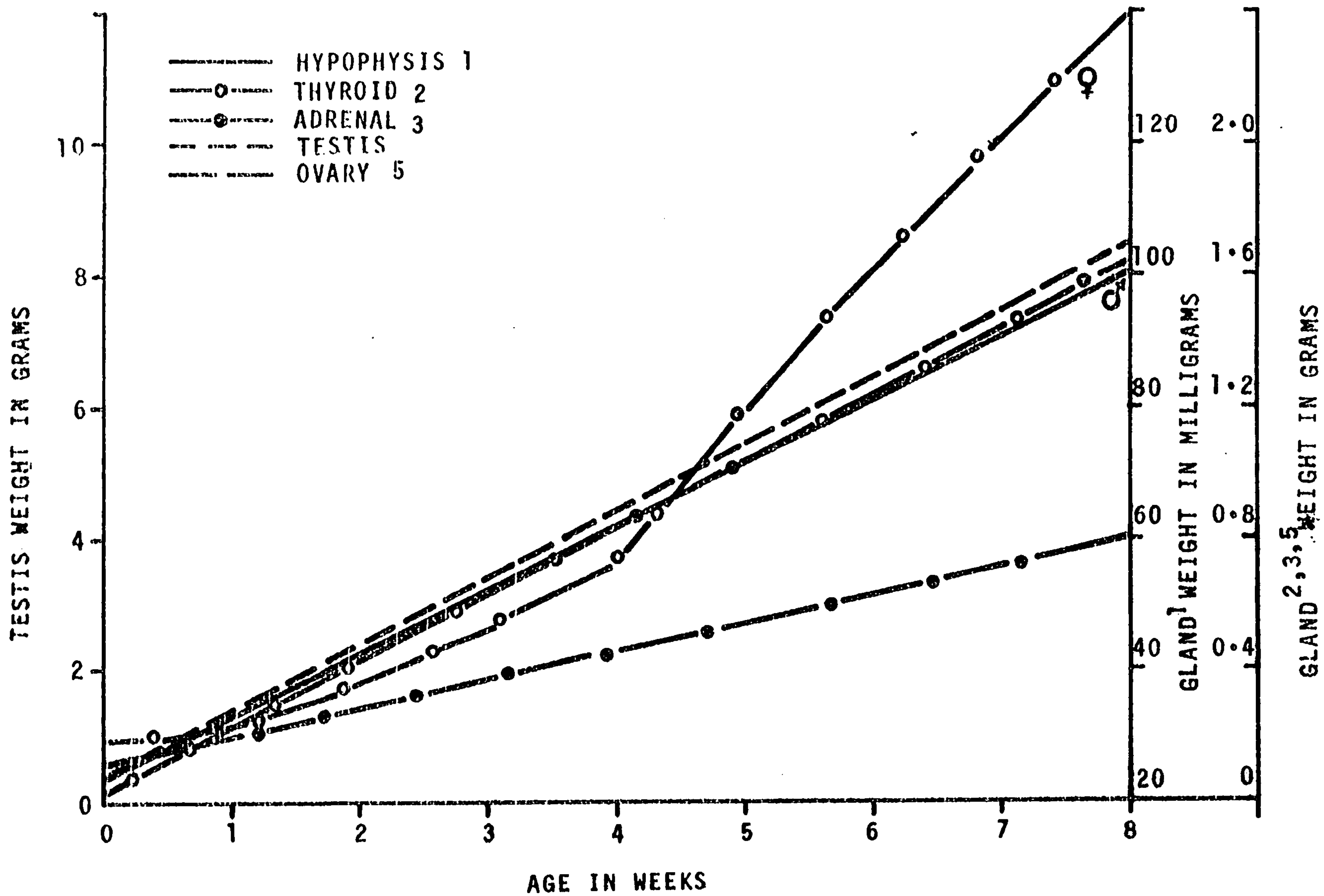
1 3 5 7 9 11 13 15

AGE IN YEARS

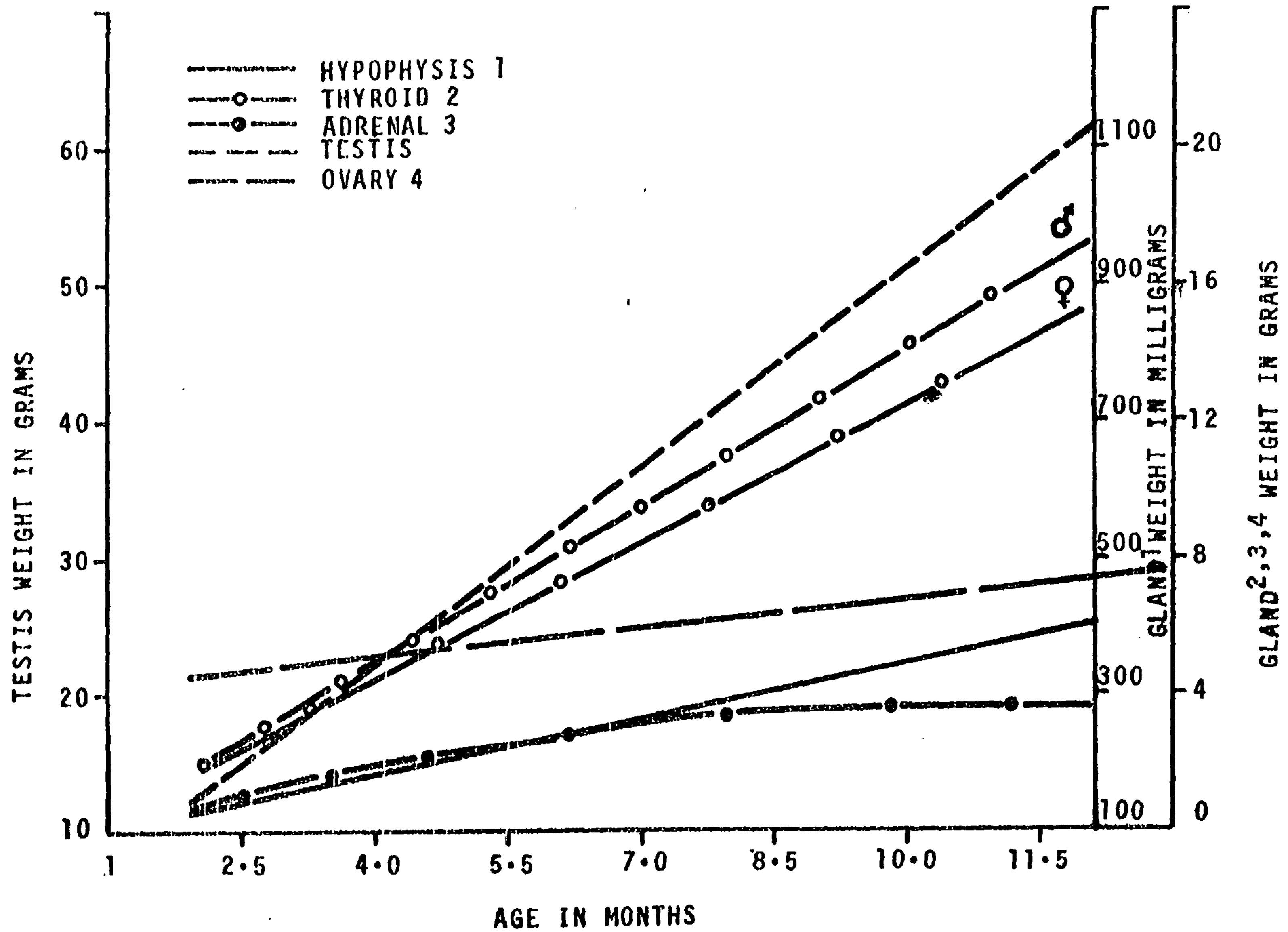
Graph 16. Absolute weights of hypophysis, thyroid, adrenal, testis and ovary from beagles versus body weight in kilograms


HYPOPHYSIS 1
THYROID 2
ADRENAL 3
TESTIS
OVARY 5
TESTIS WEIGHT IN GRAMS
0
4
8
12
16
20
BODY WEIGHT IN KILOGRAMS
0
2·5
5
7·5
10·0
12·5
15
17·5
GLAND 1 WEIGHT IN MILLIGRAMS
5
20
35
50
65
80
GLAND 2,3,5 WEIGHT IN GRAMS
0
0·4
0·8
1·2
1·6
2·0
♀
♂

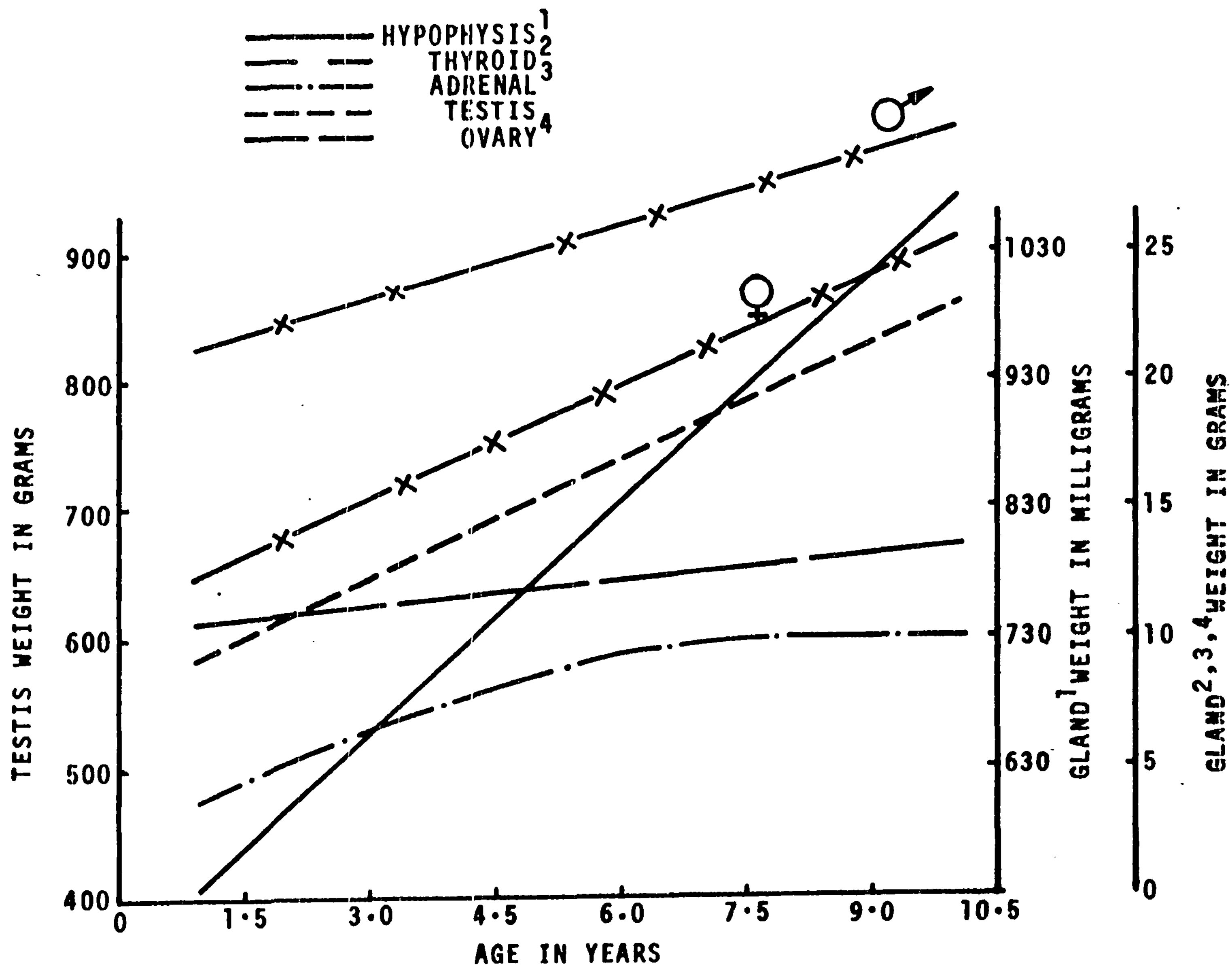
Graph 17. Absolute weights of hypophysis, thyroid, adrenal, testis and ovary from pigs versus age in weeks


HYPOPHYSIS 1
THYROID 2
ADRENAL 3
TESTIS
OVARY 5
TESTIS WEIGHT IN GRAMS
GLAND[1] WEIGHT IN MILLIGRAMS
GLAND[2,3,5] WEIGHT IN GRAMS
AGE IN WEEKS
0
2
4
6
8
10
1
3
5
7
20
40
60
80
100
120
0·4
0·8
1·2
1·6
2·0
♀
♂

Graph 18. Absolute weights of hypophysis, thyroid, adrenal, testis and ovary from pigs versus age in months

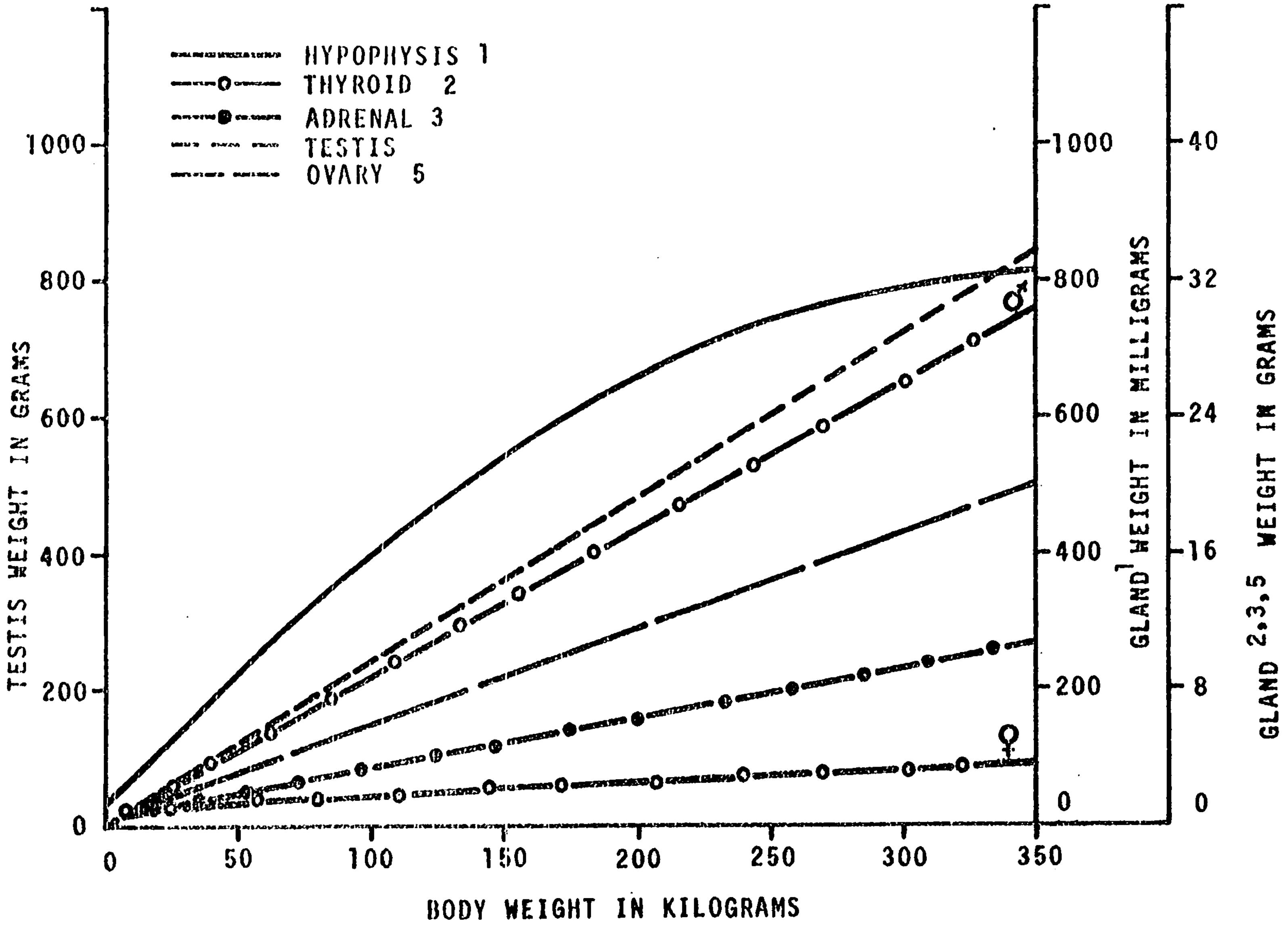

HYPOPHYSIS 1
THYROID 2
ADRENAL 3
TESTIS
OVARY 4
TESTIS WEIGHT IN GRAMS
60
50
40
30
20
10
1
2·5
4·0
5·5
7·0
8·5
10·0
11·5
AGE IN MONTHS
GLAND¹ WEIGHT IN MILLIGRAMS
1100
900
700
500
300
100
GLAND^2,3,4 WEIGHT IN GRAMS
20
16
12
8
4
0
♂
♀

Graph 19. Absolute weights of hypophysis, thyroid, adrenal, testis and ovary from pigs versus age in years



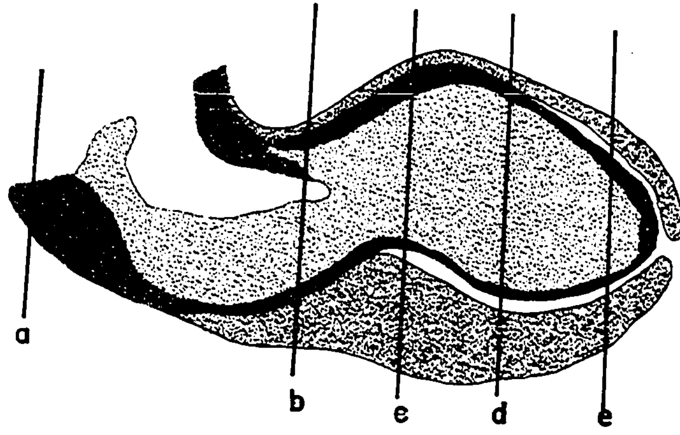
HYPOPHYSIS1
THYROID2
ADRENAL3
TESTIS
OVARY4
TESTIS WEIGHT IN GRAMS
900
800
700
600
500
400
0
1·5
3·0
4·5
6·0
7·5
9·0
10·5
AGE IN YEARS
1030
930
830
730
630
GLAND1 WEIGHT IN MILLIGRAMS
25
20
15
10
5
0
GLAND2,3,4 WEIGHT IN GRAMS

Graph 20. Absolute weights of hypophysis, thyroid, adrenal, testis and ovary from pigs versus body weight in kilograms


HYPOPHYSIS 1
THYROID 2
ADRENAL 3
TESTIS
OVARY 5
TESTIS WEIGHT IN GRAMS
0
200
400
600
800
1000
BODY WEIGHT IN KILOGRAMS
0
50
100
150
200
250
300
350
GLAND[1] WEIGHT IN MILLIGRAMS
0
200
400
600
800
1000
GLAND 2,3,5 WEIGHT IN GRAMS
0
8
16
24
32
40

APPENDIX C. PHOTOMICROGRAPHS

Figure 1. Sagittal section of the hypophysis of the dog (top) and the pig (bottom). Schematic diagram. Five different levels from which groups of 40 sections were obtained


a
b
e
d
e

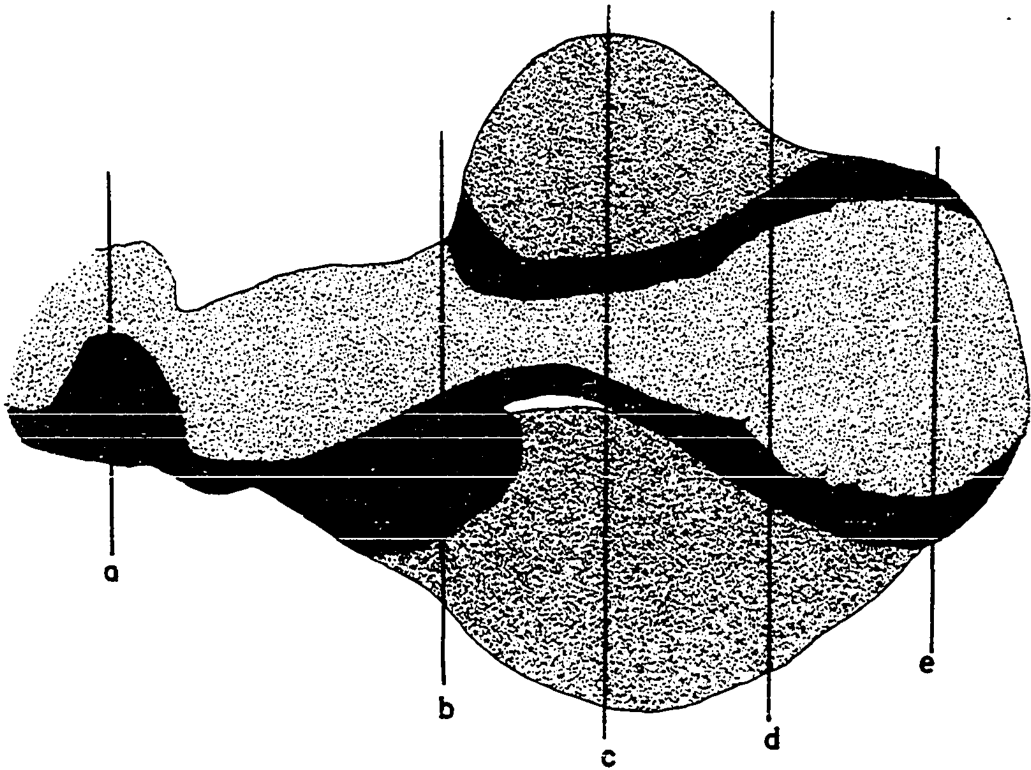
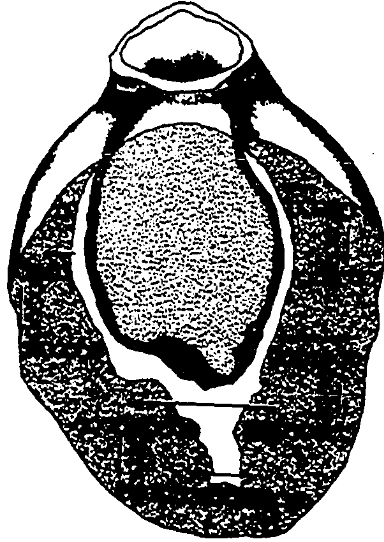

a
b
c
d
e

Figure 2. Two-dimensional view of the hypophysis of the dog (top) and the pig (bottom). Schematic diagram. Distribution of fields used for differential cell-count



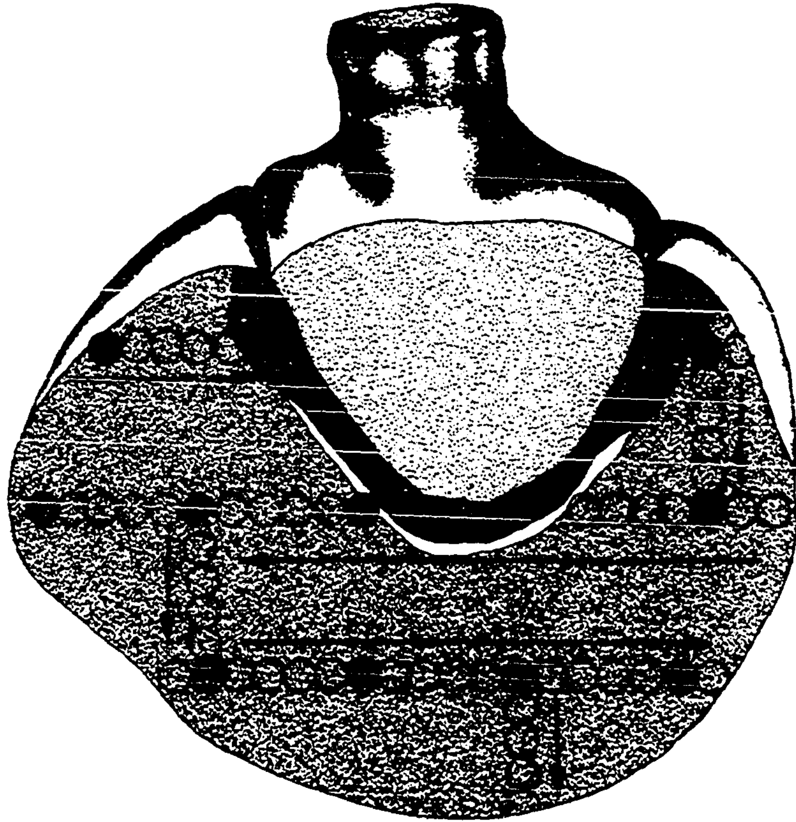
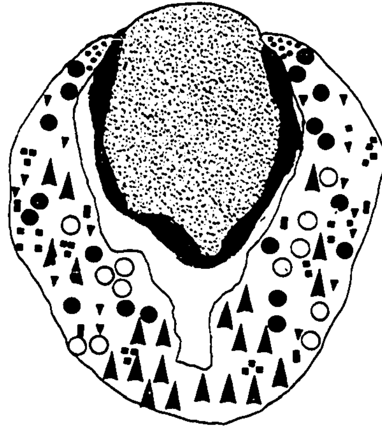


Figure 3. Topographical location of different cell-types in the canine (top) and porcine (bottom) hypophysis. Schematic diagram

- Somatotrope cell
- Lactotrope cell
- Thyrotrope cell
- FSH gonadotrope cell
- ICSH gonadotrope cell
- Adrenocorticotrope cell



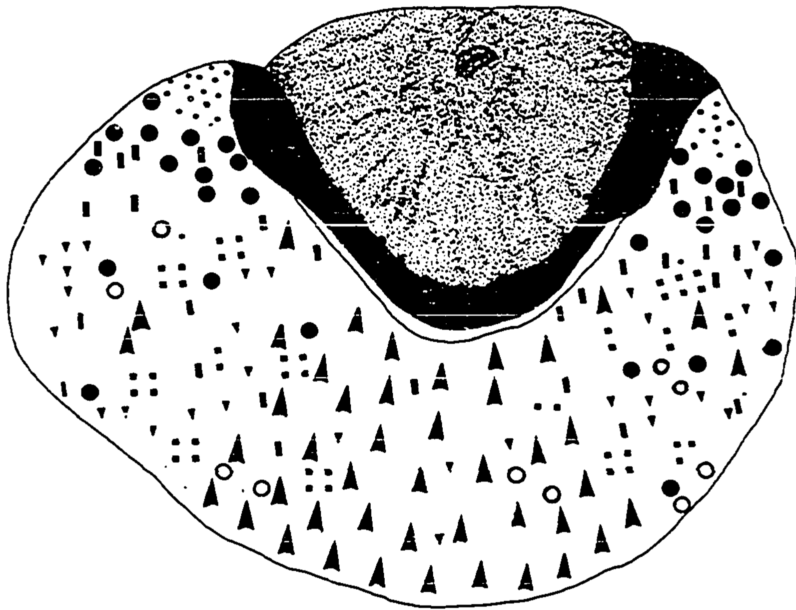
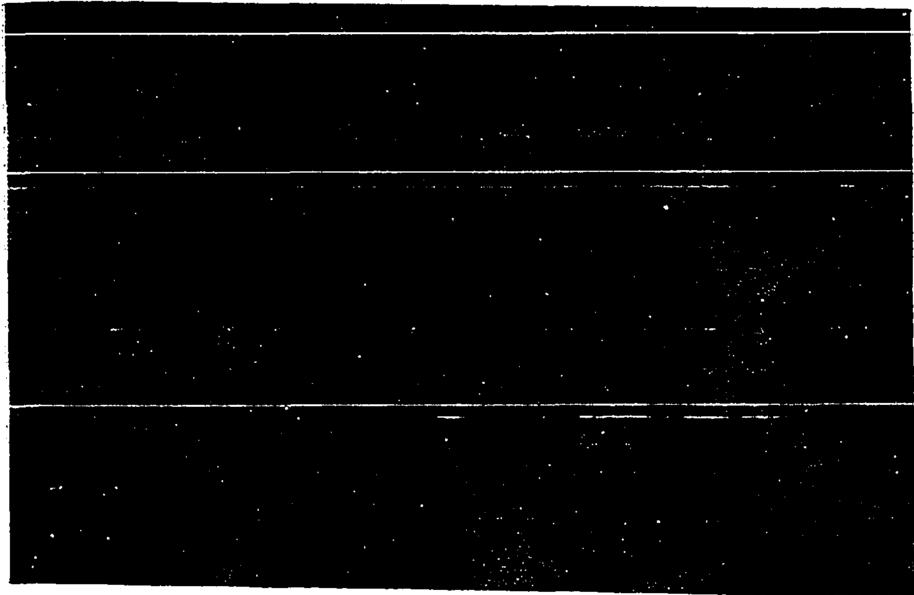


Figure 4. Pars distalis adenohypophysis. Poor development of the stroma. PAS positive FSH gonadotrope cells and aldehyde-thionin positive thyrotrope cells
Dog #B 9. Female. 1 day
Aldehyde-thionin--PAS--orange G stain
X 400

Figure 5. Pars distalis adenohypophysis. Somatotrope cell (yellow). Thyrotrope cell (light blue). PAS positive basement membrane in perivascular region
Dog #B 9. Female. 1 day
Alcian blue--PAS--orange G stain
X 1000



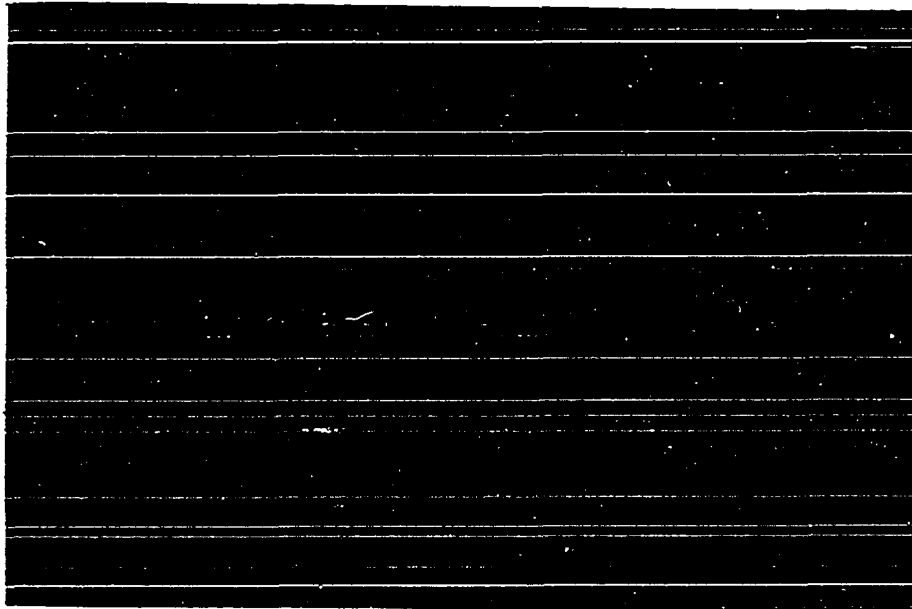


Figure 6. Pars distalis adenohypophysis. Mitosis of cells
Dog #E 76. Female. 14 days
Verhoeff's stain
X 250

Figure 7. Pars distalis adenohypophysis. Degranulated thyrotrope cell. Colloid in small capillaries
Dog #E 76. Female. 14 days
Aldehyde-thionin--PAS--orange G stain
X 250

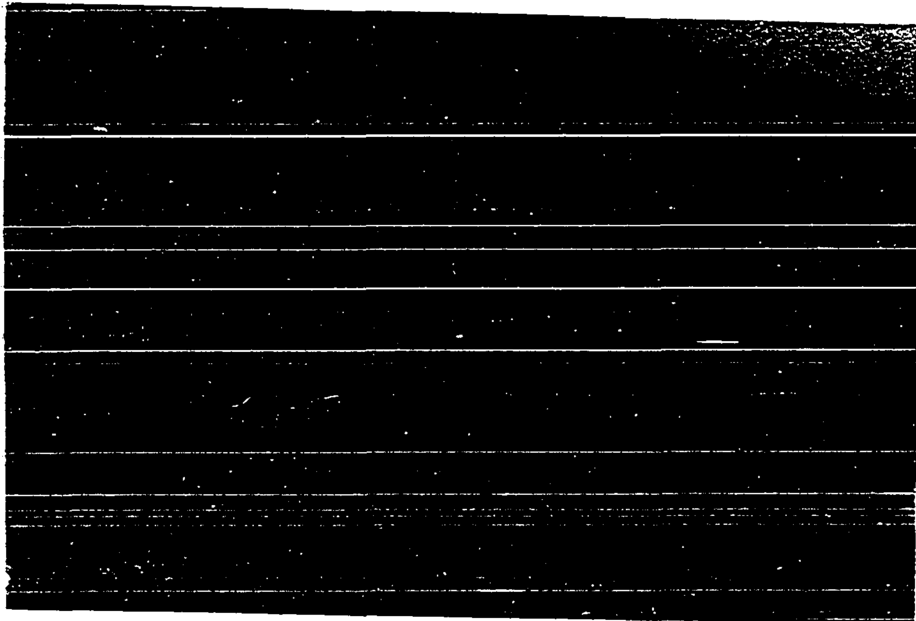


Figure 8. Pars distalis adenohypophysis. Somatotrope cell (orange). Lactotrope cell (red). Thyrotrope cell (blue)

Dog #B 29. Female. 1 month

Azocarmine--orange G--aniline blue stain

X 400

Figure 9. Pars distalis adenohypophysis. Large granulated thyrotrope cells. Location of granules on the peripheral margin of the cells

Dog #B 99. Female. 1.5 months

Alcian blue--PAS--orange G stain

X 400

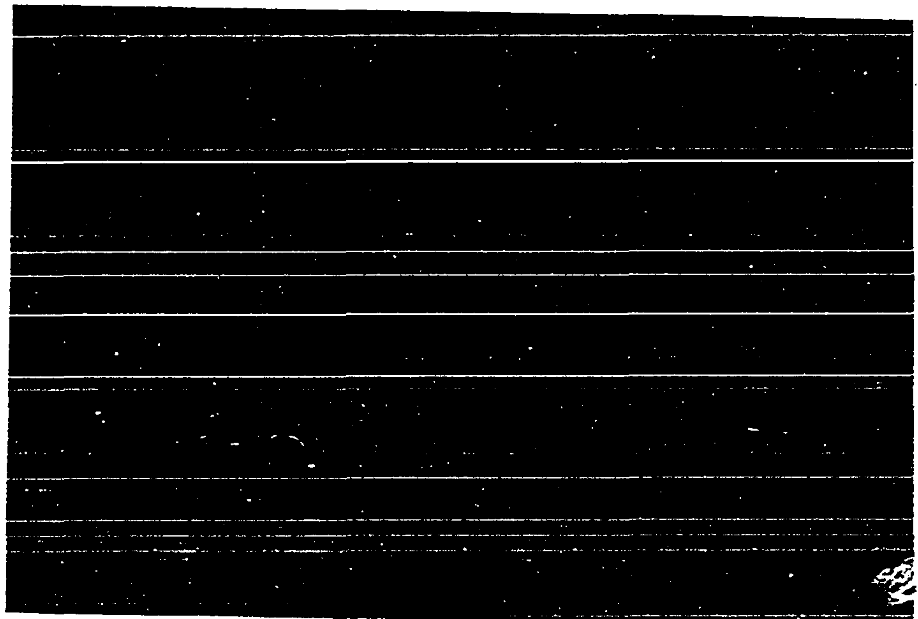


Figure 10. Pars distalis adenohypophysis. Somatotrope cell (yellow). Thyrotrope cell (blue). FSH gonadotrope cell (purple). Degranulated thyrotrope cells. Cupping of somatotrope and thyrotrope cells

Dog #100. Female. 3 months

Alcian blue--PAS--orange G stain

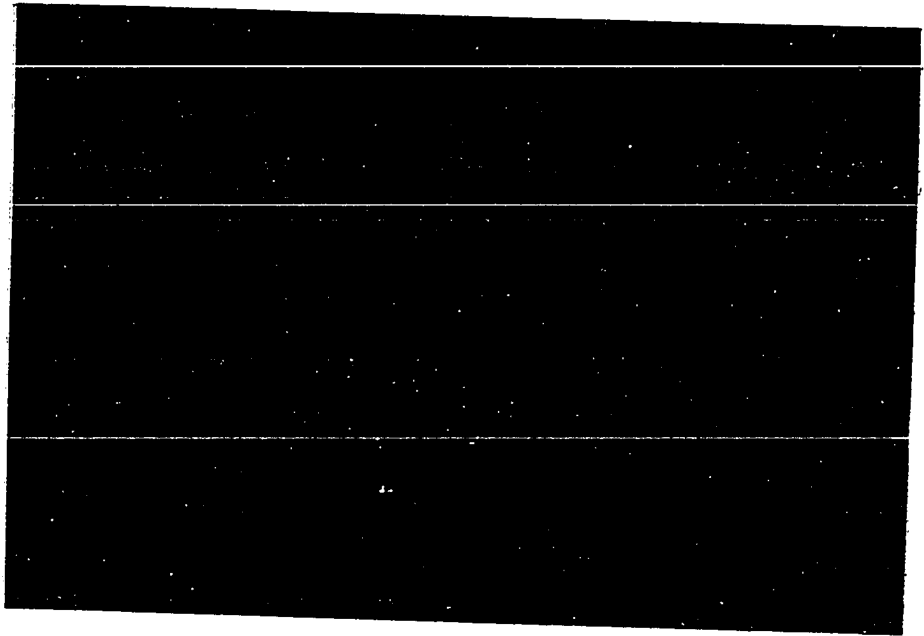
X 400

Figure 11. Pars distalis adenohypophysis. Distribution of postganglionic autonomic fibers in perivascular region

Dog #B 61. Female. 7 months

Bielschowsky's stain

X 250



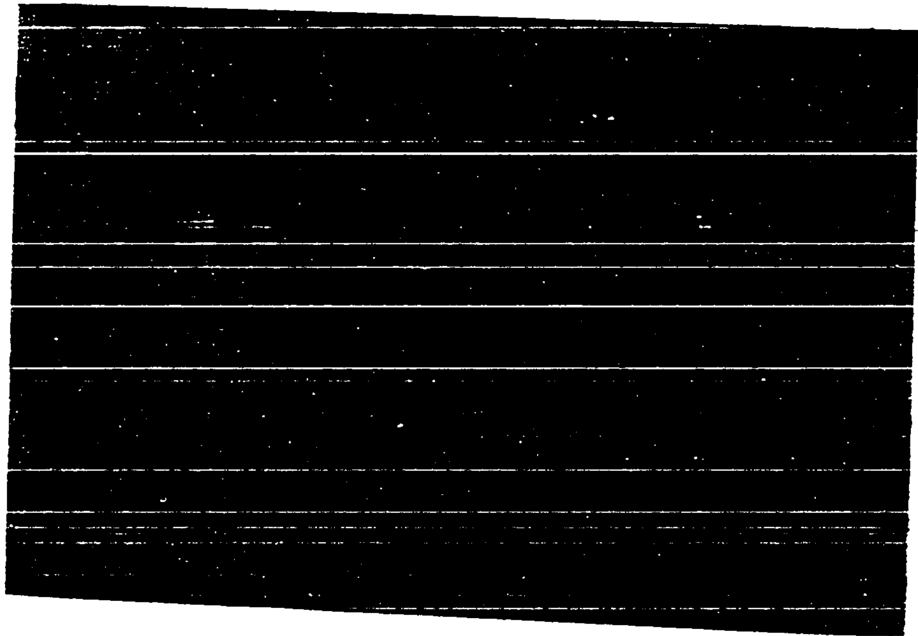


Figure 12. Pars distalis adenohypophysis. Somatotrope cell (yellow). Thyrotrope cell (blue). FSH gonadotrope cell (purple). ICSH gonadotrope cell (rose red)

Dog #B 49. Female. 8 months

Alcian blue--PAS--orange G stain

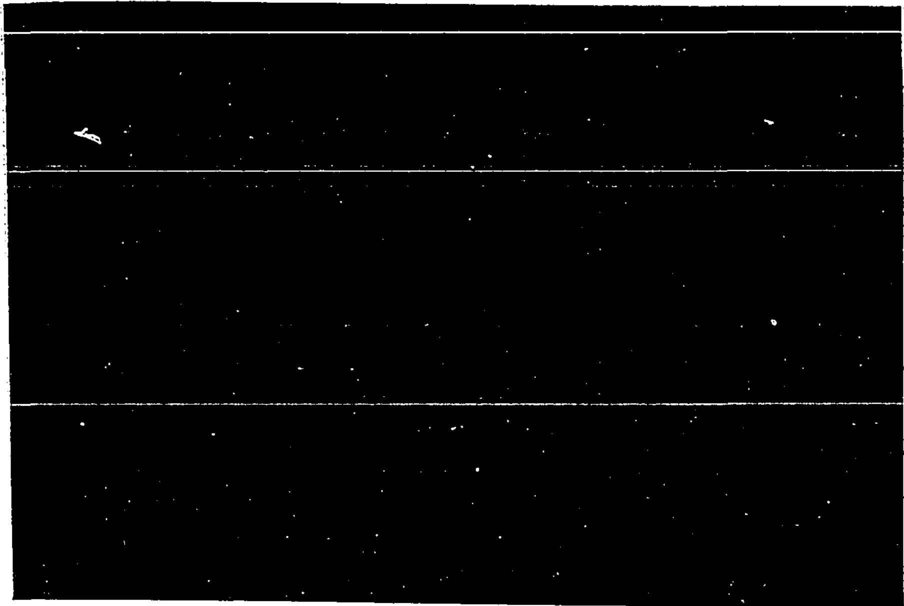
X 400

Figure 13. Pars distalis adenohypophysis. Somatotrope cell (yellow). Lactotrope cell (magenta). Thyrotrope cell (blue). FSH gonadotrope cell (purple)

Dog #B 49. Female. 8 months

Alcian blue--PAS--orange G stain

X 400



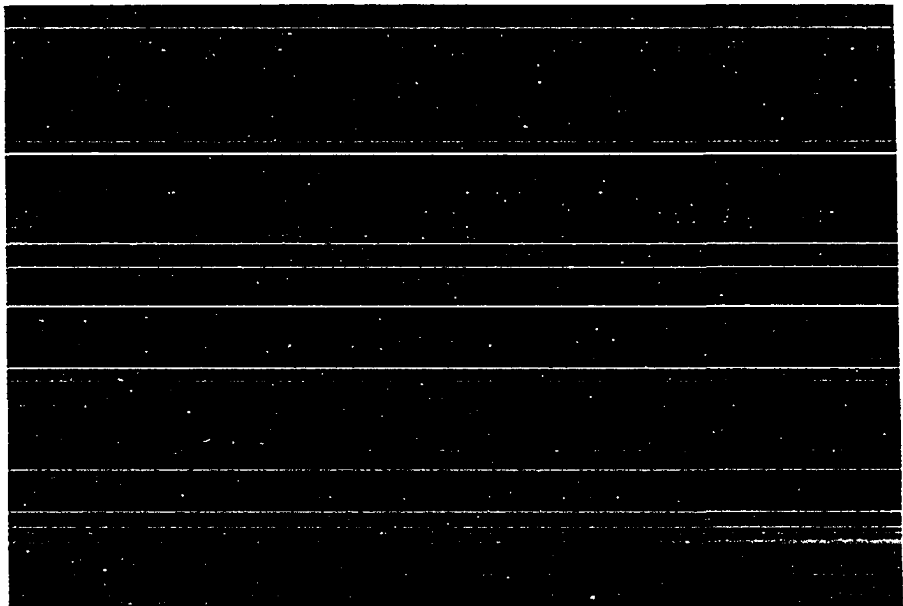
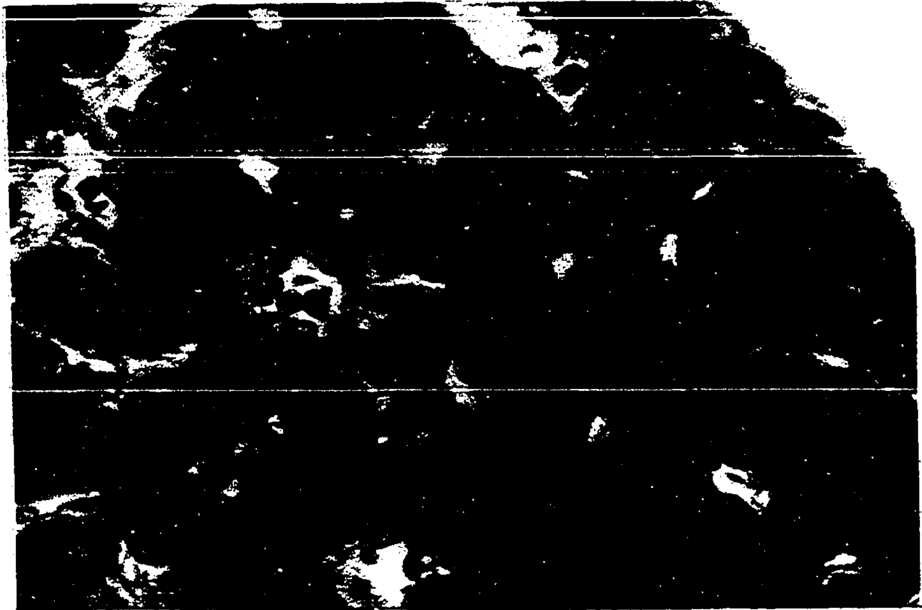


Figure 14. Pars distalis adenohypophysis. Lactotrope cell (red). Thyrotrope cell (light green). FSH gonadotrope cell (purple). ACTH cell (gray)
Dog #B 86. Female. 10 months
Aldehyde-fuchsin-trichrome stain
X 400

Figure 15. Pars distalis adenohypophysis. Typical distribution of collagen fibers in the perivascular region
Dog #B 62. Female. 1 year
Verhoeff's stain
X 250



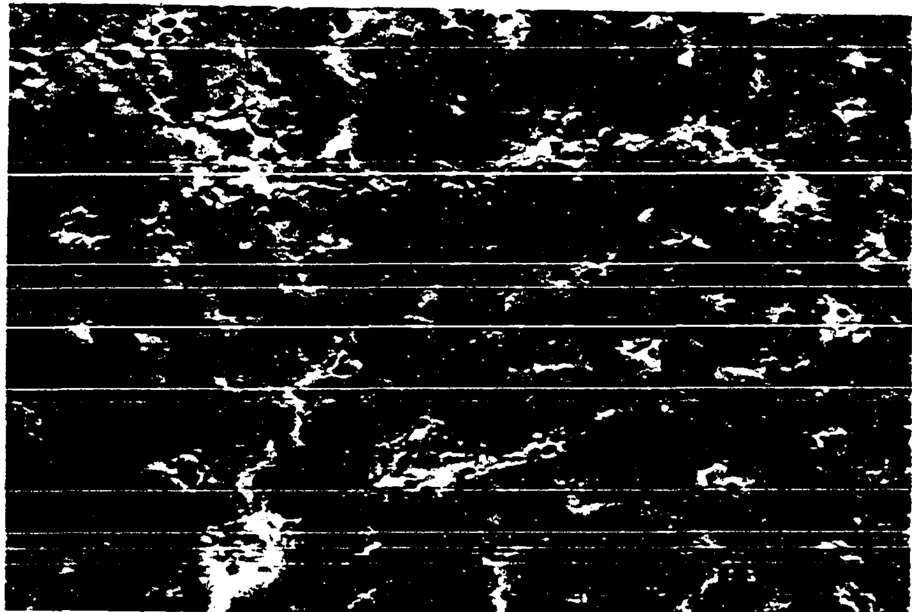
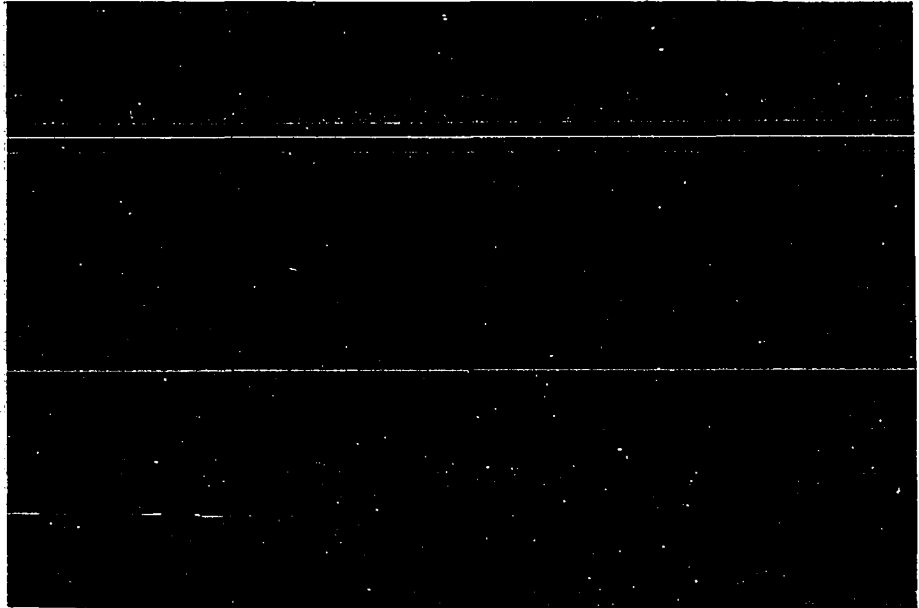


Figure 16. Pars distalis adenohypophysis. Somatotrope cell (yellow). FSH gonadotrope cell (light purple).
ACTH cell (gray)
Dog # 5FC. Male. 1 year
Aldehyde-fuchsin-trichrome stain
X 1000

Figure 17. Pars distalis adenohypophysis. Somatotrope cell (yellow). ICSH gonadotrope cell (violet).
ACTH cell (gray)
Dog # 5FC. Male. 1 year
Aldehyde-fuchsin-trichrome stain
X 1000



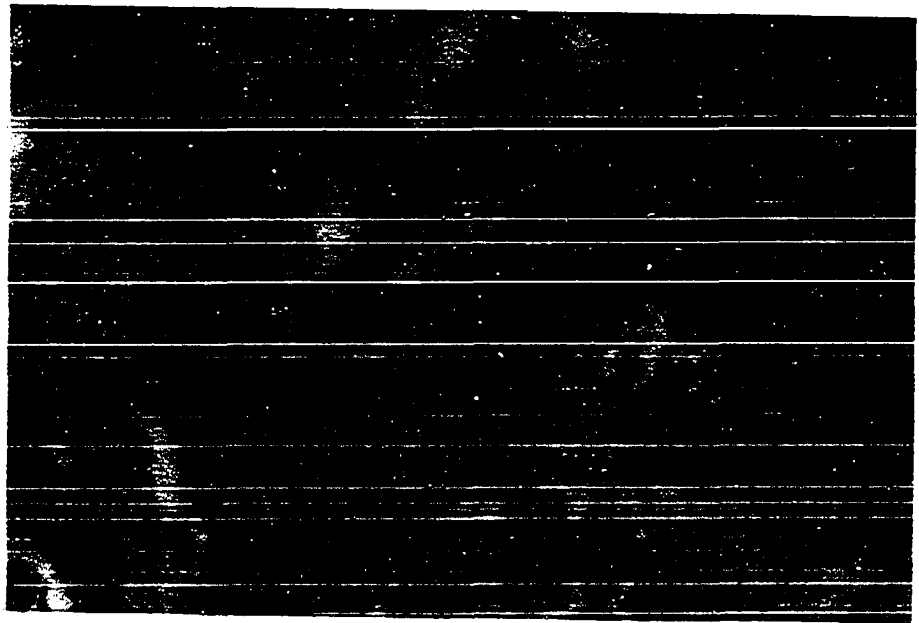
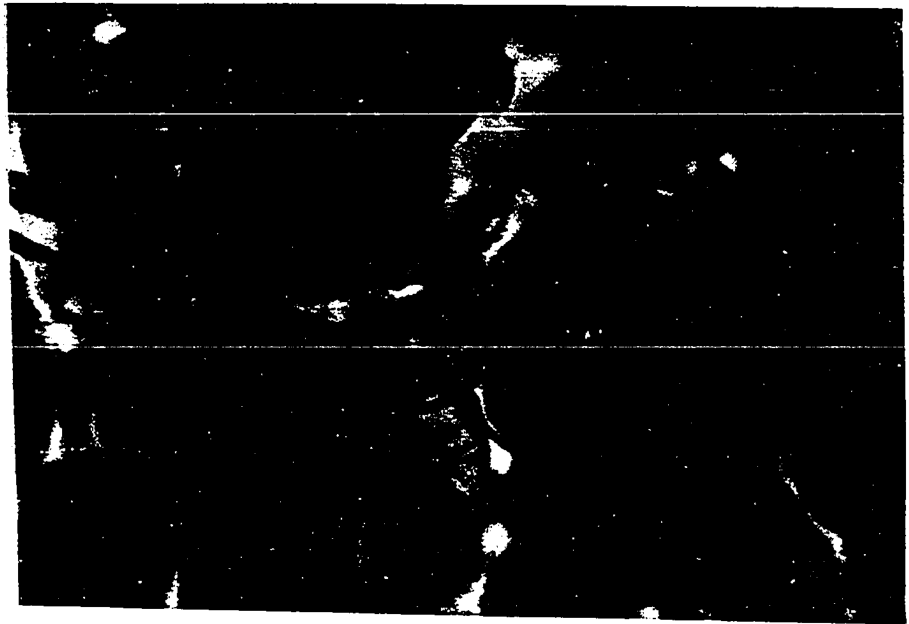


Figure 18. Pars distalis adenohypophysis. Somatotrope cell (blue-green). Lactotrope cell (brick red). Thyrotrope cell (intense blue). Negative image of Golgi body in somatotrope cell
Dog #B 128. Male. 1 year
Luxol fast blue-trichrome stain
X 1000

Figure 19. Pars distalis adenohypophysis. Somatotrope cell (yellow). Lactotrope cell (light magenta). Thyrotrope cell (blue-black). FSH gonadotrope cell (purple)
Dog #B 25. Female. 2 years
Aldehyde-thionin--PAS--orange G stain
X 1000



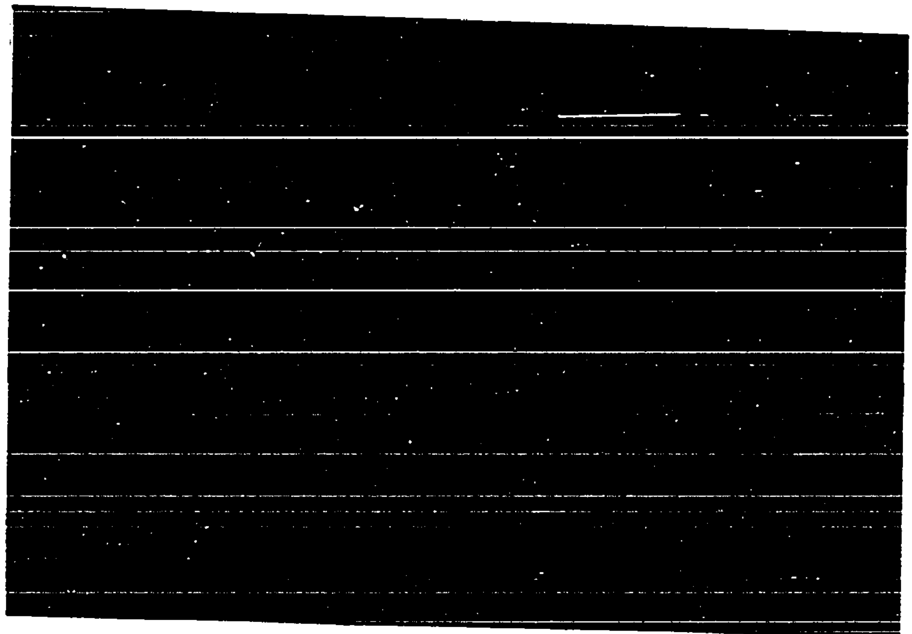
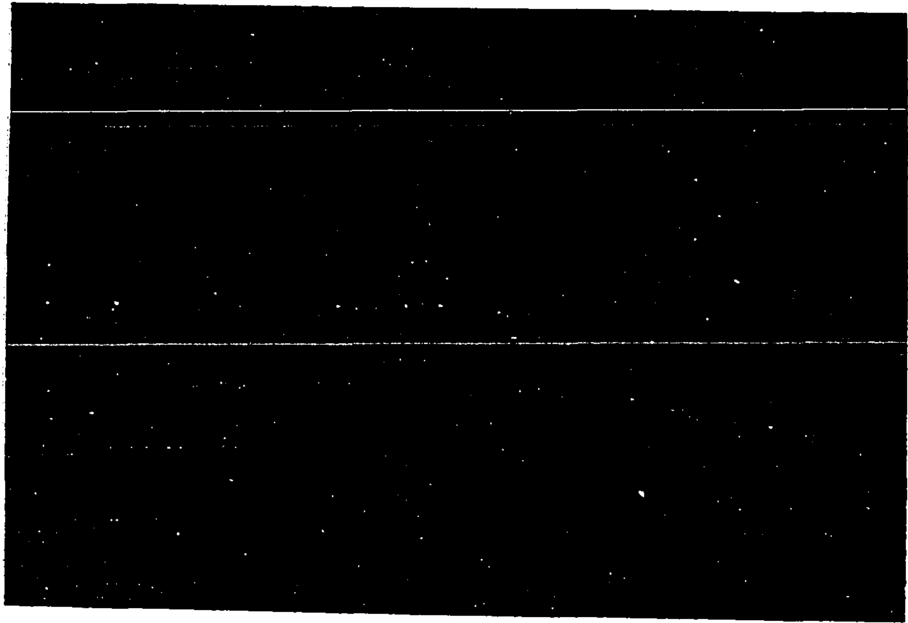


Figure 20. Pars distalis adenohypophysis. Reticular fibers in the stroma. Hyalinization of collagen fibers
Dog #B 88. Male. 3 years
Manuel's stain
X 250

Figure 21. Pars distalis adenohypophysis. Initial stages of colloid deposition in cell-cords
Dog #B 88. Male. 3 years
Alcian blue--PAS stain
X 250



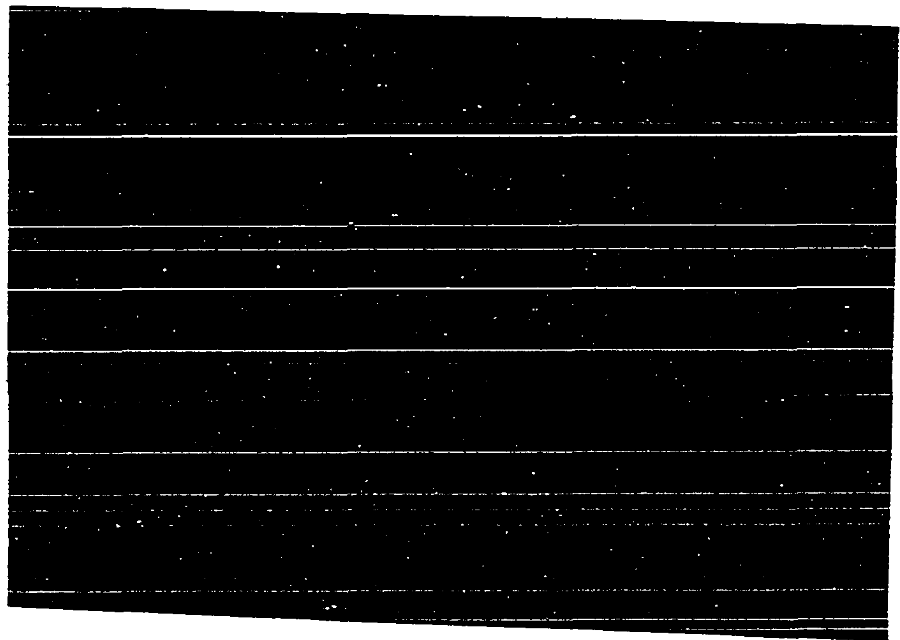


Figure 22. Pars distalis adenohypophysis. Denaturation of collagen fibers

Dog #B 88. Male. 3 years

Verhoeff's stain

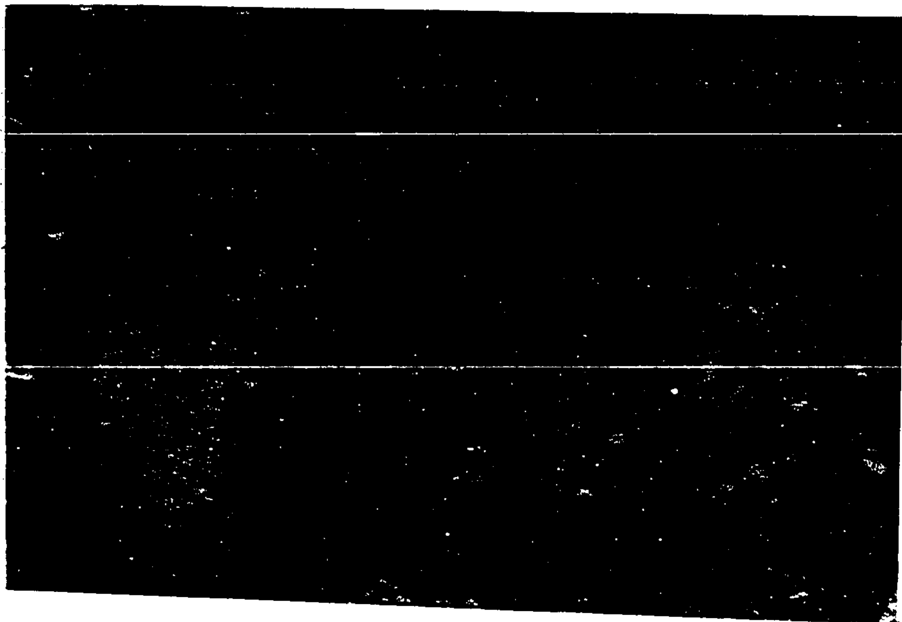
X 250

Figure 23. Pars distalis adenohypophysis. Light staining of colloid during the process of reabsorption. Peripheral vacuoles

Dog #B 51. Female. 4 years

Aldehyde-thionin--PAS--orange G stain

X 250



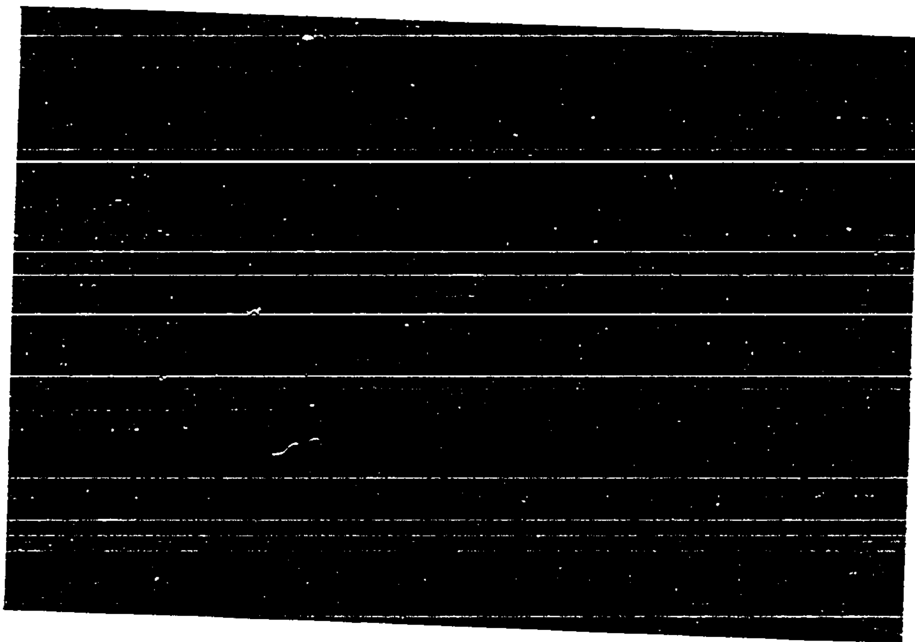
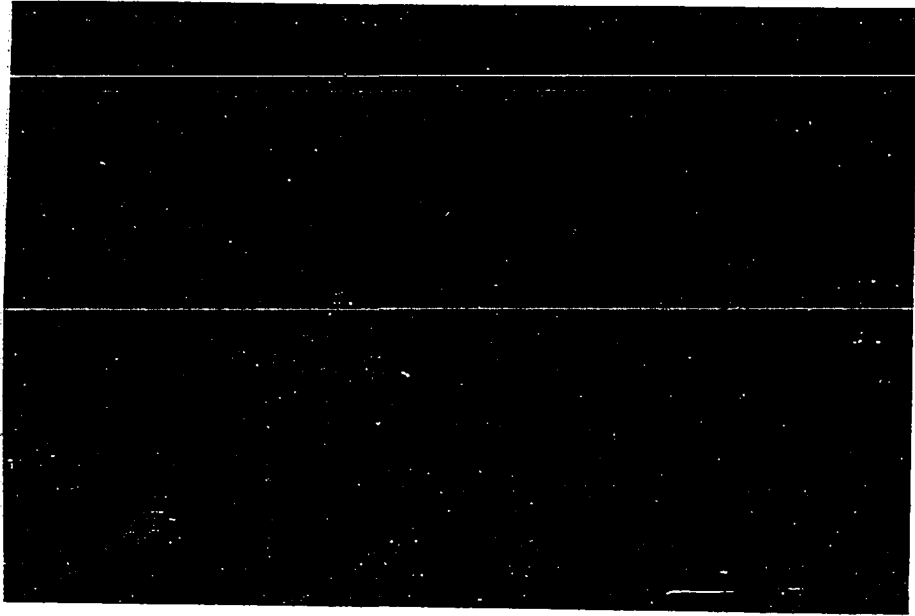


Figure 24. Pars distalis adenohypophysis. Groups of pyknotic ICSH gonadotrope cells
Dog #B 51. Female. 4 years
Alcian blue--PAS--orange G stain
X 400

Figure 25. Pars distalis adenohypophysis. Treatment with trichloroacetic acid, 2.5 percent. Loss of staining affinity of all cell-types except somatotrope cells
Dog # 071. Female. 5 years
Alcian blue--PAS--orange G stain
X 250



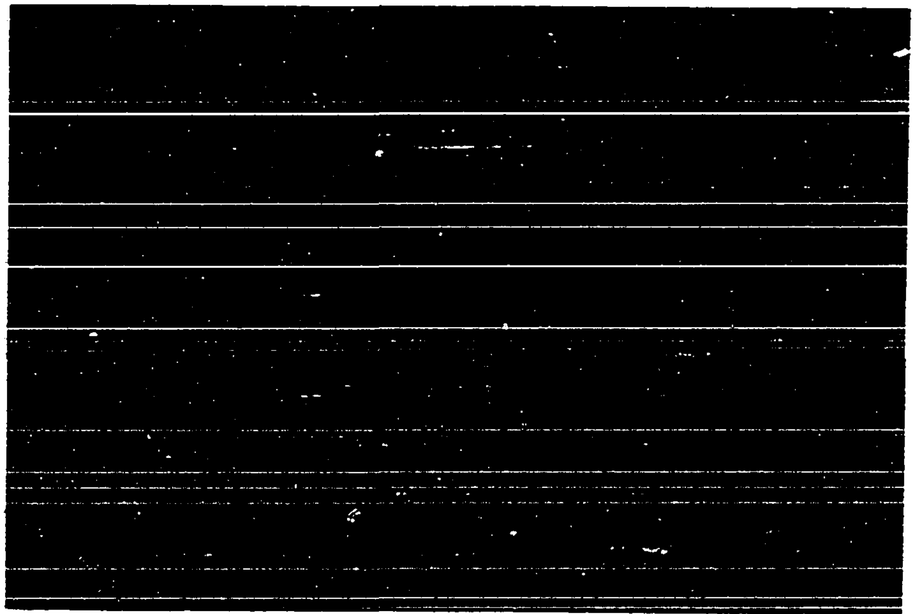


Figure 26. Pars distalis adenohypophysis. Positive metachromasia of follicular colloid

Dog #B 44. Female. 7.5 years

Modified methyl violet method

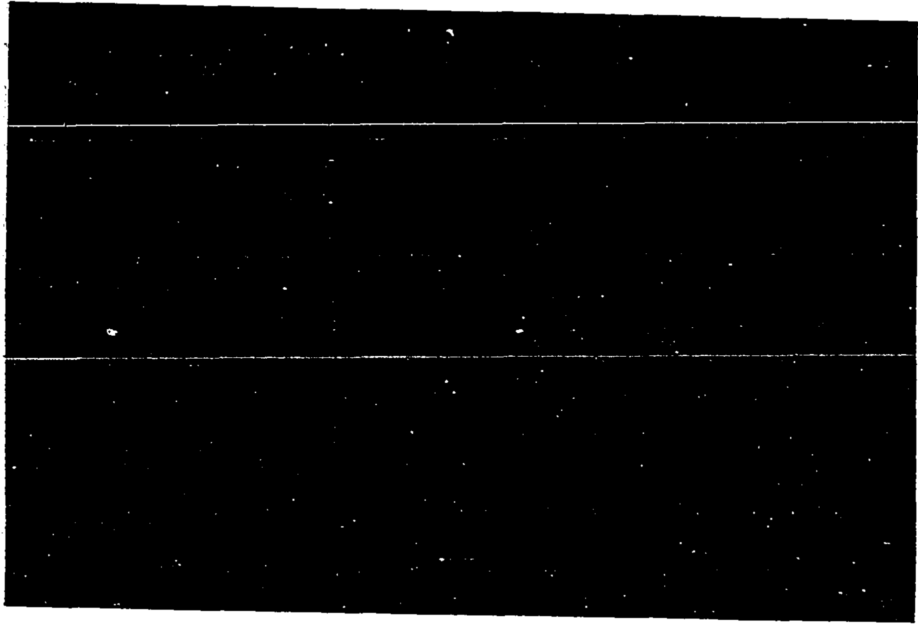
X 100

Figure 27. Pars distalis adenohypophysis. Large colloid follicles. Alcian blue staining of colloid

Dog #B 30. Female. 7.5 years

Alcian blue--PAS--orange G stain

X 250



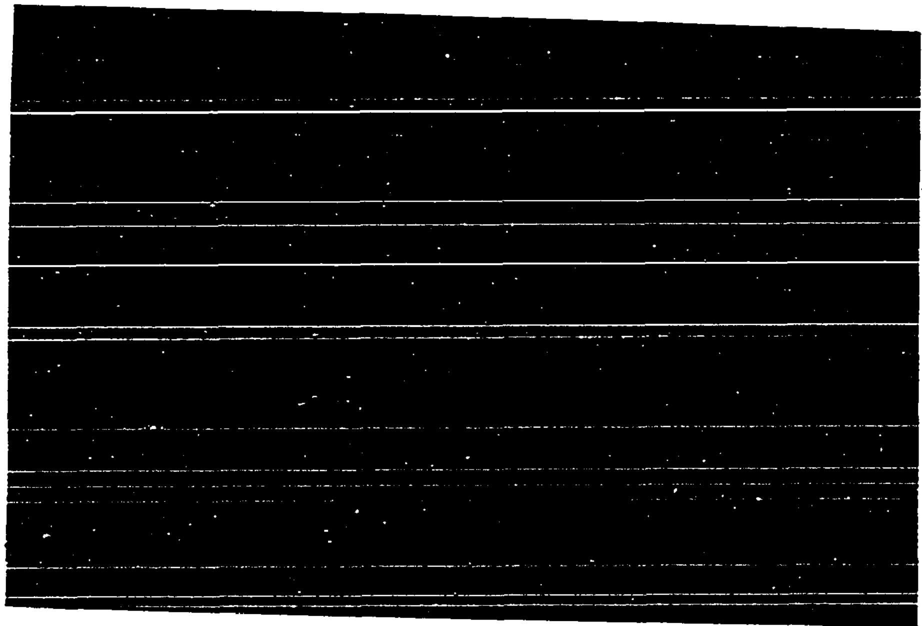


Figure 28. Pars distalis adenohypophysis. Capsule and diaphragma sellae. Collagen fibers sparse in peripheral region

Dog #B 64. Female. 10 years

Verhoeff's stain

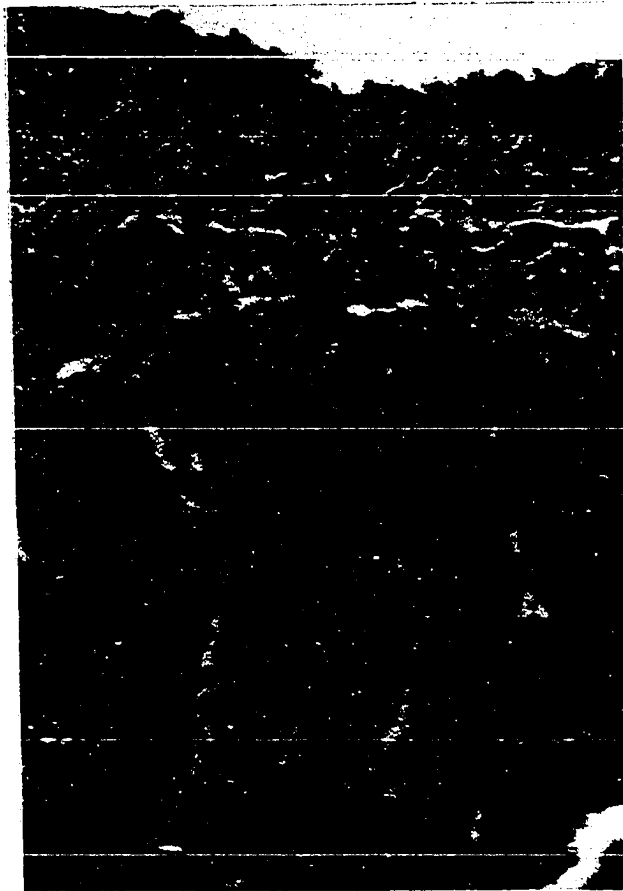
X 250

Figure 29. Adhesion of pars distalis and pars intermedia adenohypophysis. Intermittent obliteration of cavum hypophysis

Dog #B 123. Female. 11.5 years

Aldehyde-thionin--PAS--orange G stain

X 100



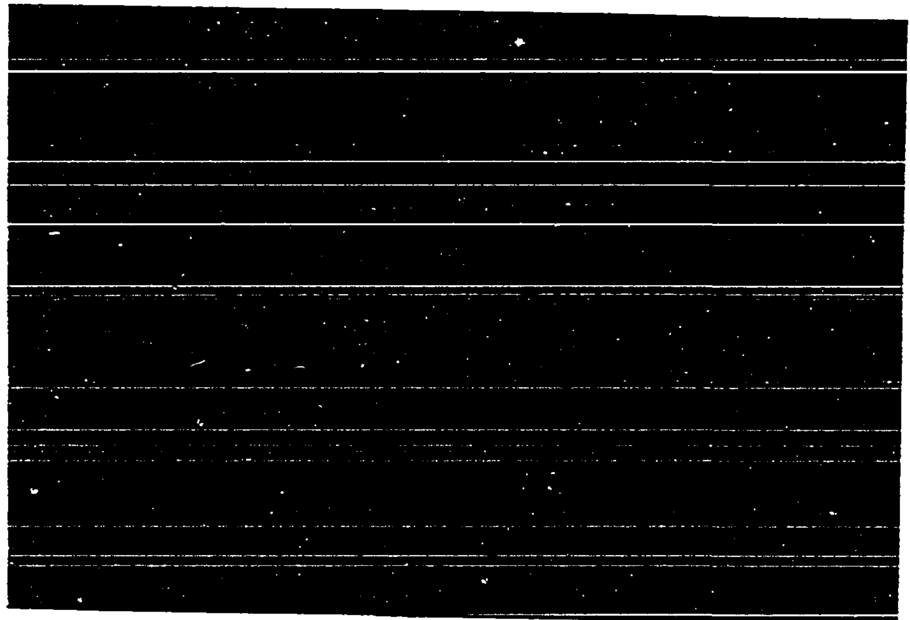


Figure 30. Pars distalis adenohypophysis. Hyperplastic chromophobic nodule

Dog #B 123. Female. 11.5 years

Aldehyde-thionin--PAS--orange G stain

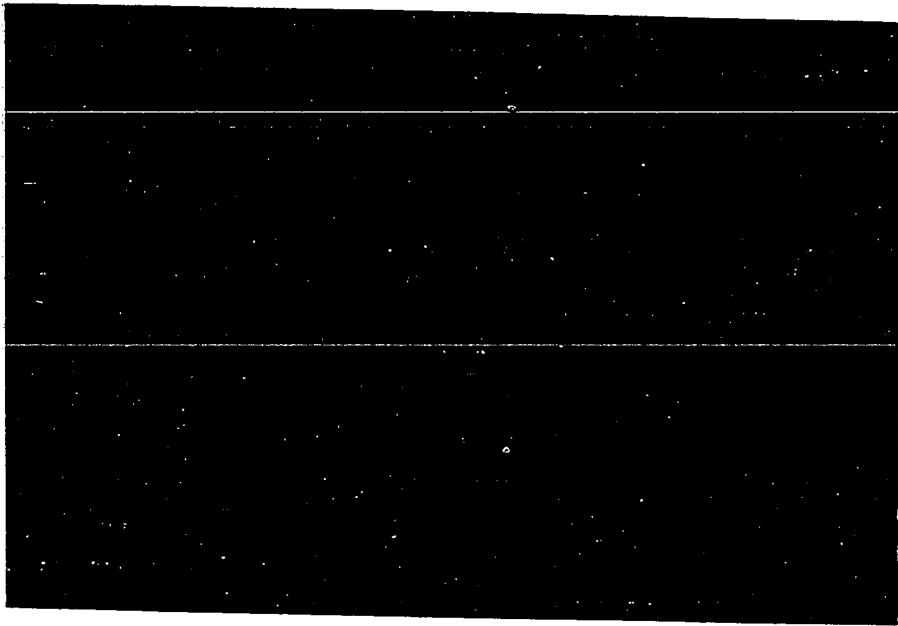
X 250

Figure 31. Pars distalis adenohypophysis. Degranulated cells. Clear delineation of cell-cords due to increased density of collagen fibers

Dog #B 123. Female. 11.5 years

Alcian blue--PAS--orange G stain

X 250



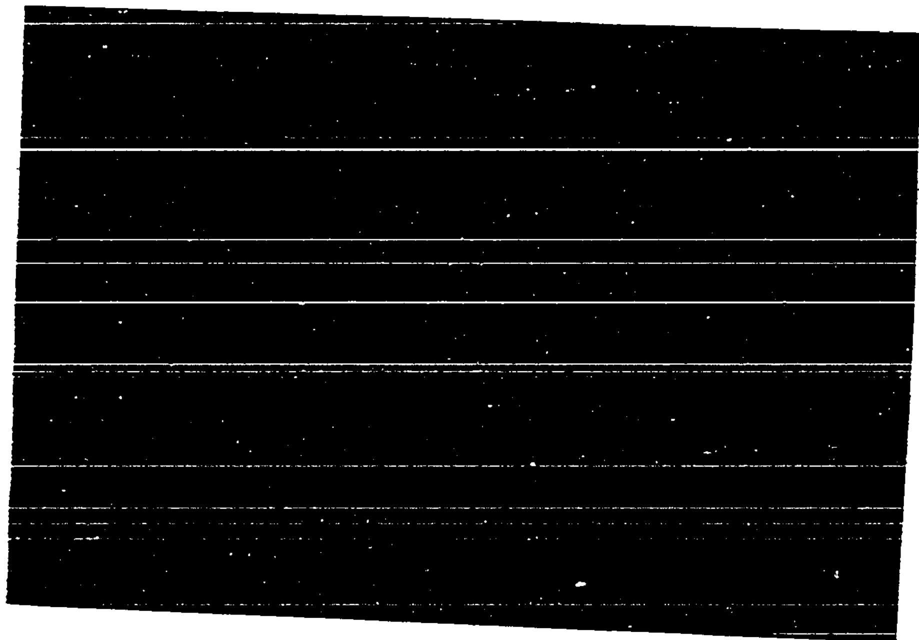


Figure 32. Pars distalis adenohypophysis. Increased density of collagen fibers

Dog #B 123. Female. 11.5 years

Verhoeff's stain

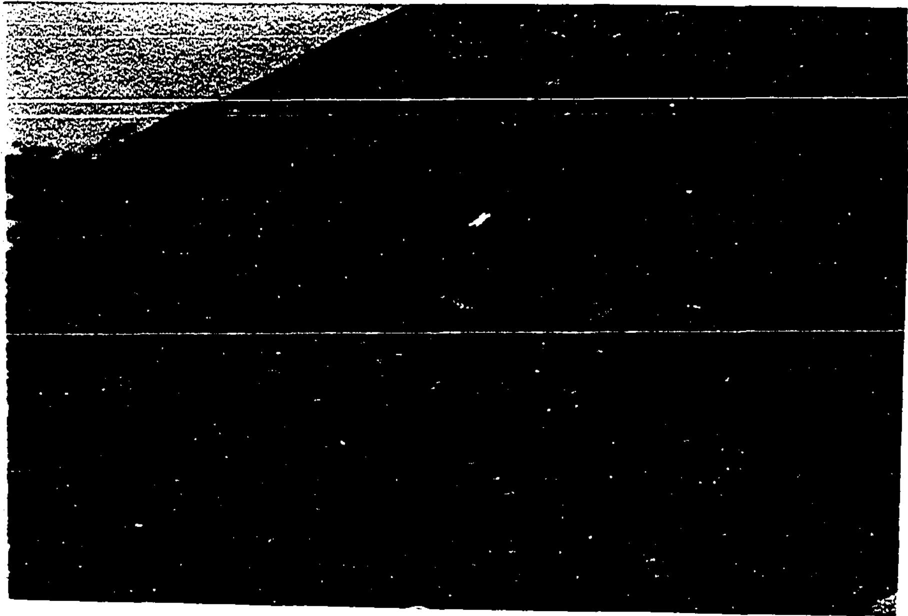
X 100

Figure 33. Pars distalis adenohypophysis. Vacuolated areas caused by necrosis of portal vessels

Dog #B 31. Female. 12 years

Aldehyde-thionin--PAS--orange G stain

X 250



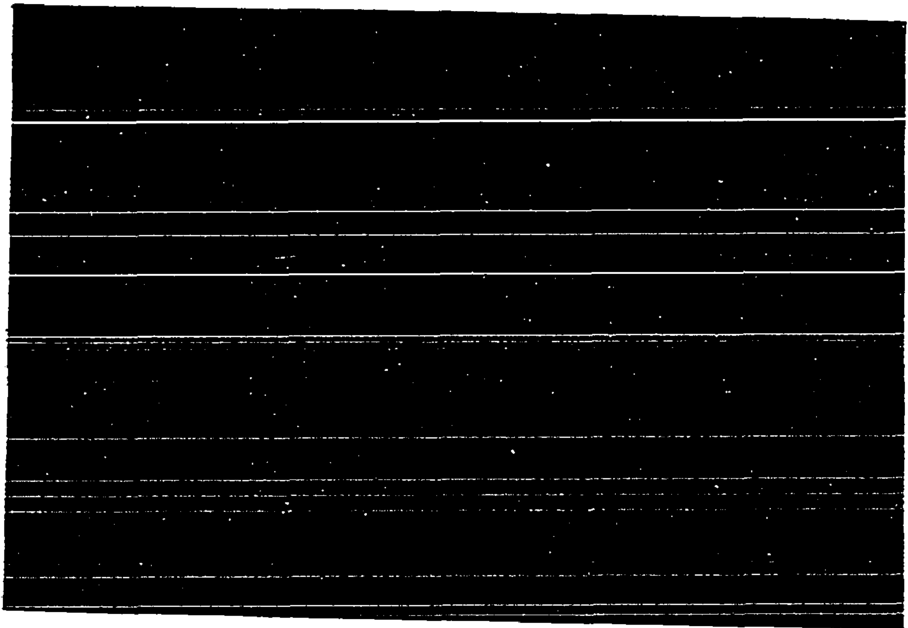
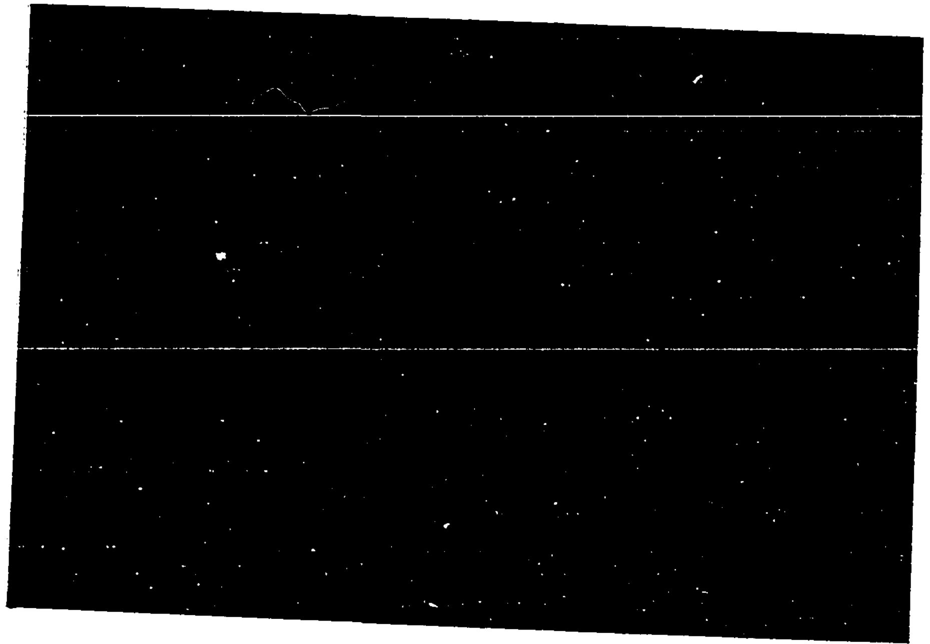


Figure 34. Pars distalis adenohypophysis. Somatotrope cell (yellow). Thyrotrope cell (blue). FSH gonadotrope cell (purple)
Dog #B 31. Female. 12 years
Alcian blue--PAS--orange G stain
X 400

Figure 35. Pars distalis adenohypophysis. Somatotrope cell (yellow). Lactotrope cell (magenta). Thyrotrope cell (blue). FSH gonadotrope cell (purple). ICSH gonadotrope cell (brick red)
Dog #B 31. Female. 12 years
Alcian blue--PAS--orange G stain
X 400



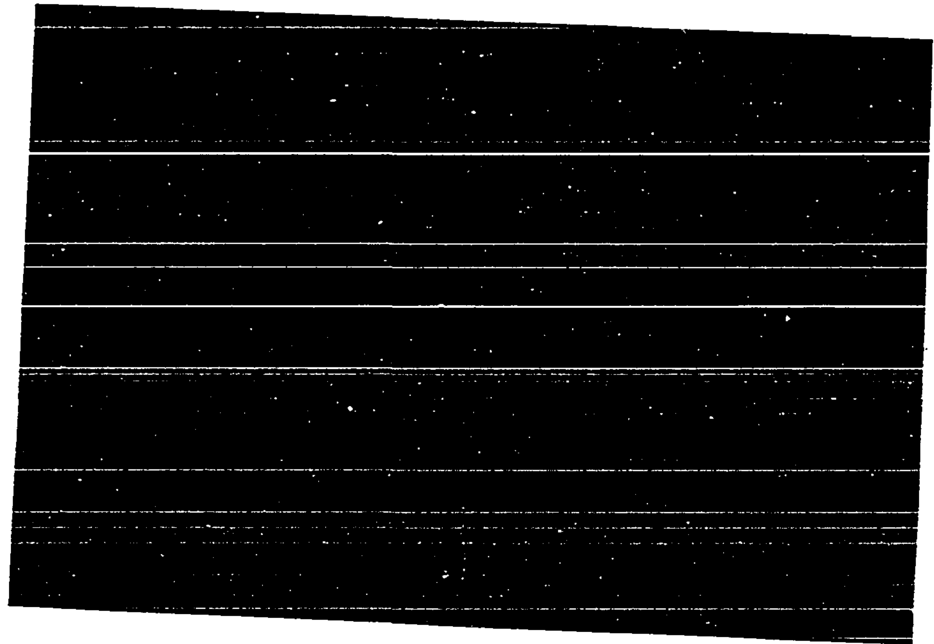


Figure 36. Pars distalis adenohypophysis. Necrosis of portal vessels

Dog #B 31. Female. 12 years

Aldehyde-thionin--PAS--orange G stain

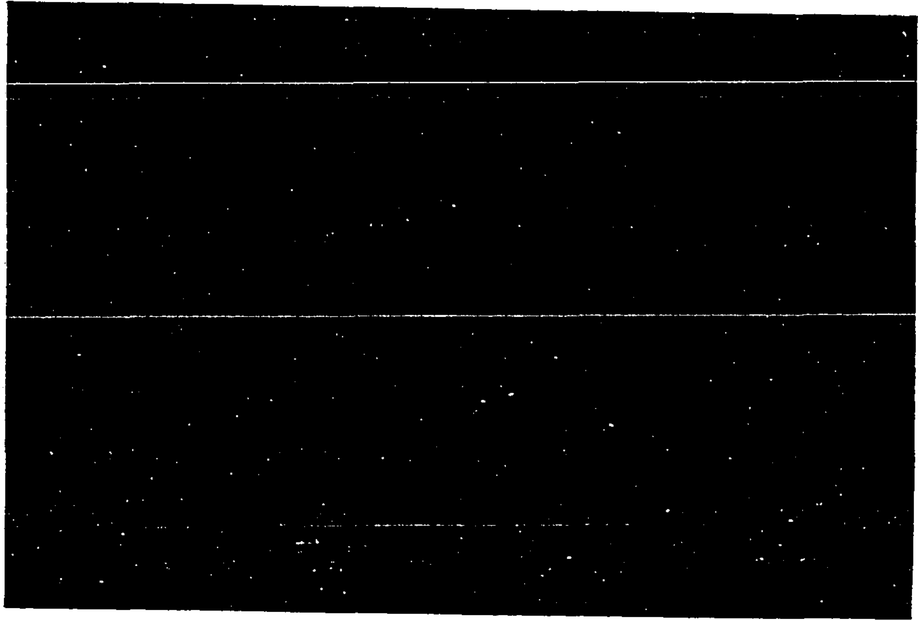
X 250

Figure 37. Pars distalis adenohypophysis. Increased density of reticulin in stroma

Dog #B 131. Female. 12.5 years

Manuel's method

X 250



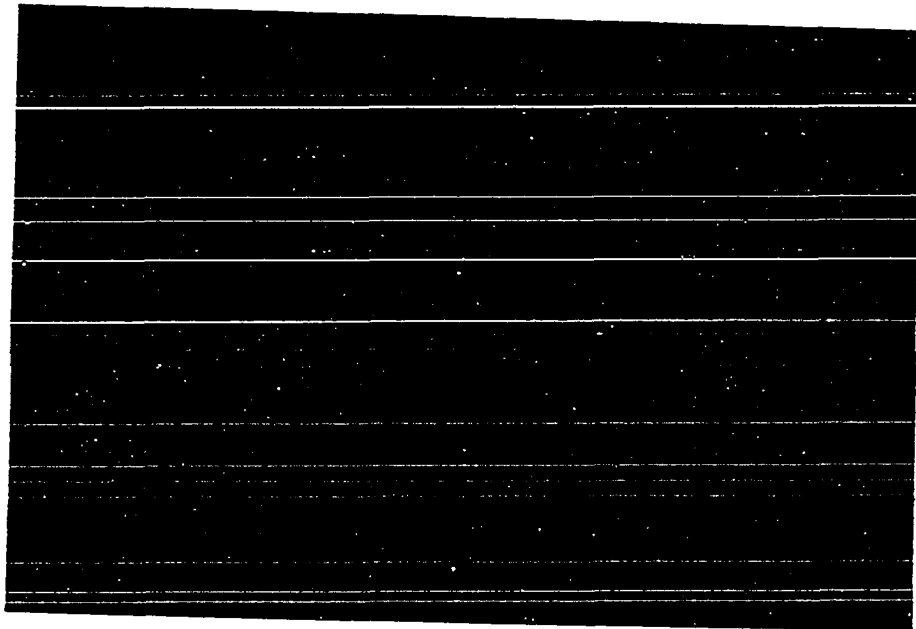
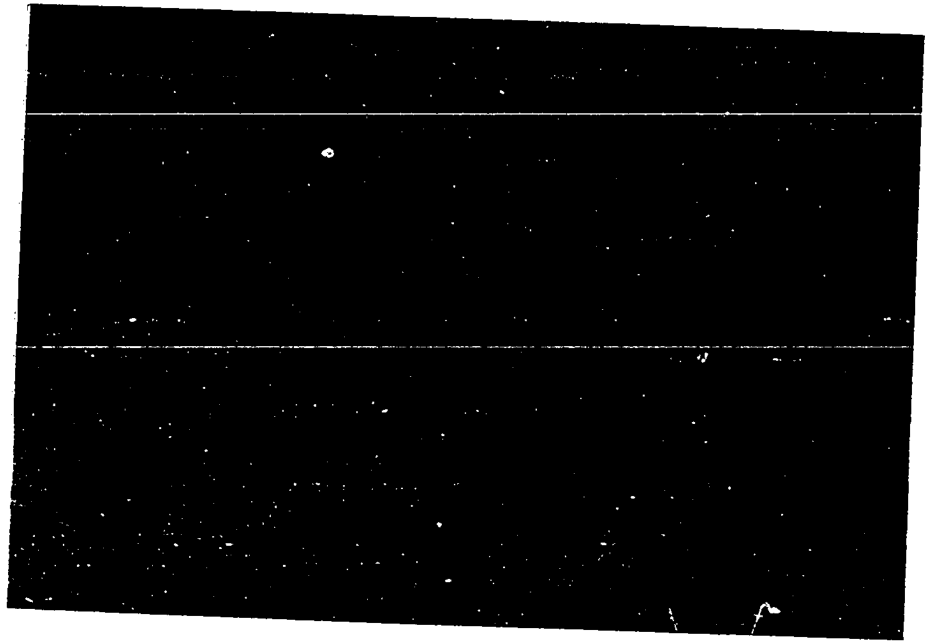


Figure 38. Pars distalis adenohypophysis. Colloid cysts.
Large hyperplastic chromophobic nodules
Dog #B 14. Female. 13.5 years
Alcian blue--PAS stain
X 40

Figure 39. Pars distalis adenohypophysis. Cyst lined with chromophobe cells. Denaturation of collagen fibers
Dog #B 14. Female. 13.5 years
Chrome alum hematoxylin stain
X 250



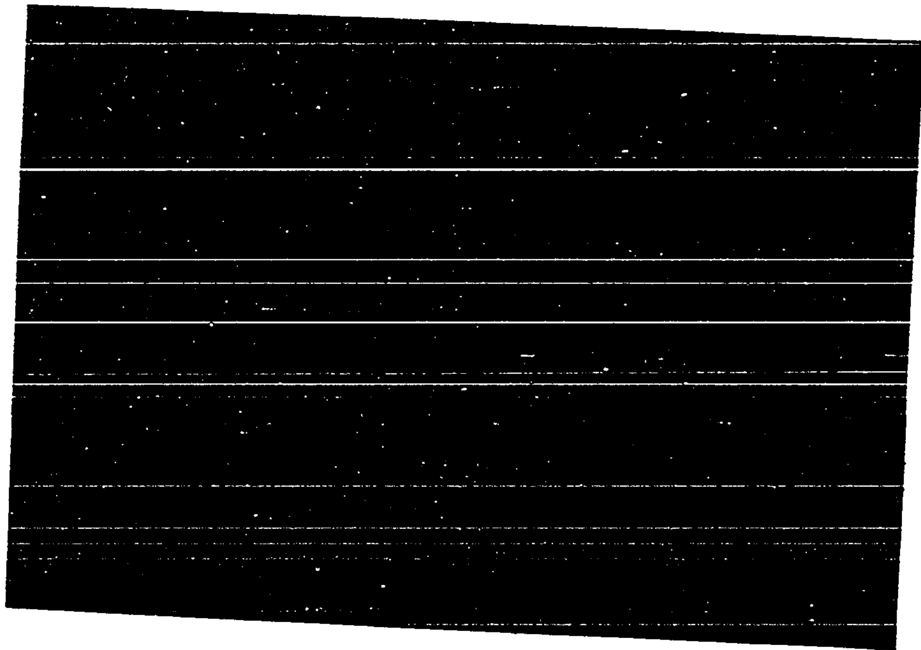


Figure 40. Pars distalis adenohypophysis. Cyst lined with ciliated cells

Dog #B 32. Male. 13.5 years

Crossman's trichrome stain

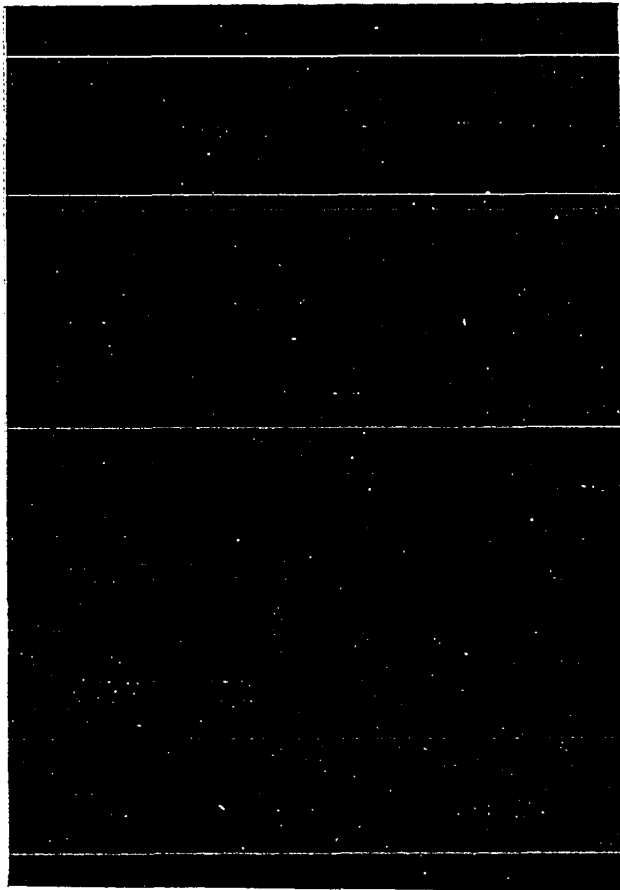
X 400

Figure 41. Pars paraneuralis. Typical structure

Dog # 068. Male. 2.5 months

Alcian blue--PAS--orange G stain

X 100



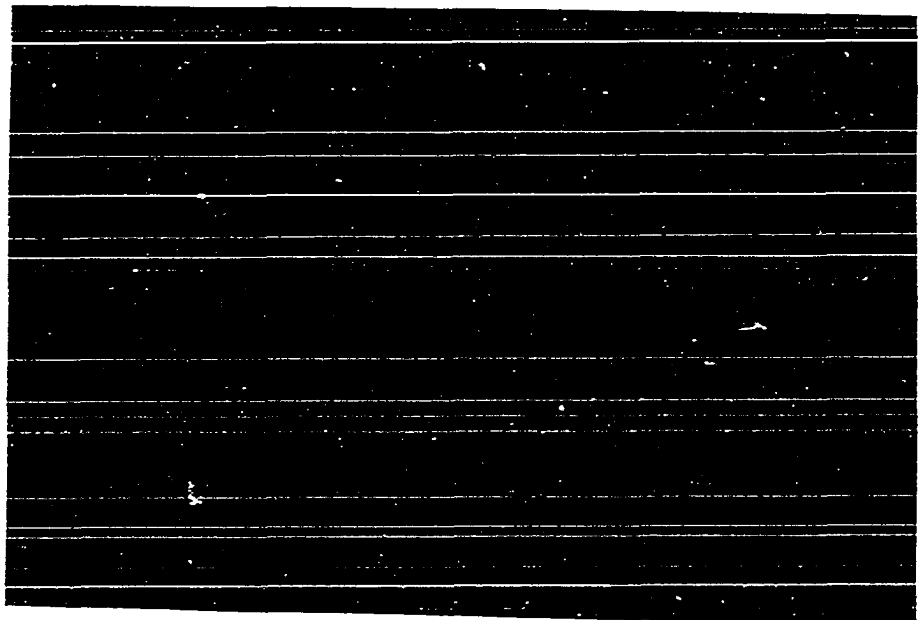


Figure 42. Pars paraneuralis. PAS positive cells and chromophobe cells

Dog # 115. Female. 6 months

Aldehyde-thionin--PAS--orange G stain

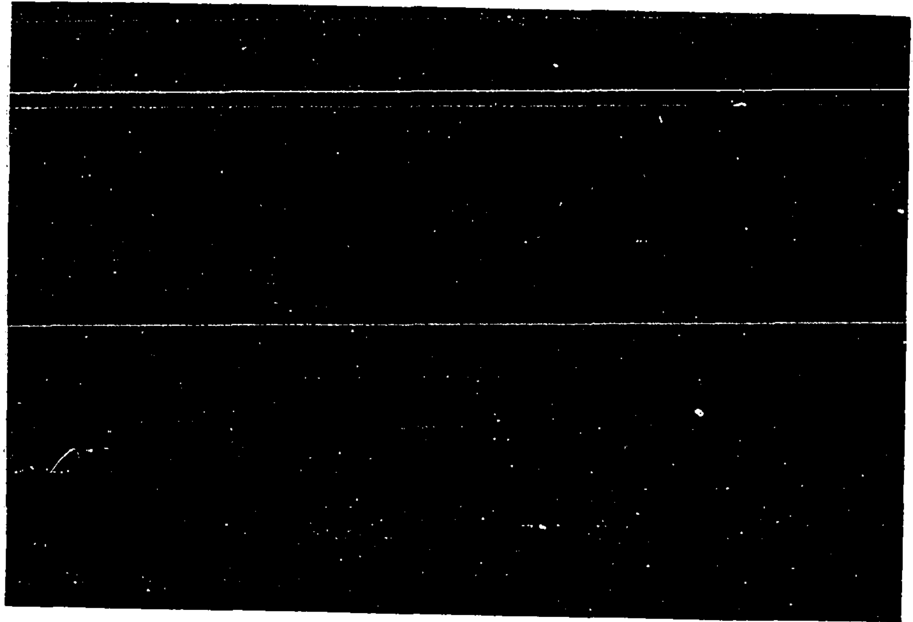
X 400

Figure 43. Pars paraneuralis. Subjacent mantel plexus. PAS positive basement membrane. Intravascular colloid infiltration

Dog #C 17. Female. 11 months

Aldehyde-thionin--PAS--orange G stain

X 250



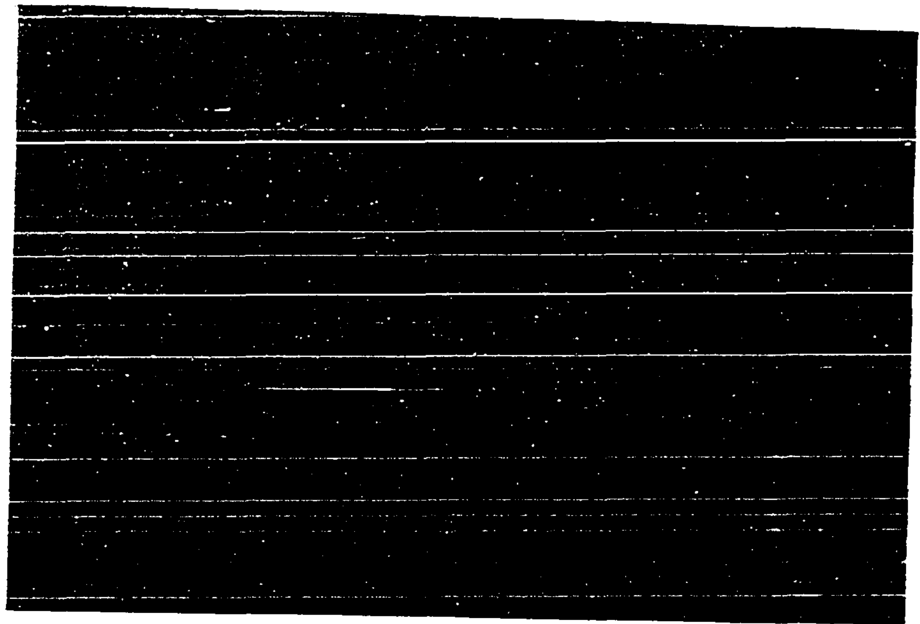
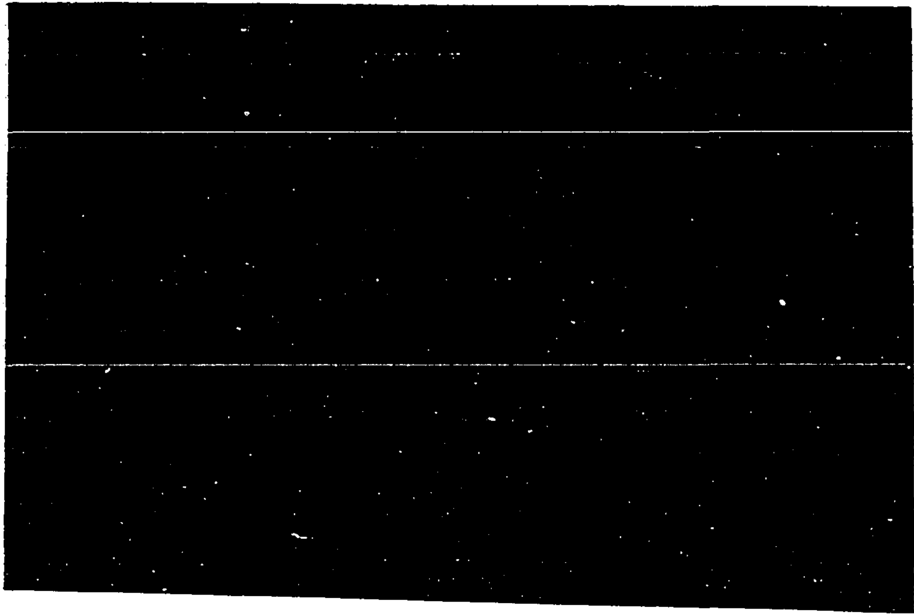


Figure 44. Pars paraneuralis. Colloid infiltration of vessels
Dog #B 80. Male. 1.5 years
Aldehyde-thionin--PAS--orange G stain
X 250

Figure 45. Pars paraneuralis. Increased number of thyrotrope cells (blue-black). Initial stages of colloid deposition in follicles. Herring body in zona interna of pars cava infundibuli
Dog #B 25. Female. 2 years
Aldehyde-thionin--PAS--orange G stain
X 250



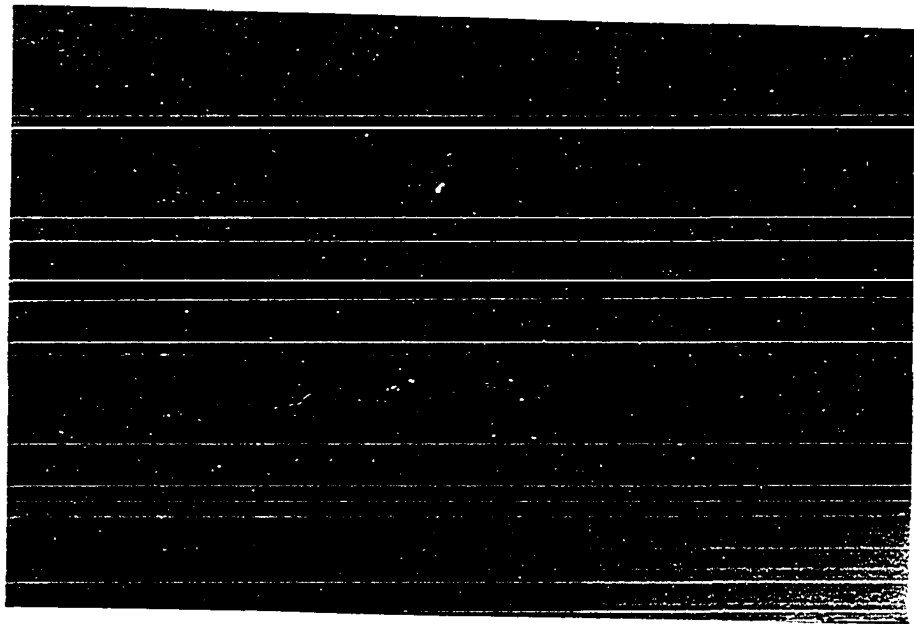


Figure 46. Pars paraneuralis. Obliteration of the cavum hypophysis. Herring body and neurosecretory material (NSM) in zona interna of pars cava infundibuli

Dog #B 51. Female. 4 years

Aldehyde-thionin--PAS--orange G stain

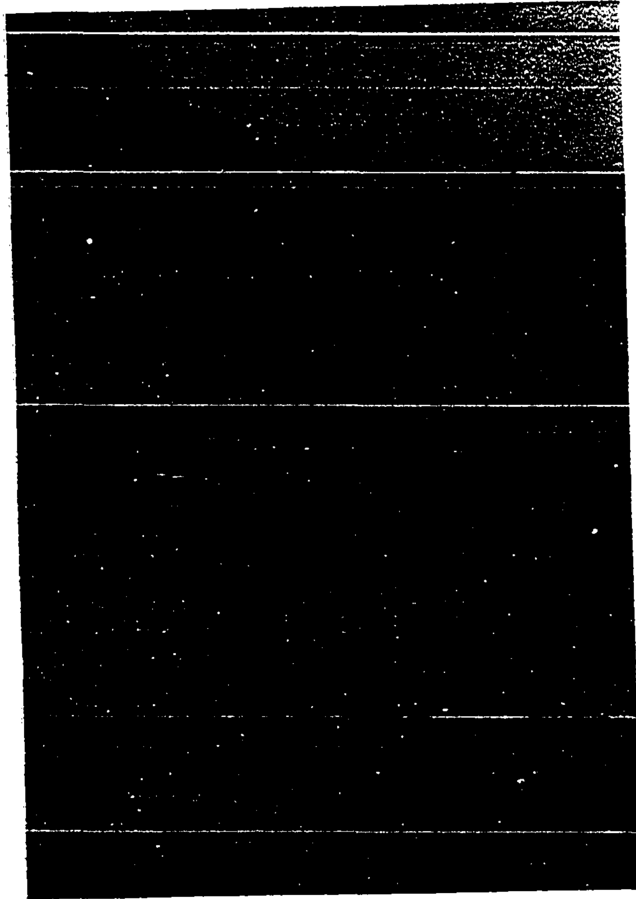
X 250

Figure 47. Pars paraneuralis. Large colloid cyst

Dog #B 31. Female. 12.5 years

Chrome alum hematoxylin stain

X 250



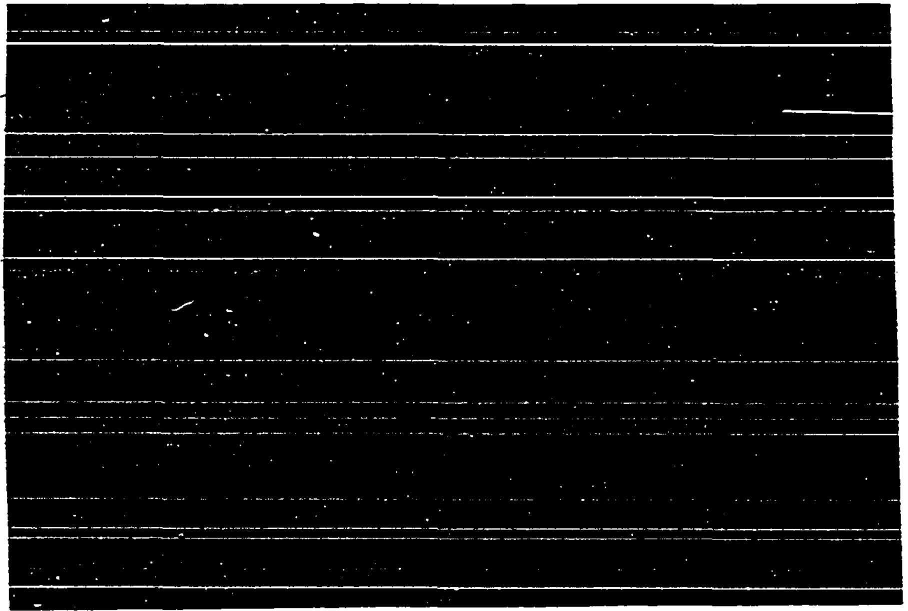
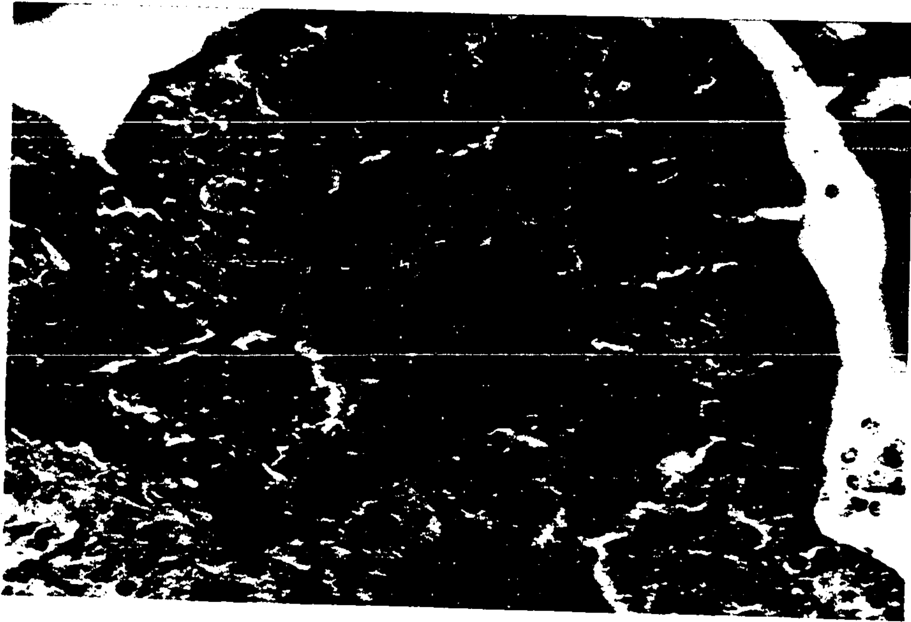


Figure 48. Pars intermedia adenohypophysis. Mitosis of undifferentiated cells
Dog #E 76. Female. 14 days
Verhoeff's stain
X 250

Figure 49. Pars intermedia adenohypophysis. Undifferentiated and light magenta cells
Dog #B 97. Female. 1 month
Aldehyde-thionin--PAS--orange G stain
X 400



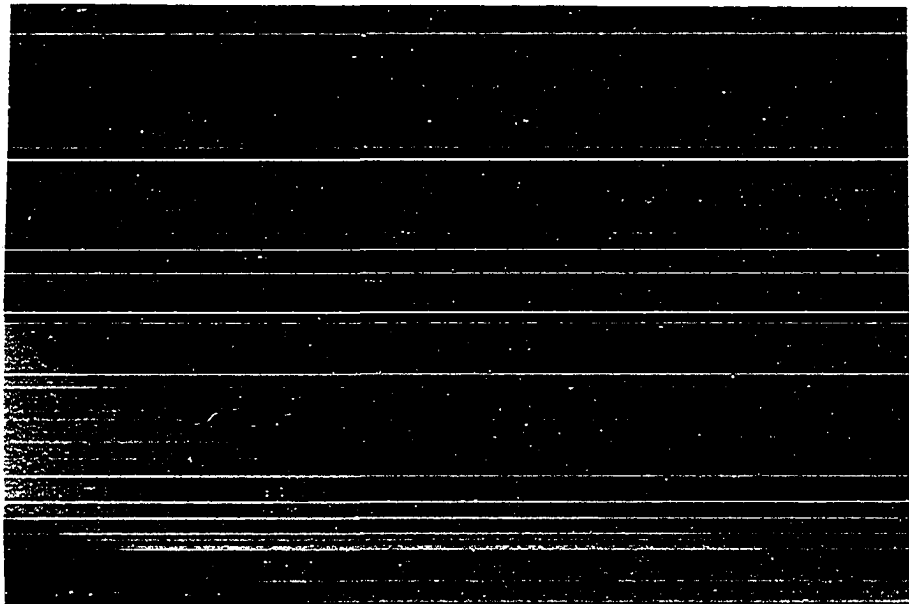


Figure 50. Pars intermedia adenohypophysis. Initial stage of colloid deposition

Dog #B 106. Female. 5 months

Alcian blue--PAS--orange G stain

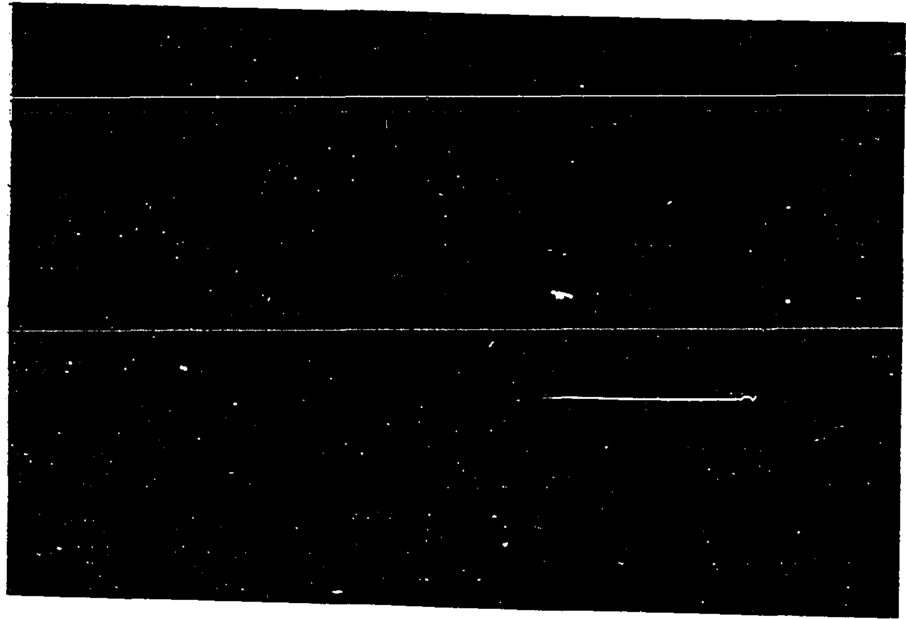
X 250

Figure 51. Pars intermedia adenohypophysis. PAS positive cells. Chromophobe cells

Dog #B 128. Male. 1 year

Aldehyde-thionin--PAS--orange G stain

X 400



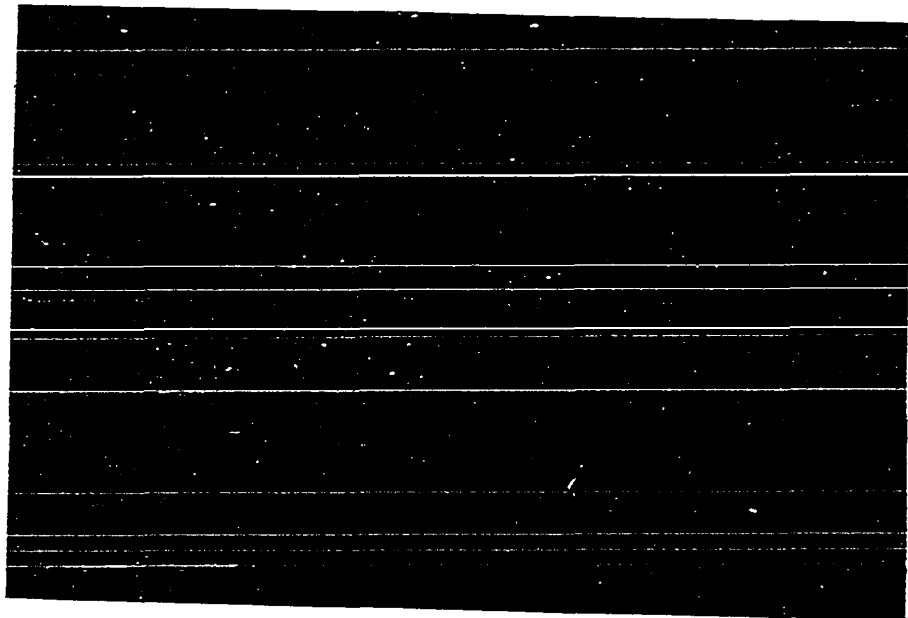


Figure 52. Adhesion between pars intermedia and pars distalis adenohypophysis. Hyperplastic pars intermedia. Increased density of collagen in pars distalis adenohypophysis

Dog #B 73. Female. 11.5 years

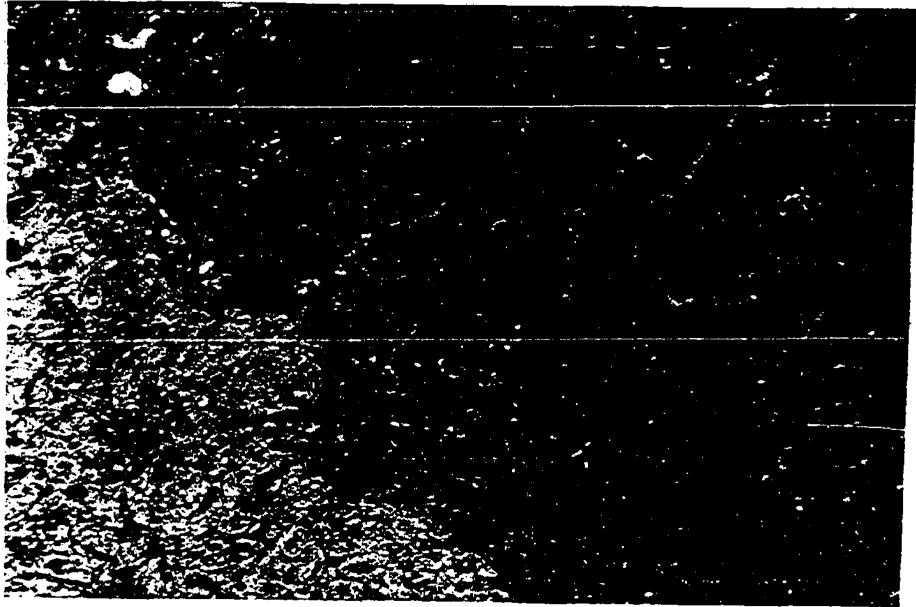
Aldehyde-fuchsin-trichrome stain

Figure 53. Radix infundibuli. Ill-defined zones. Lack of NSM. Developing pars infundibularis adenohypophysis

Dog #B 9. Female. 1 day

Chrome alum hematoxylin stain

X 250



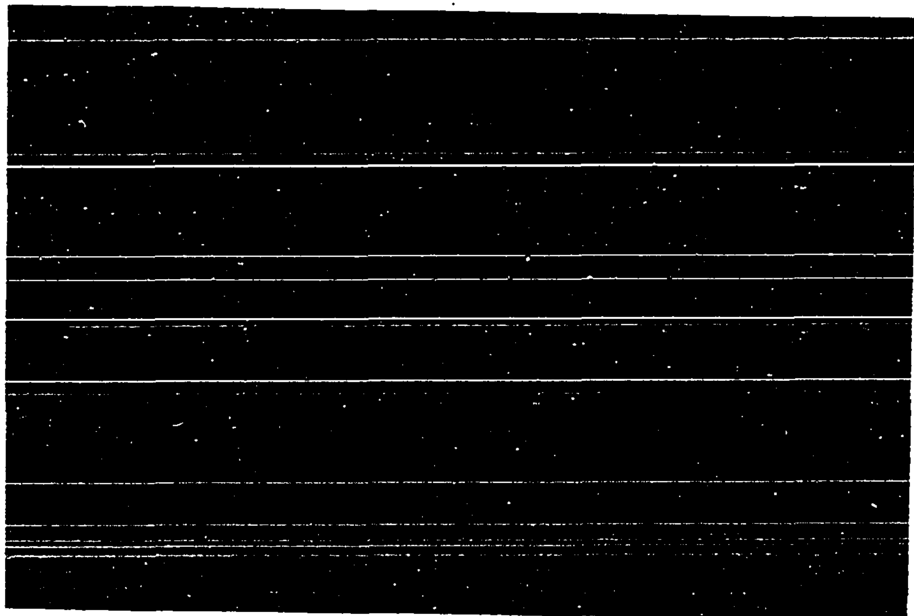
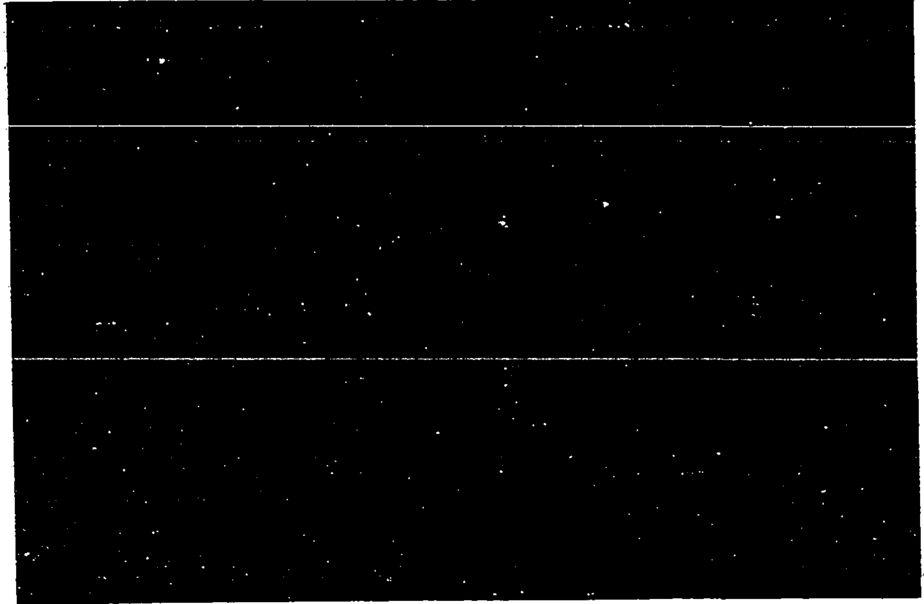


Figure 54. Radix infundibuli. Early stage of zonal arrangement of fibers. NSM sparse
Dog #E 76. Female. 14 days
Chrome alum hematoxylin stain
X 250

Figure 55. Radix infundibuli. Treatment with trichloroacetic acid, 2.5 percent. Loss of stainable NSM from neurohypophysial fibers
Dog # 071. Female. 5 years
Chrome alum hematoxylin stain
X 250



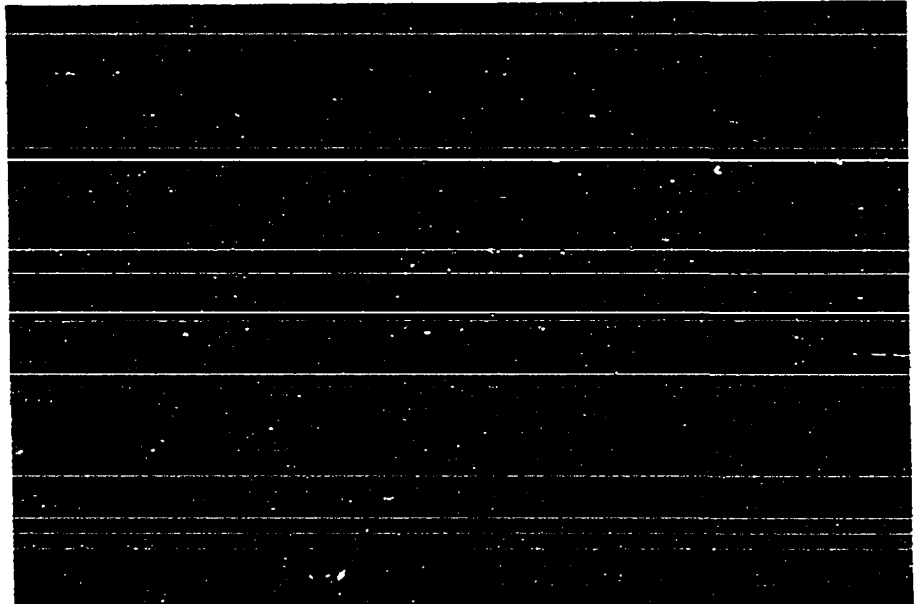
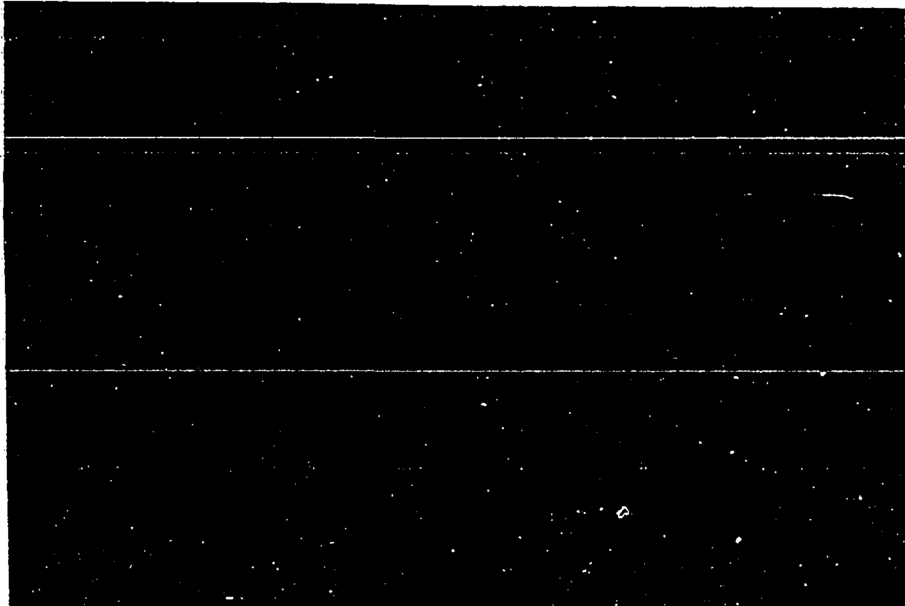


Figure 56. Radix infundibuli. Increased density of collagen fibers and matrix in the mantel plexus
Dog #B 44. Female. 7.5 years
Weigert's method
X 250

Figure 57. Radix infundibuli and pars cava infundibuli. Zona externa and zona interna. Fibrosis of mantel plexus. Ventrolateral part of supraoptic nucleus
Dog #B 31. Female. 12 years
Aldehyde-thionin--PAS--orange G stain
X 40



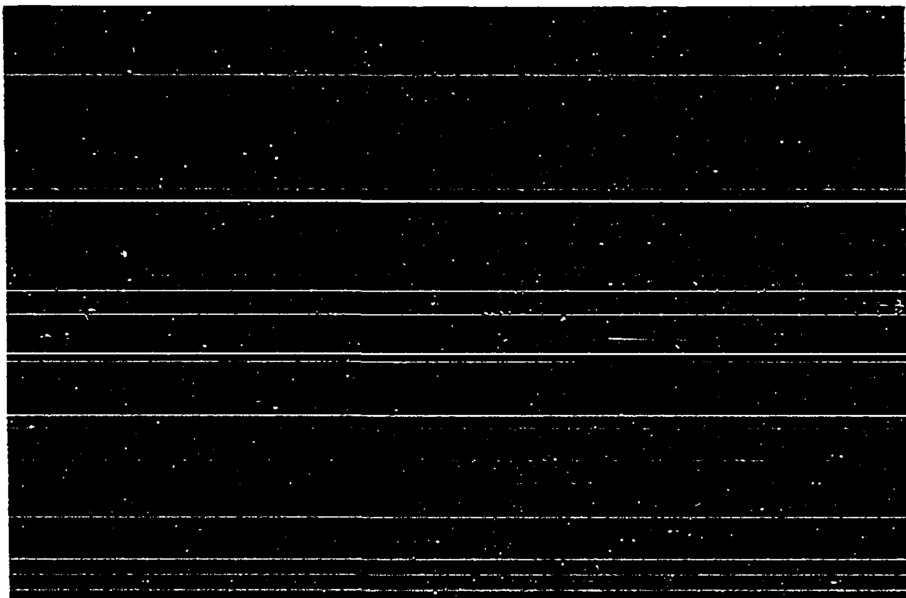


Figure 58. Pars paraneuralis. Mitosis of cells
Dog #B 99. Female. 1.5 months
Verhoeff's stain
X 400

Figure 59. Pars cava infundibuli. Ependymal layer.
Admixture of fibers of zona interna and zona externa. NSM and Herring body
Dog #B 99. Female. 1.5 months
Chrome alum hematoxylin stain
X 250

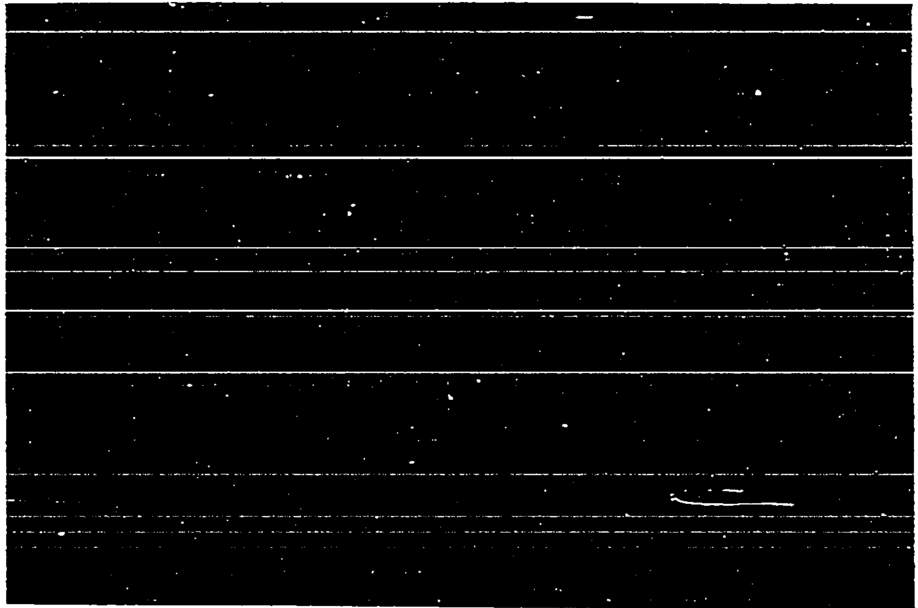
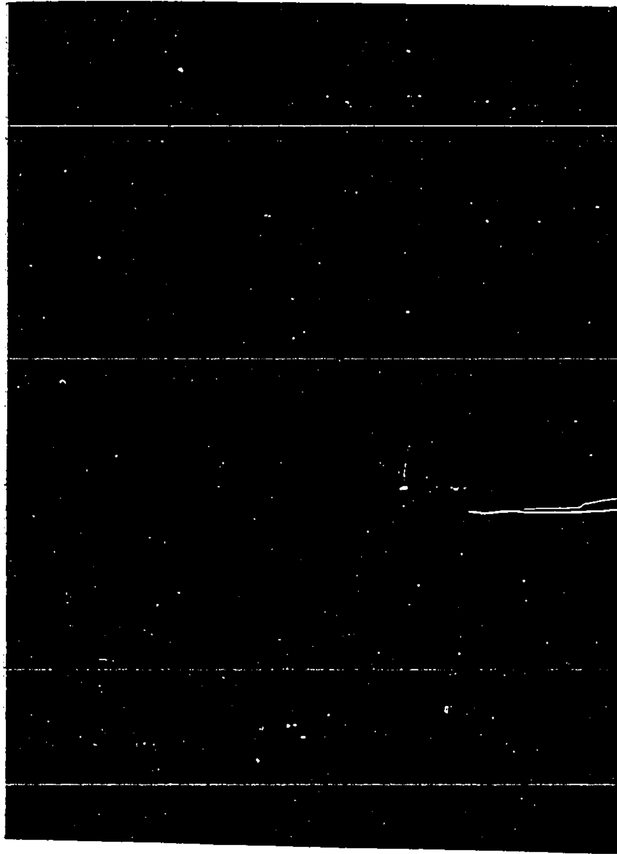


Figure 60. Pars cava infundibuli. Ependymal layer. Cellular zona interna with NSM and Herring body. Zona externa with perpendicularly oriented fibers terminating on the mantel plexus
Dog #B 26. Female. 2 years
Chrome alum hematoxylin stain
X 250

Figure 61. Pars cava infundibuli. Increased density of fibrous tissue in mantel plexus
Dog #B 44. Female. 7.5 years
Alcian blue--PAS stain
X 100



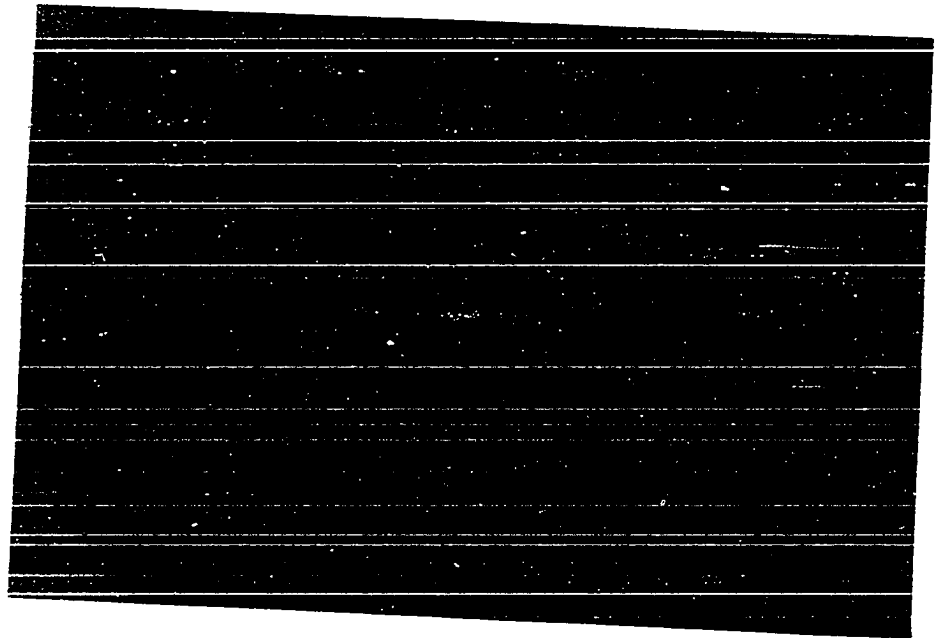
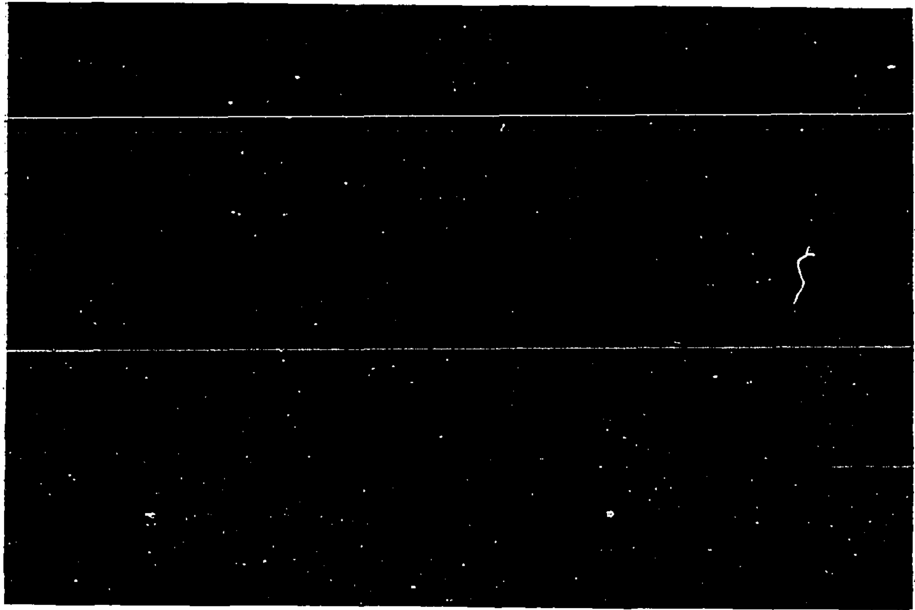


Figure 62. Pars distalis neurohypophysis and pars intermedia. Increased density of cells. Undifferentiated and chromophobe cells in pars intermedia
Dog #B9. Female. 1 day
X 250

Figure 63. Pars distalis neurohypophysis. NSM in fibers. Colloid infiltration of vein
Dog # C68. Male. 2.5 months
Alcian blue--PAS--orange G stain
X 250



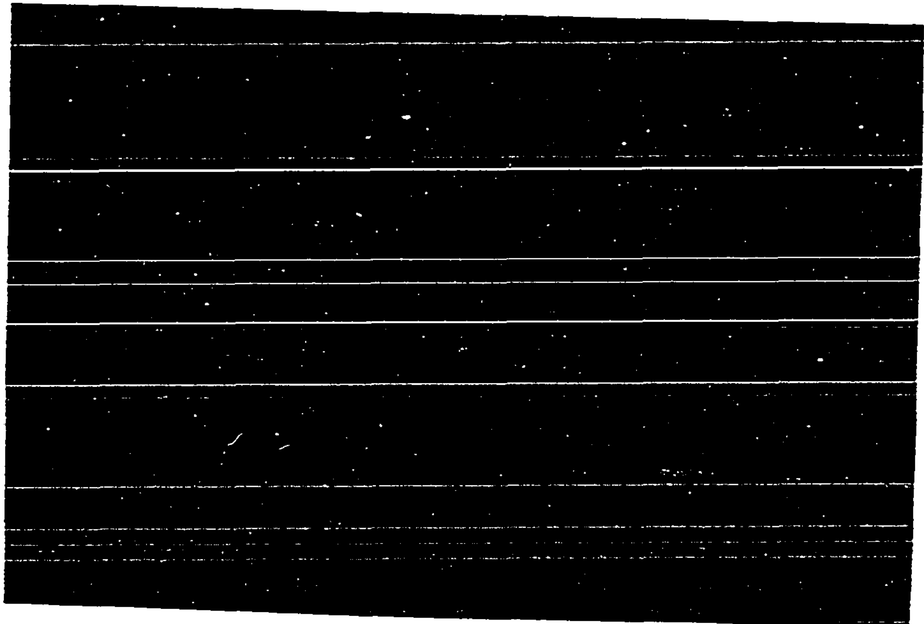


Figure 64. Pars distalis neurohypophysis. NSM and neuroglia cells

Dog #B 61. Female. 7 months

Chrome alum hematoxylin stain

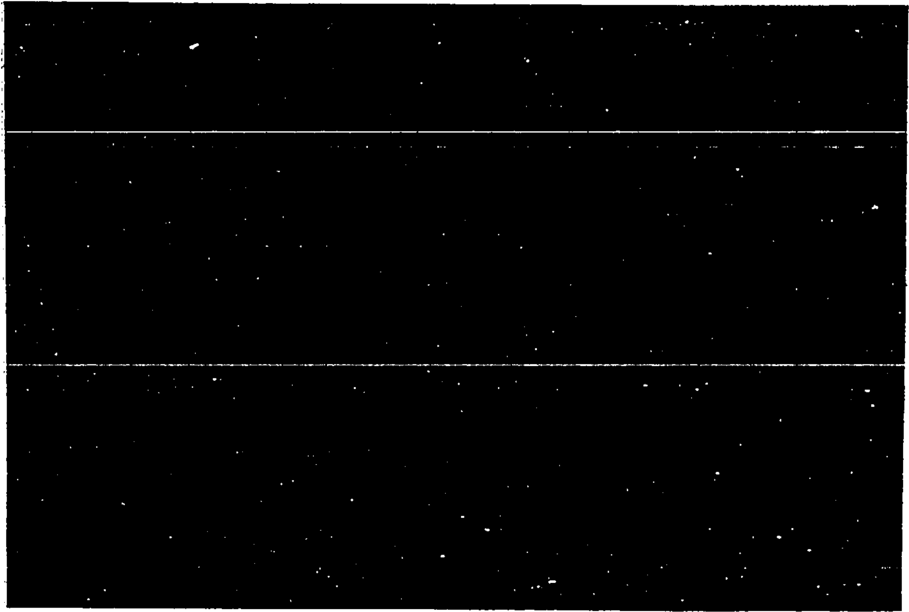
X 400

Figure 65. Pars distalis neurohypophysis. NSM and Herring bodies

Dog #B 49. Female. 8 months

Alcian blue--PAS--orange G stain

X 250



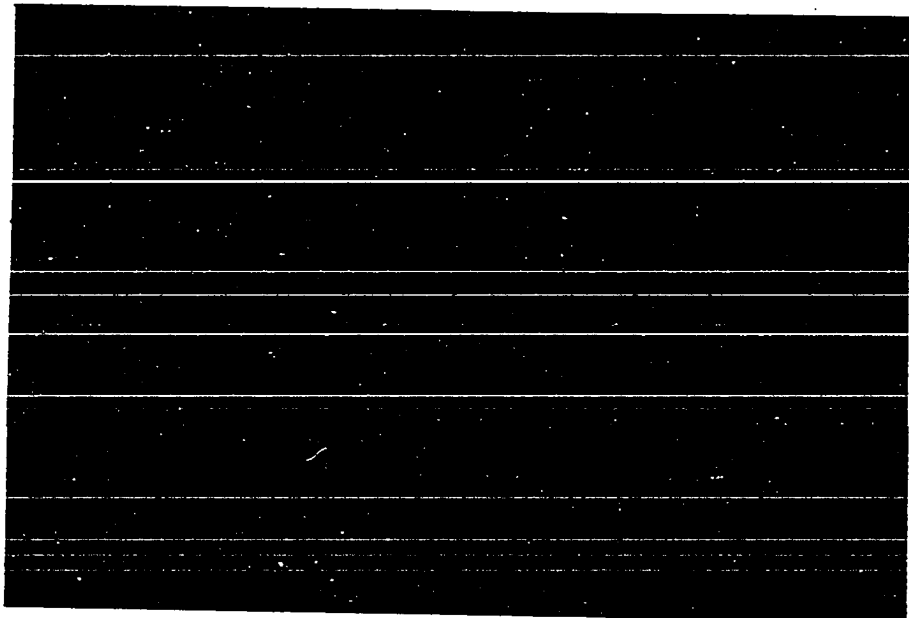


Figure 66. Pars distalis neurohypophysis. Argyrophilia of fibers

Dog #C 8. Female. 11 months

Bielschowsky's stain

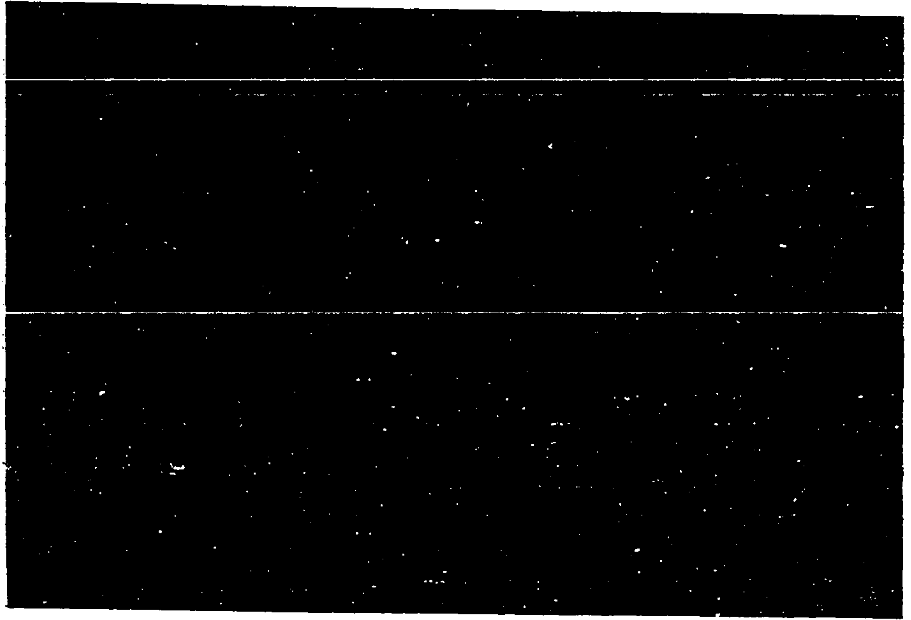
X 250

Figure 67. Pars distalis neurohypophysis. Colloid infiltration of vein. Patent artery

Dog #C 17. Female. 11 months

Alcian blue--PAS--orange G stain

X 250



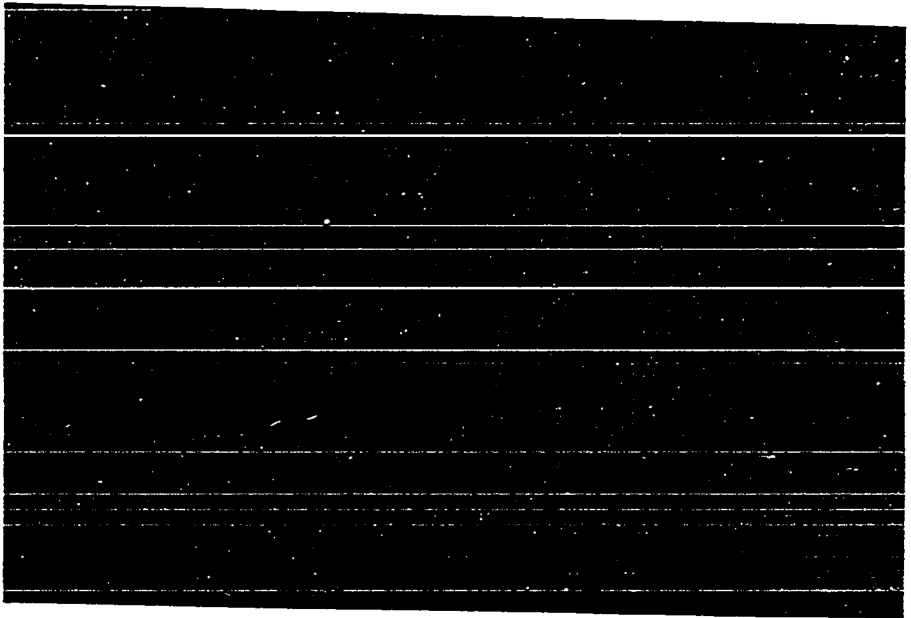
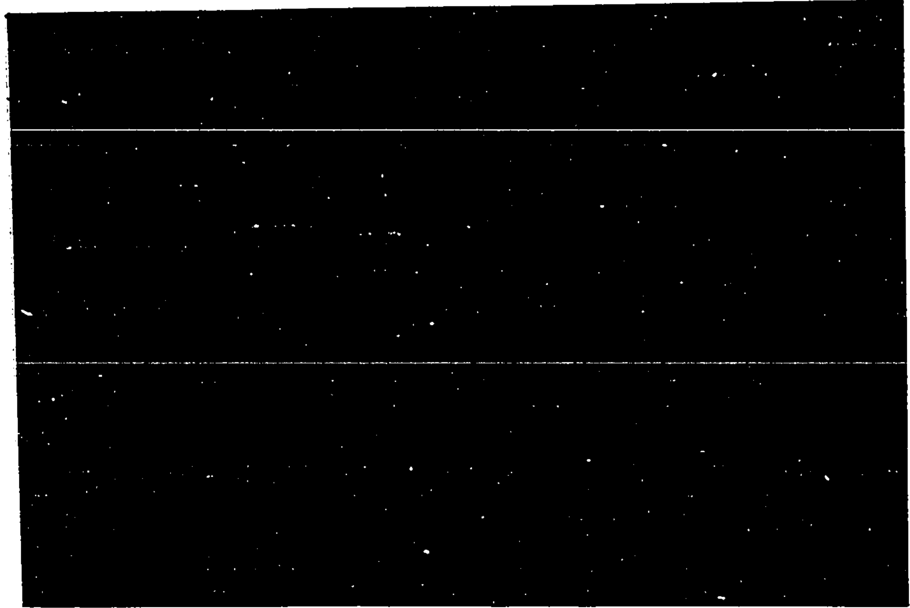


Figure 68. Pars distalis neurohypophysis. NSM and Herring bodies. Invading cell-columns of pars intermedia. Hyperplasia of intermedia cells
Dog #B 62. Female. 1 year
Alcian blue--PAS--orange G stain
X 250

Figure 69. Pars distalis neurohypophysis. NSM and Herring bodies. Termination of neurohypophysial fibers on capillary wall
Dog #B 25. Female. 2 years
Chrome alum hematoxylin stain
X 250



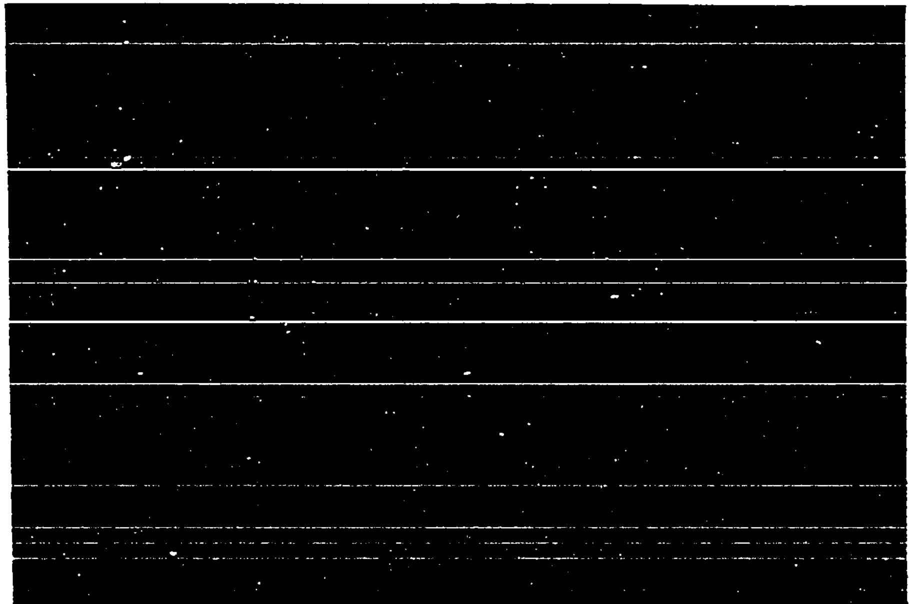


Figure 70. Pars distalis neurohypophysis. NSM in neurohypophysial fibers. Hypertrophy of pars intermedia cells

Dog #B 51. Female. 4 years

Alcian blue--PAS--orange G stain

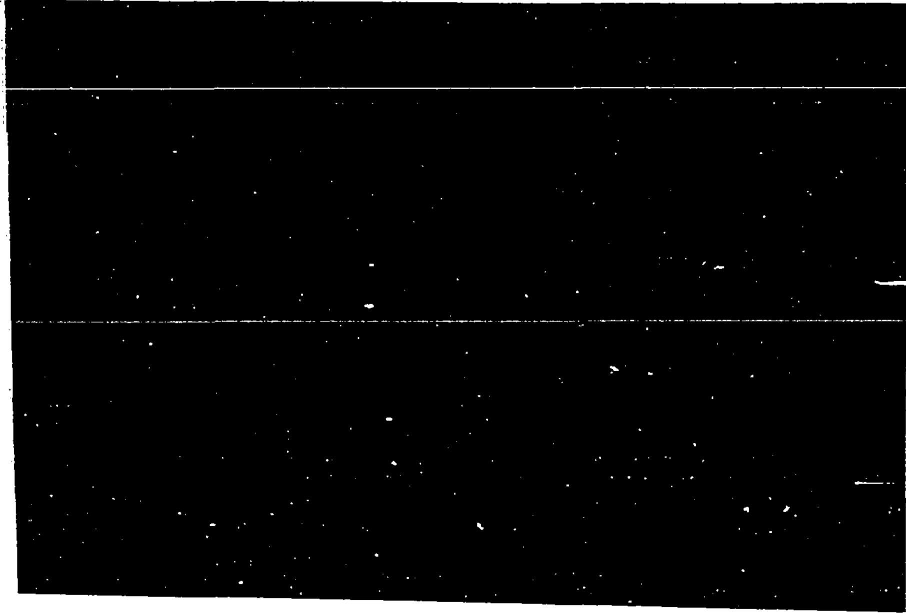
X 250

Figure 71. Pars distalis neurohypophysis. Intravascular colloid infiltration of the vein. Relatively less affection of the artery

Dog #B 44. Female. 7.5 years

Aldehyde-thionin--PAS--orange G stain

X 400



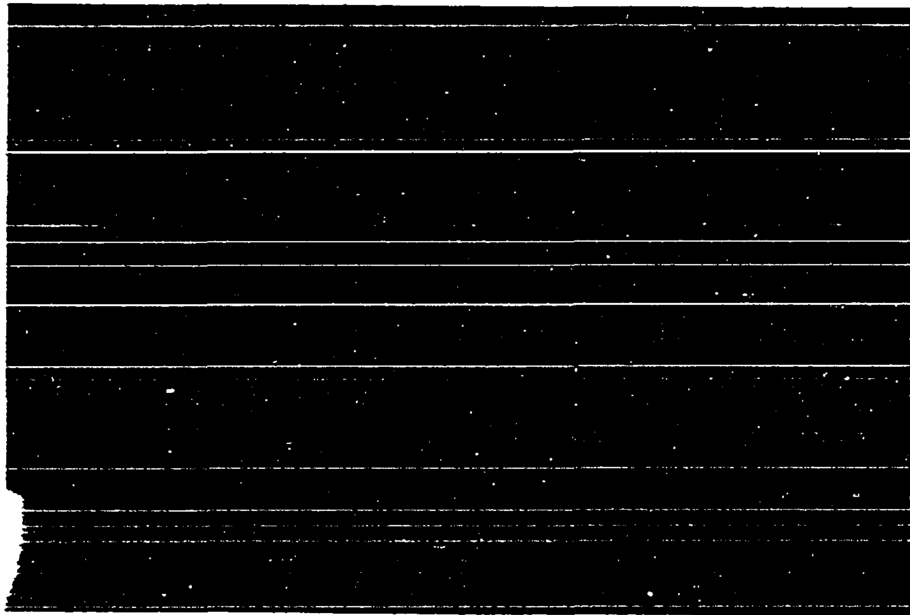


Figure 72. Pars distalis neurohypophysis. Increased density of perivascular collagen fibers

Dog #B 123. Female. 11.5 years

Verhoeff's stain

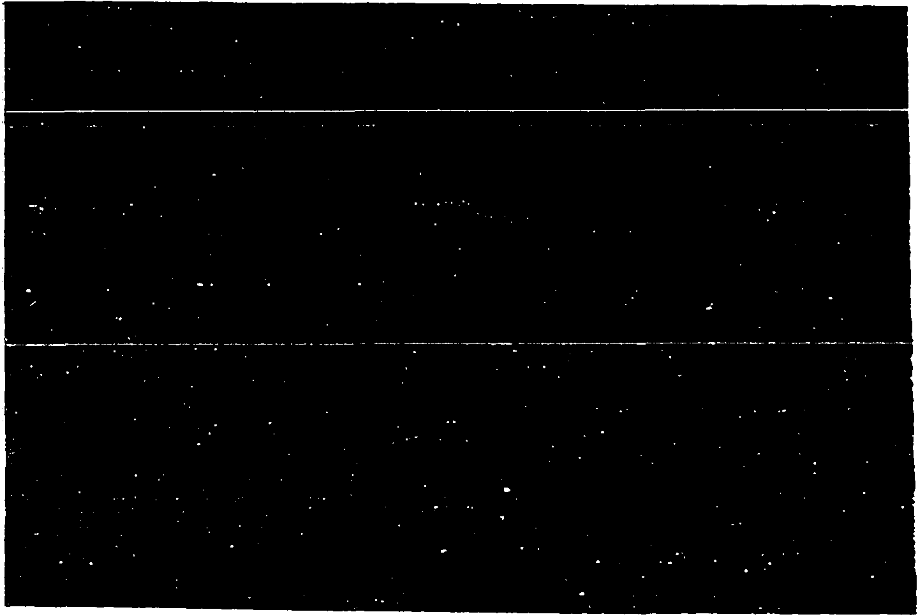
X 250

Figure 73. Pars distalis neurohypophysis. Migratory pars intermedia cells. NSM and Herring body

Dog #B 14. Female. 13.5 years

Alcian blue--PAS--orange G stain

X 250



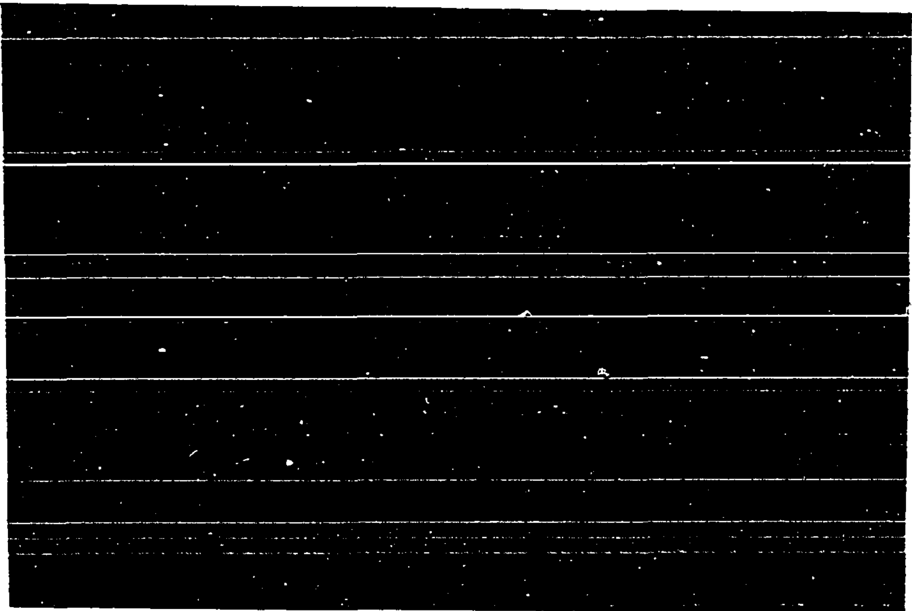


Figure 74. Longitudinal section of the hypophysis. Horizontal plane. Large size of pars infundibularis adenohypophysis on rostroventral surface. Bulbous character of the pars distalis neurohypophysis

Pig # 9000. Male. 1.5 years

Aldehyde-thionin--PAS--orange G stain

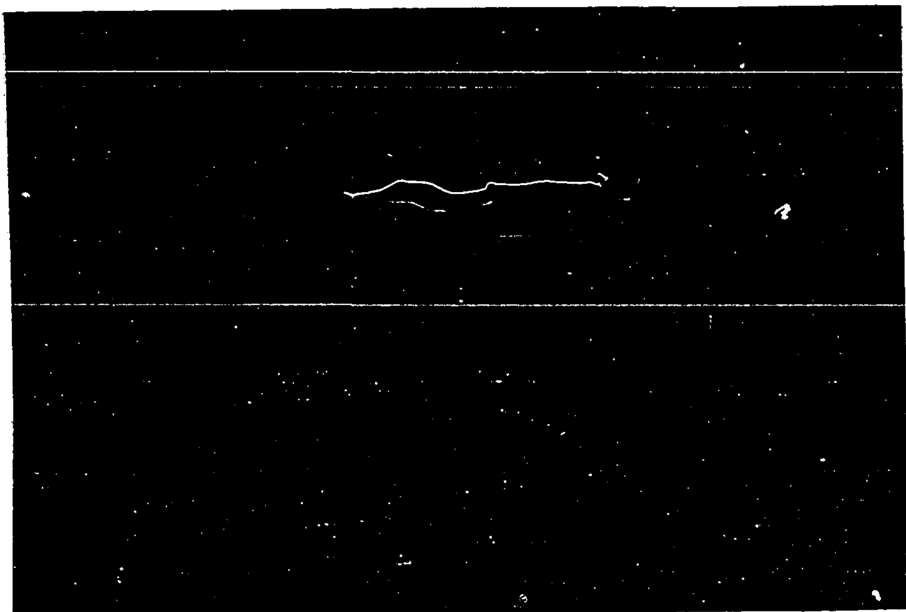
X 2.5

Figure 75. Longitudinal section of the hypophysis. Vertical plane. Inconspicuous merging of pars paraneuralis, pars intermedia and basophil zone of pars distalis adenohypophysis. Caudal hypophysial vein

Pig # FS. Male. 1.5 years

Aldehyde-fuchsin-trichrome stain

X 2.5



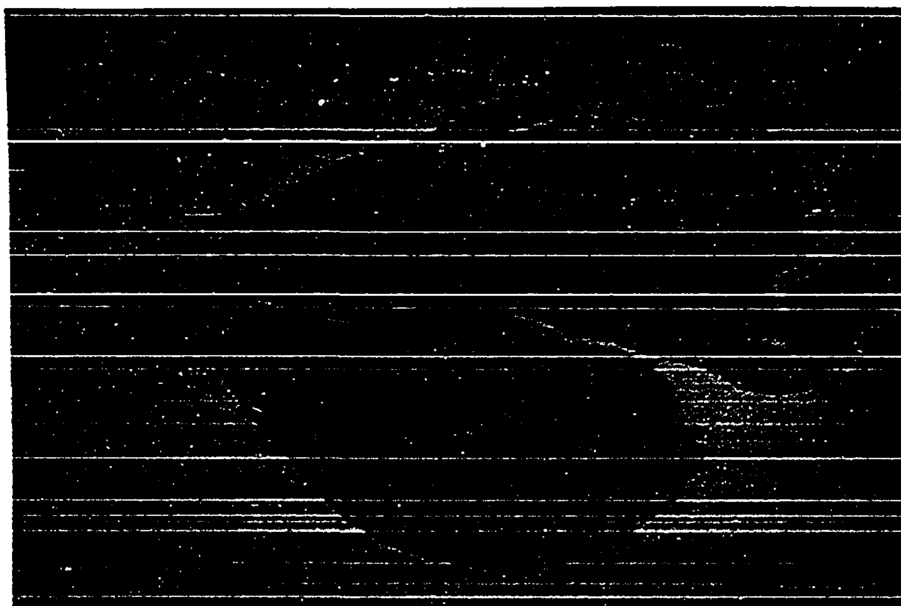
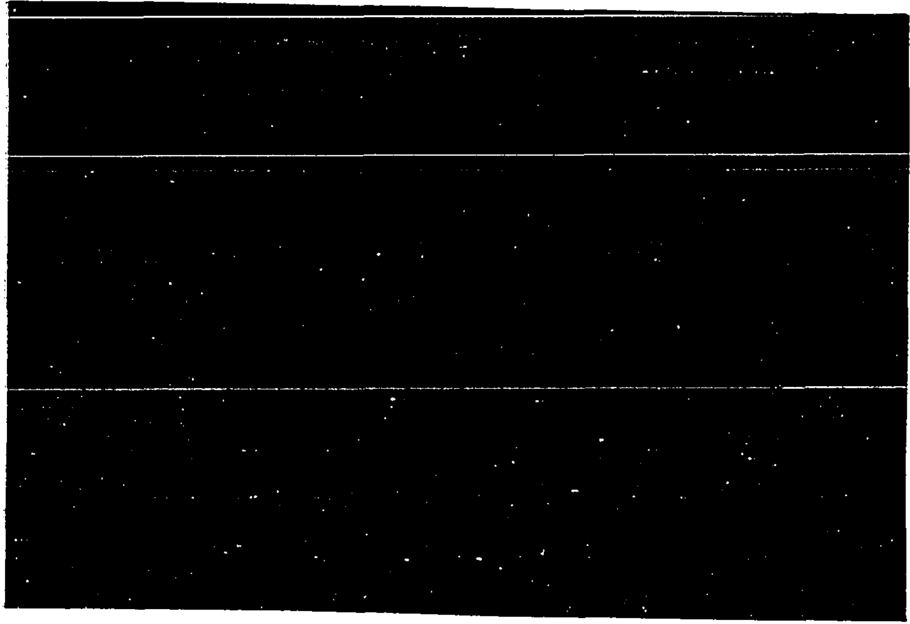


Figure 76. Cross section of the hypophysis. Basophil and acidophil zones. Peripheral condensation of NSM in pars distalis neurohypophysis
Pig # 50. Male. 1 year
Chrome alum hematoxylin stain
X 2.5

Figure 77. Cross section of the hypophysis. Lack of colloid-follicles in dorsolateral acidophil zone (right)
Pig # 50. Male. 1 year
Aldehyde-fuchsin-trichrome stain
X 2.5



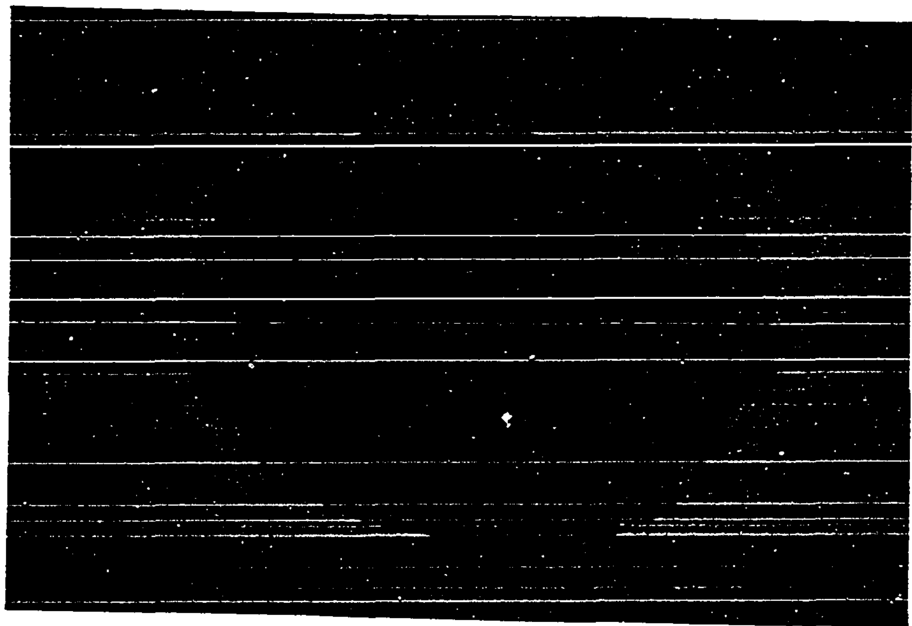
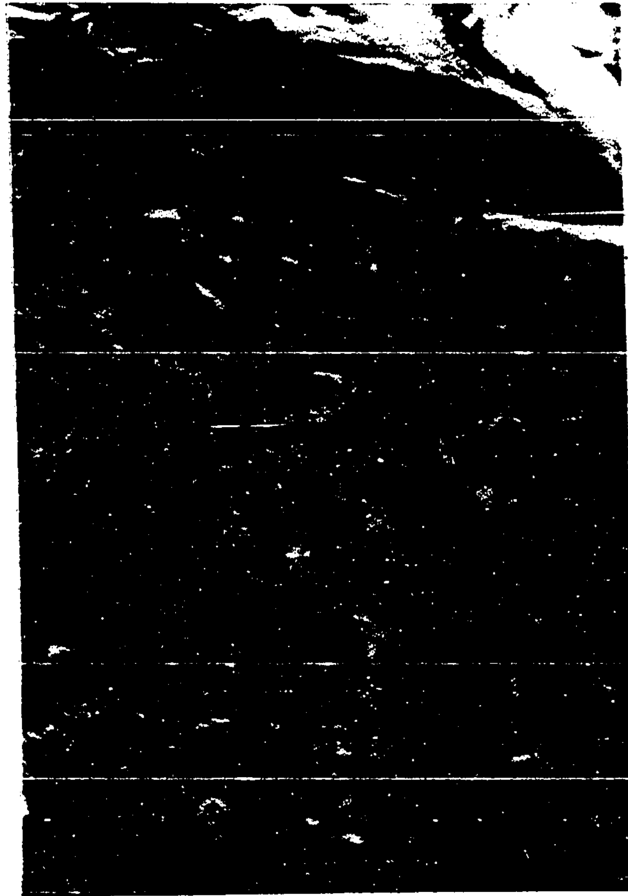


Figure 78. Pars distalis adenohypophysis. Collagen fibers encircling one or two parenchymal cells. External capsule and diaphragma sellae
Pig # 4. Female. 1 day
Weigert's method
X 250

Figure 79. Pars distalis adenohypophysis. Somatotrope cell (yellow). Thyrotrope cell (blue). FSH gonadotrope cell (magenta)
Pig # 4. Female. 1 day
Alcian blue--PAS--orange G stain
X 400



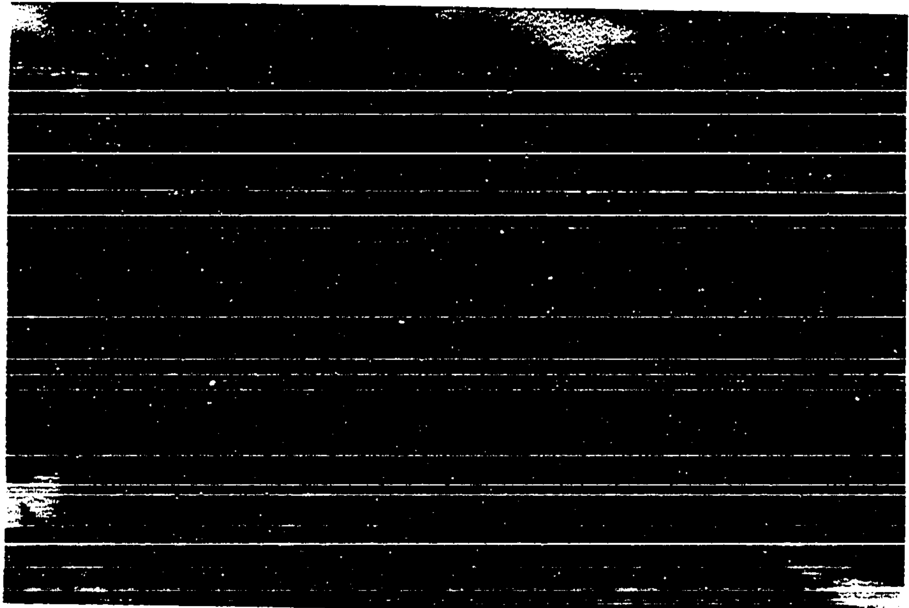
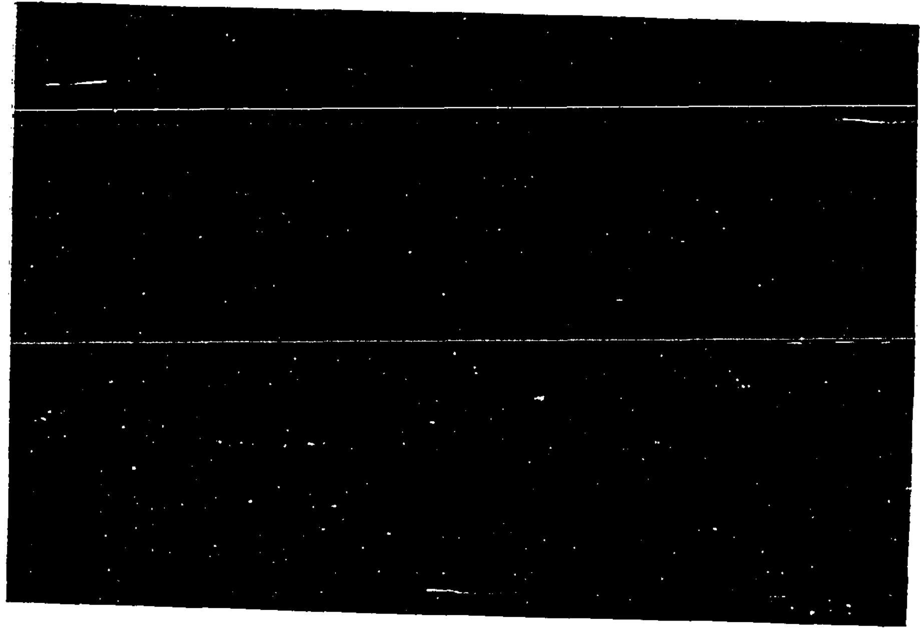


Figure 80. Pars distalis adenohypophysis. Somatotrope cell (yellow). Thyrotrope cell (blue-black). FSH gonadotrope cell (magenta)
Pig # 4. Female. 1 day
Aldehyde-thionin--PAS--orange G stain
X 400

Figure 81. Pars distalis adenohypophysis. Absence of reticular fibers in stroma
Pig # 4. Female. 1 day
Manuel's method
X 250



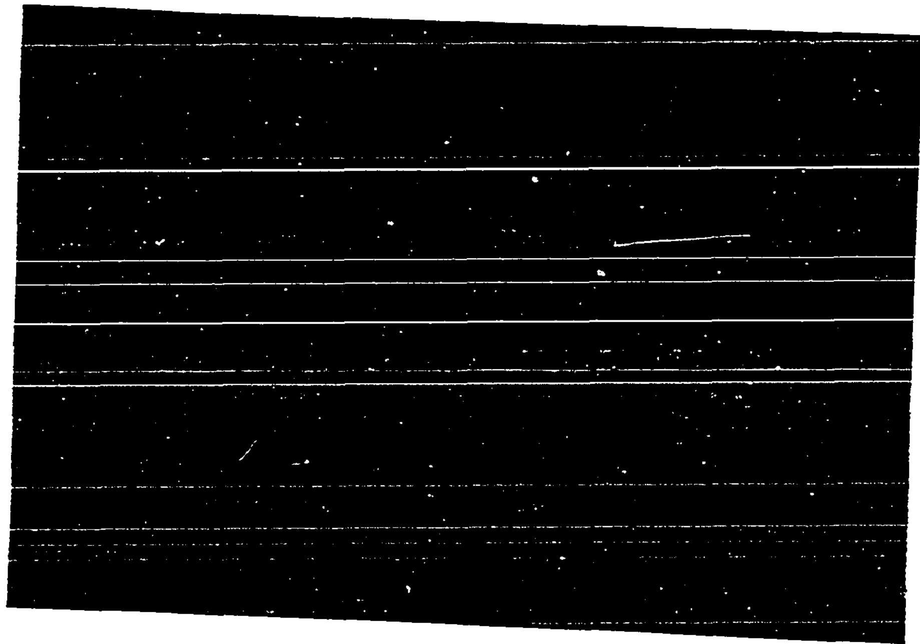
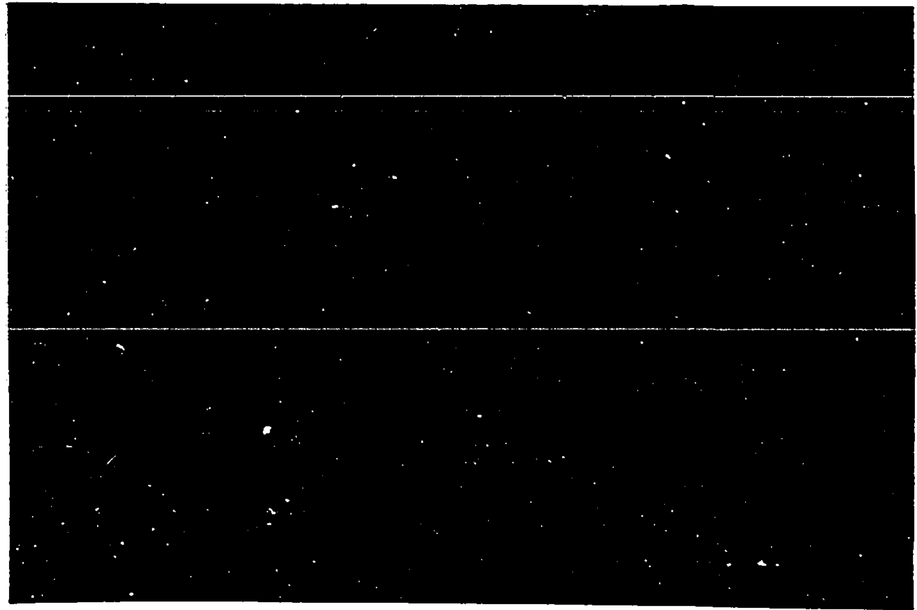


Figure 82. Pars distalis adenohypophysis. Early stages of intravascular colloid infiltration. PAS positive FSH gonadotrope cells
Pig # 1320. Male. 14 days
Aldehyde-thionin--PAS--orange G stain
X 400

Figure 83. Pars distalis adenohypophysis. Somatotrope cell (blue-green). Lactotrope cell (brick red). Thyrotrope cell (blue)
Pig # 1651. Female. 2 months
Luxol fast blue-trichrome stain
X 400



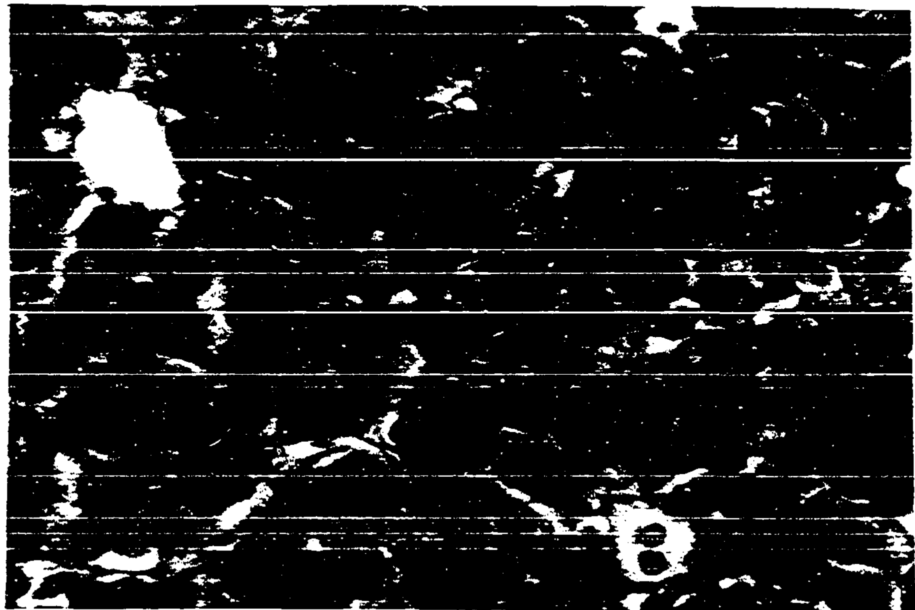


Figure 84. Pars distalis adenohypophysis. Intravascular colloid infiltration

Pig # 5250. Female. 3.5 months

Aldehyde-thionin--PAS--orange G stain

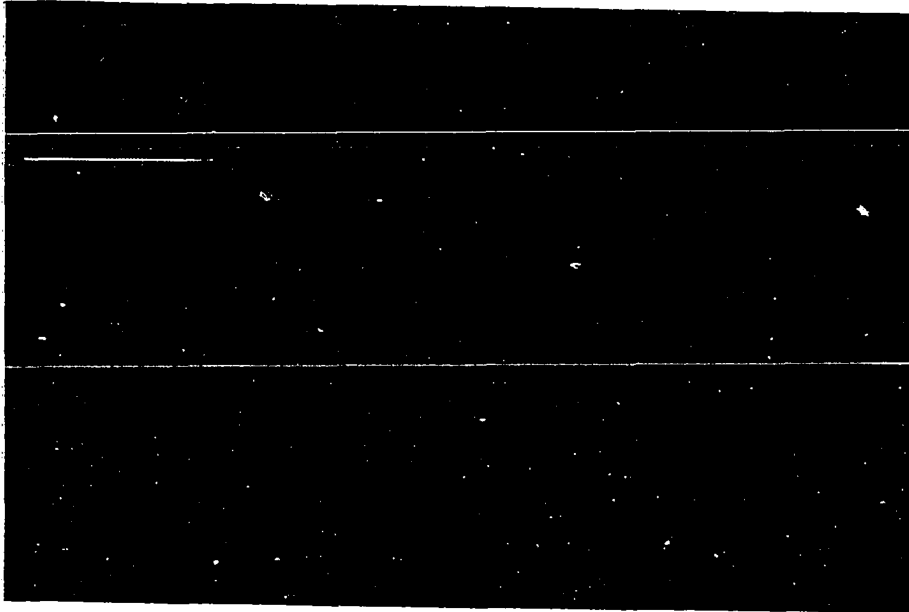
X 250

Figure 85. Pars distalis adenohypophysis. Typical arrangement of collagen fibers in stroma

Pig # 5250. Female. 3.5 months

Weigert's method

X 250



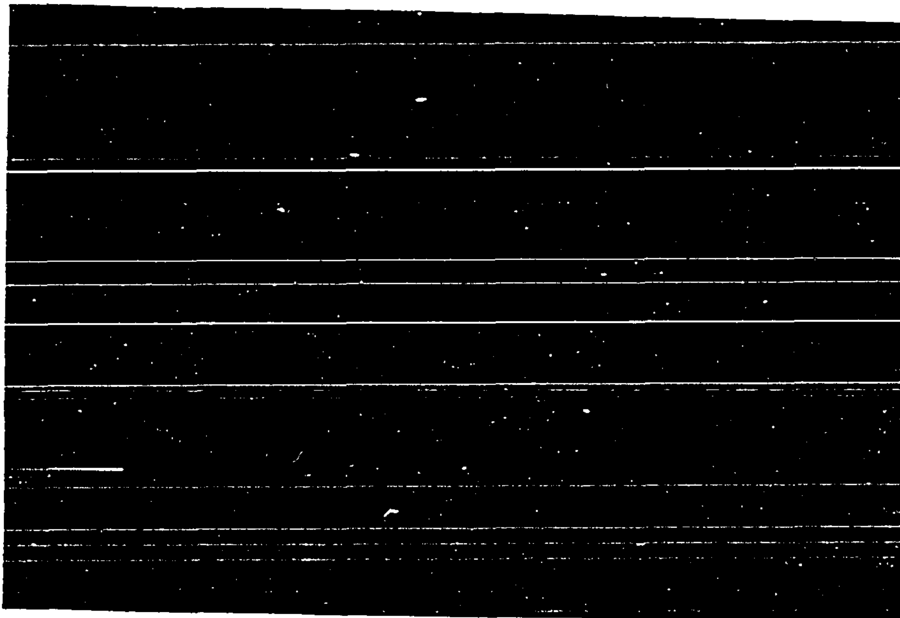
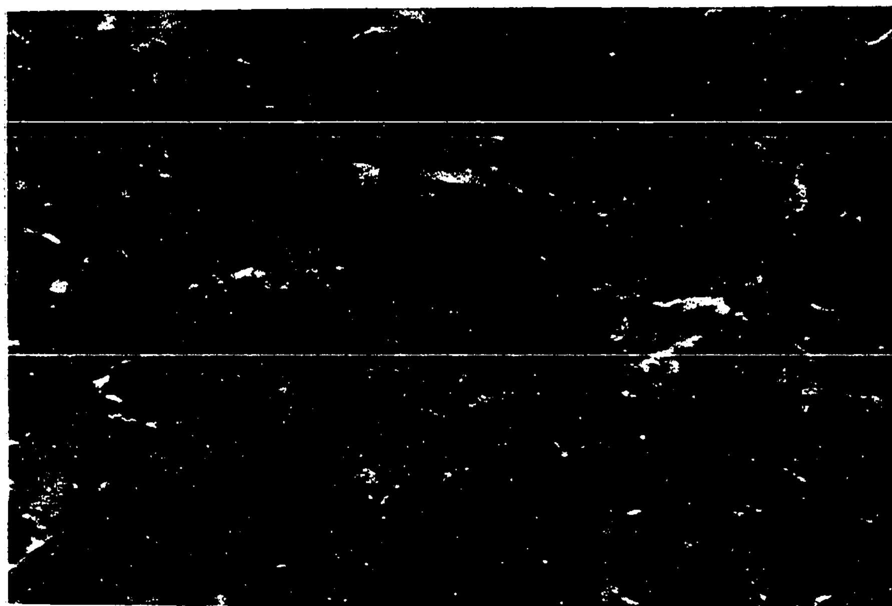


Figure 86. Pars distalis adenohypophysis. Somatotrope cell (blue-green). Thyrotrope cell (intense blue). FSH gonadotrope cell (light blue)
Pig # 5250. Female. 3.5 months
Luxol fast blue-trichrome stain
X 400

Figure 87. Pars distalis adenohypophysis. Positive crystal violet metachromasia for amyloid
Pig # 5250. Female. 3.5 months
Modified methyl violet method
X 100



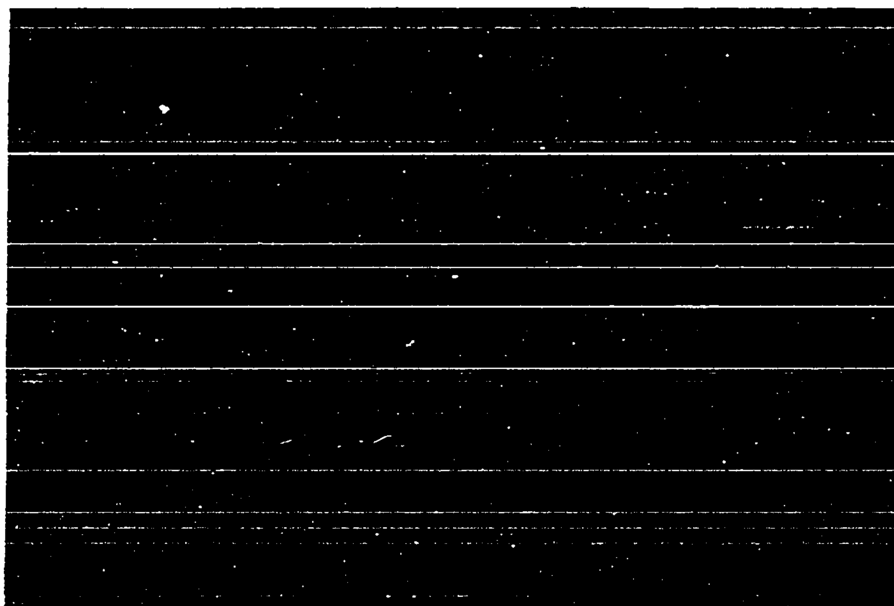
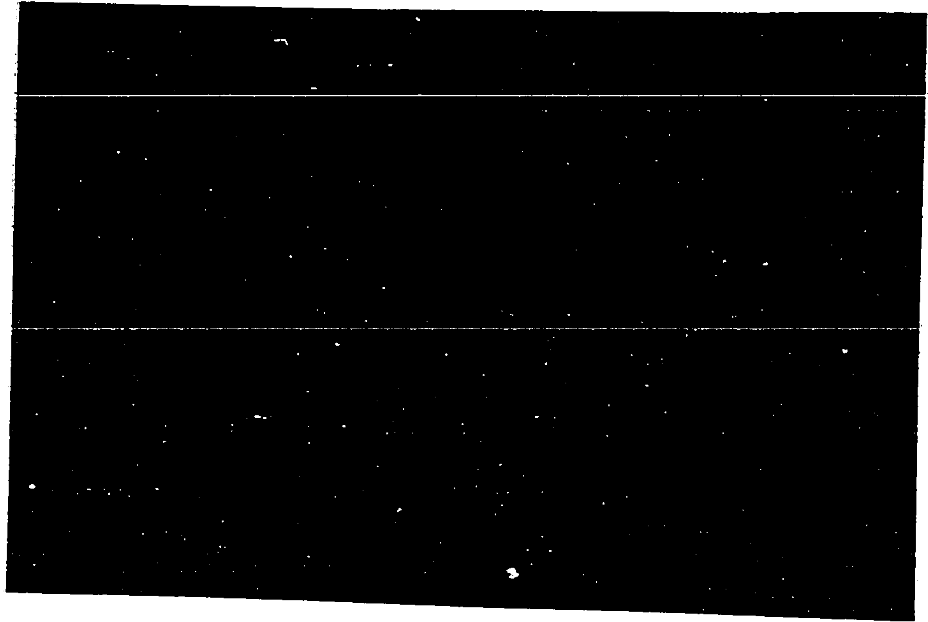


Figure 88. Pars distalis adenohypophysis. Occlusion of portal capillaries simulating septa
Pig # 9713. Female. 4 months
Aldehyde-thionin--PAS--orange G stain
X 250

Figure 89. Pars distalis adenohypophysis. Vacuoles on the periphery of occluded vessel
Pig # 9713. Female. 4 months
Aldehyde-thionin--PAS--orange G stain
X 250



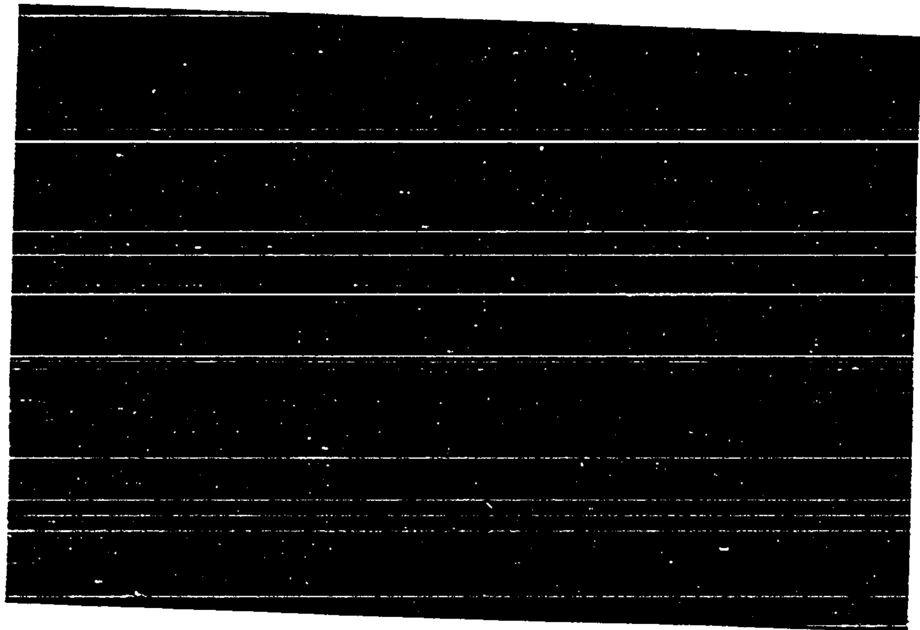
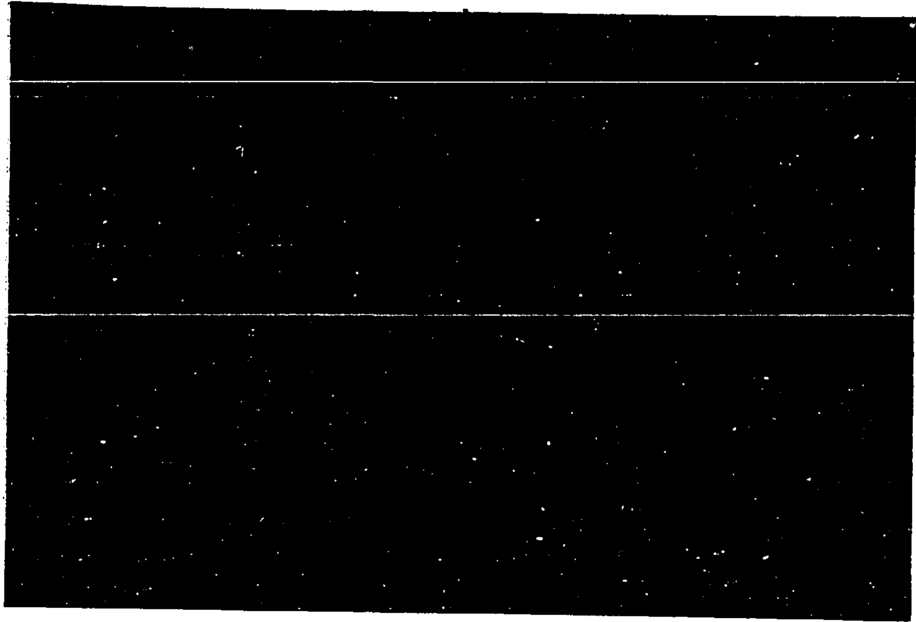


Figure 90. Pars distalis adenohypophysis. ICSH gonadotrope cells stained with lead hematoxylin
Pig # 2250. Female. 4 months
MacConaill's stain
X 400

Figure 91. Pars distalis adenohypophysis. Somatotrope cell (blue-green). Lactotrope cell (brick red). Thyrotrope cell (intense blue). FSH gonadotrope cell (light blue). ACTH cell (stellate-shaped and erythophilic)
Pig # 3923. Female. 10 months
Luxol fast blue-trichrome stain
X 400



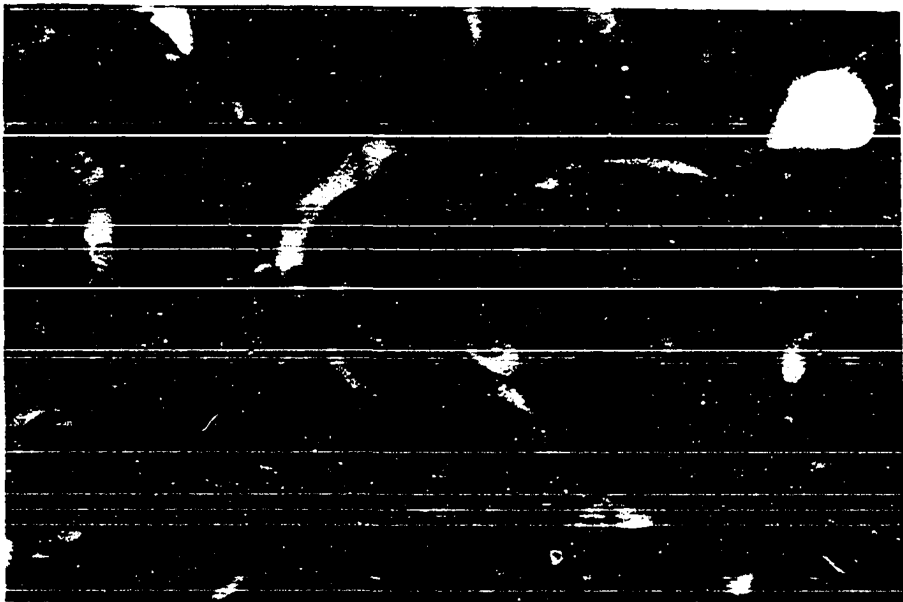
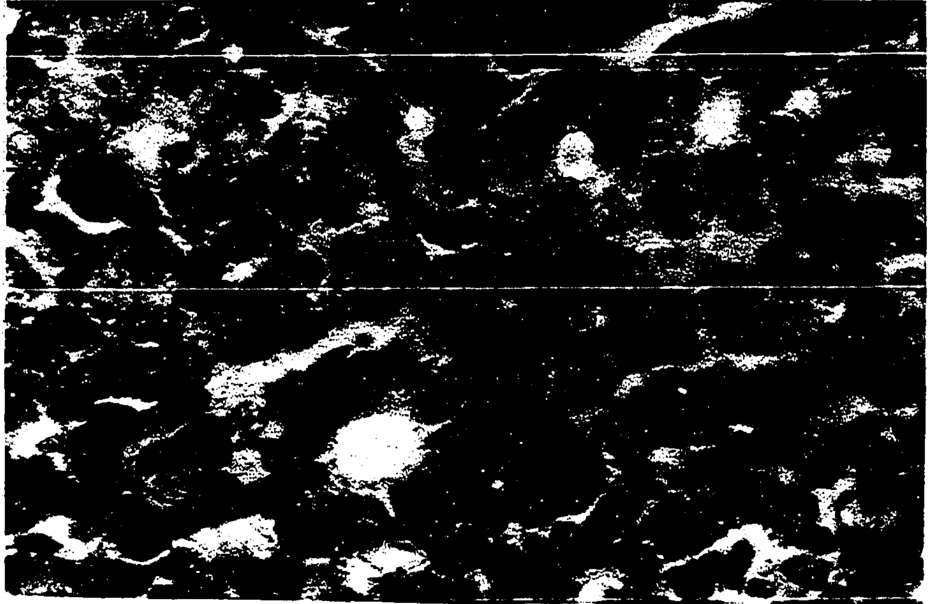


Figure 92. Pars distalis adenohypophysis. Fibrin deposition in the centrum of cell-cord as a prelude to colloid deposition
Pig # 6333. Female. 1.5 years
Mallory's phosphotungstic acid hematoxylin stain
X 400

Figure 93. Pars distalis adenohypophysis. Vacuolated FSH gonadotrope cell (purple) and ICSH gonadotrope cell (rose red)
Pig # AEC. Male. 1.5 years
Aldehyde-thionin--PAS--orange G stain
X 400



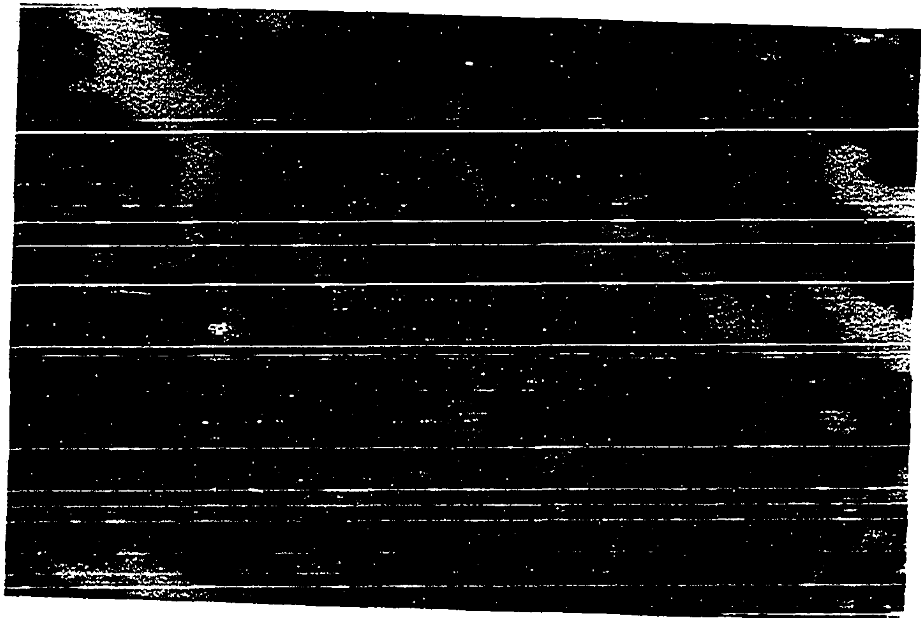


Figure 94. Pars distalis adenohypophysis. Somatotrope cell (yellow). Lactotrope cells (brick red). Thyrotrope cells (blue-black). FSH gonadotrope cells (purple). ICSH gonadotrope cell (rose red)

Pig # 1203. Male. 4 years

Aldehyde-thionin--PAS--orange G stain

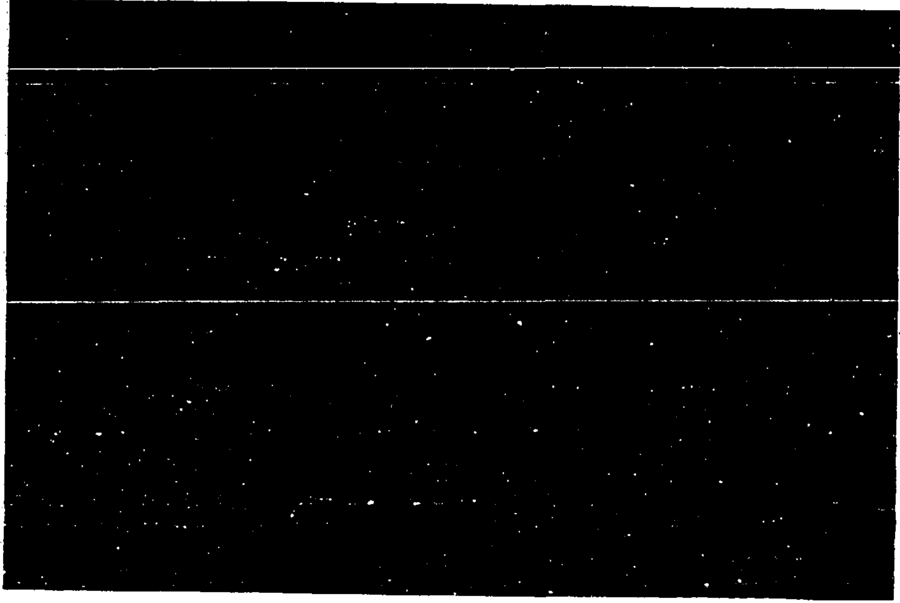
X 400

Figure 95. Pars distalis adenohypophysis. Cells undergoing autolysis

Pig # 5132. Female. 4 years

Alcian blue--PAS stain

X 250



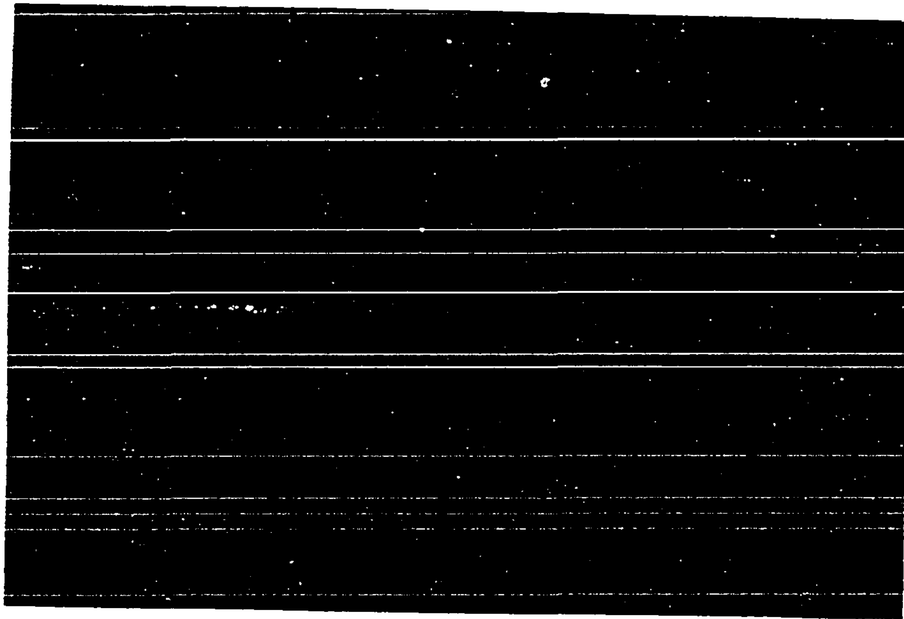


Figure 96. Pars distalis adenohypophysis. Somatotrope cell (orange). Lactotrope cell (red). Thyrotrope cell (light green). ICSH gonadotrope cell (violet)

Pig #BB-5. Female. 5.5 years

Aldehyde-fuchsin-trichrome stain

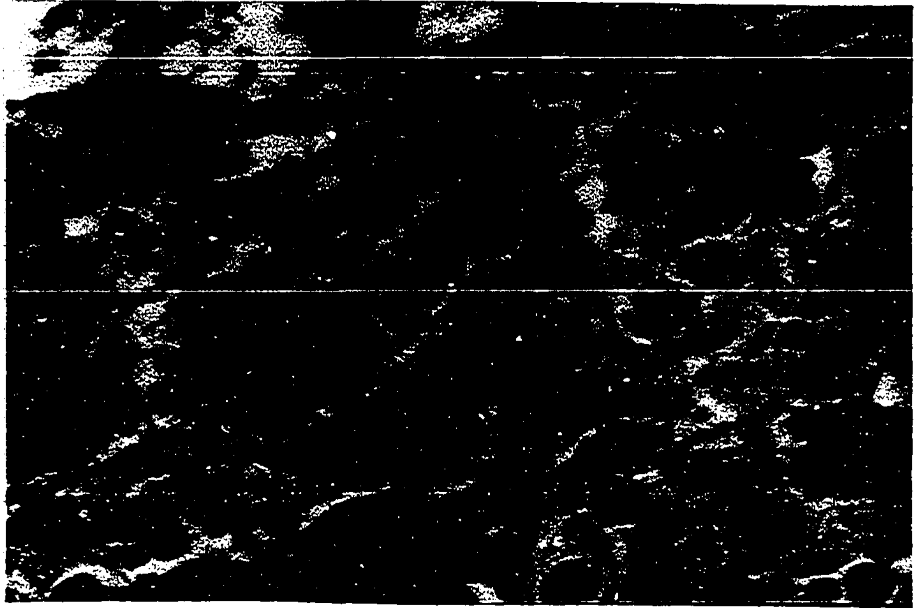
X 250

Figure 97. Pars distalis adenohypophysis. Increased density of collagen fibers. Distinct lobular arrangement

Pig # 3093. Female. 7 years

Weigert's method

X 250



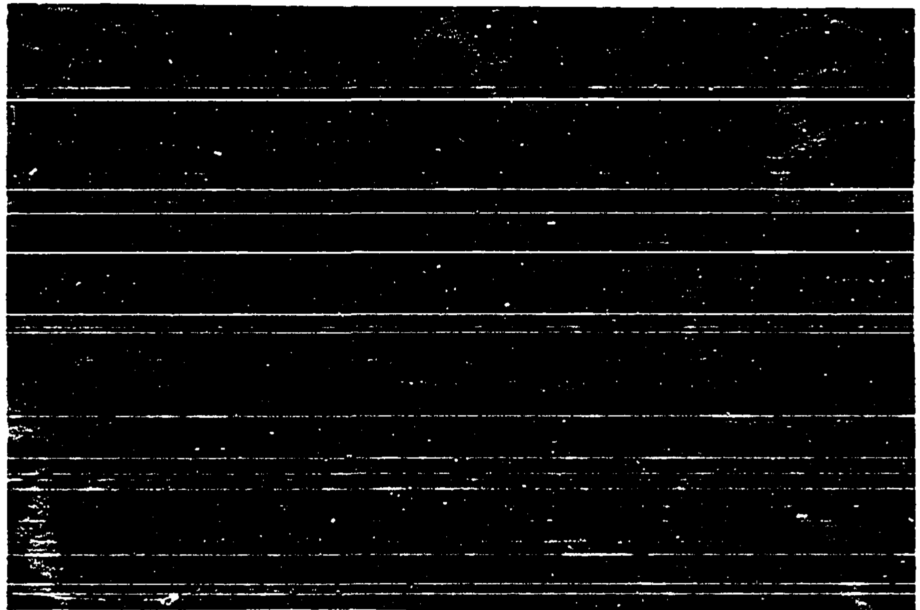
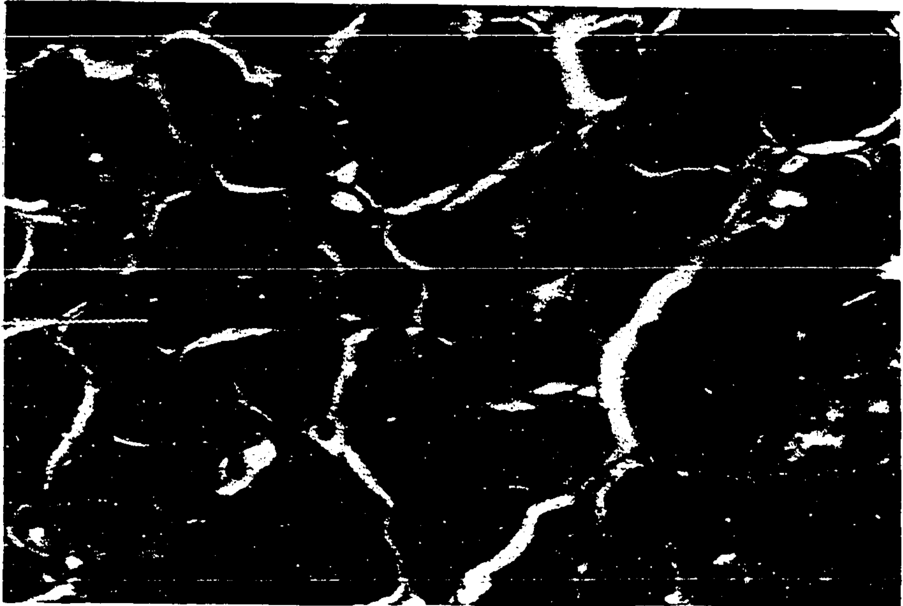


Figure 98. Pars distalis adenohypophysis. Increased number of lactotrope cells (brick red)
Pig #FH-4. Male. 7.5 years
Luxol fast blue-trichrome stain
X 400

Figure 99. Pars distalis adenohypophysis. Intercellular and follicular colloid
Pig #ET-4. Female. 7.5 years
Aldehyde-thionin--PAS--orange G stain
X 400



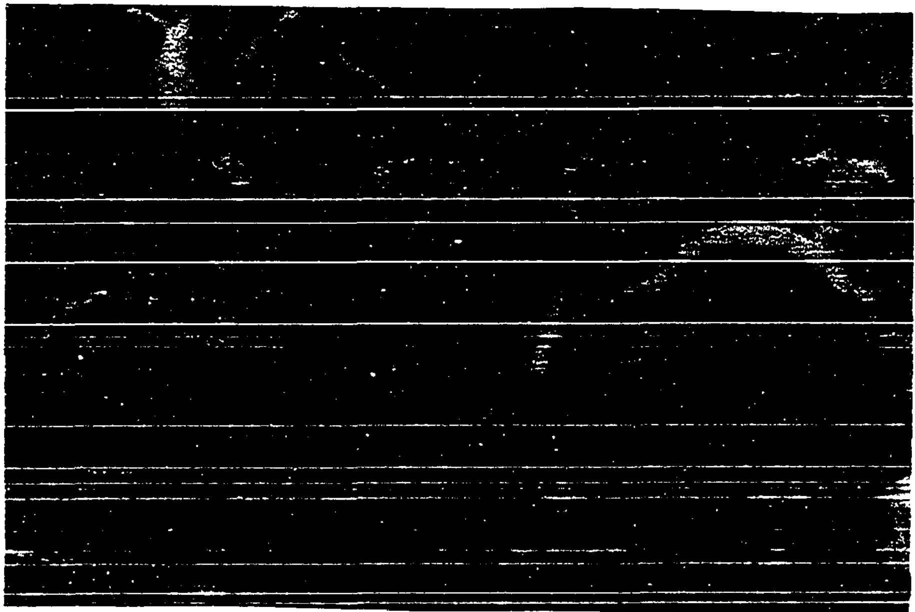


Figure 100. Pars distalis adenohypophysis. Treatment with trichloroacetic acid, 2.5 percent. ICSH gonadotrope cell (red)

Pig #D-5. Female. 8 years

Alcian blue--PAS stain

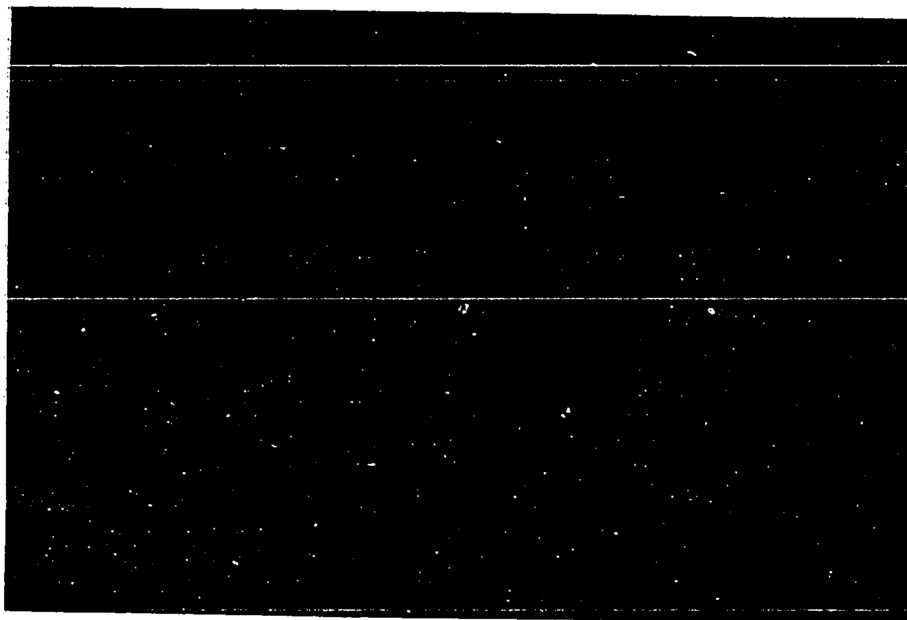
X 400

Figure 101. Pars distalis adenohypophysis. Hyperplastic chromophobic nodule. Partial occlusion of portal capillary adjacent to the nodule

Pig # N.F. Female. 8 years

Alcian blue--PAS stain

X 250



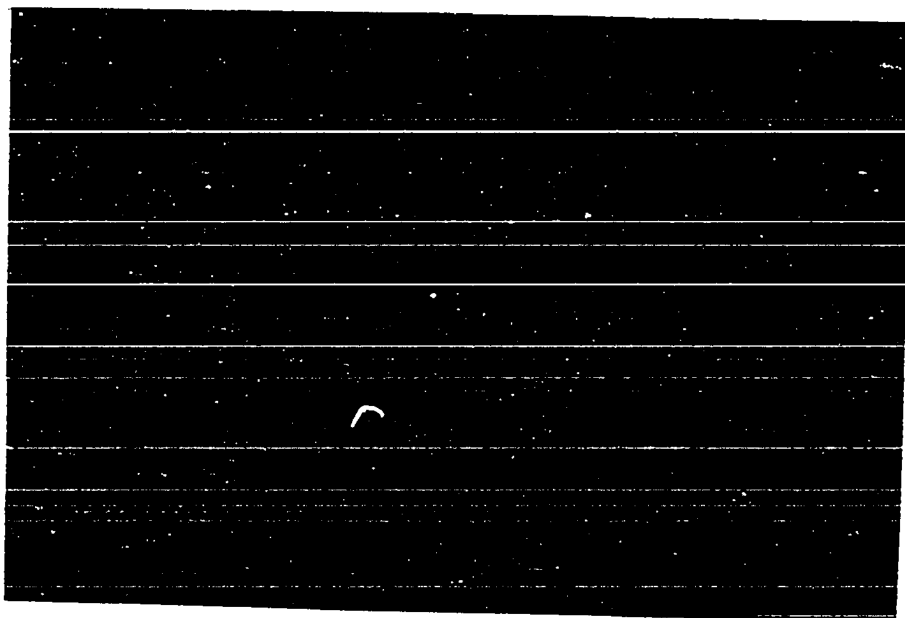
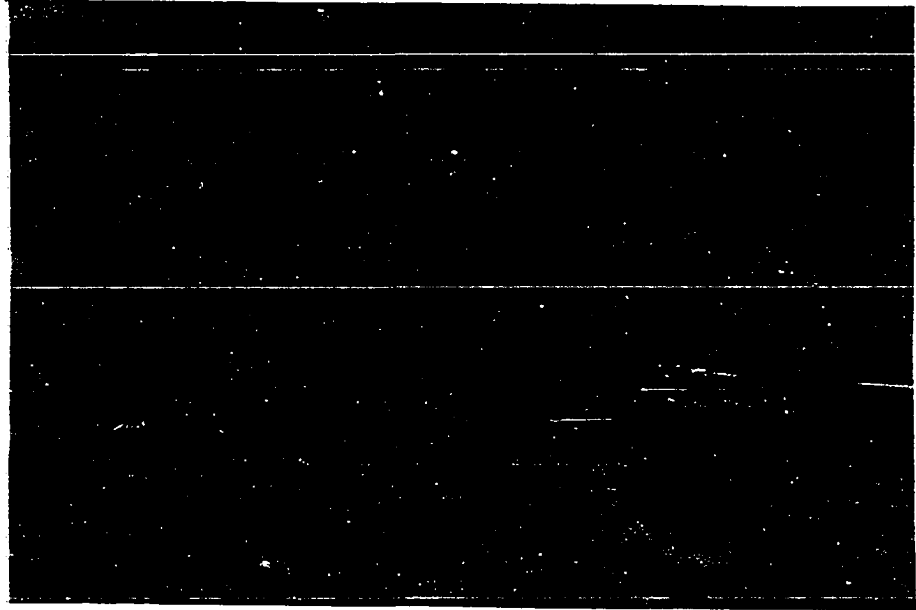


Figure 102. Pars distalis adenohypophysis. Severe colloid infiltration of veins
Pig # N.F. Female. 8 years
Aldehyde-thionin--PAS--orange G stain
X 250

Figure 103. Pars distalis adenohypophysis. Increased density of collagen fibers in the stroma
Pig # 1561. Female. 8.5 years
Aldehyde-fuchsin-trichrome stain
X 250



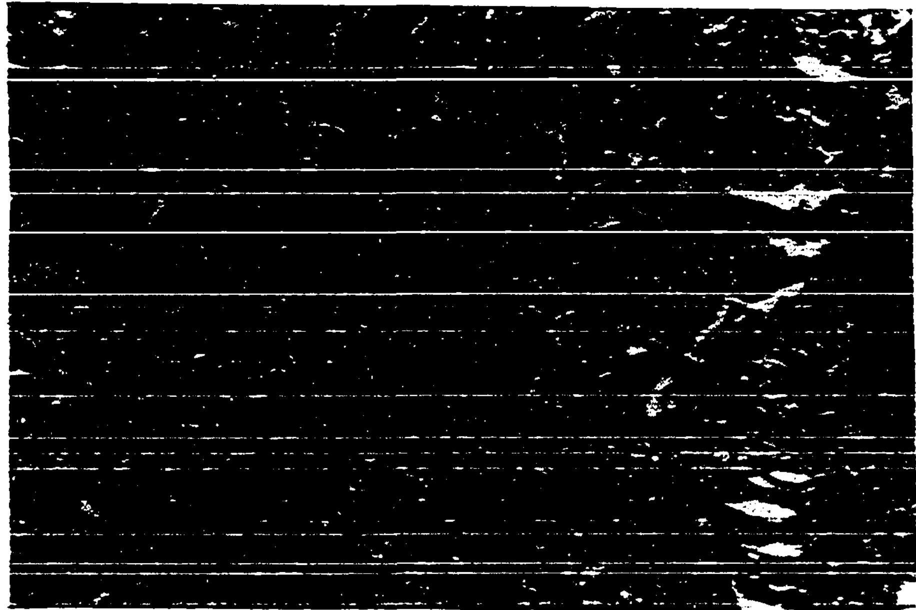
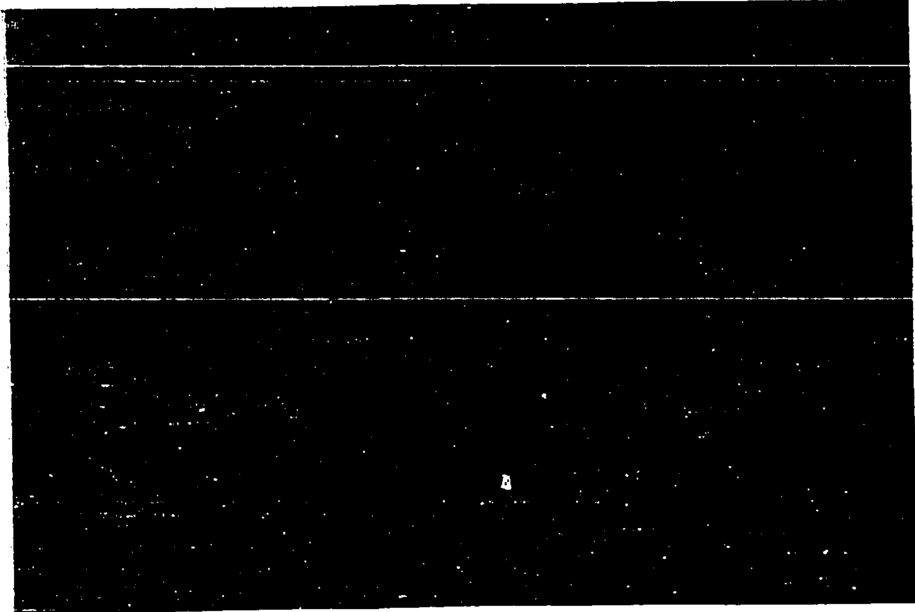


Figure 104. Pars distalis adenohypophysis. Postganglionic autonomic fibers
Pig # 1561. Female. 8.5 years
Bielschowsky's stain
X 250

Figure 105. Pars distalis adenohypophysis. Increased density and hyalinization of collagen fibers
Pig # 1561. Female. 8.5 years
Weigert's method
X 250



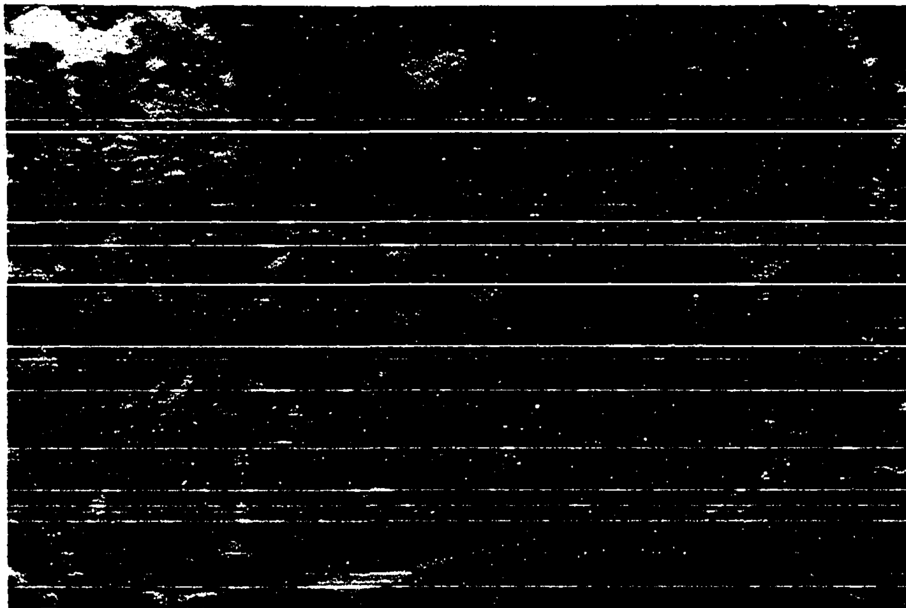


Figure 106. Pars distalis adenohypophysis. Increase in ground matrix. Alcian blue staining of the above

Pig # 1561. Female. 8.5 years

Performic acid-alcian blue

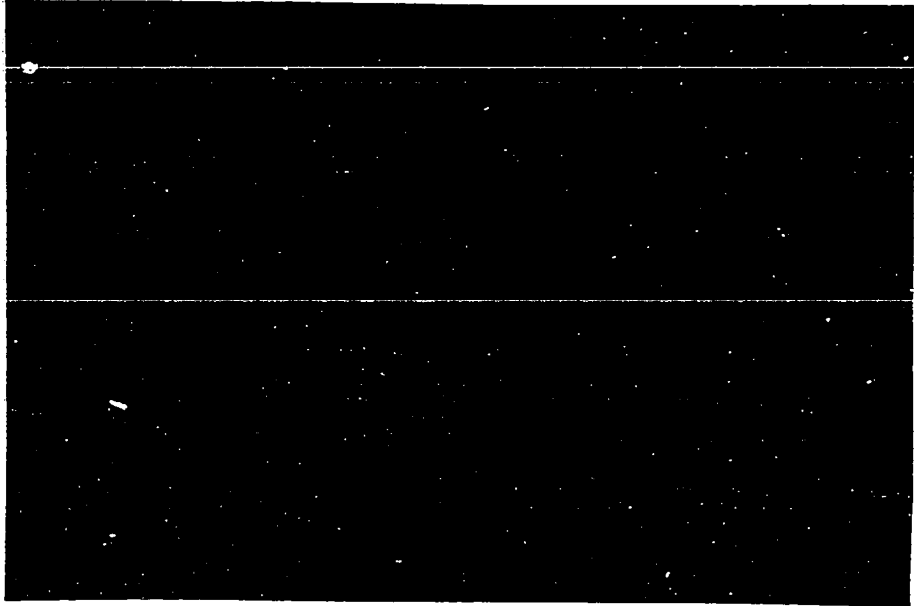
X 250

Figure 107. Pars distalis adenohypophysis. Progressive intravascular colloid infiltration. Avascular hyperplasia of chromophobe cells

Pig # 1561. Female. 8.5 years

Aldehyde-thionin--PAS--orange G stain

X 250



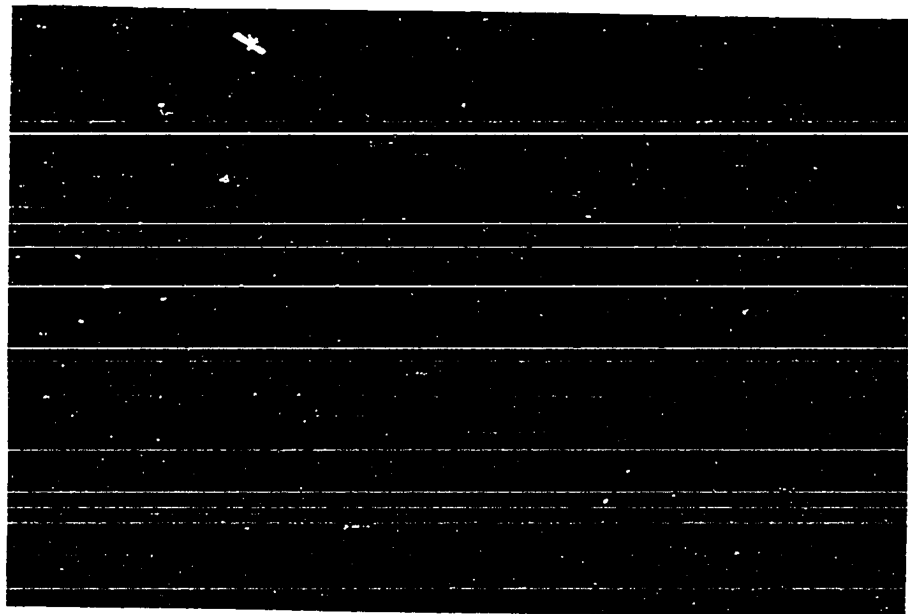


Figure 108. Pars distalis adenohypophysis. Positive staining of colloid with crystal violet metachromasia

Pig # 254. Female. 9 years

Modified methyl violet method

X 40

Figure 109. Pars distalis adenohypophysis. Follicular colloid

Pig # 254. Female. 9 years

Alcian blue--PAS stain

X 250

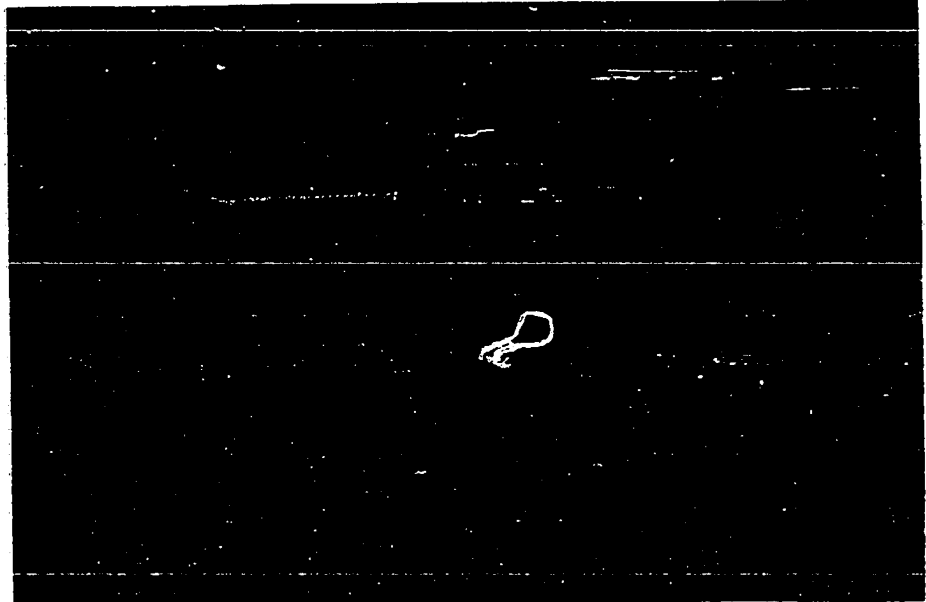
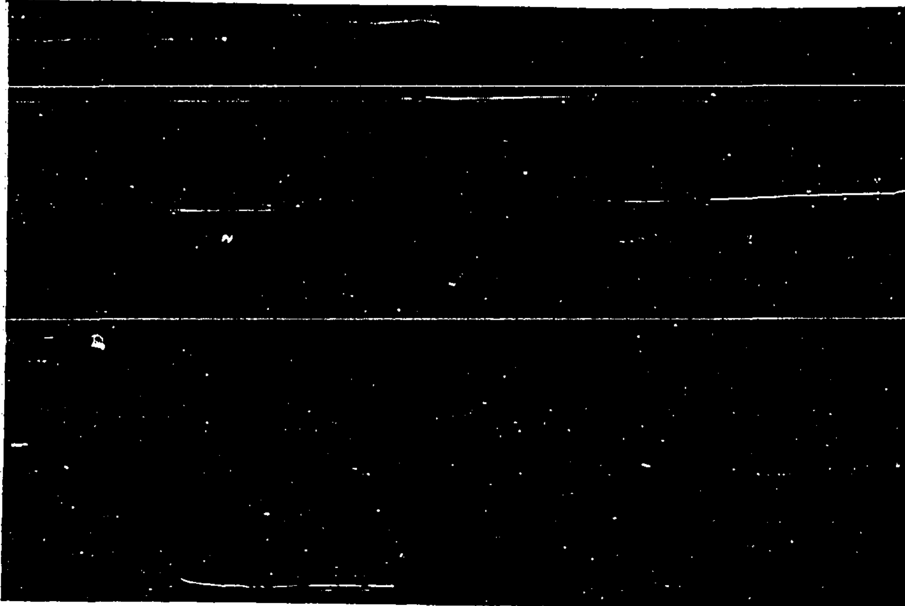


Figure 110. Pars distalis adenohypophysis. Degranulation of cells contributing to increased number of chromophobe cells. Increase in relative proportion of thyrotrope cells
Pig Merrick. Female. 10 years
Aldehyde-thionin--PAS--orange G stain
X 250

Figure 111. Pars distalis adenohypophysis. Necrosis of portal capillaries and parenchymal cells
Pig Merrick. Female. 10 years
Aldehyde-thionin--PAS--orange G stain
X 250



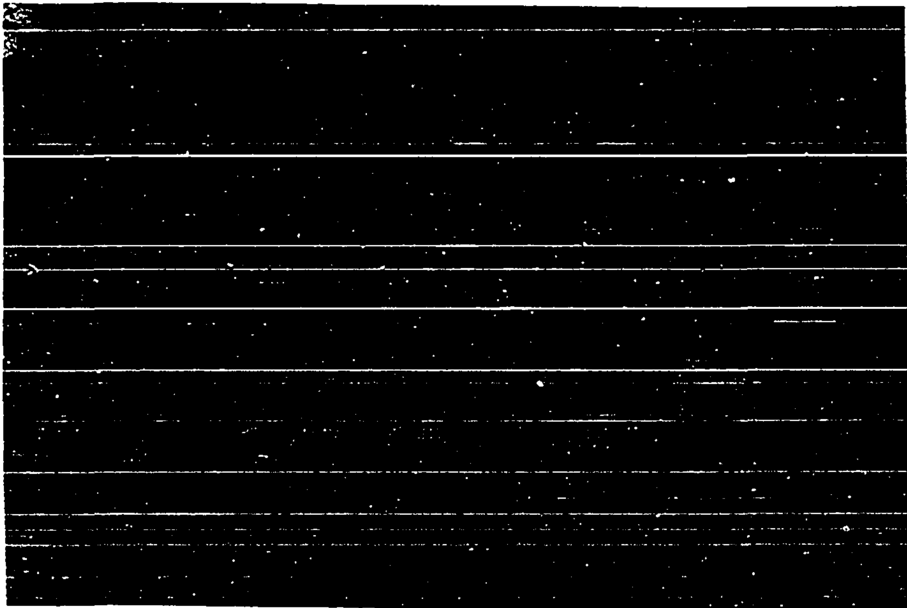


Figure 112. Pars distalis adenohypophysis. Cell-types. Increased number of chromophobe cells. Binucleated FSH gonadotrope cell (purple). Augmented size of ICSH gonadotrope cell (rose red)

Pig # 37-258. Female. 10 years

Aldehyde-thionin--PAS--orange G stain

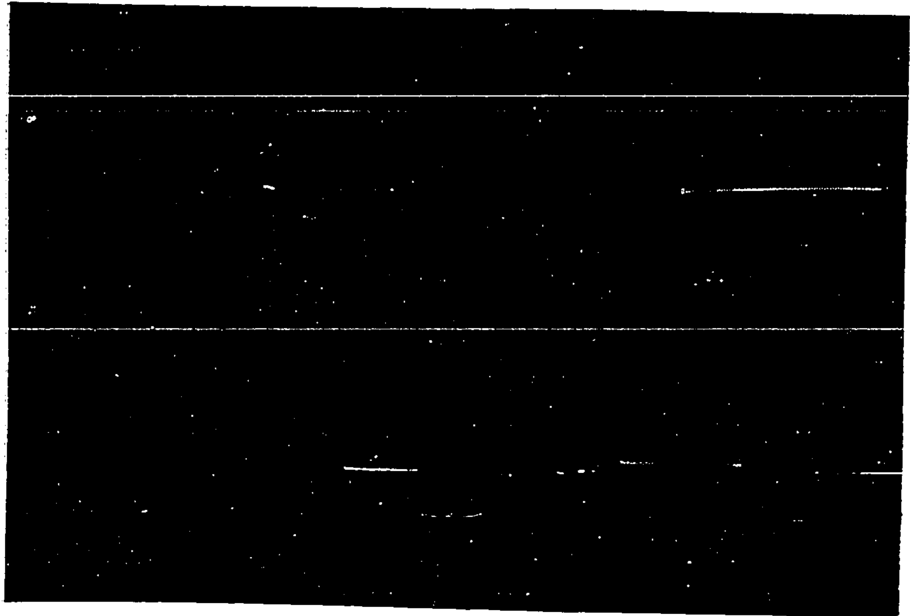
X 400

Figure 113. Pars distalis adenohypophysis. Increased density of collagen fibers in the stroma

Pig # 37-258. Female. 10 years

Verhoeff's stain

X 250



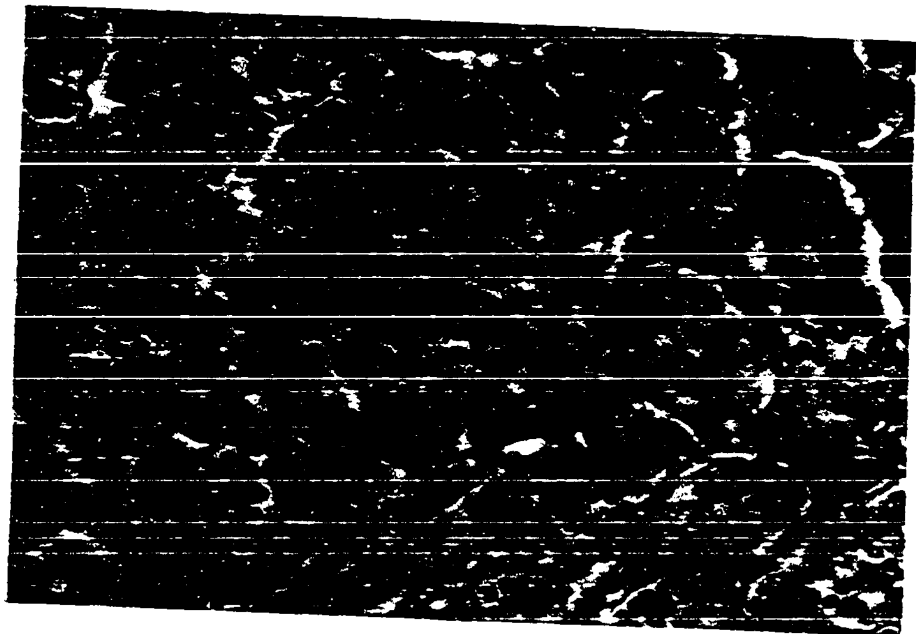
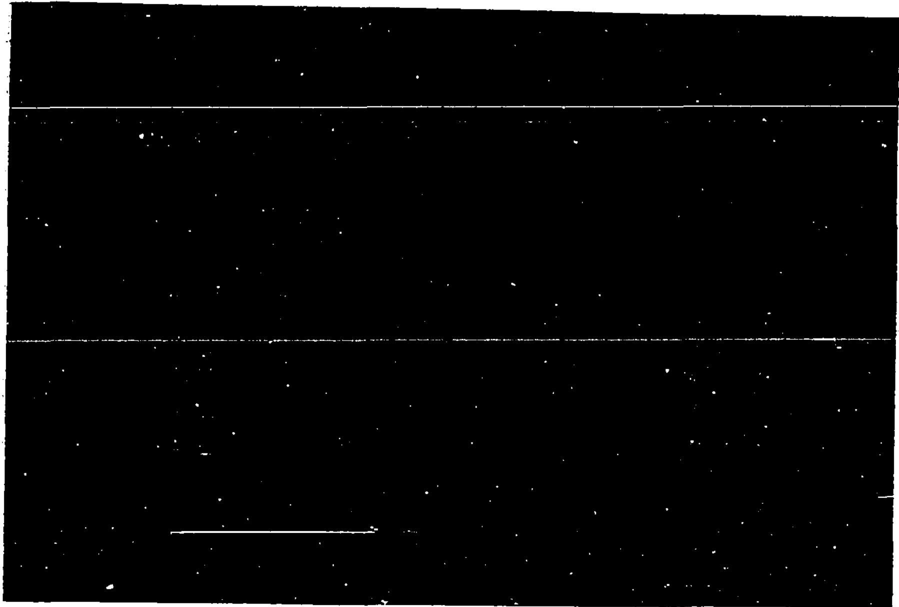


Figure 114. Pars distalis adenohypophysis. Reticular fibers in the stroma
Pig # 37-258. Female. 10 years
Manuel's method
X 250

Figure 115. Pars distalis adenohypophysis. Intracellular and interstitial colloid
Pig # 37-258. Female. 10 years
Aldehyde-thionin--PAS--orange G stain
X 1000



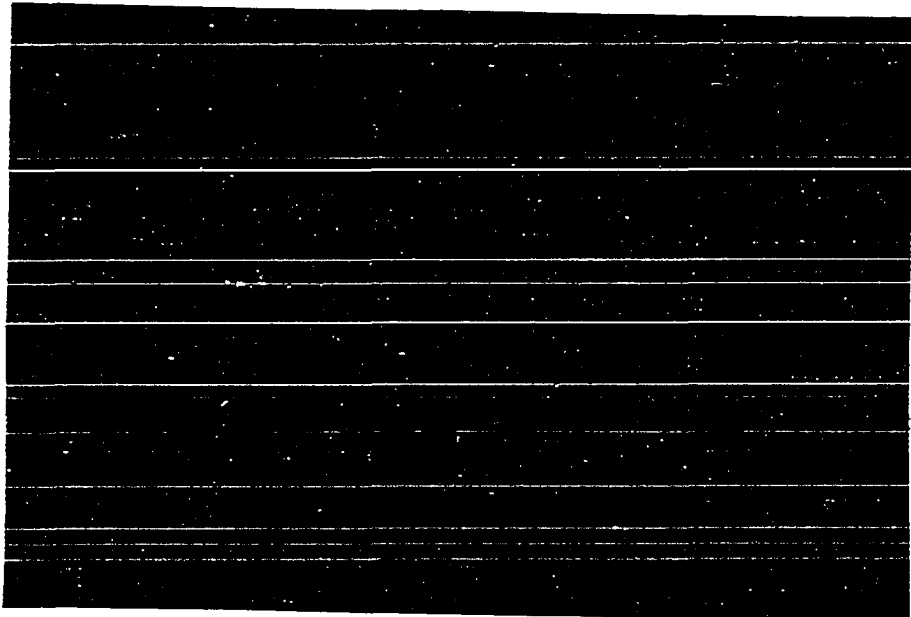
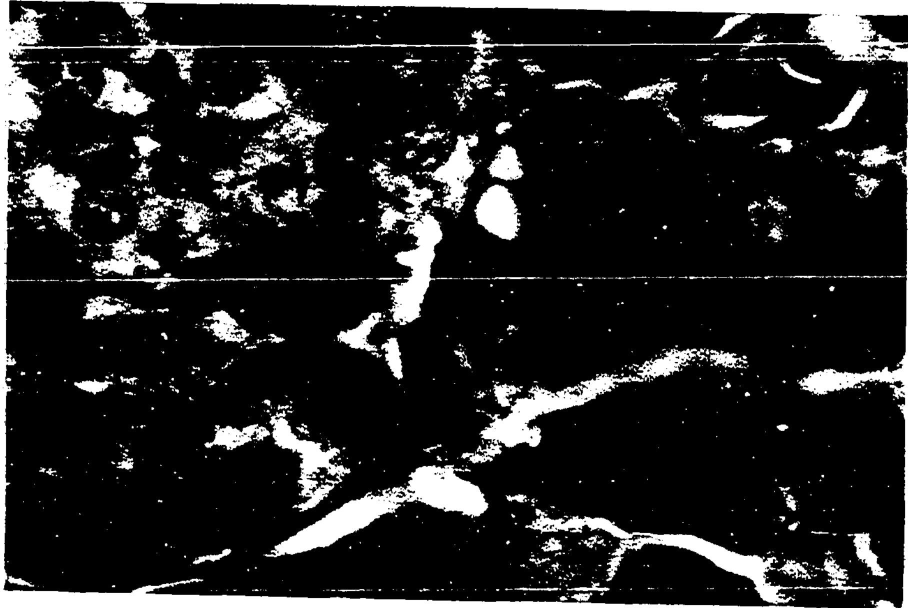


Figure 116. Pars distalis adenohypophysis. Increased size of somatotrope (blue-green) and lactotrope (brick red) cells. Cupping of somatotrope and FSH gonadotrope cells
Pig # 37-258. Female. 10 years
Luxol fast blue-trichrome stain
X 1000

Figure 117. Pars distalis adenohypophysis. Increased size of FSH gonadotrope cell (purple). Somatotrope cell (yellow). Lactotrope cell (light magenta). Thyrotrope cell (blue black)
Pig # 37-258. Female. 10 years
Aldehyde-thionin--PAS--orange G stain
X 1000



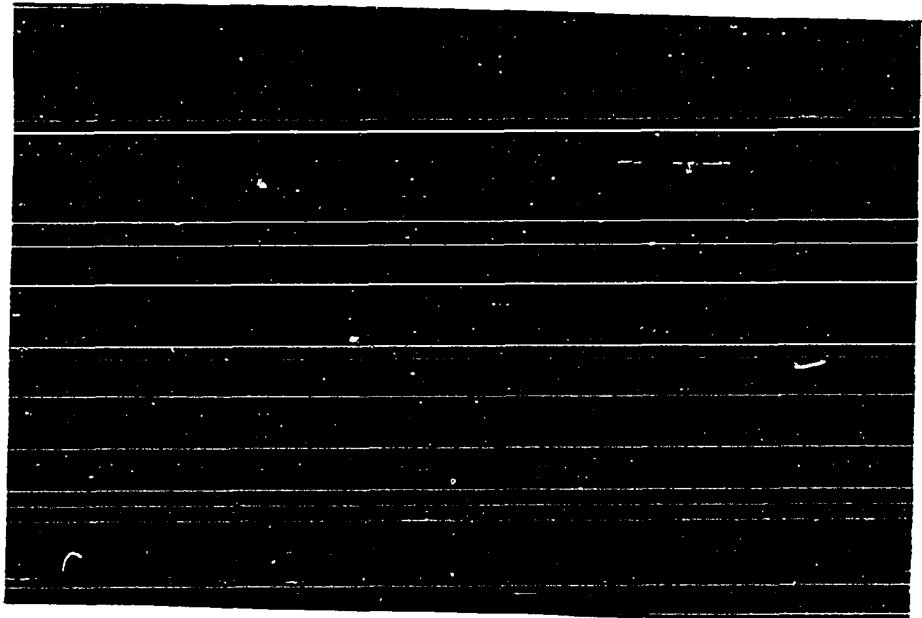


Figure 118. Pars distalis adenohypophysis. Colloid follicles

Pig Merrick. Female. 10 years

Alcian blue--PAS stain

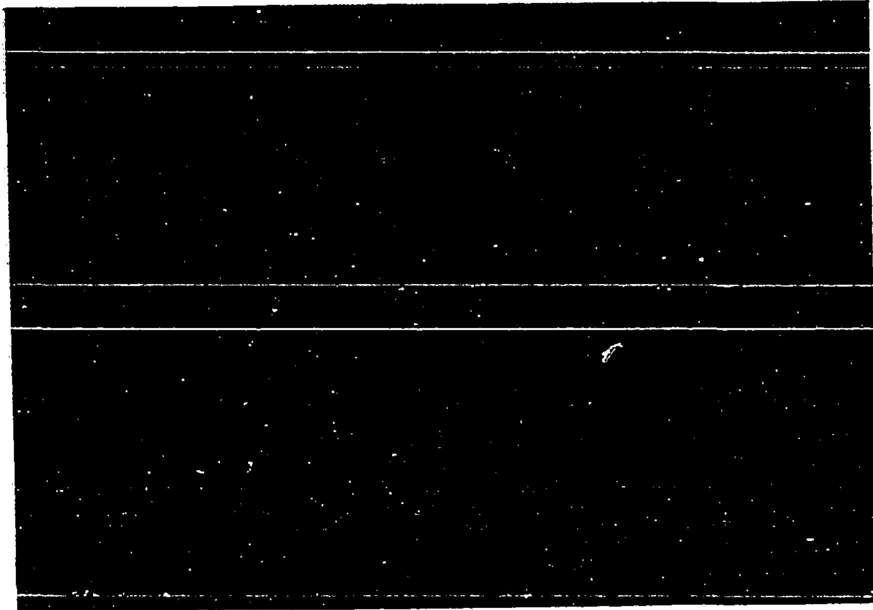
X 250

Figure 119. Pars paraneuralis. Permeation by cavum hypophysis. Similarity with homologue of the canine

Pig # 999. Male. 1 month

Chrome alum hematoxylin stain

X 250



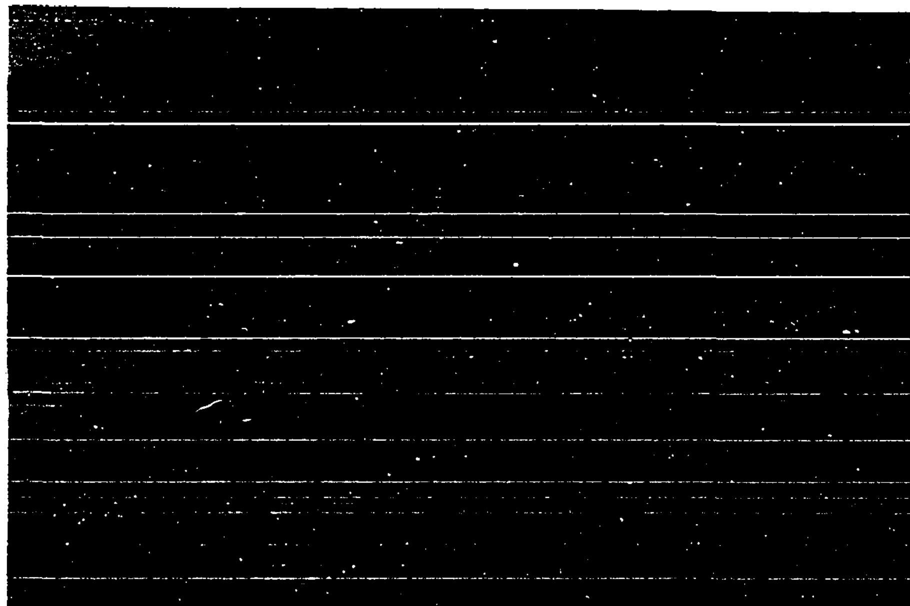


Figure 120. Pars infundibularis adenohypophysis. Intricacy of postganglionic autonomic fibers in mantel plexus

Pig # 3184. Female. 20 days

Bielschowsky's stain

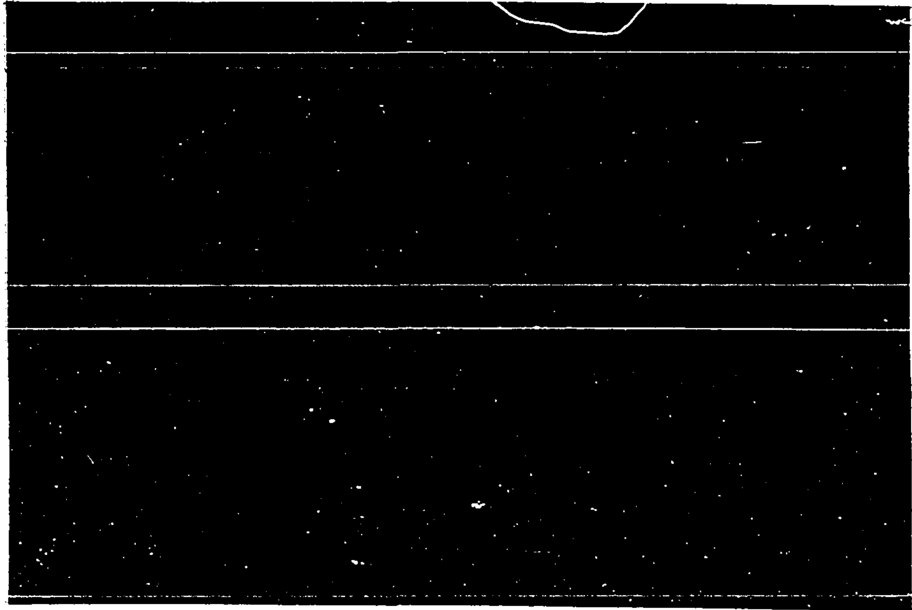
X 250

Figure 121. Pars infundibularis adenohypophysis and radix infundibuli. Cellular character of the zona interna

Pig # 5093. Female. 9 months

Aldehyde-thionin--PAS--orange G stain

X 100



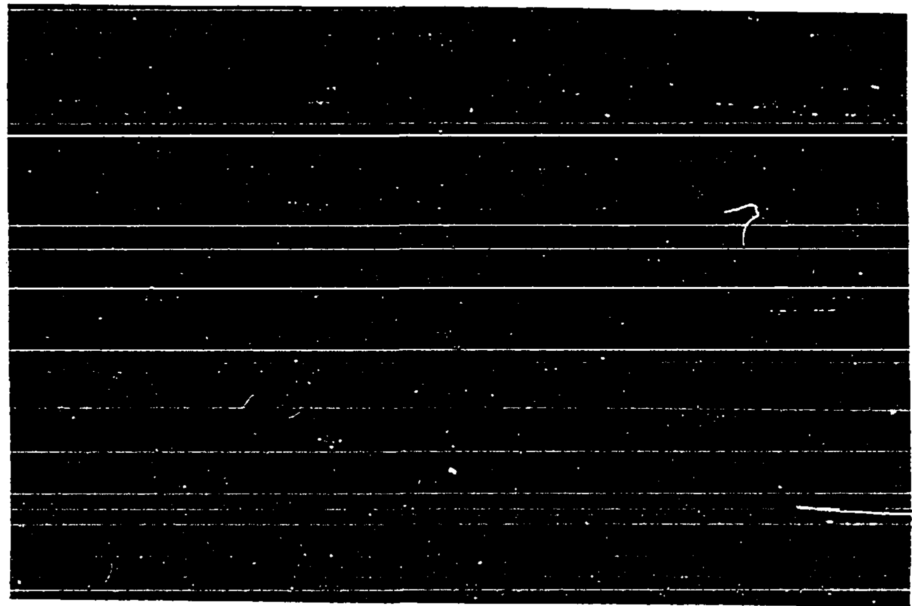
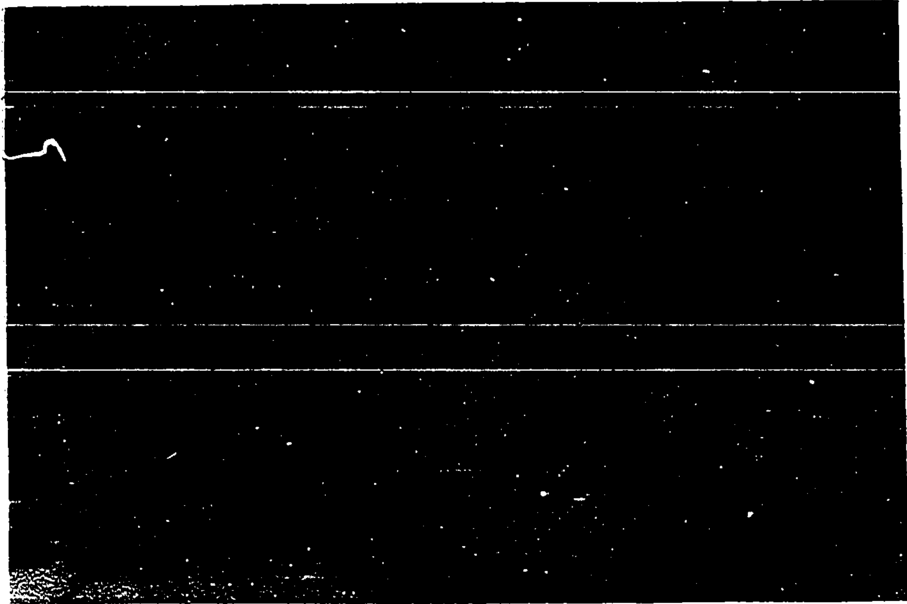


Figure 122. Pars infundibularis adenohypophysis. PAS positive cells. Colloid follicles
Pig # 1350. Female. 6 years
Alcian blue--PAS stain
X 250

Figure 123. Pars infundibularis adenohypophysis. Decrease in the number of chief cells. Large size of PAS positive cells
Pig # 9090. Female. 9 years
Aldehyde-thionin--PAS--orange G stain
X 400



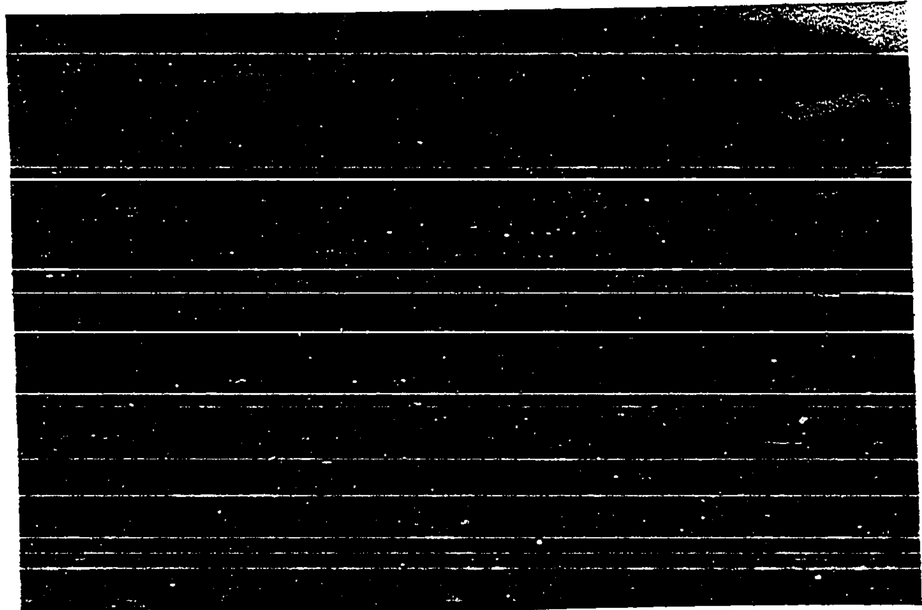


Figure 124. Pars intermedia adenohypophysis. Tributary of caudal hypophysial vein. Interstitial collagen fibers in stroma
Pig # 4471. Female. 1.5 years
Aldehyde-fuchsin-trichrome stain
X 100

Figure 125. Pars intermedia adenohypophysis. Follicular colloid. Colloid in the cavum hypophysis
Pig #FH-4. Male. 7.5 years
Aldehyde-thionin--PAS--orange G stain
X 100

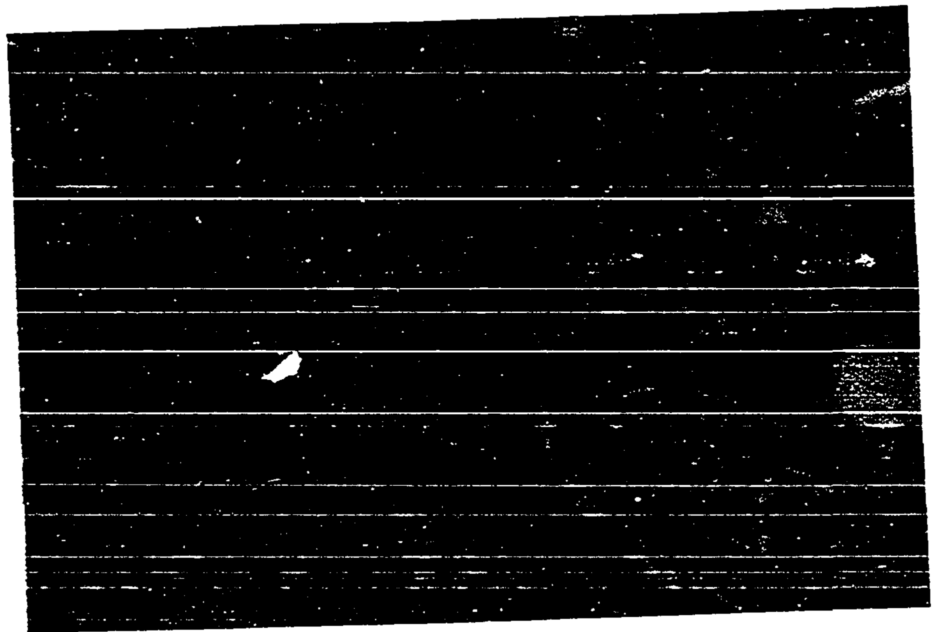


Figure 126. Pars intermedia adenohypophysis. Follicular colloid. Partial occlusion of vein. NSM and blood vessels along the peripheral margin of pars distalis neurohypophysis

Pig #FH-4. Male. 7.5 years

Alcian blue--PAS stain

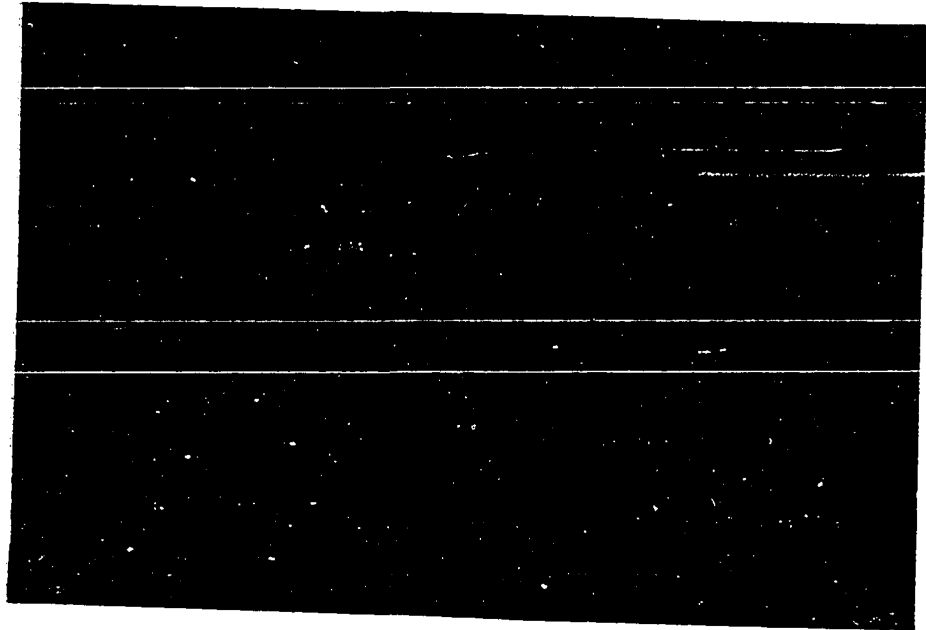
X 250

Figure 127. Radix infundibuli. Poor development of zones and mantel plexus

Pig # 4. Female. 1 day

Chrome alum hematoxylin stain

X 400



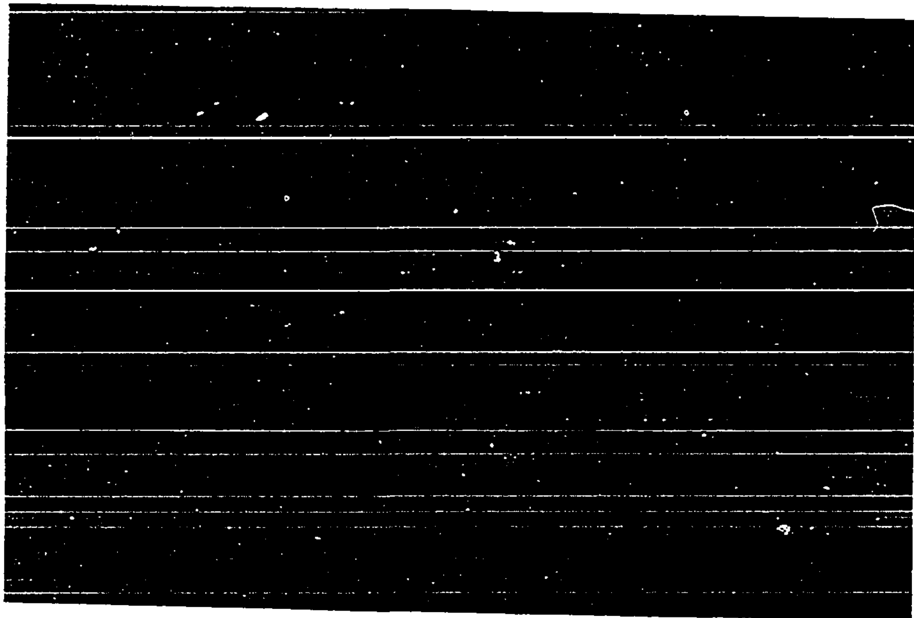
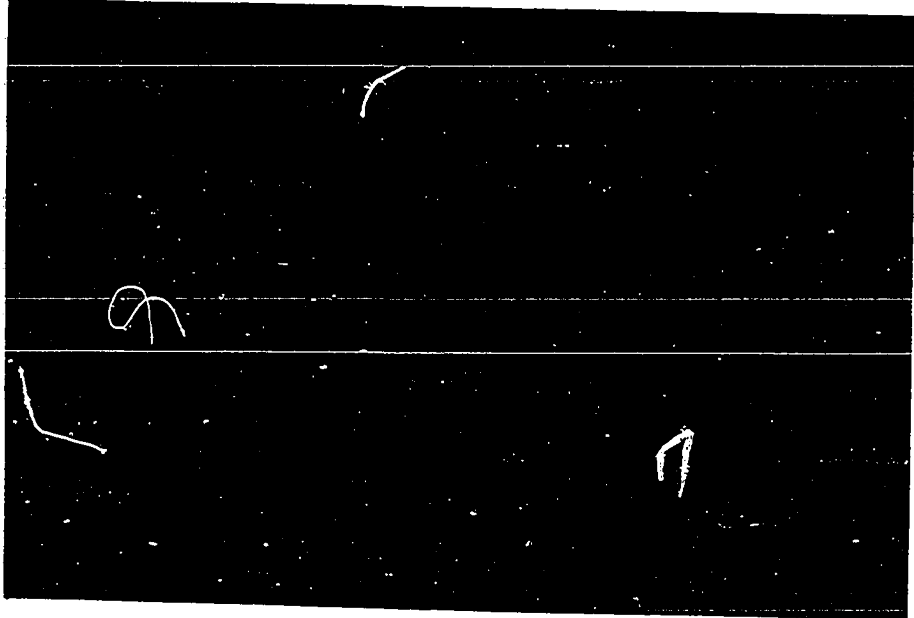


Figure 128. Radix infundibuli. Ground matrix sparse.
Typical distribution of collagen fibers in perivascular region
Pig AEC. Male. 1.5 years
Alcian blue--PAS stain
X 250

Figure 129. Radix infundibuli. Increased density of perivascular collagen fibers
Pig # 3093. Female. 7 years
Weigert's method
X 100



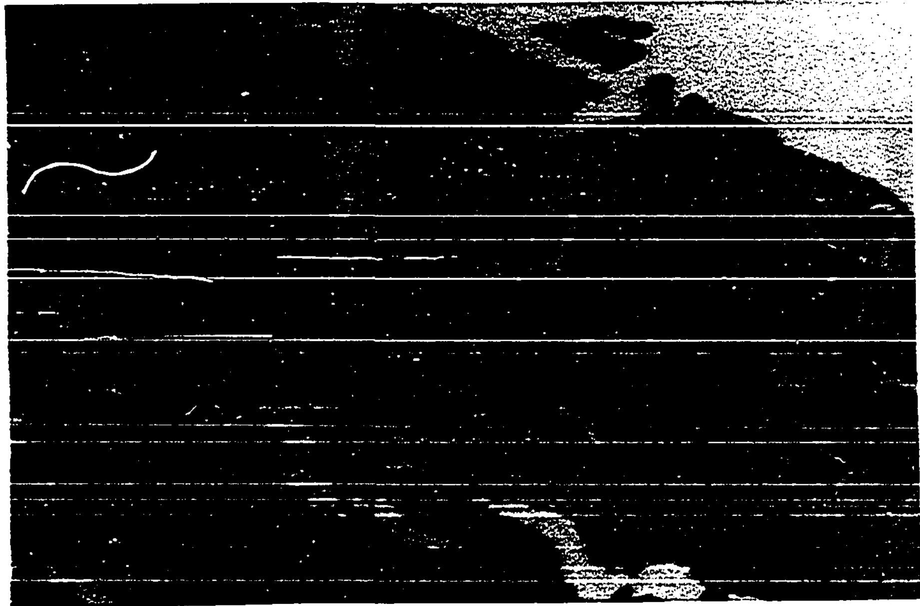


Figure 130. Radix infundibuli. Increased density of perivascular matrix and fibers. Initial stage of colloid infiltration on endothelium of the vein

Pig # 3093. Female. 7 years

Alcian blue--PAS stain

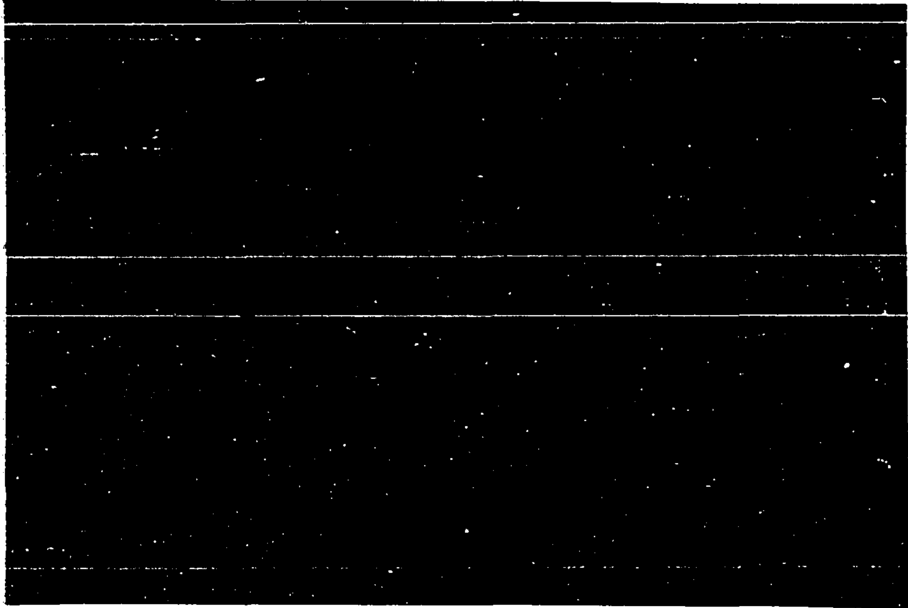
X 250

Figure 131. Radix infundibuli. Looping of blood vessels. Increased density of perivascular collagen fibers

Pig # 254. Female. 9 years

Verhoeff's stain

X 250



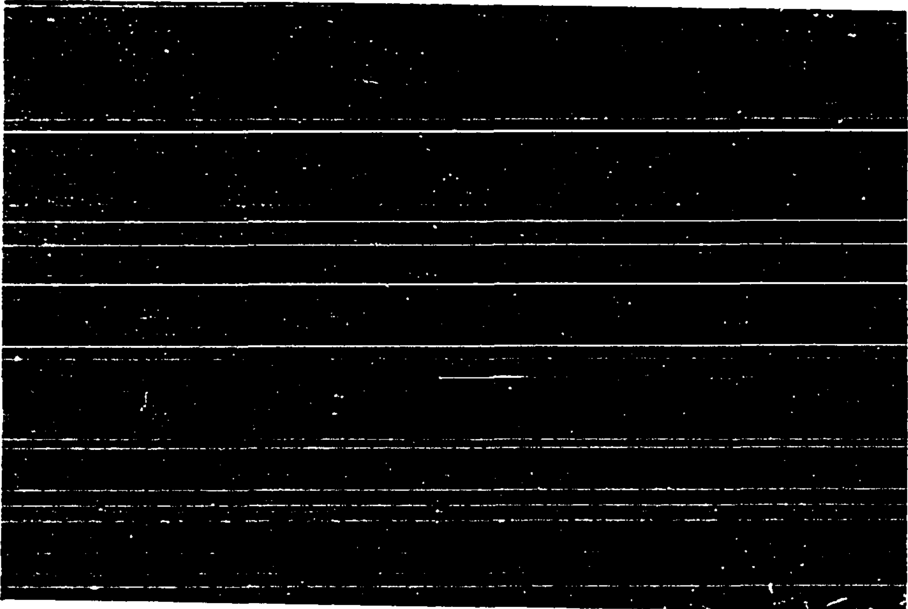
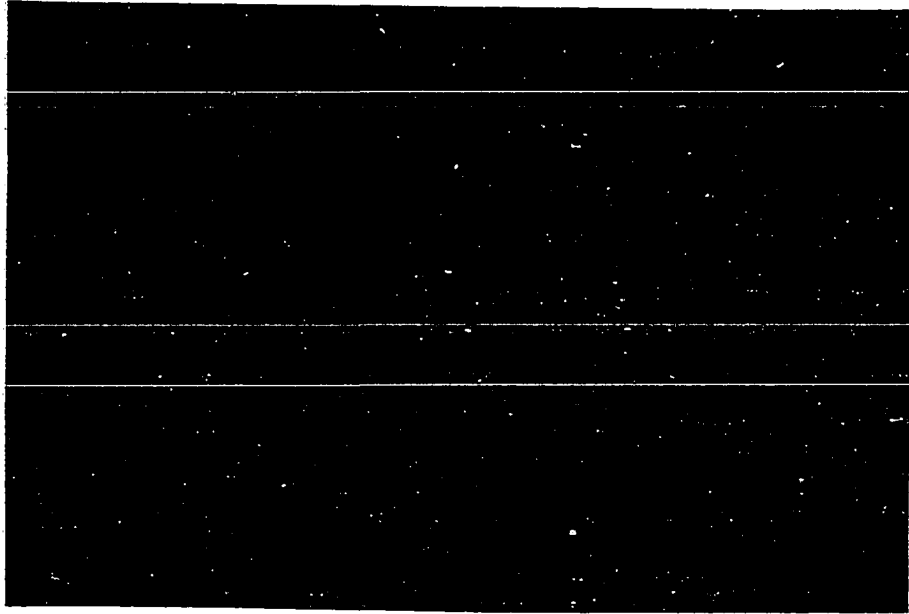


Figure 132. Radix infundibuli. Increased density of ground matrix. NSM sparse. Large pituicyte cells
Pig Merrick. Female. 10 years
Chrome alum hematoxylin stain
X 250

Figure 133. Pars compacta infundibuli. NSM in neurohypophysial fibers
Pig # 1653. Female. 1 month
Chrome alum hematoxylin stain
X 250



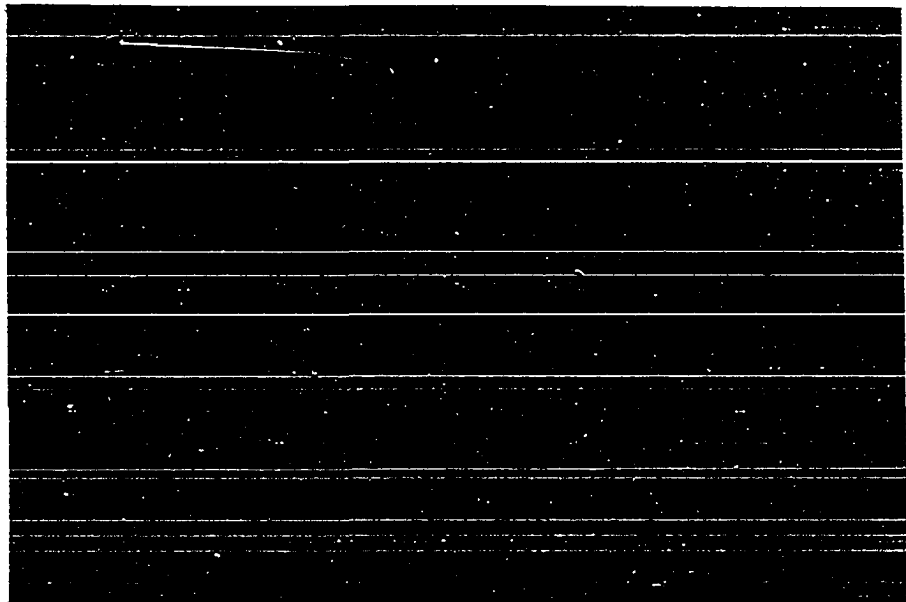
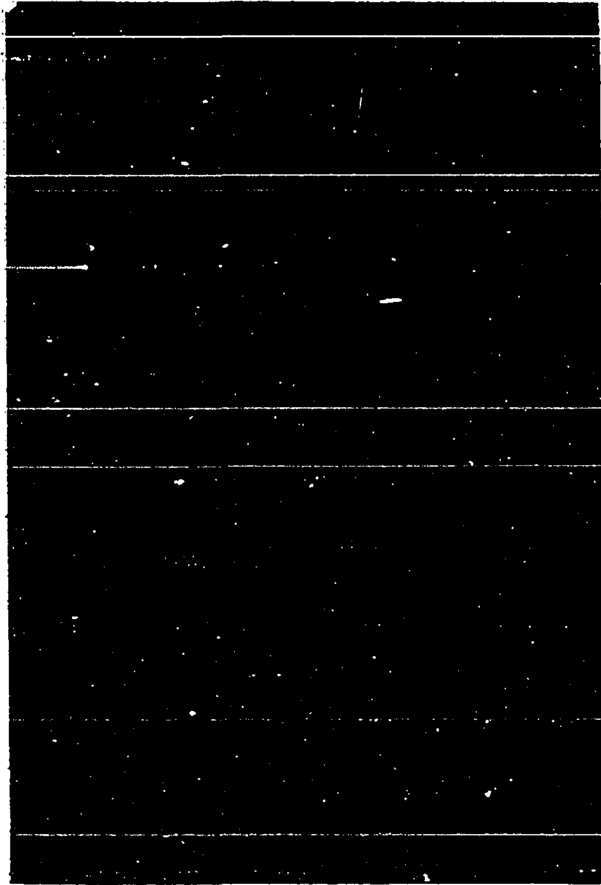


Figure 134. Pars compacta infundibuli. Typical branching of capillaries in the mantel plexus. PAS positive basement membrane
Pig # 1651. Female. 2 months
Aldehyde-thionin--PAS--orange G stain
X 250

Figure 135. Pars distalis neurohypophysis and pars intermedia. Typical distribution of NSM. Developing blood vessels. Compact cellular character of the pars intermedia
Pig # 4. Female. 1 day
Chrome alum hematoxylin stain
X 250



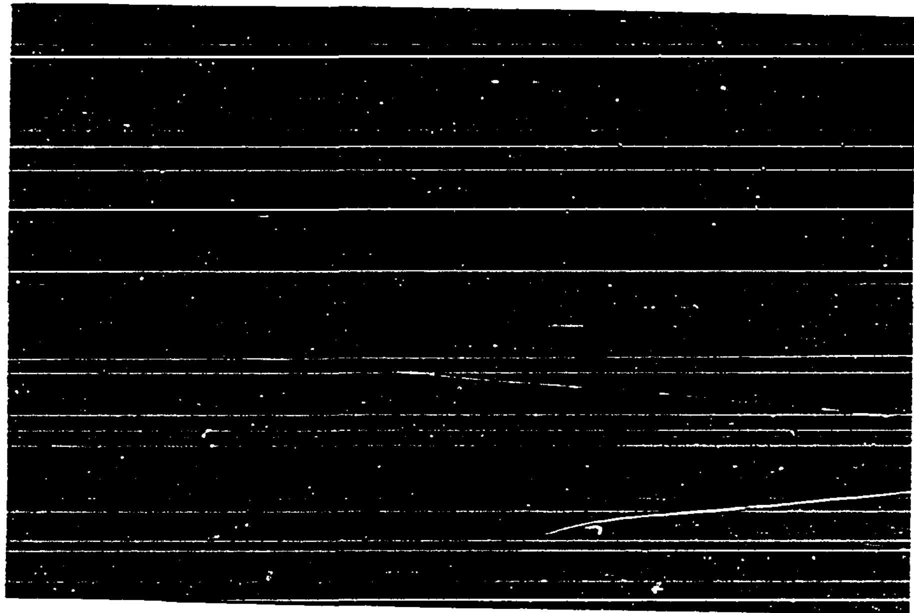


Figure 136. Pars distalis neurohypophysis. Infundibular recess. Condensation of NSM along the course of capillaries. Large vein in the pars intermedia

Pig #W-708. Female. 3 months

Alcian blue--PAS--orange G stain

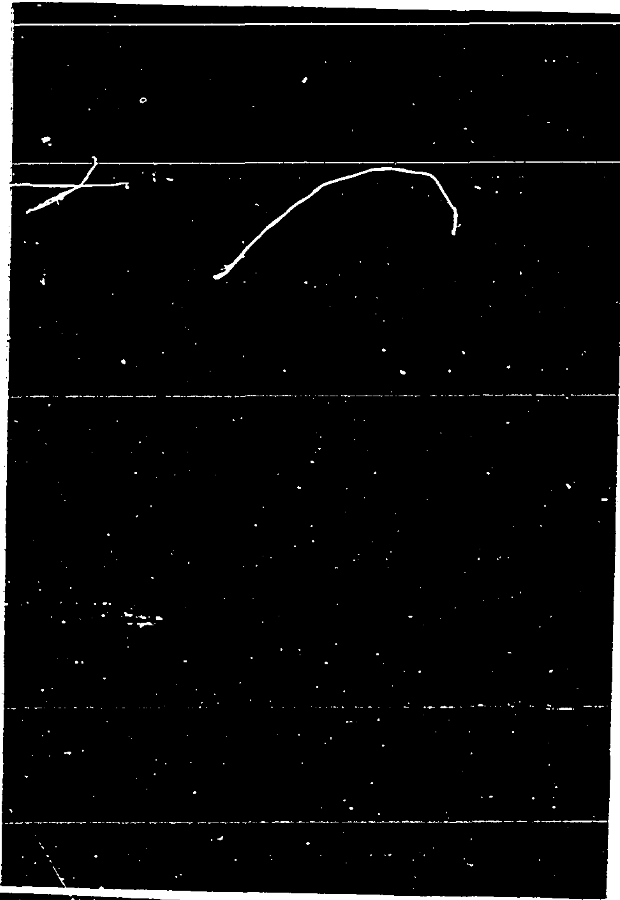
X 100

Figure 137. Pars distalis neurohypophysis. NSM and Herring body

Pig # 3923. Female. 10.5 months

Chrome alum hematoxylin stain

X 400



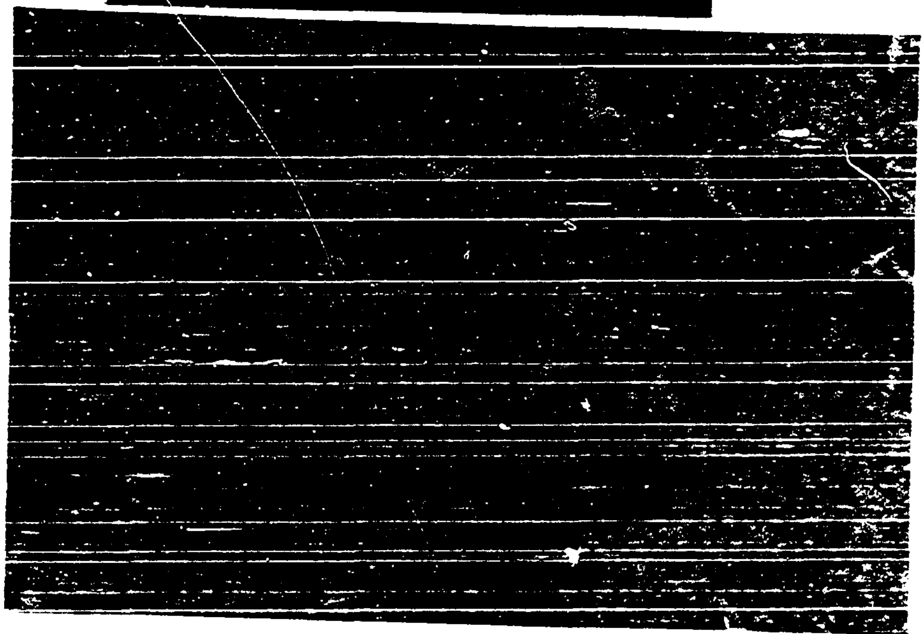


Figure 138. Pars distalis neurohypophysis. Hyaline cast in the infundibular recess. Initial stage of the replacement fibrosis

Pig # 3203. Female. 2.5 years

Aldehyde-thionin--PAS--orange G stain

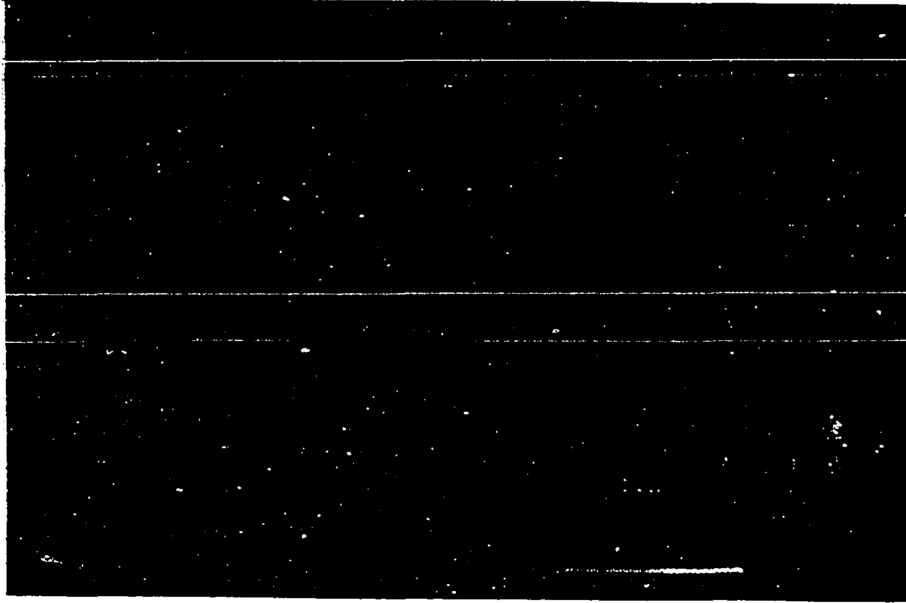
X 100

Figure 139. Pars distalis neurohypophysis. Argyrophilic nature of pre-collagen fibers in fibrous tissue

Pig # 203. Male. 4 years

Bielschowsky's method

X 250



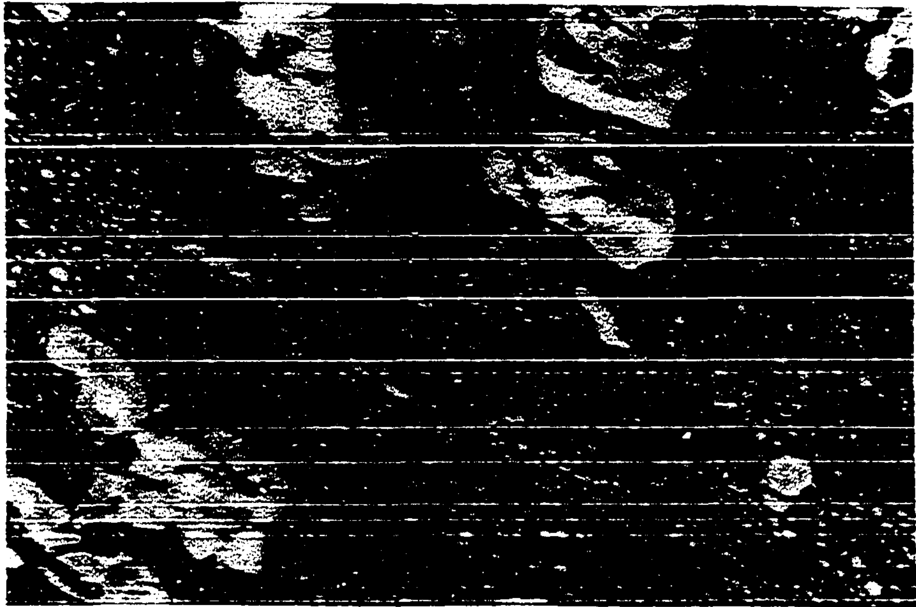


Figure 140. Pars distalis neurohypophysis. Degeneration of neurohypophysial fibers. Replacement fibrosis

Pig # 3093. Female. 7 years

Aldehyde-fuchsin-trichrome stain

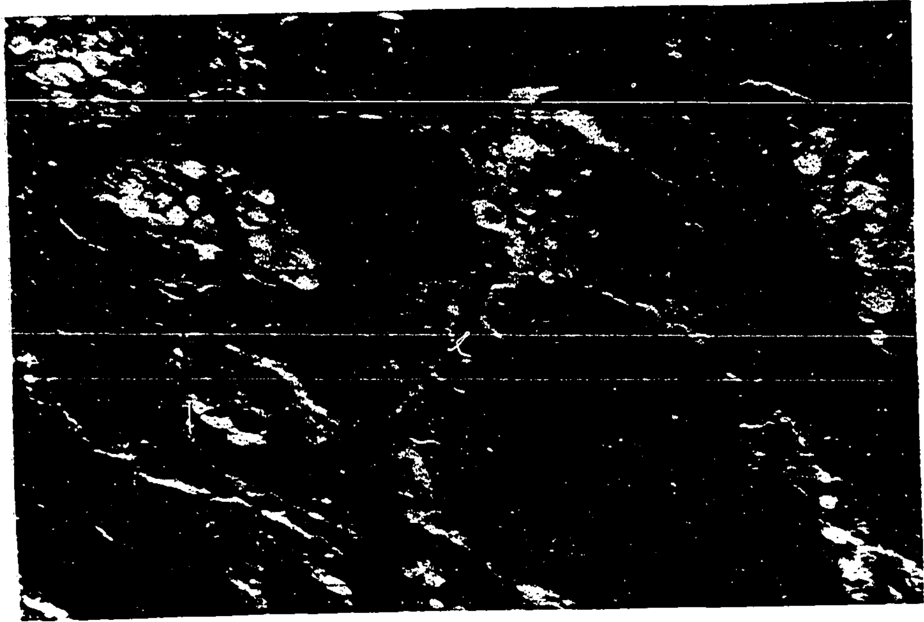
X 250

Figure 141. Pars distalis neurohypophysis. Degeneration of neurohypophysial fibers. Lack of typical staining of collagen in fibrous tissue

Fig # 3093. Female. 7 years

Verhoeff's stain

X 250



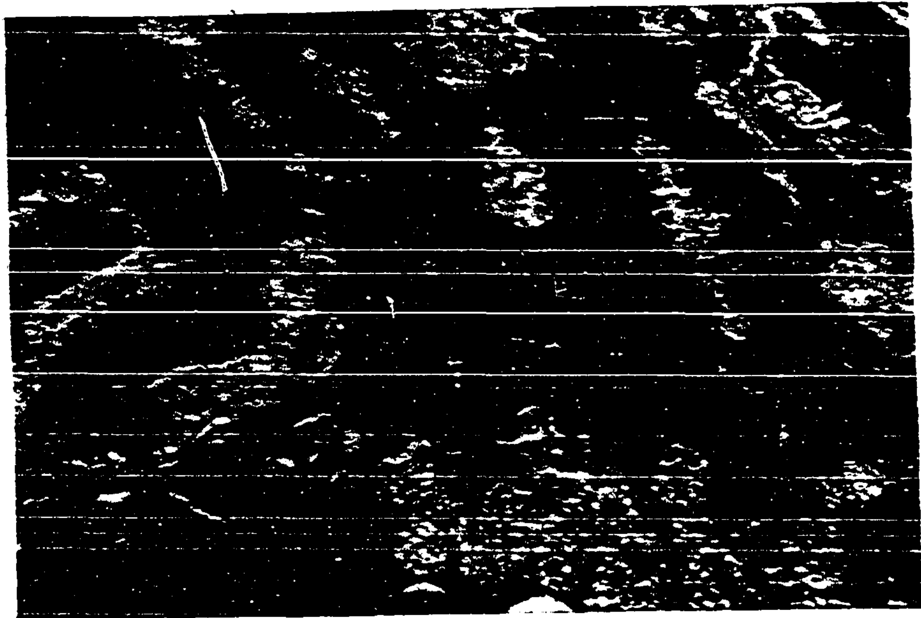
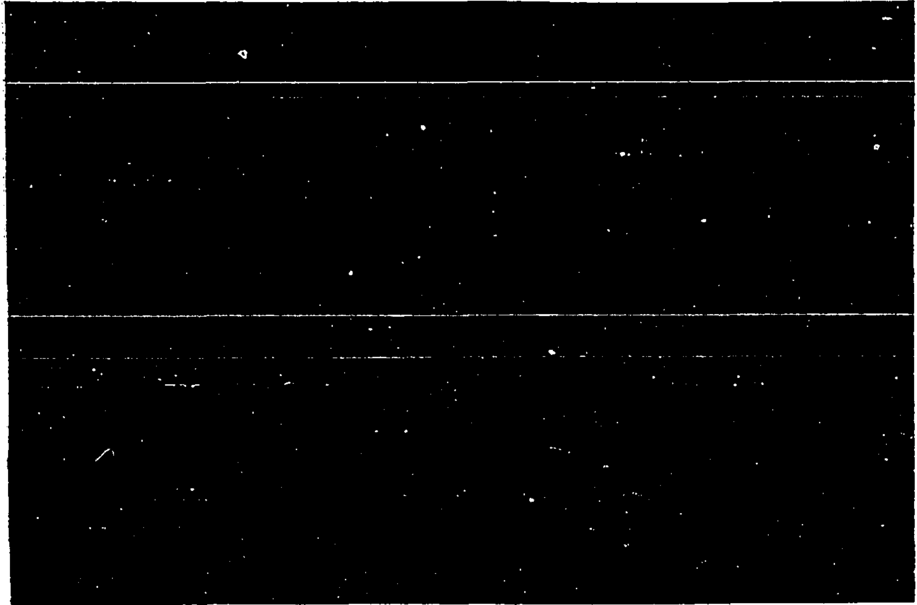


Figure 142. Pars distalis neurohypophysis. Colloid follicles in the invading cell-columns of pars intermedia. Extensive replacement fibrosis
Pig #ET-4. Female. 7.5 years
Aldehyde-thionin--PAS--orange G stain
X 250

Figure 143. Pars distalis neurohypophysis. Caudal extremity. Typical distribution of neurohypophysial fibers around the capillaries. NSM sparse
Pig Merrick. Female. 10 years
Chrome alum hematoxylin stain
X 250



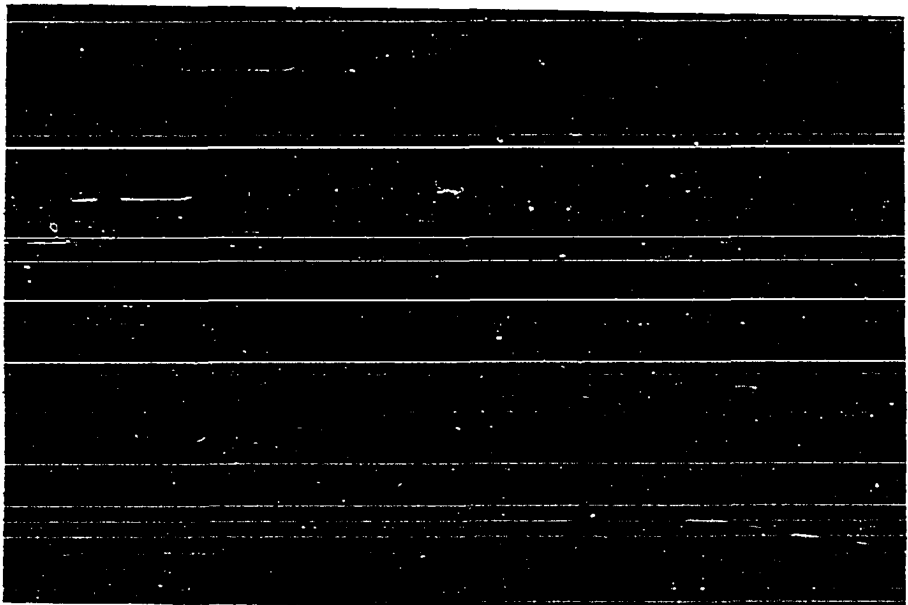
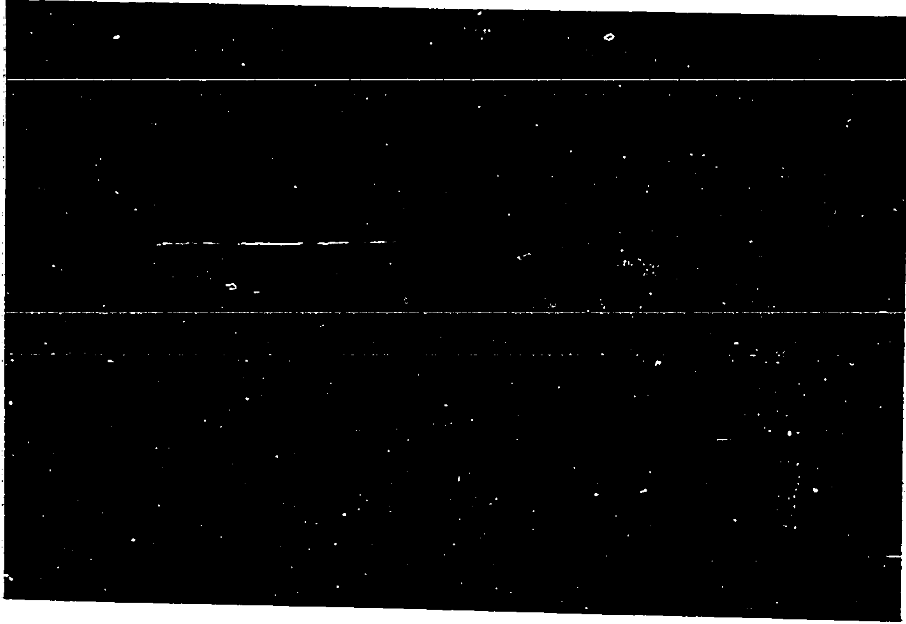


Figure 144. Paraventricular nucleus. Developing neurons.
Lack of stainable NSM
Dog #B 9. Female. 1 day
Chrome alum hematoxylin stain
X 250

Figure 145. Hypothalamohypophysial tract
Dog #E 27. Male. 4 months
Chrome alum hematoxylin stain
X 250



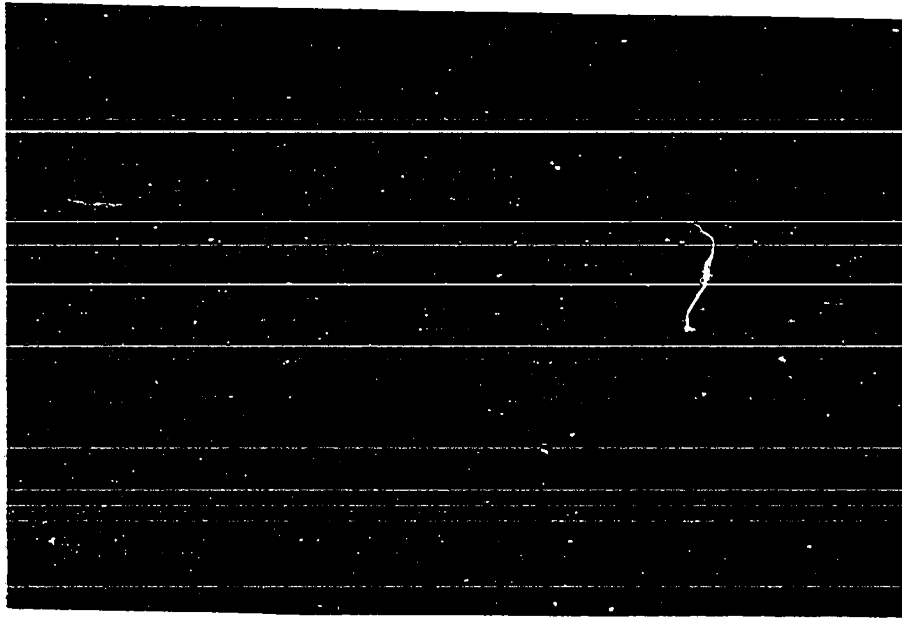
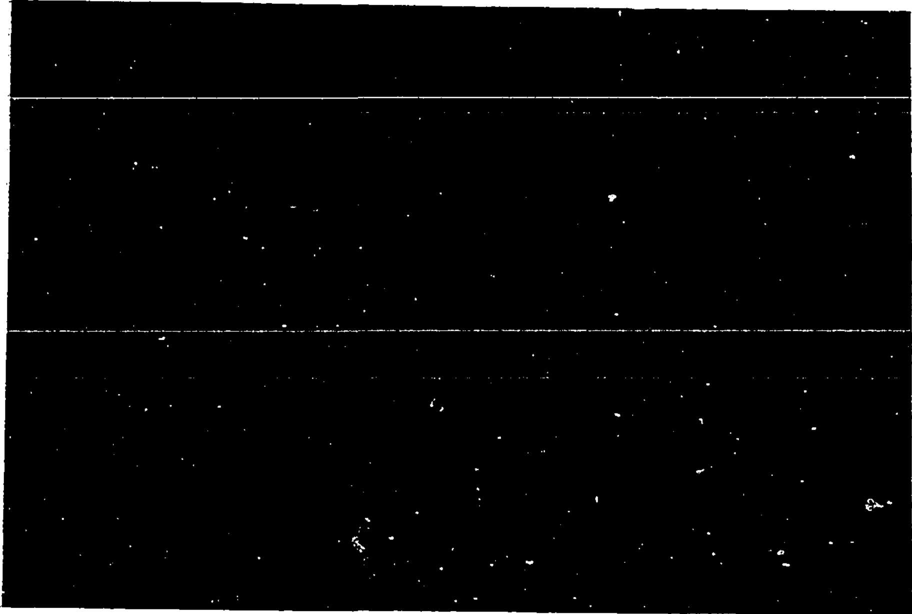


Figure 146. Paraventricular nucleus. Typical neurosecretory neuron
Dog # 047. Male. 5.5 years
Chrome alum hematoxylin stain
X 1000

Figure 147. Paraventriculohypophysial tract. Intricate pattern near supraoptic nucleus
Dog #B 63. Male. 9 years
Chrome alum hematoxylin stain
X 100



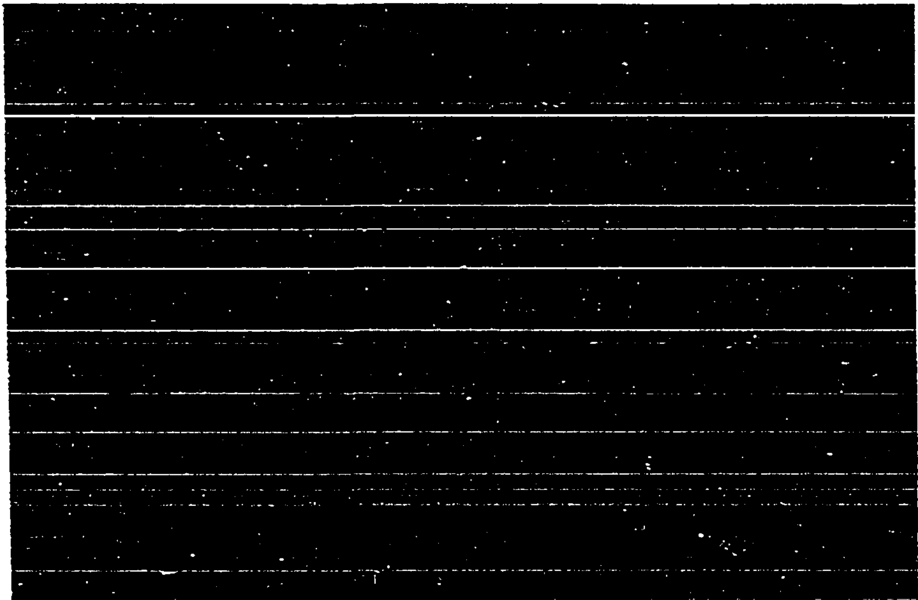
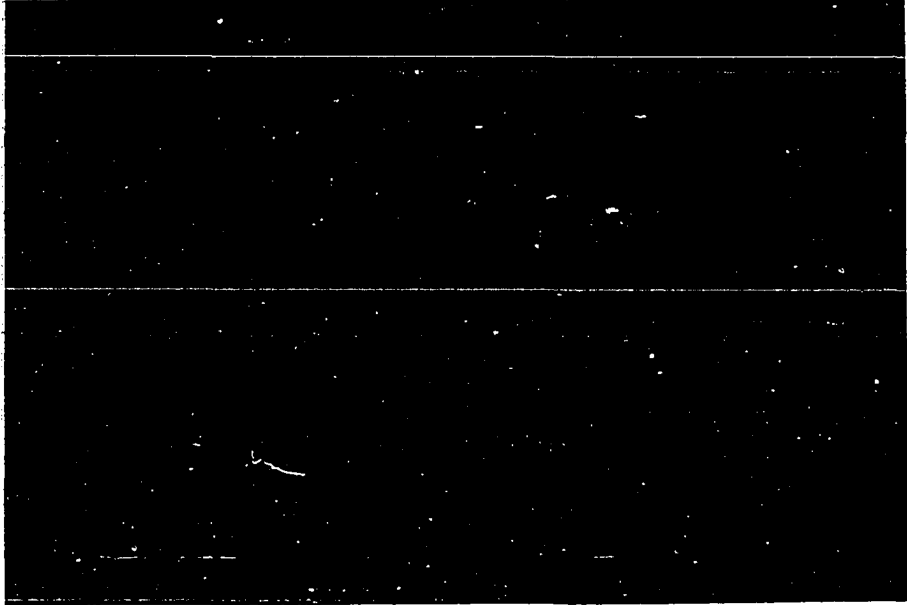


Figure 148. Supraoptic nucleus. Typical neurosecretory neuron. Eccentric nucleus. Absence of axon hillock. Nissl bodies in processes
Dog #B 63. Male. 9 years
Chrome alum hematoxylin stain
X 1000

Figure 149. Supraoptic nucleus. Ventrolateral part. Accumulated mass of NSM in cyton. Beaded axons. Decreased number of cytons
Dog #B 31. Female. 12 years
Chrome alum hematoxylin stain
X 250



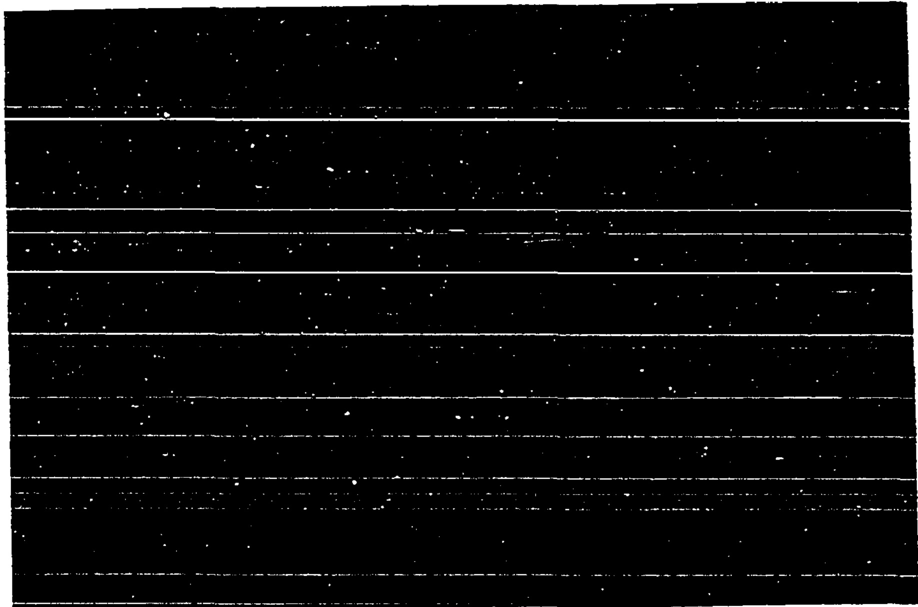
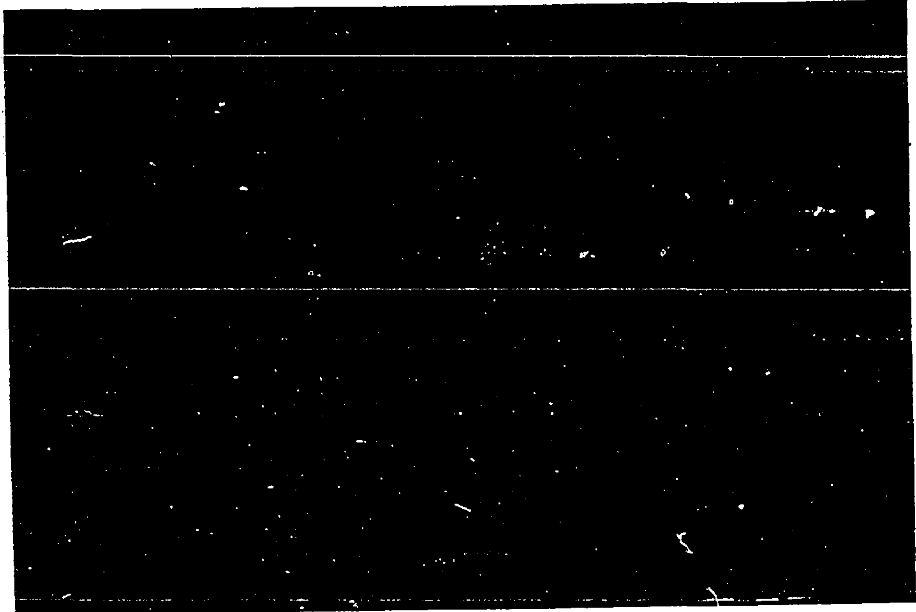


Figure 150. Paraventricular nucleus. Reduced number of cytons. Decreased concentration of NSM
Dog #B 14. Female. 13.5 years
Alcian blue--PAS--orange G stain
X 250

Figure 151. Supraoptic nucleus. NSM sparse in cytons. Reduced size of cytons. Increased density of non-neuronal fibers
Dog #B 14. Female. 13.5 years
Chrome alum hematoxylin stain
X 250



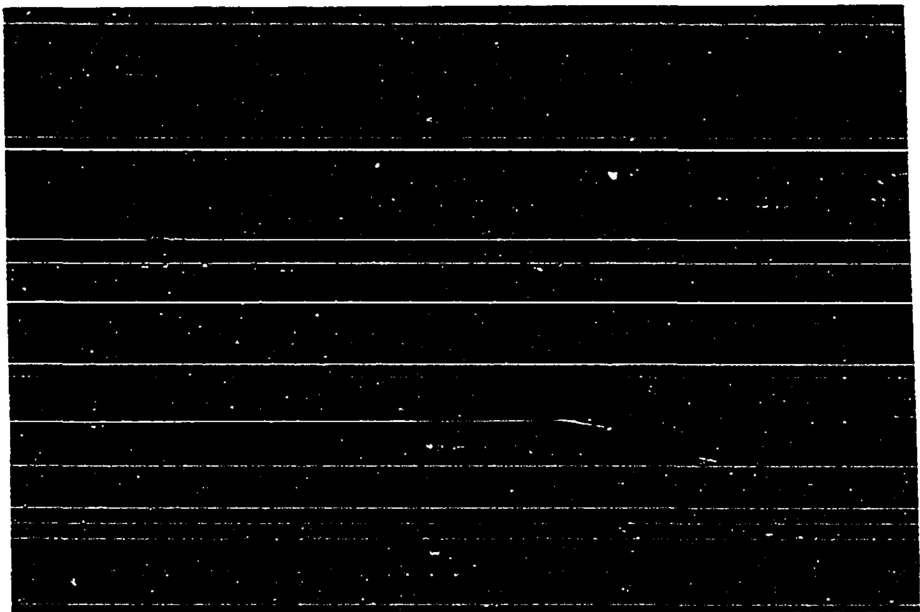


Figure 152. Supraoptic nucleus. Stainable NSM undiscernible in the cytons

Pig # 1448. Male. 3 days

Performic acid-alcian blue stain

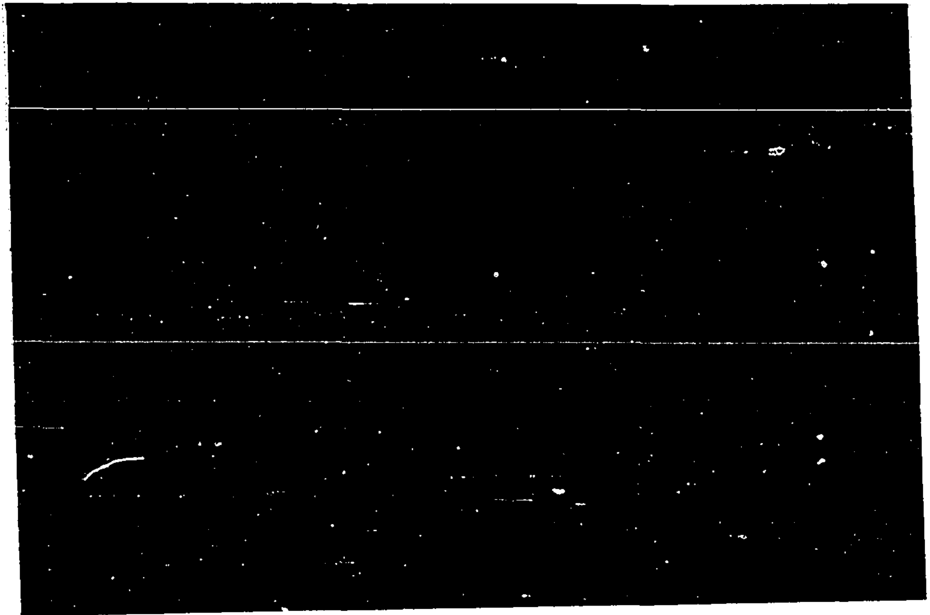
X 250

Figure 153. Paraventricular nucleus. Typical cytons. NSM stained purple

Pig # 518. Female. 1.5 months

Chrome alum hematoxylin stain

X 250



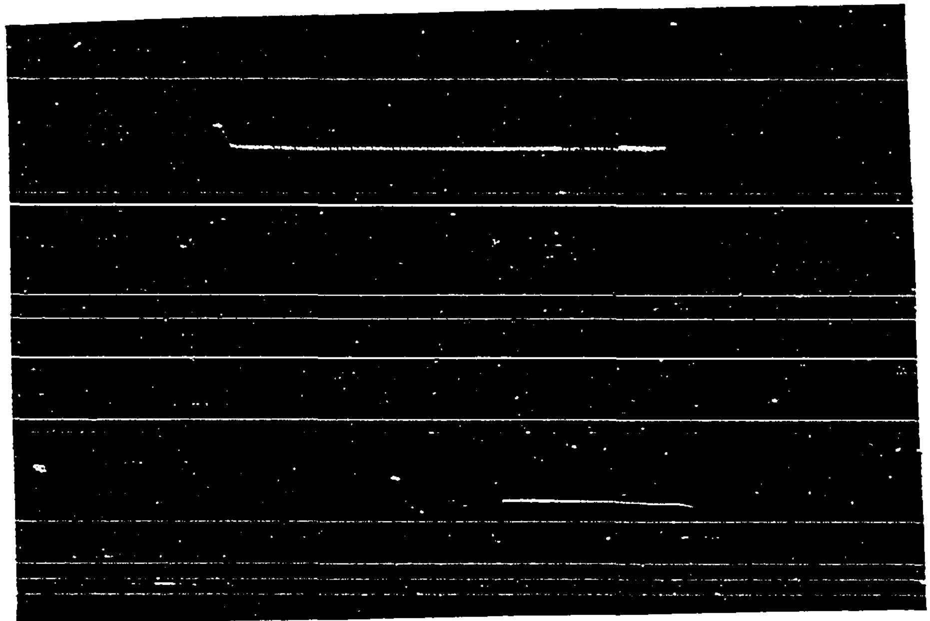


Figure 154. Supraoptic nucleus. Typical neurons. NSM stained blue

Pig # 518. Female. 1.5 months

Performic acid-alcian blue stain

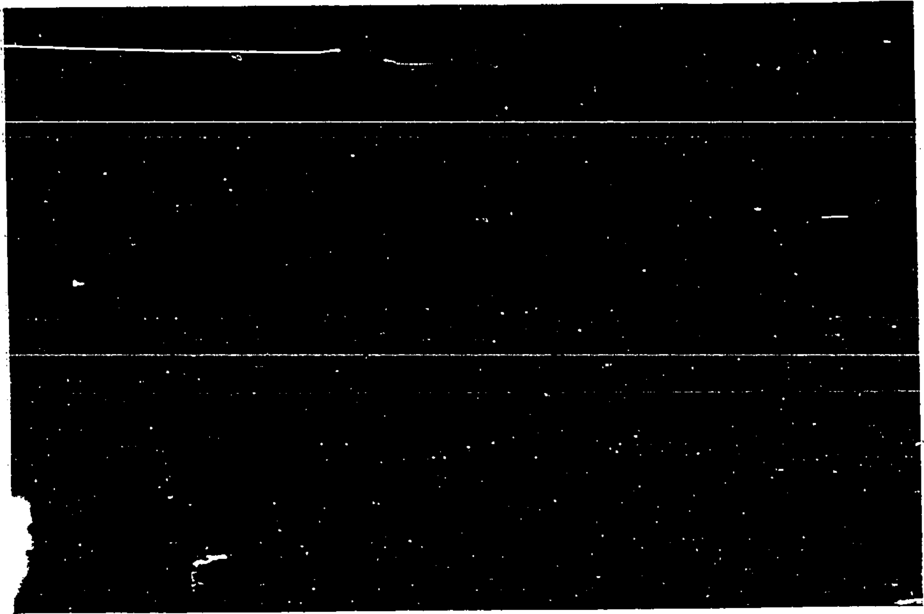
X 250

Figure 155. Supraoptic nucleus. NSM in cyton and axons. Typical neurons. Pia-glia membrane

Pig #BB-2. Female. 4.5 years

Chrome alum hematoxylin stain

X 250



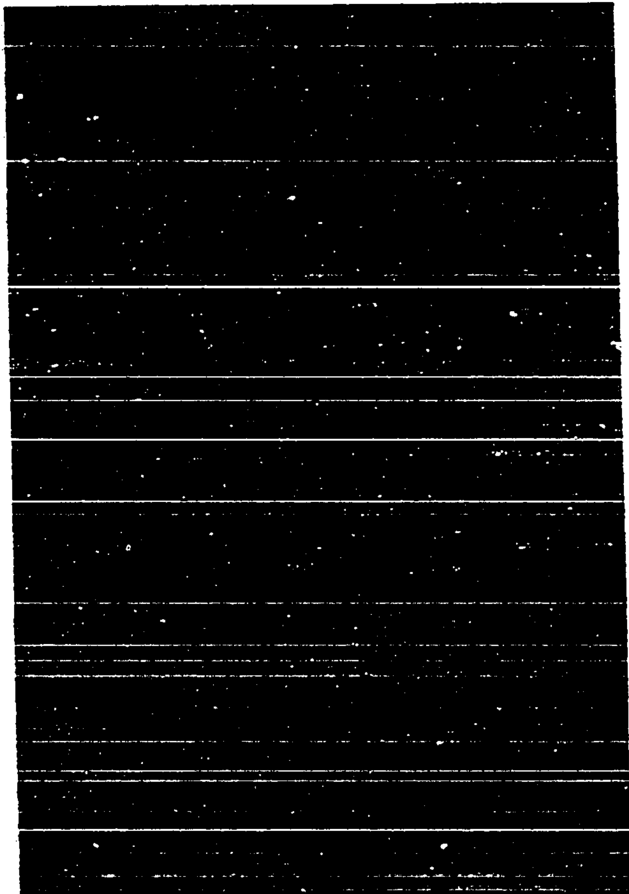


Figure 156. Hypothalamophypophysial tract. Cyst filled with NSM

Pig #BB-2. Female. 4.5 years

Chrome alum hematoxylin stain

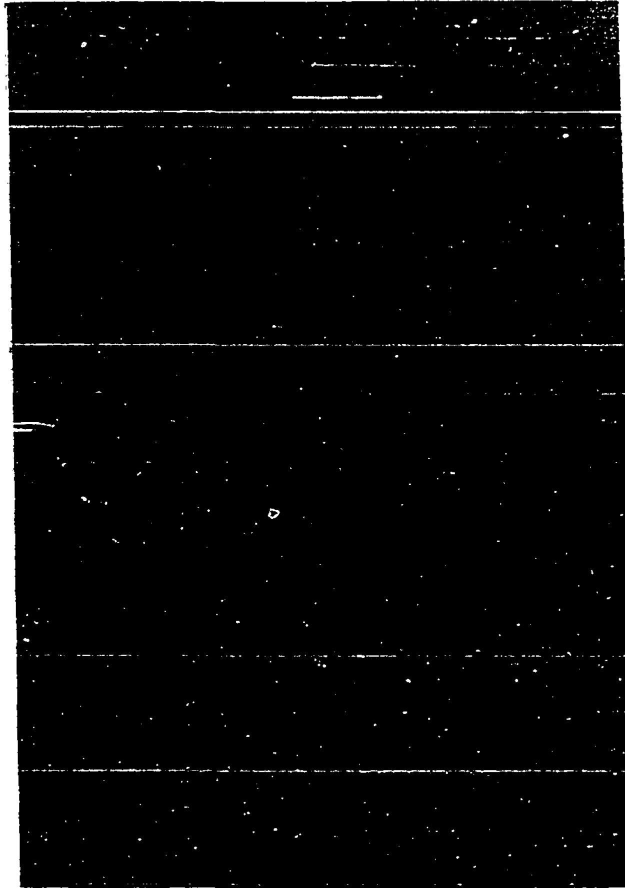
X 100

Figure 157. Paraventricular nucleus. NSM in cyton and axon. Inactive neurons

Pig #BB-5. Female. 5.5 years

Chrome alum hematoxylin stain

X 250



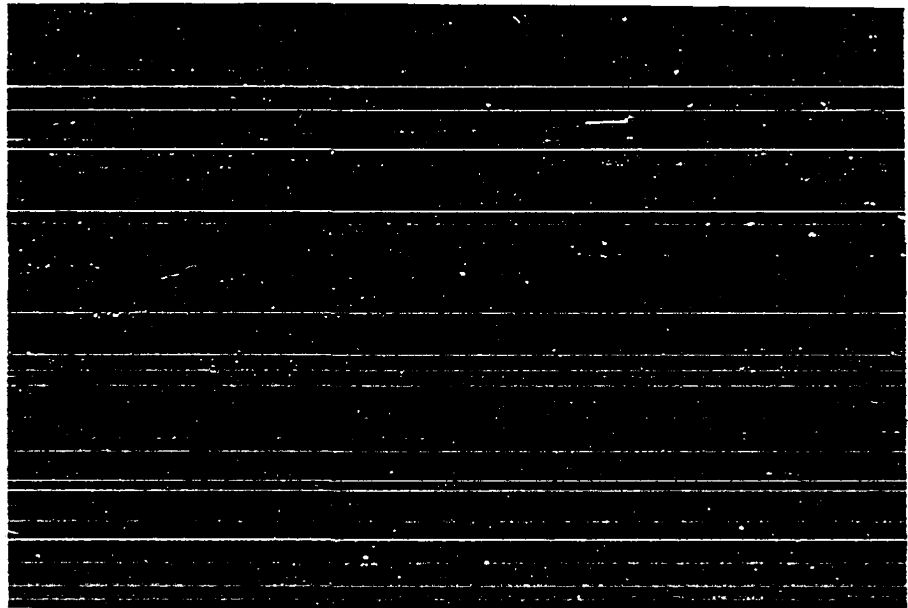
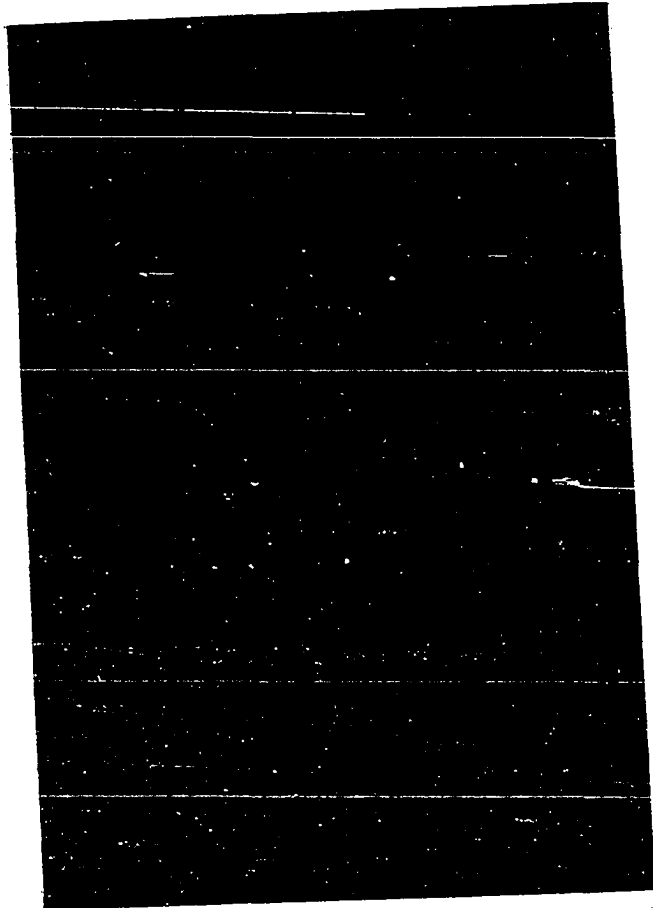


Figure 158. Paraventriculohypophysial tract. Herring bodies. Beaded axons with NSM
Pig #BB-5. Female. 5.5 years
Chrome alum hematoxylin stain
X 100

Figure 159. Paraventricular nucleus. Vacuolated neuron
Pig #BB-5. Female. 5.5 years
Chrome alum hematoxylin stain
X 250



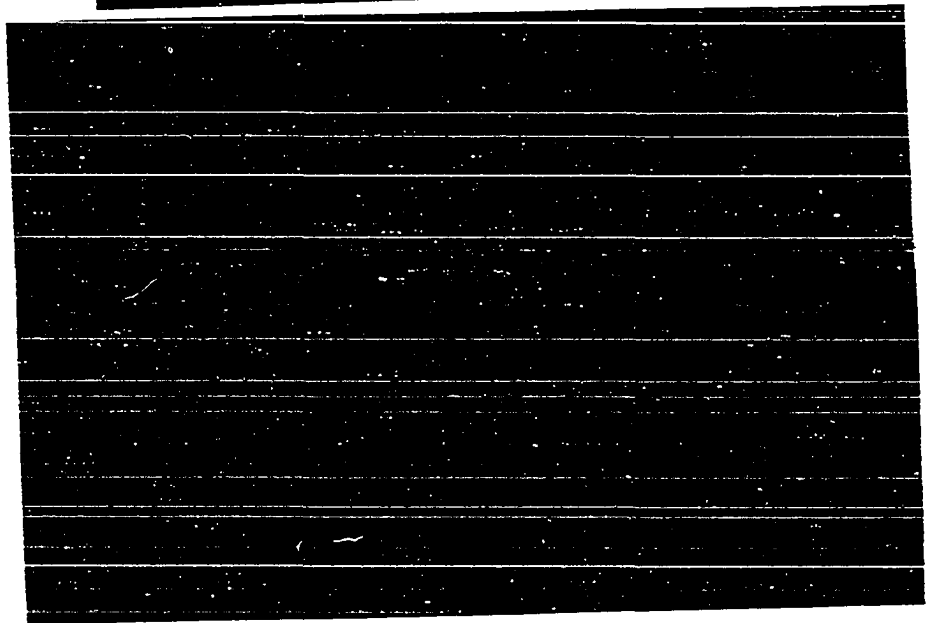


Figure 160. Paraventricular nucleus. Vacuolated neuron

Pig N.F. Female. 8 years

Performic acid-alcian blue stain

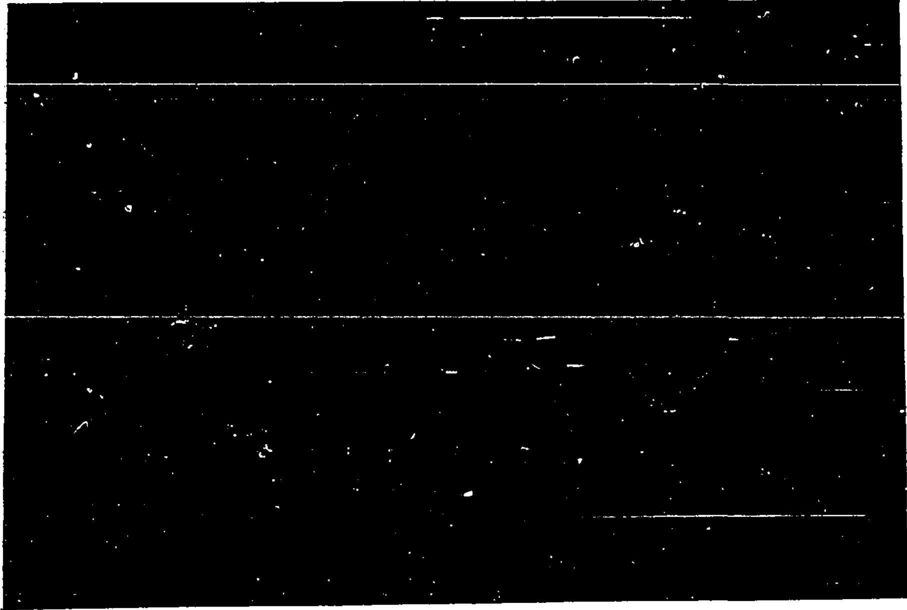
X 25

Figure 161. Paraventricular nucleus. Decreased number of cytons. Colloid occlusion of vessels

Pig Merrick. Female. 10 years

Alcian blue--PAS stain

X 400



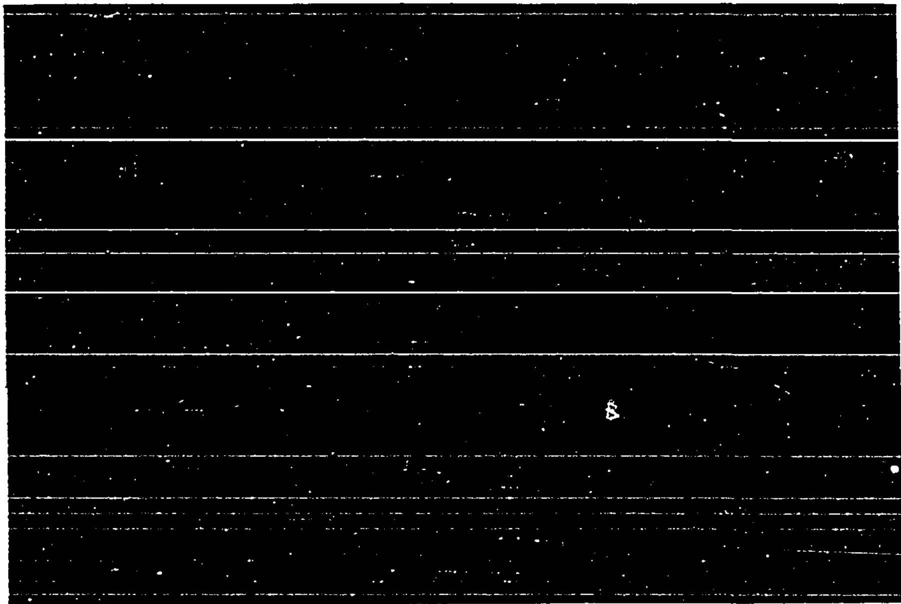


Figure 162. Supraoptic nucleus. Increased density of non-neuron fibers. NSM sparse in cytons
Pig Merrick. Female. 10 years
Chrome alum hematoxylin stain
X 250