Quantitation of corticotrophs in the pars distalis of stress-prone swine

by

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
LITERATURE REVIEW	4
Pituitary Gland	4
General morphology	4
Development	5
Blood supply	7
Staining techniques	7
Pars distalis	13
Pars tuberalis	25
Pars intermedia	28
Process of secretion	36
Neurohypophysis	41
Porcine Stress Syndrome	45
MATERIALS AND METHODS	52
RESULTS	56
DISCUSSION	64
SUMMARY AND CONCLUSIONS	72
BIBLIOGRAPHY	73
ACKNOWLEDGMENTS	85
APPENDIX	86

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INTRODUCTION

As early as 1953, there came reports (Ludvigsen, 1953; Briskey et al., 1959) of pale soft exudative (PSE) post-mortem porcine musculature which later stimulated research into the mechanisms responsible for this condition. It was noted by Judge et al. in 1966 that the animals which yielded these poor quality carcasses were less able to withstand heat stress than controls; they were thus termed stress-susceptible (SS). Subsequently, Topel et al. (1968) found that certain well-muscled swine experienced sudden death when exposed to stressful conditions and called this response porcine stress syndrome (PSS). Selye's (1959) work concerning the importance of the adrenal gland in stress adaptation led others to compare the adrenal function in animals with normal and PSE carcasses.

In 1967, Topel et al. demonstrated an inverse although not absolute relationship between levels of plasma 17-OH-corticosteroids and the degree of PSE muscle. They later (1968) demonstrated that pigs which are terminated during the last stage of the syndrome possess a high muscle lactic acid content and a low muscle pH. The carcasses show PSE properties and have low plasma 17-OH-corticosteroid levels. Topel et al. (1968) suggested that these pigs lack the ability to secrete adrenal glucocorticoids when stressed and therefore have difficulty adapting to a stressful situation. However, other reports (Weiss, 1971; Marple et al., 1972c) on plasma adrenocorticoid levels in SS pigs are not in accord with these findings.

In 1972, Marple *et al.* demonstrated that under control conditions, stress-susceptible pigs had a three to four times higher plasma adrenocorticotrophic hormone (ACTH) level than stress-resistant pigs, yet they had similar plasma corticoid levels. They concluded that SS pigs are not hampered by insufficient circulating ACTH and they suggest that high ACTH levels may indicate the stress-susceptibility of an animal (Marple et al., 1972c).

Sebranek *et al.* (1971) used dexamethasone and exogenous ACTH to show that SS pigs are less able to respond to exogenous ACTH with increased glucocorticoid levels than are stress-resistant (SR) pigs. However, in an *in vitro* experiment in 1973 (Meiburg *et al.*) both SS and SR adrenal glandular tissue responded to ACTH by increased cortisol synthesis. This paradox was apparently resolved by Marple and Cassens (1973a) who studied the metabolic clearance rate of cortisol in SS and normal pigs and concluded that the lower corticoid levels in SS swine are due to an increased rate of cortisol utilization rather than an insufficiency in production.

If the current theory concerning the mechanism for stress-susceptibility in swine is correct, one may rule out the possibility of a secondary type of adrenal insufficiency originating in the pituitary as the cause of this syndrome. The results of Marple *et al.* (1972c) demonstrating three to four fold higher plasma ACTH levels in SS pigs as compared to SR pigs under control conditions would suggest the presence of a greater number of ACTH cells within the pituitaries of SS pigs.

The purpose of this research is to test this hypothesis by performing ACTH cell-counts on median sagital sections of the anterior lobes of SS and normal pig pituitaries using light microscopy.

LITERATURE REVIEW

Pituitary Gland

General morphology

The pituitary gland or hypophysis is clearly the most complex organ of the endocrine system. It acts as a site of production or storage of at least nine hormones each of which is vitally important in the regulation of other body organs including many endocrine glands. It is for this reason it has been referred to as the "master gland" of the body.

The hypophysis lies at the base of the brain in a concavity of the basisphenoid bone, the hypophyseal fossa, a part of the sella turcica. It is suspended from the ventral surface of the forebrain by means of a short hollow stalk, the infundibulum, into which the third ventricle of the brain extends (Miller et al., 1964).

The neurohypophysis consists of the median eminence, infundibular stem, and the posterior lobe or pars nervosa. The latter is linked to the tuber cinereum of the hypothalamus by a stalk of nerve fibers designated as the infundibular stem. The tuber cinereum is located between the optic chiasma anteriorly and the mammillary bodies posteriorly and lies in the floor of the third ventricle. The proximal portion of the neurohypophysis is known as the median eminence of the tuber cinereum and lies between the optic chiasma and the origin of the pituitary stalk. The stalk consists of the portal venules from

the median eminence and the nerve fibers of the infundibular stem (Holmes and Ball, 1974). Some authors include the pars tuberalis of the adenohypophysis as part of the stalk (Rhodin, 1974).

Three major components comprise the adenohypophysis. They are the anterior lobe or pars distalis, intermediate lobe or pars intermedia, and infundibular lobe or pars tuberalis. All are composed of loose cords of epithelial cells surrounded by a rich network of capillaries and sinusoids. The structure and function of the adenohypophysis will be dealt with in detail later (Rhodin, 1974).

The porcine hypophysis is generally dorsoventrally compressed. The pars distalis is flat and broad while the intermediate lobe is very small. The infundibular recess extends well into the neural lobe, which itself is rather enlarged distally in the pig (Dellmann, 1971). The intermediate lobe is firmly attached to the rostroventral surface of the pars nervosa. A wide hypophyseal cavity separates the pars distalis from the intermediate lobe and is often filled with colloid (Trautmann, 1909).

Development

The pituitary gland is composed of two structurally distinct lobes which arise from widely different ectodermal sources. The adenohypophysis or glandular lobe is derived from Rathke's pouch, a dorsal evagination of somatic ectoderm from the primitive buccal cavity, while a process called the saccus infundibuli or infundibulum develops ventrally from the neural ectoderm of the floor of the diencephalon and eventually becomes the neural lobe or neurohypophysis.

A median unpaired outgrowth appears in the anterior portion of Rathke's pouch and becomes readily distinguishable from two smaller lateral lobes. The proximal wall of this outgrowth makes contact with the neural lobe and differentiates histologically into the pars intermedia or intermediate lobe. The distal portion which has no contact with the neural lobe becomes the pars distalis or distal lobe. The paired lateral lobes spread dorsally, enveloping the infundibulum, and form the pars tuberalis or infundibular lobe (Hanström, 1966). The lumen of Rathke's pouch commonly persists in the adult as the hypophyseal cleft lying between the pars intermedia and pars distalis (Miller et al., 1964).

The portion of the neural tube which gives origin to the infundibulum also gives rise to the hypothalamus. The neurohypophysis retains a connection with this part of the brain by means of a stalk of nerve fibers which arise from the tuber cinereum of the hypothalamus. A cavity known as the infundibular recess persists in some species and is actually an extension of the third ventricle of the brain into the infundibulum (Holmes and Ball, 1974).

Neither the adenohypophysis nor neurohypophysis develops when it is transplanted without a portion of the other indicating that, despite their development as two separate entities, each is apparently dependent upon the other for its subsequent development (Kingsbury and Roemer, 1940; White and Foust, 1944).

Blood supply

Vascularity is of major concern when considering the pituitary because it is not only essential for the supply of nutrients and removal of wastes, but it is also responsible for the transportation of glandular secretions to the target organs and tissues. However, of special importance is its role as a link by which the central nervous system can control the secretory activity of the pars distalis by way of the hypophyseal portal system. The internal carotid artery and circle of Willis contribute to the arterial supply of the pituitary by way of the superior and inferior hypophyseal arteries. Branches of the superior vessels comprise the primary supply to the adenohypophysis, although none of them directly enters this area. Instead, the blood is carried first through the pars tuberalis and subsequently into the median eminence where a capillary bed penetrates the nervous tissue. It is then conducted down the stalk via portal venules to the pars distalis where it enters a second capillary bed (Holmes and Ball, 1974).

Staining techniques

As long ago as 1844, the pituitary gland was the subject of cytological studies, however, histological techniques were rudimentary then so little was noted beyond the overall size and shape of the cells and nuclei, and the obvious presence or absence of cytoplasmic granules. The development of biological stains was of great significance to the pituitary cytologist. It allowed Flesch, in 1884, to describe

the arrangement of cells in the pars distalis as 'cellular tubes' and to note two distinct cell types, large cells with large deeply stained granules and smaller cells which did not stain. He was using osmic acid, eosin, and indigo or Weigert's chrome haemotoxylin to look at pituitaries from the horse, dog and man. The granule-containing cell type was later termed a 'chromophil' by one of his students. The unstained cells were called 'chromophobes' (Holmes and Ball, 1974).

In 1892, Schonemann used alum haematoxylin and eosin to further classify the chromophils into 'eosinophils' which stained red and 'cyanophils' which stained blue. When it appeared that most acid or basic dyes would serve the purpose, the terms 'acidophils' and 'basophils' were substituted; however, this classification is inaccurate because in many species neither alum haematoxylin nor most basic dyes stain basophils (Purves, 1966).

A stain involving only acid dyes was developed in 1900 by Mallory. Through modification it could be made to work in all species, which is a feature many methods could not claim (Purves, 1966).

A technique by which sections are stained by one method, observed, destained, and then restained by another method was employed by Maurer and Lewis in 1922 to study the staining characteristics of the same cells stained by different dyes. They demonstrated that a given cell may stain with acid or basic dyes; therefore, in the traditional sense, the terms 'acidophil' and 'basophil' are not truly accurate for describing adenohypophyseal cells. Biedermann (1927) later showed the amphoteric staining characteristics of pituitary cells by demonstrating

that their staining reaction varies according to the alkalinity or acidity of the medium in which the dyes are dissolved (Holmes and Ball, 1974).

Considerable progress was made when Cleveland and Wolfe introduced a technique based on Mallory's method (1900) for staining pituitaries of the pig, dog, rat and rabbit. After fixation in Regaud's mixture, the tissues were post-chromated in potassium dichromate and erythrosin was substituted for aldehyde-fuchsin and followed with orange G and aniline blue separately. Four cell types were readily distinguishable. They were (1) large cells with a non-granular cytoplasm which was either colorless or a pale blue, (2) dark blue cells with purple-red granules, (3) cells with large orange granules and (4) cells with large yellow granules (Cleveland and Wolfe, 1932). Later they identified a class of cells in the dog which stained selectively with erythrosin (Holmes and Ball, 1974).

Dawson and Friedgood (1938) used a modification of the Mallory connective tissue stain on rabbit and cat pituitaries to develop a technique which differentiated two classes of acidophils, one which stained red with azocarmine and the other orange with orange G.

The terms 'aurantophil' and 'erythrophil' were given to cells which stained orange and red respectively with the Herlant tetrachrome method (1960) which utilizes orange G, erythrosin, aniline blue and alizarin blue (Purves, 1966).

About the same time, Hall (1938) was studying bovine pituitaries in an effort to develop a method to further differentiate basophils.

He succeeded and found that one cell type stained deeply with aniline blue and contained granules with a great affinity for haematoxylin. These cells appeared to be widespread throughout the pars distalis while the other type of basophil, which stained weakly, predominated in the central region (Holmes and Ball, 1974).

Other dyes which were later found to be useful in differential staining of basophils are resorcin-fuchsin, kresofuchsin, aldehyde-fuchsin, Alcian blue, and aldehyde-thionin (Purves, 1966).

It is important to remember that these terms, acidophilia and basophilia, do not have the same connotation to a pituitary cytologist as to those in other fields of cytology. In this case, they refer only to the staining affinities of the secretory granules and must not be confused, for example, with the basophilia that results from ribonucleoprotein in other glandular cells. The terminological problem is made more confusing by the fact that nowadays most of the staining procedures routinely applied for pituitary cytology do not utilize an acid and a basic dye but involve mixtures of acid dyes. Furthermore, staining with many of these methods does not depend on the binding of a dye by a tissue component of opposite charge; therefore, in these cases it is impossible to ascertain the chemical nature of granules from their colors. Depending on the combination of acid dyes used, a granule of a given cell type may stain red, orange, purple or blue (Bloom and Fawcett, 1975). Nevertheless, Abolins (1952) favored the retention of the terms, 'acidophilia' and 'basophilia' with regard to the pituitary on the basis that one can

differentiate these two affinities by staining at a controlled pH, although both types of granules are actually amphoteric (Holmes and Ball, 1974).

The most significant progress in this area can be attributed to the McManus periodic-acid-Schiff (PAS) method of staining for glycoprotein. This technique, which was originally used to demonstrate mucin, is based on the concept that periodic acid acts upon the 1,2 glycol linkage of carbohydrates in tissues to produce aldehydes, which can be stained with Schiff's reagent (McManus, 1946, 1948). At present, the primary classification of pituitary chromophils is based on this reaction and thus these cells are divided into 'serous' and 'mucoid' types. The serous cells correspond to the acidophils which secrete simple protein hormones. The mucoid cells are the glycoprotein-secreting basophils. Some authors still use the terms 'acidophil' and 'basophil'; however, they are now defined in terms of the PAS reaction. The cells of the acidophil class can be further differentiated by use of acid dyes and those of the basophil class, by staining with complex basic dyes and acid dyes (Purves, 1966).

In 1950, Gomori described a paraldehyde-basic fuchsin stain which gained popularity as a routine method for pituitary staining. This is an intensely purple dye which reacts similarly to Schiff's reagent; however, it also possesses an affinity for strong sulfur acids, resulting in deep staining of highly sulfated mucopolysaccharides and other substances which are high in cysteine. Aldehyde-thionin (AT) was later introduced as a substitute for aldehyde-fuchsin

(AF), as the latter produced diffuse staining of the background in chromate-fixed tissues and tends to deteriorate quickly. Aldehyde-thionin was shown to give a dense blue-black stain with a distribution similar to that of aldehyde-fuchsin, but with little background staining, on chromate-fixed tissues. Aldehyde-thionin also withstands subsequent oxidation by periodic acid, which allows one to simultaneously demonstrate structures which are aldehyde-fuchsin negative but PAS positive. This includes certain adenohypophyseal cells (Paget and Eccleston, 1959).

Shanklin et al. (1959) demonstrated the usefulness of another dye, luxol fast blue (LFB), in selective staining of pituitary glandular cells. This acid dye shows a great affinity for the lipoprotein reactants of certain acidophilic cells of the hypophysis (Herlant, 1964).

Yamaguchi et al. (1973) studied the anterior pituitary of the pig using a combination of the aldehyde-thionin method of Paget and Eccleston (1959), PAS technique of McManus (1948) and a modification of Cleveland and Wolfe's trichrome stain (1932). They were able to distinguish all seven cell types (including follicular cells) by using this method plus a combined dialyzed-iron PAS staining method.

An important factor when staining pituitaries is the fixation procedure. Both the fixative and speed of penetration of the fixative must be considered. Baker (1958) and Ruitenberg et al. (1974) evaluated various methods and have concluded that the best all-round results are obtained using formol sublimate. Helly's (Zenker-formol)

also gave good results according to Baker, although it only rated satisfactory in Ruitenberg's findings. Ruitenberg _et al_. also tested Bouin's fixative and found it gave poor results. Perfusion was shown to be a much better method than fixation by immersion alone, apparently due to the sanguinity of the pituitary gland.

Chromation in potassium dichromate prior to staining has also proven to be effective in improving the staining affinity of the secretion granules of the adenohypophyseal cells. This technique was discussed by Cleveland and Wolfe (1932) as though it were a novel approach to staining, although in actuality it had been in practice for many years.

In recent years, pituitary cytologists have turned more and more to immunohistochemical techniques for a better understanding of this complex organ. These procedures involve induction of antibody to a certain hormone by injection of this hormone into an animal of another species. The antibody is then conjugated with fluorescent dyes or horseradish peroxidase and subsequently reacted with the tissue sections. The antigen sites are localized by fluorescence microscopy or by histochemical methods for peroxidase. The results so far have been excellent and have contributed greatly to the elucidation of the various cell types of the adenohypophysis (Bloom and Fawcett, 1975).

Pars distalis

Various methods for classifying the glandular cells of the pars distalis have been developed. Based on their affinity or lack of

affinity for dyes used in routine histologic staining, these cells are identified as chromophils and chromophobes, respectively. Chromophils are further subdivided into acidophils and basophils (Bloom and Fawcett, 1975).

Other systems of classification, such as the ones based on the Greek alphabet, have been proposed. Most recent use of this terminology is based on the system developed by Romeis (1940, cited in Purves, 1966). However, the procedure by which the adenohypophyseal cells are identified according to the specific hormone(s) which they secrete, is the one most widely accepted (Holmes and Ball, 1974).

The somatotroph or alpha cell is a type of acidophil which is often referred to as an orangeophil or aurantophil because of its strong affinity for orange G (Purves, 1966). It also stains selectively with luxol fast blue (Shanklin _et al._, 1959). According to Nakane (1970) who studied the rat pituitary using the peroxidase-labeled antibody method, somatotrophs are dispersed evenly throughout the adenohypophysis with the exception of the anterior-ventral region and an area adjacent to the intermediate lobe, where they are rare. Usually found lying along sinusoids, these cells are oval in shape and contain secretion granules approximately 300-350 mμ in diameter dispersed throughout the cytoplasm surrounding a central nucleus. In the bovine, (Mikami, 1970) these cells were observed in the peripheral areas of the pars distalis and contained secretion granules up to 450 mμ in diameter. Yamaguchi _et al._ (1973) found no characteristic distribution of these cells in the anterior lobe of the porcine pituitary.

A protein hormone composed of 188 amino acids is secreted by these cells (Bloom and Fawcett, 1975). One of its major functions is to influence the growth of chondroblasts in the epiphyseal cartilages of long bones. It is known as somatotropin or growth hormone (Rhodin, 1974). Somatotroph adenomas in children result in gigantism however; if such tumors arise subsequent to closure of the epiphyseal plates, the bones and soft tissues thicken and the condition is called acromegaly. In addition to its primary role, growth hormone is involved in protein, fat, and carbohydrate metabolism (Bloom and Fawcett, 1975).

Lactotrophs (luteotrophs in the rat) or epsilon cells comprise the other class of acidophils and are both erythrophilic (Herlant, 1964; Purves, 1966) and fuchsinophilic (Mikami, 1970). They have acquired the name carminophils due to their selective staining by azocarmine (Dawson and Friedgood, 1938; Dawson, 1963). Prolactin (or luteotropin) is the hormone which they secrete. It is a single chain polypeptide containing 205 amino acids (Bloom and Fawcett, 1975). Lactotrophs show a dramatic increase in number during and after pregnancy and function to initiate and maintain milk secretion in the mammary gland following pregnancy. The luteotropic effect of this hormone is seen in the rat (Rhodin, 1974).

Although sparse in the anterior-ventral portion of the anterior lobe, the presence of lactotrophs throughout the rat pars distalis including the region near the intermediate lobe has been reported (Nakane, 1970). They frequently surround large gonadotrophs in a

"cupping" fashion with the nucleus located at the base of the cup. Large pleomorphic or spherical secretion granules (often in excess of 800 mμ in diameter) are dispersed throughout the cytoplasm (Nakane, 1970). During lactation the rough endoplasmic reticulum (rER) undergoes extensive development, as does the Golgi complex. On weaning, lysosomes eliminate excess secretion granules; a process known as crinophagy. Lysosomes are also involved in autophagy of the protein synthetic apparatus (Bloom and Fawcett, 1975).

Mikami (1970) has found these cells to be most abundant in the peripheral regions of the pars distalis of the bovine, but there appears to be no characteristic distribution in the pig (Yamaguchi et al., 1973).

Basophils may be distinguished from the acidophils of the anterior lobe by their PAS-positivity. Despite their weak affinity for haematoxylin, these cells stain well with resorcin-fuchsin and with aniline blue of Mallory's trichrome method (Bloom and Fawcett, 1975). This class of cells includes the thyrotrophs or beta cells and the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) gonadotrophs or $delta_1$ and $delta_2$ cells respectively. Some workers include the corticotrophs or zeta cells in this group as well (Purves, 1966; Baker et al., 1970; Dubois, 1971). Thyrotrophs can be distinguished from other basophils by selective staining with Gomori's aldehyde-fuchsin (Mikami, 1970). They also stain specifically with aldehyde-thionin (Swope et al., 1970) and are the classical cyanophils (Purves, 1966). They appear blue with the aldehyde-thionin-PAS-orange G (Midgley, 1966) and performic acid-alcian blue-PAS-orange G (Adams and

Pearse, 1959) methods in contrast to the gonadotrophs which take on a reddish color following the same treatments. However, Yoshimura *et al.* (1974) applied the former technique to rat pituitaries and stated that, based on staining properties alone, it is impossible to identify the various basophilic cells of the pars distalis due to the fact that LH-gonadotrophs demonstrate a stainability intermediate between thyrotrophs and FSH-gonadotrophs. In addition, they cultured chromophobic cells of the pars distalis in a media containing a small amount of thyrotropic releasing hormone and luteinizing releasing hormone and discovered that "FSH-gonadotroph-like" cells developed from some of the chromophobes. They claim that they have demonstrated the entire life cycle of the basophil *in vitro* and suggest that the three types of basophils may actually represent different phases of the life cycle or secretory cycle of the same basophil.

Thyrotrophs are irregular or polygonal cells which are usually limited to the zone tuberalis in the anterior lobe of the pituitary in both pigs (Yamaguchi *et al.*, 1973) and cattle (Mikami, 1970). In the rat they are frequently found in clusters in the center of the gland and are rare in the peripheral regions, including the area near the intermediate lobe. They are located in the center of a cell cord and have a greater cytoplasmic volume than corticotrophs. Dispersed throughout the cytoplasm are large vacuoles containing little or no hormone. Secretion granules, some solid and some with central cores, with a diameter of 150-200 mμ are frequently observed near the plasma membrane in the rat (Nakane, 1970). In cattle they may reach a diameter of 300-400 mμ and are of moderate electron density (Mikami, 1970).

Following thyroidectomy, these cells undergo hypertrophy and a loss of secretion granules. Their previously sparse rER enlarges extensively and they become large vacuolated "thyroidectomy" cells (Mikami, 1970). Thyrotrophs secrete thyroid stimulating hormone, a basic glycoprotein which stimulates all phases of thyroxin synthesis, storage and release (Rhodin, 1974).

Gonadotrophs are among the largest cells of the anterior pituitary in many species. They are round and show a positive reaction to PAS but lack an affinity for aldehyde-fuchsin (Bloom and Fawcett, 1975). FSH-gonadotrophs stain with both red and blue acid dyes whereas LH-gonadotrophs show little affinity for acid dyes. In many techniques the latter acquire a brick red color when treated with PAS (Herlant, 1960). In the bovine pituitary, LH cells appear red or reddish-purple when stained with PAS, aldehyde-thionin, and PAS-methyl blue methods (Mikami, 1970). It has become apparent that the staining characteristics of these cells vary significantly between species (Dubois and Herlant, 1968).

LH-gonadotrophs show a distribution nearly identical to FSH-gonadotrophs in most species studied. In the pig, they are dispersed throughout the pars distalis although they are especially abundant in the zona tuberalis (Yamaguchi et al., 1973). They are found in the central area of the distal lobe in the bovine (Mikami, 1970) and Nakane (1970) reports an abundance of these cells in the anterior-ventral portion of the pituitary gland in the rat although they are numerous throughout the pars distalis (including the area adjacent

to the pars intermedia). LH and FSH-gonadotrophs possess similar cytological characteristics as well. Secretion granules range from 200-300 mμ in diameter (Nakane, 1970; Mikami, 1970). In 1970, Nakane was able to localize both LH and FSH simultaneously within the same cell. He found that gonadotrophs in the periphery of the rat pituitary gland were oval in shape and contained large vacuoles and hormone-filled secretion granules throughout their cytoplasm. These cells showed a tendency to react with both antisera indicating the presence of both LH and FSH. Gonadotrophs near the center of the gland usually contained only one of the hormones. Those in which only FSH was localized tended to be angular in shape and their secretion granules were usually located in the vicinity of the plasma membrane (Nakane, 1970). More recently, both FSH and LH have been localized within the same secretion granule using the peroxidase labeled-antibody technique (Moriarty et al., 1976).

FSH is a water-soluble glycoprotein hormone (Bloom and Fawcett, 1975) which stimulates the growth and maturation of ovarian follicles in the female. In the male it is responsible for stimulating spermatogenesis (Rhodin, 1974). LH, or interstitial cell-stimulating hormone (ICSH) as it is called in the male, is a glycoprotein hormone (Bloom and Fawcett, 1975) which functions in the female to induce ovulation and to stimulate development of the corpus luteum. In the male it stimulates the interstitial cells of the testis to produce testosterone (Rhodin, 1974).

Two classes of cells comprise the chromophobes: the corticotrophs and the precursor cells. They are called chromophobic because they do not stain readily with routine histologic staining techniques; however, many of the cells identified as such by light microscopy may actually be partially degranulated chromophils (Bloom and Fawcett, 1975). The precursor cells are small and contain little cytoplasm and a few small granules seen only with the electron microscope. They are believed to make up a reserve population of relatively undifferentiated cells possibly capable of later differentiation into acidophils and basophils (although this has not been proven conclusively) (Rhodin, 1974).

Despite their classification as chromophobes, corticotrophs are actually chromophilic. They have been termed acidophils by some (Racadot, 1963; Herlant, 1964; Ricci and Russolo, 1973) and described as basophilic by others (Purves, 1966; Baker et al., 1970; Dubois, 1971). Although they show little affinity for acid dyes according to Purves (1966), they have been found to be PAS-positive in all species studied (Racadot, 1963; Baker et al., 1970). Ricci and Russolo (1973) used the fluorescent antibody technique to localize ACTH in the canine pituitary and reported fluorescent groups of acidophils. Based on cytochemical analysis performed on the same sections or mirror sections, they concluded that corticotrophs contain erythrosinophilic secretion granules. They explained that it is extremely difficult to achieve a consistent staining classification of corticotrophs because they show varying staining characteristics depending

on their functional state. They suggest that researchers who sacrifice animals without taking sufficient precautions or following anesthesia will observe basophilic corticotrophs due to a decrease in secretion granules and an increase in basophilic rER. These workers will detect few, if any, erythrosinophilic granules. Corticotrophs are often mistakenly classified as chromophobes due to the fact that efforts to achieve distinctive staining frequently fail because the secretion granules are small, sparse and are located just beneath the plasma membrane, so they are often barely visible with the light microscope (Bloom and Fawcett, 1975).

In 1957, Farquhar incorrectly identified the angular cells lining follicles of the pars distalis as corticotrophs. She claimed that under conditions resulting in pituitary storage of ACTH, the size of the follicles and amount of colloid within them showed significant increases and suggested that the material within the follicles was stored ACTH (Farquhar, 1957).

A type of cell known as the "adrenalectomy cell" was discovered by Siperstein in 1963. Light microscopy and autoradiography was used to detect a specific type of chromophobe (having a rapid protein turnover rate) which developed only following adrenalectomy. This cell-type had irregular outlines and cytoplasmic processes which extended to the perivascular spaces (Siperstein, 1963). Later, using the electron microscope, it was shown that despite their apparent chromophobicity, these cells actually contained secretion granules approximately 200 mμ in diameter. (Siperstein and Allison, 1965).

More recently, Siperstein and Miller (1970) have studied the effects of acute and chronic administration of cortisol on the cells of pituitaries of intact and adrenalectomized rats at the electron microscope level. Their results confirmed their original findings. They showed that the adrenalectomy cell is without doubt the site of ACTH secretion in adrenalectomized rats and that using the same criteria, one can identify the cells which are responsible for ACTH production in normal rats. Their conclusions were based on the fact that adrenalectomy resulted in hypertrophy and conversion of normal ACTH cells to adrenalectomy cells and that following acute blockage of ACTH secretion by cortisol administration, an accumulation of granules occurred only in cells identified as corticotrophs and as adrenalectomy cells. Under conditions of chronic administration of cortisol, the cells identified as corticotrophs and as adrenalectomy cells were the only ones to undergo atrophy. They suggest that the initial effect of cortisol on ACTH secretion is prevention of secretion of ACTH by the corticotroph rather than inhibition of ACTH synthesis. This is based on the fact that 14 hours after cortisol administration, a marked accumulation of secretion granules was observed in both ACTH and adrenalectomy cells (Siperstein and Miller, 1970).

Corticotrophs, as described by Siperstein and Miller (1970), are irregular in shape and often located at the center of a cell cord. Their long and ramifying cytoplasmic processes extend between neighboring cells to terminate near or on capillary walls. Aligned in a

single row along the periphery of the cell are dense, smooth, homogeneous secretion granules approximately 200 mμ in diameter. According to Nakane (1970), who identified these cells by immunohistochemistry, ACTH cells are distributed in much the same manner as the somatotrophs. In fact, corticotrophs are often seen in the center of a cord in juxtaposition to several somatotrophs. They are stellate cells which contain mitochondria, ER, and a Golgi complex clustered about the nucleus. In Nakane's opinion, corticotrophs and thyrotrophs cannot be distinguished by ultrastructural characteristics alone; immunochemical techniques are necessary. In cattle, corticotrophs are most abundant in the central pars distalis (Mikami, 1970) as they are in the pig, although they are often observed in the zona tuberalis as well in the latter species (Yamaguchi et al., 1973).

ACTH, the straight-chained polypeptide composed of 39 amino acids (Bloom and Fawcett, 1975) secreted by the corticotrophs, acts as a stimulus to induce the release of glucocorticoids from the zona fasciculata of the adrenal cortex (Rhodin, 1974). Its biological activity resides in the first 24 amino acids from the N-terminal end (Brobeck, 1973). In addition to secretion of this hormone, there is evidence that corticotrophs in the sheep and pig anterior pituitaries simultaneously synthesize ACTH, beta-MSH and liptropic hormone (Dubois, 1972; Dubois and Graf, 1973; Dessy and Herlant, 1975).

The last major cell-type found in the pars distalis is the follicular cell. It was first described by Farquhar in 1957 but was

incorrectly identified as the source of ACTH (Farquhar, 1957). It is characterized by an absence of secretion granules and an irregular shape. Surrounding small intercellular cavities which are sealed off by junctional complexes, the follicular cells resemble the marginal cells which line the hypophyseal cleft. Both of these cell-types have been studied in the mouse at the electron microscope level and have been shown to be involved in digestion of waste material from other cells (Dingemans and Feltkamp, 1972).

Small peptides produced by neurosecretory cells in the hypothalamus are liberated into the perivascular spaces of the median eminence. Here they enter the hypothalamo-hypophyseal portal system which transports them to the pars distalis where they cause release of specific hormones by the adenohypophyseal endocrine cells. It is thought that there are six of these chemically distinct releasing hormones, corticotropin-releasing factor (CRF), thyrotropin-releasing factor (TRF), LH-releasing factor (LH-RF), FSH-releasing factor (FSH-RF), growth hormone releasing-factor (GH-RF), and prolactin-releasing factor (PRF or LTH-RF); however, it is not yet certain if there are actually two separate releasing hormones to stimulate the release of FSH and LH (Bloom and Fawcett, 1975). Matsuo et al. (1971) have reported that they have determined the structure of a decapeptide which they claim to be LH/FSH-releasing hormone. Crighton (1973) states that incubation of pituitary tissue with this decapeptide induces release of both LH and FSH. The exact mechanism by which the releasing hormones exert their effect on the anterior

pituitary cells is not known but Zolman (1974) suggests that the releasing hormone binds to the plasma membrane of the glandular cell and, according to Bloom and Fawcett (1975), this may result in an increase in cyclic AMP concentration within the cell which in turn may influence the release of hormone.

Hormones which inhibit the release of prolactin (Dhariwal et al., 1968) and growth hormone (Dhariwal et al., 1969) have been isolated from hypothalamic tissue and have been called prolactin-inhibiting factor (PIF) and somatostatin respectively.

Pars tuberalis

Relatively little attention has been given the pars tuberalis despite its widespread occurrence in mammals, birds, reptiles and amphibians. It is the least understood portion of the pituitary with respect to functional significance (Holmes and Ball, 1974).

The pars tuberalis forms a narrow sleeve of tissue separated from the infundibular stem and median eminence only by a thin layer of connective tissue (Cameron and Foster, 1972). It is adjacent to, and in most mammals, continuous with the main mass of adenohypophyseal tissue (Bloom and Fawcett, 1975). Longitudinally arranged epithelial cell cords and colloid-containing follicles interspersed with connective tissue comprise the bulk of the pars tuberalis. It is traversed by the major arterial supply to the anterior lobe and

the hypothalamo-hypophyseal portal system and is therefore the most vascular region of the entire pituitary. These vessels pass through or lie at the junction of the pars tuberalis and the surface of the hypothalamus (Holmes and Ball, 1974).

Cameron and Foster (1972) studied the rabbit pars tuberalis and discovered that two cell-types predominate, the most common being the 'tuberalis cell.' This type has rounded contours, pale electron-translucent cytoplasm, a few small secretory granules (about 100 nm. in diameter) and a fairly large ovoid nucleus.

The 'interstitial cell' is the second cell-type; its most distinguishing features are long cytoplasmic processes which often embrace 'tuberalis cells'. These processes are fairly electron-dense due to the numerous microfilaments contained within them. According to Cameron and Foster (1972), these 'interstitial cells' are similar to those described for the pars intermedia however, they appear to be more developed and more numerous in the pars tuberalis.

In addition to these two major cell-types, numerous fibroblasts, plasma cells, and macrophage-like cells with pseudopodia were seen particularly at the junction between the pars tuberalis and infundibular stem.

Evidence that some cells of the pars tuberalis secrete luteinizing hormone (LH) was produced by Midgley in 1966. He used immunohistochemistry to localize LH within cells of the pars anterior and pars tuberalis. Both these cells were aldehyde-fuchsin and PAS-positive. Legait (1969) reported a decrease in the volume of the

pars tuberalis in addition to stimulation of testicular interstitial tissue in males and ovarian tissue in females following injections of extracts of the tuberalis into male and female pre-pubertal rats. He also concluded that LH is secreted by cells of the pars tuberalis.

Cameron and Foster (1972) demonstrated evidence of neurosecretory innervation of the 'tuberalis cells' using chrome alum-haematoxylin to show 'beaded' nerve fibers and neurosecretory material within the pars tuberalis. At the electron microscopic level, they observed several 'bouton'-like structures containing clusters of mitochondria, numerous microvesicles (similar to synaptic vesicles) and dense membrane-bound neurosecretory granules. These structures were observed in close association with the plasma membrane of 'tuberalis cells'.

The role of the pars tuberalis in mammals remains uncertain. At present, it is thought to produce some, as yet, unidentified hormones. It has been noted that secretory products produced by the 'tuberalis cell' would most likely enter capillary loops originating from the primary vascular plexus and therefore would pass into the immediate vicinity of sites of release of hypothalamic releasing factors into the circulation of the pituitary. Cameron and Foster (1972) wonder "could the role of a hypothetical secretion lie here?"

Finally, the pars tuberalis lies in close association with tanycytes (modified ependymal cells which may act as a link in transporting materials from the hypothalamus to the portal vascular system) of the basal hypothalamus. This relationship has led to the hypothesis that the pars tuberalis may exert some influence on the activity of these cells (Holmes and Ball, 1974).

Pars intermedia

Much work has been done concerning the role of the pars intermedia in lower vertebrates. It is the source of melanophore and melanocyte stimulating hormone (MSH), which controls color adaptation in these animals. However, it is only recently that researchers have begun to successfully elucidate its role in mammalian species.

The pars intermedia is not present in all species, although it exists in varying degrees of development in most mammals, reptiles and amphibians. It is lacking in birds, although MSH or a similarly acting substance has been isolated from the pars distalis of these animals (Holmes and Ball, 1974).

In most mammals, the pars distalis is separated from the neurohypophysis by a cleft which is lined on its juxtaneural side by multilayered epithelium composed predominantly of basophilic cells. These cells comprise the pars intermedia. The human is an example of a mammal in which the cleft usually becomes discontinuous in post natal life and is later represented by a region of colloid-containing cysts called Rathke's cysts (Bloom and Fawcett, 1975). If the cleft is absent, the cells of the intermedia intermingle with those of the pars distalis (Holmes and Ball, 1974).

The intermediate lobe, despite its function as an endocrine organ, is poorly vascularized in most species. Although vessels may traverse areas of continuity between the pars distalis and pars intermedia, in other areas the cleft acts as a barrier preventing direct passage between the two (Holmes and Ball, 1974). Capillaries

can be observed in the pars intermedia of the pig but in most mammals, particularly the rat, this area is almost avascular. In addition, some species demonstrate loops of capillaries in the intermedia which connect with the hypothalamo-hypophyseal portal system. It is postulated that these may be the link between the hypothalamus and the intermediate lobe by way of which melanocyte releasing factor (MRF) and melanocyte release inhibiting factor (MRIF) are transported (Howe, 1973).

The cells of the pars intermedia are usually arranged in several layers producing a stratified appearance, however in some species, lobulation can be observed. Groups of cells separated by membranous septa as well as follicles containing an amorphous PAS-positive material are fairly common (Holmes and Ball, 1974).

At least two cell-types, usually three, can be observed using light microscopy. Type I cells may be polygonal, prismatic, rounded or cuboidal in shape and contain a large vesicular nucleus. Ultrastructurally and biochemically the type I cell exhibits characteristics of active metabolic processes. It shows changes indicative of altered secretory activity in response to stimuli and is commonly recognized as the source of MSH (Howe, 1973). It stains with basic dyes and contains fine secretory granules, about 200-300 mμ in diameter, which are frequently polarized towards the basal lamina and exhibit varying degrees of PAS and AF positivity. However, in this case the carbohydrate staining reaction is not indicative of the chemical nature of the hormone secreted by these cells (Howe, 1973;

Holmes and Ball, 1974; Bloom and Fawcett, 1975). According to Holmes and Ball (1974) electron microscopy demonstrates the presence of two types of parenchymal cells within this class: they are light cells and dark cells. The former contain numerous clear vesicles and small dense granules. They also possess a fairly well-developed Golgi apparatus. In contrast, the dark cells contain very few if any clear vesicles and numerous free ribosomes. Their Golgi complex is even more developed than that of the light cells. Romeis (1940, cited in Holmes and Ball, 1974) believed that these two forms actually represent different functional states of the same cell-type. Kobayashi (1964) noted that dehydration produces variations in electron density of the secretion granules; this suggests that the function of these cells is controlled by neurosecretory material of the posterior pituitary. Later, it was observed (Kobayashi, 1965) that these cells changed morphologically in response to stress and adrenalectomy, and so it was concluded that there may be a close functional relationship between the pars intermedia and the adrenal cortex. However, even varying fixation methods produced differing results with these cells, contributing to the confusion (Porte et al., 1971). With the light microscope, one could make no clear distinction between the two types using PAS staining and it was found that the difference is not due to the presence of glycogen because the reaction was not destroyed by pretreatment with diastase (Holmes and Ball, 1974). Within the cell, the proteinaceous hormone is thought to be bound to glycoprotein granules, thereby producing the carbohydrate

staining reaction. This cell-type is the most numerous in the pars intermedia (Bloom and Fawcett, 1975).

Type II cells are less abundant, small, angular, strongly PAS-positive cells containing a hyperchromatic nucleus (Howe, 1973). They have less cytoplasm and contain smaller granules (about 100-130 mμ in diameter) compared to type I cells. They resemble the ACTH cells of the pars distalis, and it is thought that they may secrete ACTH, partly because they increase in number following adrenalectomy (Holmes and Ball, 1974). Nakane (1970) reported localization of ACTH antibodies to cells of the intermedia using immunohistologic studies. However, because ACTH and MSH share a common sequence of amino acids, it is possible that the results were due to a cross-reaction of ACTH antibodies with MSH. Phifer and Spicer (1970) then generated antibodies to a portion of the ACTH molecule which contains no amino acid sequence in common with any known MSH. Using the immunoglobulin-peroxidase bridge technique, they found that in the cow, pig and rat, some intermedia as well as some pars distalis cells were selectively immunostained. A faint staining appeared in the rabbit pars intermedia. They concluded that ACTH is present in the pars intermedia of the species studied.

Stoeckel et al. (1971) have studied the mouse hypophysis and discovered that the rostral zone of the intermediate lobe consists almost entirely of putative corticotrophs which show reactive changes subsequent to adrenalectomy. Their ultrastructural characteristics are in accord with those of corticotrophs in the pars distalis, and

the corticotrophic nature of these cells was supported by the development of rER and the frequent presence of granular condensations within the saccules of the Golgi complex following adrenalectomy. These are signs of increased secretory activity. Identical changes were observed in the pars distalis. A rich vasularity exists in this rostral zone along with numerous cysts deriving from the hypophyseal cleft. There is a narrow transitional zone where corticotrophs of the rostral zone lie in direct contact with MSH cells. These two cell-types were also seen in close association with one another along the neural lobe, although they were always separated from the nervous tissue by an epithelial basal lamina. However, dorsally, the corticotrophs are in direct contact with axons of the hypophyseal stalk, due to the lack of a basal lamina. In addition, synaptoid contacts existed between these cells and nerve fibers which contained dense core vesicles. Such structures were present only in the rostral zone, and Stoeckel _et al_. suggest that these cells repond differently to various stimuli in comparison with corticotrophs in other portions of the adenohypophysis. Due to the intimate relationship with the capillaries of the hypothalamo-hypophyseal portal system, these rostral zone corticotrophs are the first cells to contact CRF released in the median eminence and therefore have an advantage in being rapidly influenced by the vascular and neural pathways.

Stoeckel _et al_. (1971) also observed larger cells with more regular outlines, predominantly in the periphery of the rostral zone. These were easily identified as gonadotrophs as they contained an

abundance of secretion granules and dilated cisternae of ER filled with weakly electron dense material.

Earlier, Stutinsky (1958, cited in Holmes and Ball, 1974) had reported both aminergic and neurosecretory nerve fibers in the pars intermedia of the horse, ox and pig and suggested a dual innervation of the pars intermedia in general.

In 1972, Moriarty and Halmi used unlabeled antibody and soluble peroxidase-antiperoxidase complex to demonstrate the presence of ACTH in the secretion granules and vesicles of both light and dark cells of the rat pars intermedia at the electron microscope level. They found definite immunohistochemical differences between the corticotrophs of the intermedia and distalis. The Golgi complex and its granules stained intensely in the ACTH cells of the pars distalis, yet they did not stain at all in the corticotrophs of the pars intermedia. The immunohistochemical staining intensity for ACTH was found to be much stronger in the ACTH cells of the pars intermedia than in those of the pars distalis (Moriarty and Halmi, 1972).

A corticotrophin-like intermediate lobe peptide (CLIP) was discovered in 1973, using radioimmunoassay. Higher concentrations existed in the pars intermedia than in the adenohypophysis, suggesting that perhaps the intermedia plays an even more important role in ACTH secretion than the anterior lobe (Scott _et al_., 1973).

However, by removing the adenohypophysis or neurohypophysis from rats and then measuring plasma ACTH and corticosterone following stress and basal plasma ACTH three weeks after adrenalectomy, Greer _et al_.

(1975) have shown that the posterior lobe of the pituitary does not secrete functionally significant amounts of ACTH. (The posterior lobe includes both the pars intermedia and pars nervosa.) In addition, hypophyseal peptidases have been reported which split ACTH into alpha-MSH and other fragments suggesting that immunoreactive ACTH may be simply a precursor of CLIP and alpha-MSH. Therefore, ACTH demonstrated by radioimmunoassay to be in the pars intermedia may never reach the blood as biologically active ACTH. Allen _et al_. (1974) have also found that there is a several-fold higher ratio of posterior to anterior lobe ACTH with the N-terminal antibody than with the $alpha^{11-24}$ antibody indicating a greater correlation of the $alpha^{11-24}$ than of the N-terminal antibody with specific ACTH activity.

Moriarty and Moriarty (1975) and Moriarty _et al_. (1975) have reported the presence of bioactive ACTH in the intermediate lobe of the rat. They suggest that ACTH secretion from this portion of the pituitary is not appreciably regulated by plasma glucocorticoid levels and that only neurogenic stress results in depletion of ACTH from neuro-intermediate lobes.

Ependymal cells are the third cell-type in the pars intermedia. They are acidophilic and weakly PAS-positive. These cells may be flattened, low cuboidal or columnar and are often ciliated. They line the surface of the intermedia which borders the hypophyseal cleft (Holmes and Ball, 1974). These cells lack immunopositivity for MSH and ACTH; however, they show a high level of enzymatic

activity. Some suggest they play a role in the production of colloid which fills cysts of the pars intermedia and the cleft (Howe, 1973). Others believe that the colloid within the cleft originates from the anterior lobe and that these cells are more likely to be involved in transport than secretion (Vanha-Perttula and Arstila, 1970).

It is thought that the hypothalamus normally exerts an inhibitory effect on the pars intermedia and that the paraventricular nucleus is probably somehow involved. Removal of the intermediate lobe from the influence of the central nervous system results in hypertrophy and hypersecretion of MSH (Howe and Thody, 1969). In addition, Kastin and Schally (1967) have shown that there is a negative feed-back mechanism by which the blood level of MSH itself influences the release of MSH from the pars intermedia and have concluded that part of this feedback is directly exerted upon the pituitary gland. Evidence of a negative feedback control of MSH by adrenal glucocorticoids similar to that exerted on ACTH secretion has been presented by Liddle (1969, cited in Howe, 1973). However, Kraicer _et al_. (1973) have produced contradictory results. They report no effect on MSH secretion in response to modification of circulating corticosteroid levels by either adrenalectomy or cortisone administration. Presently, these two views are irreconcilable.

The role of the pars intermedia has been the focal point of many studies in recent years. Legait and Legait (1964) have suggested that the mammalian intermediate lobe is in some way involved with an animal's ability to survive in arid conditions. This is partly based

on the fact that desert-dwelling species possess a relatively large intermedia compared to other mammals. In the mink, dehydration results in an increase in the number of cells of the pars intermedia which stain intensely with PAS. Rats appear to have an even greater capacity to adapt to the effects of salt-loading than the mink. After four to seven days of salt-loading, the cells of the rat pars intermedia had returned to normal proportions, indicating adjustment to a new level of activity. The number of PAS-positive cells of the mink intermedia, on the other hand, was still elevated eleven days after initiating treatment (Legait and Legait, 1964). However, Howe (1973) points out that one cannot be sure that the intermedia is responding primarily to the salt or osmotic stress or secondarily to hormones released from other glands such as the neurohypophysis or adrenal cortex.

Process of secretion

The same general pathway of protein synthesis that is currently accepted for protein-secreting cells is thought to apply to the protein-secreting cells of the anterior pituitary. That is, secretory proteins are synthesized on ribosomes, transferred into the cisternae of the rough endoplasmic reticulum (rER), transported via transitional elements to the immature face of the Golgi complex and finally, concentrated and packaged into vesicles which are released from the mature face as secretion granules. However, present findings indicate that the condensation step is slightly modified in adenohypophyseal cells. Initially, condensation of small packages of secretory

material within Golgi cisternae occurs (rather than in condensing vacuoles) and eventually, the mature secretion granule is formed by aggregation of several of the small Golgi-derived packages (Smith and Farquhar, 1966).

Adenohypophyseal cells release their hormones into the extracellular space via a process called exocytosis or granular extrusion (Farquhar, 1961; Smith and Farquhar, 1966). The granular membrane fuses with the plasma membrane and the secretory product is released from the cell. This occurs primarily on cellular surfaces facing either the perivascular space or the intercellular extension of the basement membrane (Farquhar, 1961; Pelletier _et al._, 1971).

Uebeberg and Hohbach (1972) dealt with the question of exocytosis in normal anterior pituitary cells. They found that granules are released either separately or several together and that vacuoles containing granules which appear to be within the cytoplasm are actually shown in serial section to be located in the extracellular space. The granules do not enter the bloodstream but dissolve, losing some of their electron density. Their lysis and the release of hormone is thought to be due to a change in the medium and to the process of diffusion. Uebeberg and Hohbach (1972) also suggest that there may be other mechanisms of hormonal release besides granular extrusion.

Due to the apparent lack of an increase in cell surface area when secretion is extensively stimulated, one may assume that the extra plasma membrane resulting from fusion of granular membranes

must somehow be removed. Pelletier (1973) used horseradish peroxidase (HRP) as an intracellular tracer to investigate whether excess membranes formed during the process of exocytosis are reutilized. He used cyclic AMP (cAMP) derivatives to stimulate degranulation of somatotrophs and mammatrophs and then observed an increase in vesicles at the periphery of the cell as well as hypertrophy of the Golgi apparatus. (It is thought that the cAMP acts as a mediator of the effects of hypothalamic releasing factors.) He found that the increase in Golgi vesicles was not prevented by inhibition of protein synthesis and therefore, the vesicles apparently originate from pre-existing membranous proteins. Based on the time course of incubation with HRP, Pelletier (1973) concluded that many vesicles are formed from the membranes of expelled granules, since the uptake of HRP is directly related to the amount of exocytosis. Within one hour most of the HRP-containing vesicles had migrated to the Golgi region and soon after, HRP-containing Golgi saccules were detected in somatotrophs. He suggests that the granular membranes are added directly to the Golgi saccules to replace the membranes lost during the formation of secretion granules. The Golgi membranes in the mammotrophs were not labeled with HRP, although positive Golgi vesicles, granules and possibly lysosomes were abundant. This indicates that there is a transfer of HRP directly to the granules during their formation. In short, there is strong evidence that membranes of secretory granules are reutilized for the formation of new secretory granules (Pelletier, 1973).

Several persons have reported the existence of microtubules and microfilaments within the cytoplasm of anterior pituitary cells and have suggested that these structures may play a role in cell secretion (Pelletier and Bornstein, 1973; Labrie *et al*., 1973; MacLeod *et al*., 1973; Horvath *et al*., 1973; Shiino and Rennels, 1974; Tseng and Kittinger, 1974; Shiino *et al*., 1974; Shiino and Rennels, 1975). Horvath *et al*. (1973) suggest that these microfilaments may influence intracellular migration of secretion granules and exocytosis.

Pelletier and Bornstein (1973) also suggest that the microtubules may play a role in cytoplasmic mobility and granule movement. However, ultrastructural observations and radioimmunoassays of somatotrophs and mammotrophs following incubation with colchicine or vincristine (both of which disrupt microtubules) demonstrated that microtubules apparently were not essential for prolactin and growth hormone secretion (MacLeod *et al*. 1973).

Shiino *et al*. (1974) have perfected a method for preserving microtubules in anterior pituitary cells. They reported an abundance of microtubules in mammatrophs of lactating mice in peripheral areas of cytoplasm as well as in the Golgi region and in very close association with secretion granules and mitochondria. These observations support the theory that microtubules are involved in the migration of granules and also suggest a possible role in the movement of mitochondria.

More recently, Shiino and Rennels (1974) have observed paracrystalline aggregates of microtubules in mammatrophs and somatotrophs

from untreated chinchillas. They were not detected in cell types other than acidophils. Cells containing these structures frequently showed aberrant forms of secretion granules, sometimes with microtubular or fibrous elements in close contact with the ends of the distorted granules. The presence of these granules, in addition to a scarcity of cytoplasmic microtubules in the cells which contained paracrystalline aggregates, have led these workers to conclude that these aggregates may be related to an abnormality in the secretory process.

In 1975, microtubules were successfully demonstrated in thyrotrophs of rat pituitary glands. They were most numerous in early stages of development of thyroidectomy cells and were located in close proximity to mitochondria, endoplasmic reticulum, secretion granules and membranes of Golgi complexes. These observations indicate a possible role of microtubules in degranulation or other cellular functions associated with the hypersecretory state (Shiino and Rennels, 1975).

Regulation of the secretory process in the cells of the anterior pituitary was investigated by Smith and Farquhar (1966) by studying mammatrophs in lactating rats during lactation and following removal of the suckling young. The techniques used involved determination of the nature and content of lytic bodies and the localization of acid phosphatase (AcPase). They discovered an intracellular disposal mechanism which acts to turn over secretory protein when secretion is suppressed. Mammotrophs from the continuously lactating

animals contained abundant rER, a large Golgi complex with many forming secretion granules and a few lytic bodies, primarily multivesicular and dense bodies. However, the lactating animals which had been separated from their young for more than twelve hours, thereby inhibiting production and discharge of prolactin, showed progressive involution of the protein synthetic apparatus with sequestration of endoplasmic reticulum and ribosomes in autophagic vacuoles. They also showed incorporation of secretion granules into multivesicular and dense bodies. The contents of the lytic bodies were progressively degraded over a period of 24-72 hours, which provided evidence that in the glandular cells of the anterior pituitary, lysosomes function in the regulation of secretion by destruction of excess secretory products.

Neurohypophysis

Three main components comprise the neurohypophysis: they are the median eminence of the tuber cinereum in the basal hypothalamus, the infundibular stem and the infundibular process or pars nervosa. Populations of cells called pituicytes and a multitude of processes from extrinsic secretory neurons compose the bulk of this organ. Approximately 100,000 unmyelinated nerve fibers originating in the hypothalamus and terminating at various levels of the neurohypophysis make up the major portion of the infundibular stem. They are collectively known as the hypothalamohypophyseal tract. More specifically, the cell bodies of these neurons reside in the supraoptic

nucleus (above the optic chiasma) and the paraventricular nucleus (in the wall of the third ventricle), and portions of the tuber cinereum. Neurosecretory granules, 100-300 mμ in diameter, are synthesized within the perikarya of these secretory neurons. The abundant rER (Nissl substance) is believed to be the site of hormone synthesis. Subsequently, the hormones are bound to a carrier protein called neurophysin and passed to the Golgi complex where they are packaged in membrane-bounded electron dense secretion granules. These then pass peripherally through the nerve fiber. (Numerous microtubules contained within the fiber may facilitate this transport.) The terminal arborizations of the axons abut directly on the basal lamina of the capillaries, which are lined by an extremely attenuated fenestrated endothelium. The hormones are thought to be released from the neurosecretory granules within the nerve endings and traverse the perivascular space and endothelium in molecular dispersion.

Spherical masses of highly variable size which stain deeply with chrome alum-haematoxylin can be seen along the nerve fibers of the hypothalamohypophyseal tract with the light microscope. These are local accumulations of neurosecretory material within the axoplasm. With the electron microscope they appear as dilated portions of the axons, densely packed with 100-300 mμ granules. After transection of the hypophyseal stalk, stainable neurosecretory material and detectable hormone in the pars nervosa distal to the lesion disappear, while a concomitant accumulation of both appear in the stalk proximal to the transection. Neurosecretory material synthesized

in the supraoptic and paraventricular nuclei is transported along the nerve fiber and is stored in their terminals in the pars nervosa.

Pituicytes are similar in structure to the neuroglial cells of the central nervous system. They have cytoplasmic processes which embrace nerve fibers, Herring bodies and nerve terminals. Some pituicytes contact the perivascular space, the significance of which is unknown (Rhodin, 1974; Bloom and Fawcett, 1975). Various functions from merely supportive to an actively metabolic role involved with the terminal stages of the neurosecretory process have been proposed for the pituicyte; however, no definite conclusions have yet been made (Olivieri-Sangiacomo, 1973).

The neurohypophysis functions as a site of storage and release of two cyclic polypeptide hormones, oxytocin and vasopressin or anti-diuretic hormone (ADH). Oxytocin stimulates the contraction of uterine smooth muscle and myoepithelial cells in the alveoli of the mammary gland, thus mediating the milk ejection reflex in response to suckling in lactating animals. Contraction of smooth muscle within the walls of small blood vessels is the result of vasopressin secretion. Vasopressin also acts on the distal and collecting tubules of the kidney where it stimulates the reabsorption of water, thereby producing an anti-diuretic effect (Rhodin, 1974; Bloom and Fawcett, 1975).

The proximal portion of the neurohypophysis or median eminence is primarily involved with the transfer of releasing hormones from the hypothalamus into the portal blood for transport to the adenohypophyseal cells. However, the neurosecretory fibers passing to the pars nervosa also traverse this region (Holmes and Ball, 1974).

Porcine Stress Syndrome

A condition exists in market-size pigs which results in sudden unexpected deaths and a reduction in pork quality. It has been called 'porcine stress syndrome' (PSS) (Topel et al., 1968) and occurs predominantly in heavily-muscled animals following periods of stress. Pale, soft, exudative (PSE) post-mortem musculature, first reported by Ludvigsen (1953) and Briskey et al. (1959), develops in some SS pigs, however, others produce dark carcasses. It has been suggested that the postmortem muscle characteristics depend upon the animal's ability to adapt to stress and the strength and duration of the stress (Topel, 1969).

The significance of the adrenal gland in stress adaptations (Selye, 1959) has led to comparisons of adrenal function and the pituitary/adrenal axis between SS and SR swine. Cassens et al. (1965) observed large lipid masses in the zona reticularis of some SS pigs and postulated that they may be representative of a degenerative change. Later, Howe et al. (1969) noted that these masses were induced by fluctuating environmental temperatures or low humidity and theorized that there is a relationship between the rapid anaerobic glycolysis of striated muscle and these adrenal alterations. In 1972, Ball et al. reported excessive lipid deposits in the inner cortex of one-third of the SS pigs studied. Large masses of lipid were observed in only ten percent of the normal meat-type pigs, and none in fat-type pigs which displayed no signs of stress-susceptibility.

An adrenocortical insufficiency in SS swine was suggested by Judge et al. (1966) based on findings that these animals excrete lower concentrations of urinary corticoids than normal pigs.

A negative correlation between plasma 17-hydroxy corticosteroid (17-OHCS) levels and the degree of PSE muscle characteristics was reported by Topel et al. in 1967. This encouraged Topel and Merkel (1967) to investigate the effects of exogenous adrenal corticoids on plasma 17-OHCS levels and porcine carcass traits. Although adrenal atrophy and suppressed 17-OHCS levels were attained, PSE muscle did not result. In addition, they found that following normal handling, control pigs showed plasma corticoid levels comparable to those previously reported (Topel et al., 1967) for pigs producing dark, firm, dry carcasses. Untreated control pigs who experienced minimal handling had even lower levels indicating that pre-slaughter handling may greatly influence circulating 17-OHCS concentrations and possibly the condition of the carcass. A year later they demonstrated that pigs which are terminated in the last stage of the syndrome show low plasma 17-OHCS levels, high muscle lactate concentrations and low muscle pH and produce PSE carcasses. Thus, Topel et al. (1968) concluded that "these pigs lack the ability to secrete adrenal glucocorticoid hormones when stressed and therefore, have difficulty adapting to a stressful situation."

Marple et al. (1969) reported conflicting results. They noted no significant relationship between levels of plasma corticoids at death and postmortem quality of the muscle. However, they produced

adrenal insufficiency in normal swine by chronic exogenous corticoid treatment followed by abrupt withdrawal and found that these animals became less able to tolerate exercise and excitement. Nevertheless, they could not conclude whether the adrenal insufficiency apparent in SS pigs was of primary (adrenal) or secondary (pituitary) origin.

In 1971, Sebranek et al. used dexamethasone to suppress endogenous ACTH and then injected exogenous ACTH into the ear veins of SS and SR swine. The SS pigs showed a lesser ability to respond to exogenous ACTH with increased glucocorticoid production. Meiburg et al. (1973) did not agree. They concluded that SS pigs are fully competent to synthesize cortisol based on an in vitro study in which the cortisol synthetic response to ACTH did not differ between SS and SR porcine pituitary tissues.

Marple et al. (1972c) studied the pituitary/adrenocortical axis in SS and SR swine under various conditions. They observed that under control conditions, SS pigs had a three to four fold higher plasma ACTH level than SR pigs yet they had similar plasma corticoid levels. The mean plasma adrenocorticoids to mean plasma ACTH ratio in SS pigs was one-third to one-fourth that of the SR pigs indicating that either there is a lesser adrenal response to ACTH in SS pigs or they have an increased rate of adrenocorticoid metabolism. Under stress, they saw a two fold increase in plasma ACTH and lower plasma corticoid levels in both types of animals. This is in conflict with Topel et al. (1967) and Judge et al. (1968) who had previously reported lower plasma and urinary corticoid levels

in SS swine. Marple et al. (1972c) concluded that plasma adrenocorticoid levels alone are not a reliable estimate of the stress-susceptibility of a pig and that the plasma ACTH concentration is influenced by the environment but is nevertheless always higher in SS animals. Thus, under basal conditions or a controlled stress, circulating ACTH concentrations could be indicative of the stress-susceptibility of an animal. The plasma corticoid/plasma ACTH ratio (under similar conditions) may also serve as an index of adrenal responsiveness to ACTH or the rate of metabolism of adrenocorticoids. Due to the high levels of endogenous plasma ACTH among SS pigs, it was believed that these animals do not suffer a secondary (pituitary) type of adrenal insufficiency but more likely one of an acute primary (adrenal) origin.

Additional evidence which could be interpreted as indicating that SS swine are capable of synthesizing and releasing adrenocorticoids in response to ACTH but at a lower rate than SR pigs was presented by Sebranek et al. (1973). An equivalent dosage of dexamethasone was less effective in suppressing ACTH release in the SS pigs than in the SR animals. A possible abnormality of the ACTH-corticosteroid feedback mechanism was proposed. These authors suggested that,while under a high degree of stimulation by ACTH, the adrenal cortex in SS pigs functions at a low level which is apparently not adequate to maintain homeostasis.

Topel et al. (1973) reported that SS swine respond to stress with an increase in plasma corticoids at a slower rate than SR pigs,

but he suggested that either decreased functional capacity of the adrenal cortex or an excessive corticoid clearance rate could cause this condition.

Ball _et al_. (1974) have produced results which cast doubt on the role of adrenal hypofunction in the pathogenesis of PSS. They produced such a state by the exogenous administration of glucocorticoids and studied the major adrenocortical changes. Their findings bore a limited resemblance to those observed in SS pigs.

The metabolism of cortisol in these two types of swine was studied by Marple and Cassens in 1973. SS pigs had a cortisol turnover rate three times that of the SR pigs and a metabolic clearance rate five times as great. Although the cause of this major difference in body metabolism of adrenocorticoids is unknown, a possible malfunctioning of the liver and skeletal muscle was proposed.

In order to explain the rapid disappearance of labeled cortisol from the circulation of SS pigs, Marple _et al_. (1974) measured the corticosteroid binding activity in these and SR swine. SS animals had a higher mean corticoid-binding globulin (CBG) level as well as a significantly lower mean cortisol-CBG association constant. Although they found no correlation between cortisol, CBG-binding activity or CBG-cortisol association constant and specific carcass traits, they did observe greater levels of plasma cortisol and CBG-binding activity along with a larger number of death losses due to stress in Poland China swine (a breed in which PSS is frequently seen). Nevertheless, based on work relating to muscle metabolite

and serum enzyme levels to pork quality scores, they have concluded that CBG-binding levels are not particularly accurate indices of stress-susceptibility in pigs.

A study was conducted by Kraeling et al. (1975) to determine whether the rate of post-mortem glycolysis in the longissimus muscle could be altered by varying the level of activity of the anterior pituitary. A direct relationship between the two was discovered, suggesting that pigs which demonstrate PSE post-mortem muscle properties may hypersecrete one or more anterior pituitary hormones prior to exsanguination.

There is evidence (Weiss, 1971; Topel and Staun, 1972; Topel et al., 1974) that SS swine produce and utilize epinephrine at a greater rate than SR pigs under stressful conditions.

Epinephrine activates the first step of glycogenolysis via cyclic AMP and results in an increase in blood glucose. During severe stresses, anaerobic pathways are drawn upon to supply energy. Oxidized nicotinamide-adenine-dinucleotide (NAD) (which is required for glycogen breakdown) is regenerated by conversion of pyruvate to lactate. Muscle lactate rapidly accumulates and diffuses into the blood faster than lactate can be converted to glucose in the liver. This results in acidosis. This is the major cause of death from PSS and the high level of muscle lactate is involved in the development of PSE carcass traits (Topel et al., 1974). High blood glucose levels observed in SS pigs during and after stress (Weiss, 1971) indicate that these animals have excessive stimulation by catecholamines

for breakdown of glycogen (Topel et al., 1974). Epinephrine administration prior to slaughter results in deterioration of meat properties. Although it is not certain whether hypersecretion of this hormone is the cause, accompaniment or result of increased stress-susceptibility, under emotional stress it can at least encourage the development of quality faults (Fischer et al., 1974). It is not known whether conversion of lactate to glycogen is impaired in SS pigs (Weiss et al., 1974).

Growth hormone and thyroid hormones have also been implicated in the etiology of PSS; however, as of now, a causal relationship can neither be presumed nor denied (Fischer et al., 1974).

MATERIALS AND METHODS

Pituitaries from a total of forty-five pigs were analyzed; twenty of these were from control or stress-resistant (SR) animals. The remaining twenty-five were known to be stress-susceptible (SS) as assayed by their response to halothane anesthesia. Both sexes and a variety of breeds were represented, all animals being between three and seven months old at time of slaughter.

Pigs were killed by exsanguination by jugular transection. The pituitary glands were collected and placed in fixative within ten to twenty minutes subsequent to slaughter. In most cases, Zenker's formalin was the fixative used although a few pituitaries were fixed in Helly's solution. Following embedding in paraplast, the tissues were sectioned at a thickness of six microns (6 μ). As close to median sagital sections as possible were used for the cell-counts. Several sections were mounted on each slide to later allow a choice of the best stained sections for the counts. Adjacent groups of sections were mounted serially on consecutively numbered slides. The same staining technique was then applied to all slides bearing the same number.

Three different methods were used to stain the tissues. They are:

1. Trichrome stain of Cleveland and Wolfe (1932) as modified by Das (1971).

Following post-chromation in five percent potassium dichromate solution, the sections were dehydrated and then stained in a pre-warmed acidified solution of luxol fast blue, 0.1 percent solution in 95 percent ethanol. Differentiation was accomplished using a 0.05 percent lithium carbonate solution.

2. Azocarmine G, orange G, aniline blue method (Dawson and Friedgood, 1938) as modified by Das (1971).

   Staining in azocarmine was carried out for 2.5 hours at 57° C. A one percent solution of aniline oil in 95 percent ethanol was used to achieve differentiation.

3. Aldehyde-thionin, periodic acid-Schiff's, erythrosin, orange G, aniline blue method (Yamaguchi et al., 1973).

   This is actually a combination of the aldehyde-thionin method of Paget and Eccleston (1959), PAS technique of McManus (1948) and a modification of Cleveland and Wolfe's trichrome stain (1932).

The tinctorial appearance of the various cell-types following staining with each of these methods is as follows:

1. Luxol fast blue-trichrome stain

   somatotrophs - blue-green

   lactotrophs - brick red

   thyrotrophs - intense blue

   FSH gonadotrophs - light blue

   corticotrophs - dull red (erythrophilic)

   LH gonadotrophs - violet

2. Azocarmine G - orange G - aniline blue stain

    somatotrophs - orange

    lactotrophs - red

    thyrotrophs - blue

    corticotrophs - no stain

3. Aldehyde-thionin - PAS - erythrosin - orange G - aniline blue stain

    somatotrophs - red-orange

    lactotrophs - yellow

    thyrotrophs - blue-purple

    LH gonadotrophs - blue-purple

    FSH gonadotrophs - red-purple

    corticotrophs - light purple or light blue

    follicular cells - light blue or no stain

Cell counts were achieved using the following method. A grid reticle, five millimeters square (divided into 100 smaller squares), was inserted into the 10X ocular of the microscope. Using the 100X oil immersion lens, every fifth field of every fifth row in the central region of the pars distalis was examined and both the number of corticotrophs and the total number of cells within each field was recorded. The cells contacting the upper and left-hand margins were included in the count; those contacting the lower and right-hand margins were not. The decision as to whether or not to count a particular cell as an ACTH cell was based on the color, shape and relative size of the cell, distribution of granules, and location

of the cell within a cell cord. Doubtful cells were not counted as corticotrophs.

Experimental and control tissues were subjected to three staining techniques to compare the average number of corticotrophs per unit area that resulted from the factorial combination of the above factors. Stress resistance was the whole plot factor and staining procedure was the subplot factor of the split-plot experiment.

One section from each slide and ten fields per section were examined in this manner, and the percentage of corticotrophs per section was calculated. These data were subjected to a statistical analysis for a split-plot experiment where the primary interest was in the interaction of stress-resistance and staining techniques as well as possible main effects. The analysis of variance had the following degrees of freedom for the respective sources of variation.

Analysis of Variance

Source of variation	Degrees of freedom
Stress group	1
Pigs within group	43
Stain	2
Stress x stain	2
Remainder	86

RESULTS

The percentage of corticotrophs in the pars distalis of SS swine was found to be significantly higher than in that of SR animals. Fields from sections stained with luxol fast blue-trichrome (stain 1) appear in Figures 1 and 2. Those stained with azocarmine G-orange G-aniline blue (stain 2) can be seen in Figures 3, 4, 5 and 6. In Figures 7 and 8, sections stained with AT-PAS-erythrosin-orange G-aniline blue (stain 3) are shown. Raw data are shown in Tables 1 through 4. A significant ($P < 0.01$) stress x stain interaction was observed, which is evident in the mean values found in Table 5 and shown in Graph 1. Stain 3 revealed a less than significant increase in the number of ACTH cells in stress-prone pigs over the controls. However, a significant ($P < .01$) increase in corticotrophs in SS pigs was demonstrated by the other two stains employed, which was of sufficient magnitude to preclude the possibility that the difference between SS and SR pituitaries was an artifact of staining technique.

Seventy-two pituitaries were stained; however, only forty-five of these were considered satisfactory for analysis. The remainder produced consistently poor results as the cells either failed to demonstrate an affinity for the various dyes or showed unusual tinctorial characteristics, preventing certain differentiation between cell-types. The cause of this is unknown although possible storage near a heat source prior to histologic preparation may be involved.

Table 1. Percentage of corticotrophs in the pars distalis of stress-resistant swine as determined by use of three different staining techniques.

Pig number	Stain 1	Stain 2	Stain 3
C1	3.25	2.00	2.50
C2	4.05	3.27	4.29
C3	4.46	2.45	2.03
C4	4.00	4.73	2.61
C5	4.14	3.07	1.99
C6	3.60	2.10	1.38
C7	3.27	2.53	4.14
C8	2.17	2.11	1.43
C9	2.47	2.63	1.32
C10	3.85	2.53	2.10
C11	4.26	3.03	2.67
C12	2.92	3.27	2.65
C13	3.73	3.53	2.38
C14	2.29	2.84	1.83
C15	2.76	3.18	3.29
C16	2.48	2.41	2.70
C17	3.40	3.73	3.33
C18	5.11	3.20	3.80
C19	5.65	4.55	3.80
C20	4.58	5.59	2.58

Table 2. Percentage of corticotrophs in the pars distalis of stress-susceptible swine as determined by use of three different staining techniques.

Pig number	Stain 1	Stain 2	Stain 3
S1	6.12	3.23	1.97
S2	7.32	6.51	3.68
S3	6.25	5.23	1.92
S4	4.51	6.55	3.66
S5	4.00	3.45	3.60
S6	4.80	3.90	2.74
S7	3.45	5.26	4.48
S8	5.33	5.23	4.29
S9	5.92	8.14	3.60
S10	5.77	4.44	2.14
S11	6.34	7.97	3.33
S12	6.11	6.12	3.42
S13	5.93	7.33	3.66
S14	4.14	4.00	2.00
S15	3.20	5.71	1.94
S16	3.79	4.35	4.35
S17	4.88	5.77	2.87
S18	3.55	2.88	2.19
S19	3.68	2.63	2.84
S20	4.83	5.15	3.57
S21	4.23	4.38	4.03
S22	5.07	5.41	3.62
S23	5.48	5.60	3.45
S24	3.03	2.96	2.11
S25	2.98	3.53	2.07

Table 3. Number of corticotrophs counted per number of cells counted in the pars distalis of stress-resistant swine as determined by use of three different staining techniques.

Pig number	Stain 1	Stain 2	Stain 3
C1	5/154	3/150	4/160
C2	6/148	5/153	7/163
C3	7/157	4/163	3/148
C4	7/175	8/169	4/153
C5	6/145	5/163	3/151
C6	5/139	3/143	2/145
C7	5/153	4/158	6/145
C8	3/138	3/142	2/140
C9	4/162	4/152	2/152
C10	6/156	4/158	3/143
C11	6/141	5/165	4/150
C12	4/137	5/153	4/151
C13	7/188	6/170	4/168
C14	4/175	5/176	3/164
C15	4/145	5/157	5/152
C16	4/161	4/166	4/148
C17	5/147	5/134	5/150
C18	7/137	4/125	6/158
C19	7/124	7/154	6/158
C20	7/153	9/161	4/155

Table 4. Number of corticotrophs counted per number of cells counted in the pars distalis of stress-susceptible swine as determined by use of three different staining techniques.

Pig Number	Stain 1	Stain 2	Stain 3
S1	9/147	5/155	3/152
S2	12/164	11/169	6/163
S3	10/160	9/172	3/156
S4	6/133	11/168	6/164
S5	6/150	6/174	5/139
S6	7/146	6/154	4/146
S7	5/145	8/152	6/134
S8	8/150	9/172	6/140
S9	9/152	14/172	5/139
S10	9/156	6/135	3/140
S11	9/142	11/138	5/150
S12	11/180	9/147	5/146
S13	8/135	11/150	6/164
S14	6/145	5/125	3/150
S15	4/125	8/140	3/155
S16	5/132	6/138	6/138
S17	6/123	9/156	5/174
S18	5/141	4/139	3/137
S19	5/136	4/152	4/141
S20	7/145	7/136	5/140
S21	6/142	6/137	6/149
S22	7/138	8/148	5/138
S23	8/146	7/125	5/145
S24	5/165	4/135	3/142
S25	5/168	6/170	3/145

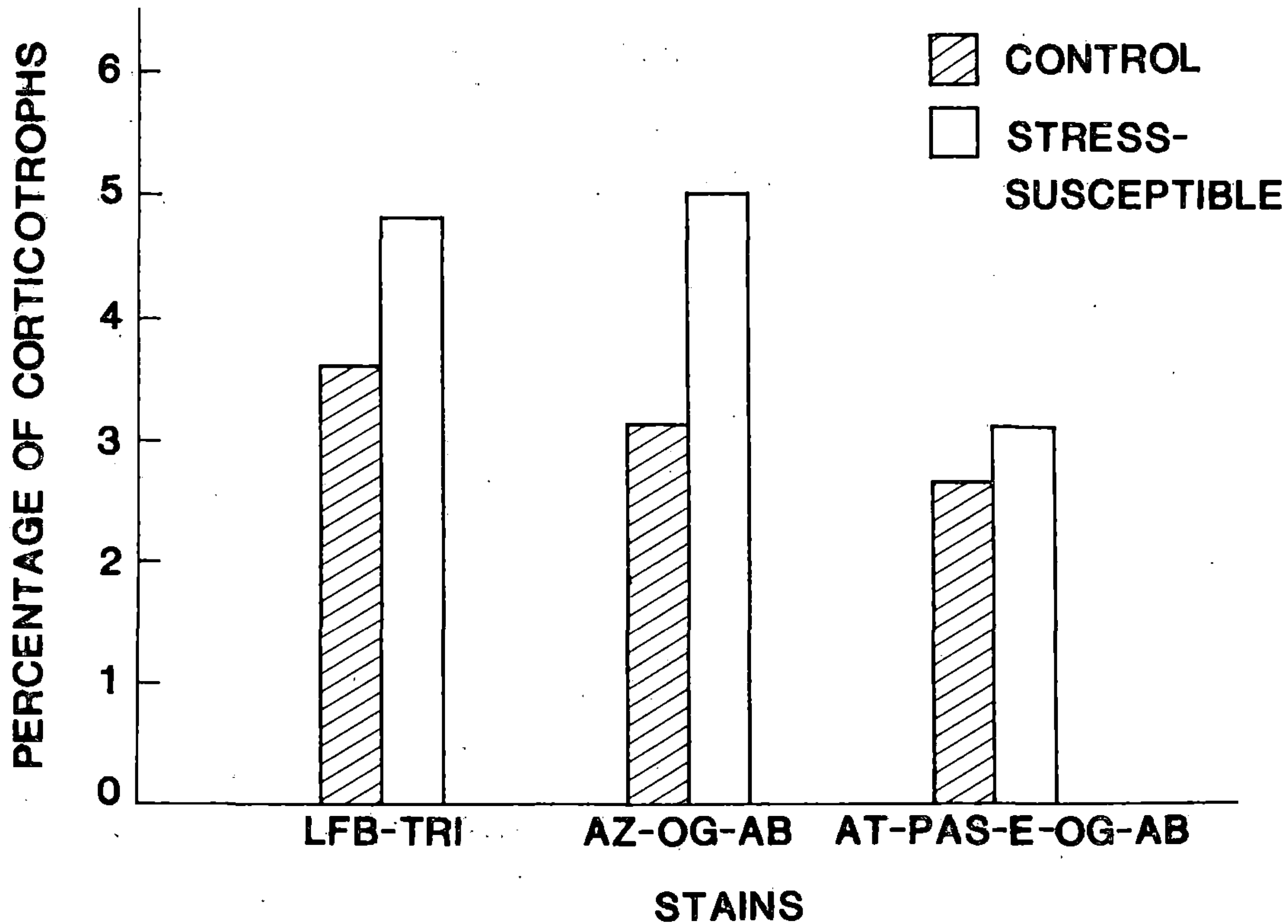
Table 5. Means for percentage of corticotrophs.

Treatment	Stain	N	Means
Control (SR)	1	20	3.6220
Control (SR)	2	20	3.1375
Control (SR)	3	20	2.6390
SS	1	25	4.8284
SS	2	25	5.0300
SS	3	25	3.1012
			LSD = .53

Table 6. Analysis of variance for variable percentage of corticotrophs.

Source	Degrees of freedom	Sum of squares	Mean square	F-value
Trt	1	46.968271	46.9682711	21.01278
Pig (trt)	43	96.114650	2.235244	
Stain	2	54.493068	27.2465341	37.81955
Trt*stain	2	11.371574	5.6857869	7.89216
Residual	86	61.957425	0.7204352	
Corrected Total	134	270.904988	2.0216790	

Graph 1. Percentage of corticotrophs found in the pars distalis of stress-resistant and stress-susceptible pigs when the tissues were stained by using (1) luxol fast blue-trichrome, (2) azocarmine-orange G-aniline blue, (3) AT-PAS-erythrosin-orange G-aniline blue.


PERCENTAGE OF CORTICOTROPHS
6
5
4
3
2
1
0
CONTROL
STRESS-
SUSCEPTIBLE
LFB-TRI
AZ-OG-AB
AT-PAS-E-OG-AB
STAINS

Corticotrophs were stellate cells, usually found at the center of a cell cord and having long cytoplasmic processes extending to or near the perivascular space. Distribution of the secretion granules was more clearly visible using stain 3 than stain 1.

Due to the intensity of stain 1, erythrosin appeared to be distributed throughout the cytoplasm, although a greater concentration in the periphery was expected. This stain imparted a dull red color to these cells. Lactotrophs also stained with erythrosin but took on a brick red hue. Lactotrophs often "cupped" other cell-types, particularly gonadotrophs. Somatotrophs stained selectively with luxol fast blue and appeared bluish-green, although a yellowish cast was frequently detected, possibly attributable to their affinity for orange G. Also easily distinguishable were the thyrotrophs, which stained strongly with aniline blue producing a deep blue color. The gonadotrophs were light blue to purple due to their varying affinities for erythrosin, orange G and aniline blue.

After application of stain 2, the corticotrophs were fairly easily differentiated due to their chromophobic appearance. Lactotrophs took on a reddish tint due to their affinity for azocarmine, and somatotrophs stained a bright orange. The basophils were not differentiated and all stained blue.

Corticotrophs appeared light purple or light blue following staining with method 3. The follicular cells took on a very similar, if not identical color. Prolactin cells appeared more of an orangeish yellow rather than the distinct yellow expected and were not

always clearly distinguishable from somatotrophs which stained reddish orange. Both thyrotrophs and LH-gonadotrophs appeared deep blue-purple; however, the former were more irregular in shape. FSH-gonadotrophs stained a vivid red-purple.

DISCUSSION

Rather low figures were obtained for the percentage of corticotrophs in the pars distalis of normal swine in comparison to results from other studies. Das (1971) has also conducted cell counts on the adenohypophysis and has reported approximately 5.5 percent corticotrophs in this region of the anterior pituitaries of pigs between 6 months and one year of age. In this study it was found that the staining methods occasionally resulted in certain cells staining in a manner similar to that of corticotrophs therefore casting some doubt on the identity of particular cells. Due to the possibility of additional, as yet unknown, effects of stress-susceptibility on other adenohypophyseal cells besides corticotrophs, it was decided to strictly exclude all ambiguous cells from the cell-counts. Some of the specific problems encountered in differentiating corticotrophs are discussed here.

Using stain 1, lactotrophs and corticotrophs stain similarly due to the presence of erythrosinophilic secretion granules within both these cell-types. Although it was usually easy to distinguish between brick red and dull red, in some cases it was not, so cell-shape and location became particularly important when differentiating these two cell-types using this stain.

According to Ricci and Russolo (1973), a corticotroph may appear either erythrosinophilic or basophilic depending on its functional state. If an animal was stressed at the time of slaughter,

the decrease in secretion granules and increase in rER would render the corticotrophs more difficult to detect using method 1. In addition, the great intensity of this particular stain obscured the clear area usually observed around the nuclei of corticotrophs due to the absence of secretion granules in this area.

Stain 2 presented the fewest problems, although distinction between the chromophobic corticotrophs and degranulated forms of other cell-types was sometimes difficult to make. The most accurate data were probably obtained by using this technique.

Corticotrophs and follicular cells stained similarly with stain 3, but this produced only minor confusion. The possibility of confusing degranulated thyrotrophs with corticotrophs was more serious. According to Nakane, these cells cannot be differentiated with the electron microscope without using immunochemistry. Both are usually located at the center of a cell cord and contain peripheral secretion granules; however, thyrotrophs tend to have more voluminous cytoplasm (Nakane, 1970). Prior to degranulation, thyrotrophs appear bluish purple in color and are easily identified. In my opinion, the reason why fewer ACTH cells were counted in sections stained with method 3 as compared to the data obtained using the other techniques is that some corticotrophs could not be distinguished from degranulated thyrotrophs. Nevertheless, the same degree of caution was used when examining both SS and SR pituitaries thereby minimizing any effect it may have had on comparisons of cell-counts between the two.

Measurements of cell size were not made because of the difficulty in determining accurate cell-boundaries of the highly irregular corticotrophs. Their long, ramifying, cytoplasmic processes extending between other cells made measurements of this type impracticable. However, it appeared that corticotrophs in the pars distalis of SS swine may have indeed been larger than those observed in the control pituitaries. The extremely high concentrations of plasma ACTH in SS pigs (Marple et al., 1972c) indicates that these cells could be expected to be hypertrophied in these animals. If this is so, one may question whether the results of these cell counts are caused by hyperplasia or hypertrophy of corticotrophs in SS pigs. Larger cells would be expected to appear in a greater number of sections and so would have an increased chance of being counted.

Although ACTH cells have been identified immunocytochemically in the intermediate lobe, a study of this area was not conducted because of the difficulty encountered in differentiating cells in this region by selective staining. Also, more recent evidence indicates that the pars intermedia does not secrete functionally significant amounts of this hormone (Greer et al., 1975).

The primary cause of PSS and PSE musculature is obscured by a plethora of clues; however, numerous theories have been proposed. One possibility is that there may be a defect in the long loop feedback mechanism by which circulating adrenocorticosteroids act at the level of the anterior pituitary and hypothalamus to inhibit secretion of ACTH. Evidence to support this theory was produced by

Sebranek et al. (1973) who reported that a greater dosage of dexamethasone is required to suppress ACTH secretion in SS pigs. This would explain the extremely high levels of ACTH in the plasma of SS pigs during stress as well as under basal conditions (Marple et al., 1972c). The resulting hypersecretion of cortisol by the adrenal in SS pigs under normal conditions would be expected to result in increased deposition of muscle glycogen (Aberle and Merkel, 1968; Marple et al., 1969) presuming the cortisol has an opportunity to act prior to inactivation and subsequent excretion. Indeed, the most significant difference between PSE muscle and high quality carcasses is the higher initial muscle glycogen concentration in PSE muscle (Briskey et al., 1958, cited in Briskey et al., 1959.) Furthermore, moderate exercise prior to slaughter, which significantly reduces initial muscle glycogen, results in decreased PSE postmortem muscle characteristics (Briskey et al., 1959). In addition, stress-prone pigs which survive after they collapse and are sacrificed 24 hours later show a high muscle pH and produce dark carcasses (Topel, 1969) because they have greatly reduced or eliminated their muscle glycogen and therefore, have reduced postmortem glycolysis. A report in 1969 (Marple et al.) that there is no relationship between plasma corticosteroid levels and the degree of PSE muscle after slaughter is probably explained by extremely rapid clearance of these hormones in SS swine, which could prevent detection of a relationship between these two factors. During stress, the abundant glucocorticoids would stimulate gluconeogenesis to

restore blood glucose which had been metabolized to produce energy and to replace liver and muscle glycogen stores. The high levels of lactate produced by anaerobic glycolysis in muscle would stimulate release of glucocorticoids by the adrenal cortex (Übersicht, 1974). The glucocorticoids would enhance conversion of lactate to glucose. The acidosis which occurs in SS pigs during stress, however, indicates that this conversion does not proceed at an adequate rate. Therefore, significant amounts of lactate accumulate, are released into the blood, and eventually cause death. This theory might be tested by administration of large doses of exogenous ACTH and glucocorticoids together to determine whether this could induce PSE postmortem muscle. An investigation of the effects of adrenalectomy and high levels of corticosteroids on plasma ACTH levels could determine whether a defect exists in the feedback mechanism specifically.

Another theory is that there is an abnormally high clearance rate of corticoids in SS pigs (Marple and Cassens, 1973a). This rapid metabolism could be caused by a defect in the liver hepatocytes. For example, an overactive or hypertrophied smooth endoplasmic reticulum could produce an increase in the rate of steroid inactivation (Orreneus _et al._, 1965). Ultrastructural morphometric studies of hepatocytes might reveal such an abnormality if it exists.

The high initial levels of muscle glycogen indicate that cortisol is probably functioning properly before being excreted. In the absence of stress, energy is produced by aerobic glycolysis, in which glucose is utilized to yield pyruvate. However, during stress anaerobic glycolysis becomes prevalent, and pyruvate therefore is reduced to lactate. A defect in the lactate dehydrogenase system which prevented conversion of lactate to pyruvate could account for lactate buildup in the face of abundant cortisol levels. Perhaps this is an area which should be investigated.

The problem could also lie at the level of the skeletal muscle cell. Studies by Swatland and Cassens (1972, cited in Marple and Cassens, 1973b) and Sair (1970) indicate that during muscle hypertrophy the mass of anaerobic fibers increases faster than the mass of red fibers. Pigs with PSE muscle have a greater percentage of white anaerobic fibers than red aerobic fibers (Merkel, 1971). It may be that while man has genetically selected for larger muscle fibers (Joubert, 1956) he has inadvertently developed an individual with reduced aerobic efficiency. Cooper _et al_. (1969) and Merkel (1971) have found that PSE muscles not only have larger fibers but also fewer capillaries per square millimeter of muscle, thereby predisposing them to anoxia. In addition, mitochondria of muscle cells from SS pigs have lower oxygen consumption due to inhibited phosphorylation (Eikelenboom, 1972). The SS animals therefore

shift much more readily to anaerobic glycolysis for the production of energy, resulting in increased lactate levels. It is also believed that the decreased capacity for oxidative phosphorylation causes a lower level of energy-rich phosphates at the time of death, which is an important factor in the development of PSE muscle (Eikelenboom and van den Bergh, 1973).

Hypersecretion of one or more anterior pituitary hormones has been implicated as the primary cause of PSS and the development of PSE muscle. Experimental variation of the level of pituitary activity produces a change in the rate of postmortem glycolysis; specifically, an increase in pituitary activity results in an increase in blood glucose and blood lactate (Kraeling et al., 1975). Thus, adenohypophyseal hormones such as ACTH and growth hormone may act synergistically to increase muscle glycolysis. Although the exact mechanism of this action is not yet understood, such an effect would be in line with such other effects of these hormones on carbohydrate metabolism as increasing the plasma and tissue glucose concentrations to produce energy.

Finally, the mechanism which triggers the porcine stress syndrome may be a hyperactive adrenal medulla. Excessive secretion of epinephrine causes an increase in muscle glycolysis and elevated lactate levels; it also stimulates the secretion of glucocorticoids by the adrenal cortex and release of ACTH from the pituitary. While the high levels of epinephrine in SS pigs (Weiss, 1971; Topel and

Staun, 1972; Topel *et al.*, 1974) may be either a cause or a symptom of this syndrome, it is certain that the stimulation of glycogenolysis which these levels would induce must exacerbate the metabolic problems described above.

Although it would have been interesting to know what effects, if any, age, weight, sex and breed have had on the results of this research, such an analysis was not possible because the materials studied were inherited and such information concerning each animal did not accompany the tissues and is at this point unattainable.

SUMMARY AND CONCLUSIONS

Light microscopy was used to show that sections of the pars distalis of stress-prone pigs contain a significantly greater percentage of corticotrophs than those from SR or control pigs. This result is in accord with the findings of Marple et al. (1972c) who have demonstrated three to four fold higher plasma ACTH levels in SS pigs as compared with SR animals. Due to the complex relationship of the endocrine system with this syndrome and the development of PSE post-mortem muscle, it is difficult to be certain whether hypersecretion of ACTH by the anterior pituitary is a cause or an effect of this syndrome. The possibility of a defect in the feedback mechanism of the adrenal cortex on the pituitary is discussed. Abnormal clearance of cortisol is suggested as a possible cause of hypersecretion of ACTH and the development of PSS. The effects of secretion of large amounts of epinephrine and growth hormone are also discussed as possible precursors of stress-susceptibility in pigs. A causal relationship between PSS and possible disfunction at the level of the muscle and/or liver is also suggested. Further investigations are necessary to test these theories before any definite conclusions as to the etiology of this condition can be made.

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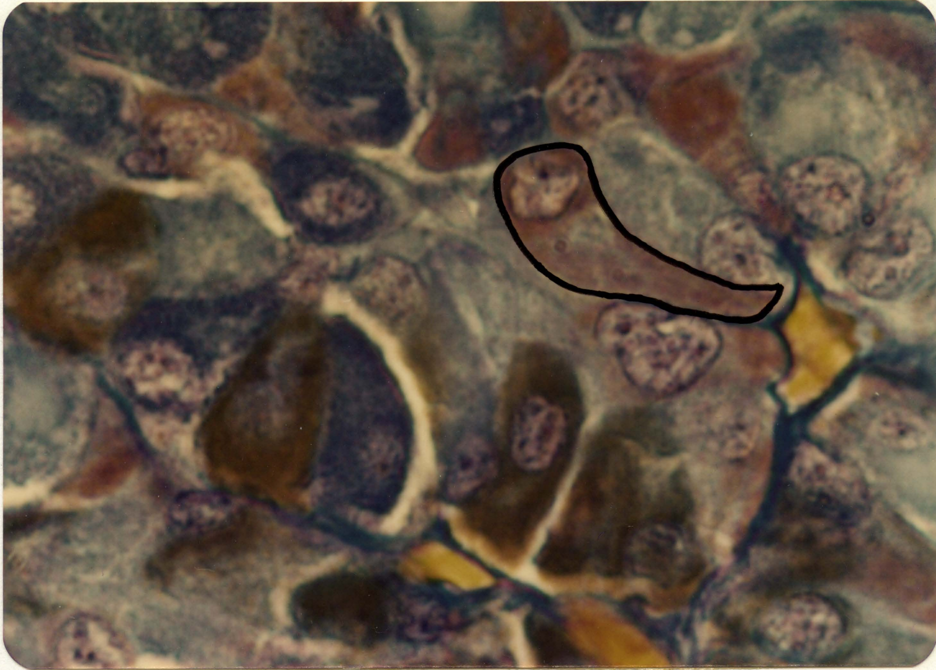
APPENDIX

Figure 1. Pars distalis of control pig approximately 6 months old (#C3). The outlined corticotroph is stellate and erythrosinophilic. Note nucleus located at center of cell cord and cytoplasmic process extending to pericapillary space. Due to intense staining, the perinuclear area, which actually lacks secretion granules, is also stained. Brick red lactotrophs are also shown; these could be mistaken for corticotrophs if identification were based solely on color of cell.
Luxol fast blue-trichrome stain.
X1000

Figure 2. Pars distalis of an SS pig approximately 6 months old (#S4). Corticotroph (outlined) is stellate and erythrosinophilic. A cytoplasmic process extends to a pericapillary space. The perikaryon is also stained in this cell. Note the nucleus of a subjacent cell visible through the cytoplasm of the corticotroph at the dotted line. Similarly staining lactotrophs (brick red) can also be seen.
Luxol fast blue-trichrome stain.
X1000



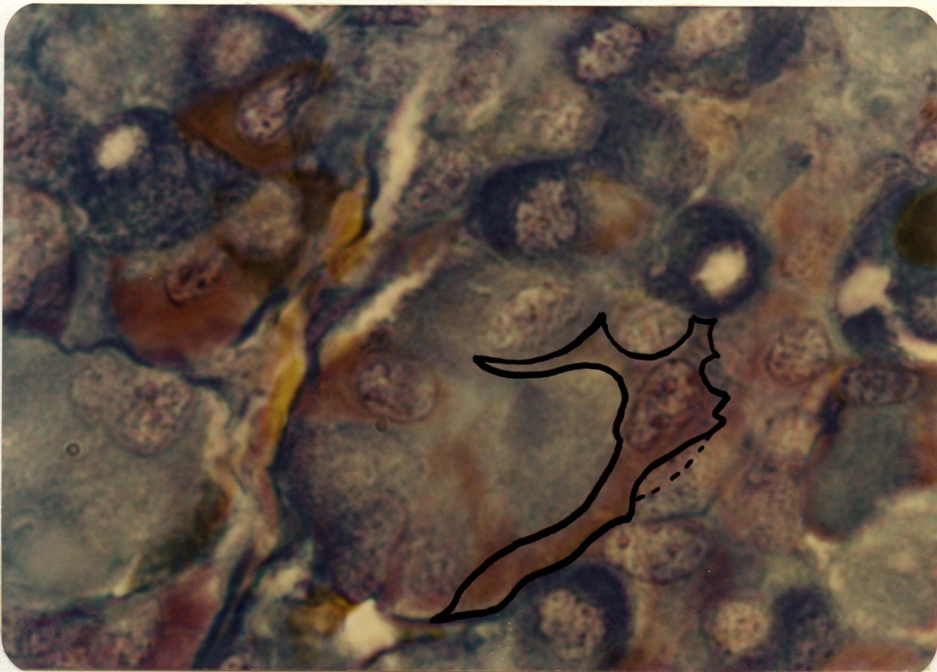
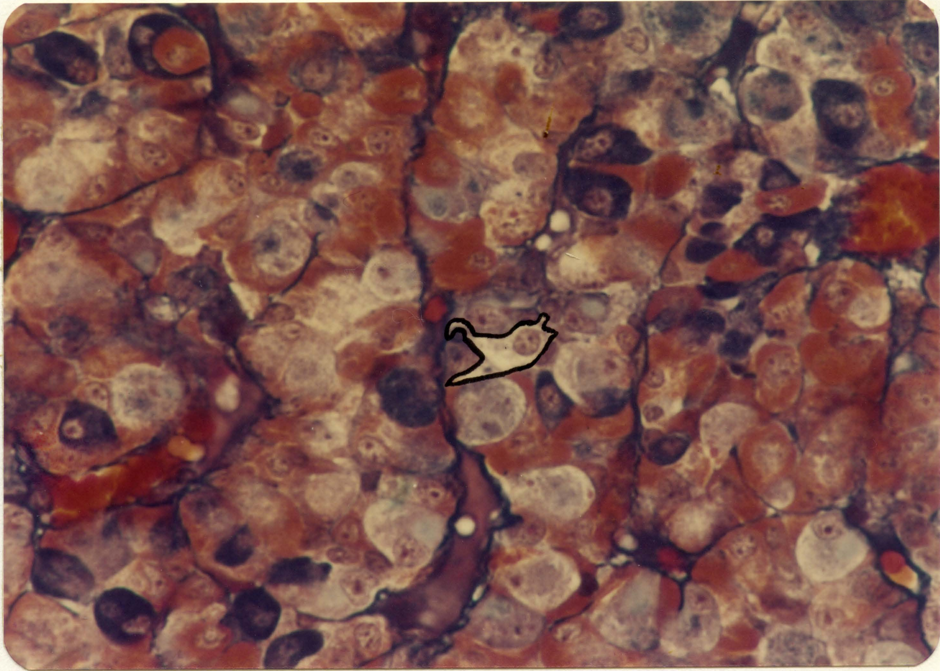


Figure 3. Pars distalis of an SS pig approximately 6 months old (#S13). A corticotroph is outlined and appears chromophobic. Its cell body is at the center of a cell cord, and processes extend to the nearby pericapillary space. Azocarmine G-orange G-aniline blue stain. X400

Figure 4. Corticotroph shown in Figure 3, here at higher magnification.
Azocarmine G-orange G-aniline blue stain.
X1000



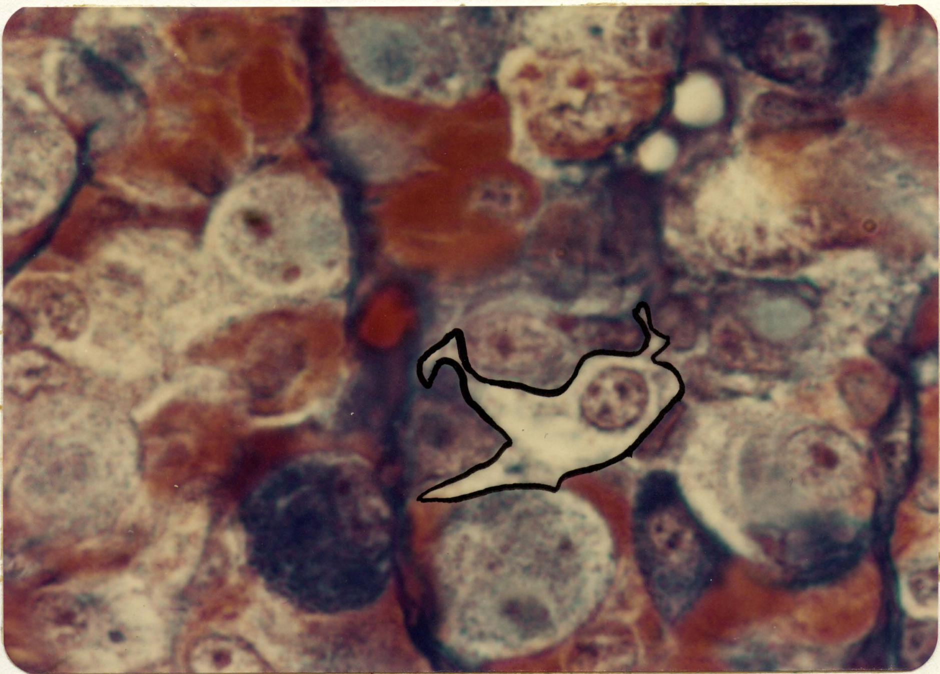
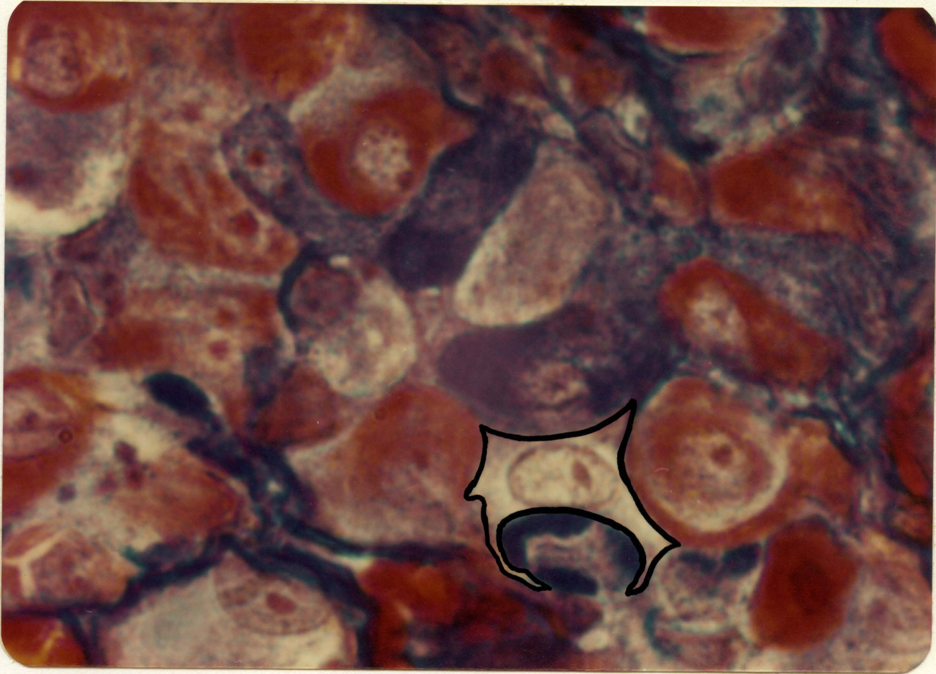


Figure 5. Pars distalis of a control approximately 6 months old (#C2). Corticotroph (outlined) is stellate and chromophobic. A process extends toward pericapillary space. Azocarmine G-orange G-aniline blue stain. X1000

Figure 6. Pars distalis of control animal approximately 6 months old (#C6). A stellate, chromophobic corticotroph is outlined.
Azocarmine G-orange G-aniline blue stain.
X1000



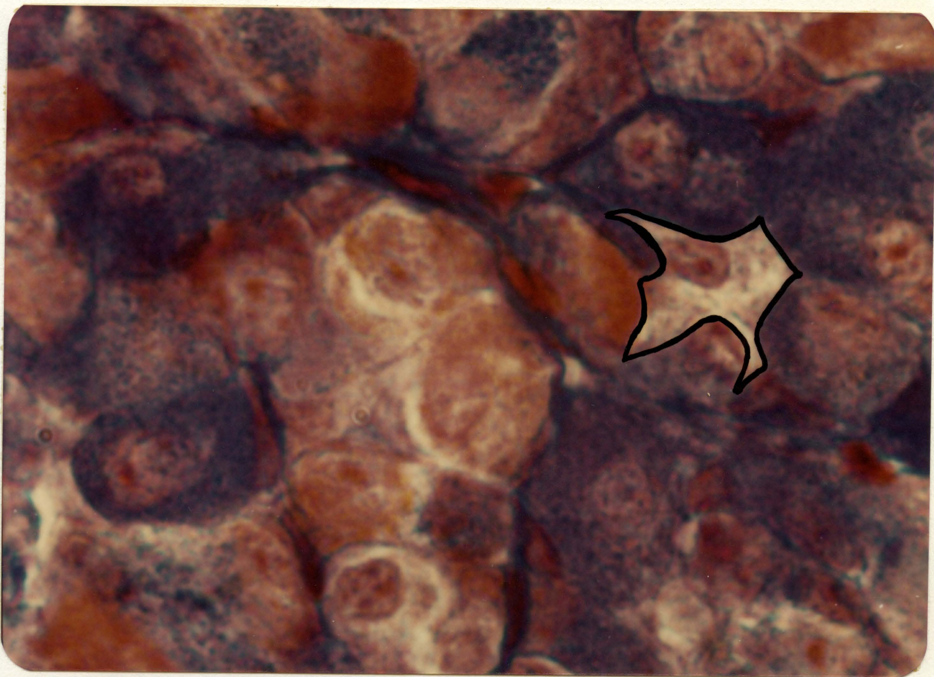


Figure 7. Pars distalis of a stress-prone pig approximately 6 months old (#S16). Corticotroph (outlined) is stellate and light purple in color. Note secretion granules in cell periphery and similar staining of degranulated portion of adjacent thyrotroph (blue-purple). Cell body of corticotroph is centered in the cell cord. Dotted line indicates a possible cytoplasmic process. AT-PAS-erythrosin-orange G-aniline blue stain. X1000

Figure 8. Pars distalis of control approximately 6 months old (#C2). Outlined corticotroph is stellate and light purple in color. Note location at center of cell cord and processes extending between other cells. AT-PAS-erythrosin-orange G-aniline blue stain. X1000