HYPOTHALAMIC AND HYPOPHYSEAL CHANGES ASSOCIATED WITH FEEDING M.A.T.C.H. (I.C.I. 33828 NON-STEROID COMPOUND) FOR ESTRUS SYNCHRONIZATION IN SWINE

by

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INTRODUCTION

Animal production is important because it provides the best protein source for human diet requirements, and in many parts of the world a fast growing population increases at a greater rate than the production of animal protein.

Animal production is greatly dependent upon efficient reproduction. Much effort, time and money has been spent on the improvement of domestic animals for milk and meat production. The greatest benefits of these improvements will not be realized unless a high degree of fertility is accomplished. The importance of reproductive efficiency has been demonstrated by the revelation of how artificial insemination can increase the utilization of fertile males of high quality genetic characteristics which make them very suitable for increasing animal production.

Estrus synchronization is needed to increase even further the influence of artificial insemination for production improvements and to make breeding operations more efficient.

Increasing demand for estrus synchronization in connection with artificial insemination in swine has stimulated the development of several methods for heat control. The best known of these are weaning of lactating sows, utilization of progestational compounds and administration of gonadotropins. One of the most promising drugs that has been used for this purpose is I.C.I. 33828, 1-alpha-methylallylthiocarbamoyl -

2 methylthyocarbamoyl hydrazine, commonly called M.A.T.C.H.[1] This compound is interesting because it is neither a steroid nor a gonadotropic material. Because of promising preliminary work with this compound it would not be surprising if this drug was released for general use in the near future. For this reason it is important to understand the mechanism of action. The exact mechanism of action of M.A.T.C.H. is not well understood at the present time, but it has been hypothesized that the drug has its effect by blocking the release of gonadotropins from the pituitary gland by inhibiting the production of releasing factors for (F.S.H.) and luteinizing hormone (L.H.) in the hypothalamus. This hypothesis would explain the inhibition of heat that occurs following the use of this drug.

Because of the relationship between the nervous system and reproductive system and the probable site of activity of this drug, it is important to understand the function of the hypothalamus and the pituitary gland in relation to the use of M.A.T.C.H. in estrus synchronization.

A great proportion of the neuro-endocrinological research concerning gonadotropin releasing factors has been done in experimental laboratory animals in which the effects vary considerably from one species to another.

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[1] M.A.T.C.H. stands for 1-alpha-methylallylthiocarbamoyl - 2 methylthyocarbamoyl hydrazine, I.C.I. 33828, Ayerst Laboratories, Veterinary Medicine Division, New York, N. Y. 10017.

The increasing amount of research demands a necessity for more data and information which permit a better understanding and a more accurate evaluation of the ductless glands, especially the pituitary gland which plays such an outstanding role in modern medicine as far as endocrine glands are concerned. Also, for any research type or pathological material experiment, it is necessary to determine the range of variability of apparently healthy individuals for proper evaluations. This is especially true in reference to studies of the effect of M.A.T.C.H. It is very important to study this situation in the species on which it might be used, in this case swine.

This study, therefore, was undertaken to determine the effects of M.A.T.C.H. on the pituitary and hypothalamus during its use in swine for estrus synchronization in order to understand its functional activity more completely.

REVIEW OF LITERATURE

Estrus Synchronization with I.C.I. 33828

The idea of estrus synchronization introduces the possibility of being able to plan and control the production of swine in connection with artificial insemination. Much of the earlier work accomplished with steroidal compounds used for estrus synchronization produced discouraging practical results because of undesirable side effects.

In 1961 research men of Imperial Chemical Industries were investigating a derivative of hydrazine which is in no way related to the naturally occurring sex hormones (steroids) (Polge, 1965b), I.C.I. 33828 formula: $CH_2 = CH.\ CH.\ NH.\ CS.\ NH.\ NH.\ CS.\ NH.\ CH_3$. CH_3

The surprising result noted when the drug was given to birds was inhibition of the pituitary (Polge, 1965b). Later the new drug, when used in rats, was also found to inhibit the output of pituitary gonadotropins. The estrus cycles of these rats were suppressed while they were kept on medication but the animals returned to normal estrus after suspending the treatment. Experimenting with chickens it was found that small quantities of the drug could affect egg production and moulting (Sykes, 1963). Experiments in pigs started in 1963 at the Agricultural Research Council's Unit of Reproductive Physiology and Biochemistry in Cambridge. Further

experiments were done in other countries under farming conditions (Groves, 1965).

When this compound was orally fed at the rate of 1 mg per kg of body weight per day, only two out of a hundred pigs treated came in heat during the medication period. After medication was suspended all the animals came into heat within ten days. The heats occurred between the fifth and seventh day after treatment in 90% of the individuals tested. (Marshall, 1967; Polge, 1965a; Groves, 1965)

The results up to the present indicate that the average litter size is normal and no adverse effect on the piglets is caused by this drug. No undesirable embryonic mortality effects were evident and the ovaries manifested no abnormalities such as cystic follicles. (Groves, 1965; Polge, 1965b; Hafez et al., 1966)

Medication started at the beginning of the luteal phase does not disturb the corpora lutea which continues functioning until the end of the second week (sixteenth day of the cycle) (Polge, 1965b). This is because, once formed, maintenance of the C.L. does not depend on a pituitary stimulus. The part of the cycle which is affected is the follicular one which means that if animals are put on the drug soon after they have been in heat, treatment with I.C.I. 33828 should last for at least 19 or 20 days in order to synchronize estrus in groups of pigs with randomly distributed cycles

(Hafez et al., 1966). If the drug is medicated too near to the expected heat, estrus and ovulation will not be prevented.

General Properties of M.A.T.C.H.

Mechanism of action

The precise mechanism of action is not yet known. It does not affect the ovary directly because the animals which have been on medication will respond normally when gonadotropins are injected, but it is clear that M.A.T.C.H. suppresses the release of pituitary gland activity. The inhibitory effect of M.A.T.C.H. upon F.S.H. or L.H. or both gonadotropins caused by M.A.T.C.H. has not been well established (Edgren and Peterson, 1964). The compound reduces the blood calcium level by decreasing the amount of estrogen production.

M.A.T.C.H., when given to laying hens, decreased blood calcium level from 20 mg/100 ml to 15 mg/100 ml. When the compound was removed from the diet the blood calcium level rose again (Sykes, 1963). I.C.I. 33828 is reported to inhibit gonadotropins in rats and to have minimal effect on thyroid activity. Doses of 20 to 100 mg/kg per day for 12 days produced unpigmented bands on the feathers of dark-colored fowls during treatment. This shows the action of the drug on melanic product formation or deposition (Wright, 1963). M.A.T.C.H. fed to pregnant mares did not change the blood level of gonadotropins which means that M.A.T.C.H. has

no effect on placental gonadotropins (Schmidt-Elmendorff et al., 1962). Assay experiments made in pituitary tissue proved that I.C.I. 33828 caused an F.S.H. decreasing effect upon the hypophyseal gonadotropin (Brown, 1963).

Effects of M.A.T.C.H.

Decrease of appetite, slight depression and development of lethargy have been observed in some animals, but these effects have not upset the general health and the withdrawal of the treatment brings about a complete recovery from the side effects (Marshall, 1967). M.A.T.C.H. will inhibit growth in the rat. This suggests a relationship of M.A.T.C.H. action and inhibitory effects on somatotropin releasing hormone (Cargill-Thompson, 1963). Urine excretion in hens is increased and water consumption in pigs is increased also. This relates with the diuretic action of M.A.T.C.H. by blocking the release of anti-diuretic hormone from the neuro hypophysis (Polge, 1965b).

M.A.T.C.H. has inhibitory action upon milk production in sows fed the drug, but when exogenous oxytocin was administered milk production was brought back to normal. After these results it was concluded that M.A.T.C.H. did not suppress lactogenic activity but did inhibit the milk ejection reflex (Gerrits and Johnson, 1965b).

The following findings have been reported by Marshall (1967).

1) The total weight of the reproductive tract decreases steadily during the treatment period, but rapidly increases with the onset of the synchronized estrus.

2) The time of ovulation in M.A.T.C.H. synchronized estrus was slower than in normal non-treated animals. Ovulation occurred from the third to fourth day.

3) M.A.T.C.H. synchronization appears to cause the proestrus period to be very prominent, with marked swelling of the vulva.

4) The M.A.T.C.H. synchronized estrus period is prolonged, lasting four to five days.

5) Treatment with M.A.T.C.H. causes a copious amount of muco-purulent vaginal discharge, composed primarily of eosinophils during the first two days of the synchronized estrus.

6) The occurrence of side effects due to M.A.T.C.H. treatment, such as lethargy and anorexia, appears to vary between individual animals.

7) There are some differences in the uteri of M.A.T.C.H. synchronized animals and untreated animals during and shortly after estrus. These differences might possibly explain the slight decrease in conception rate and litter size sometimes observed in animals treated with M.A.T.C.H.

The Pituitary Gland

The hypophysis is a reddish and ovoid structure measuring from 6 to 10 mm of transversal diameter and from 5 to 6 mm of antero-posterior diameter in swine. It weighs an average of 0.34 gms. It is connected with the base of the cerebrum by a tube called the infundibulum and it is situated in the hypophyseal fossa of the sphenoid bone, called the sella turcica. It is the key to the internal secretion

glands and keeps an intimate relationship with the superior centers of the vegetative nervous system. It also has a multiplicity of interrelations with other endocrine glands and functions.

Development of the pituitary gland

The pituitary consists of two parts differing in origin, function and structure. The pars nervosa comes from an out-pouching of the floor of the diencephalon. The pars distalis originates from a dorsal evagination of the stomodeum (in embryo, the pars buccalis) of Rathke's pouch that lies close to the pars nervosa and fuses with it and later becomes separated, leaving in between an empty space which is called the cleft. The wall of Rathke's pouch that fuses with the pars nervosa undergoes little further growth and forms the pars intermedia. Lateral proliferations of the pars buccalis surround the infundibulum to form the pars tuberalis which extends to the tuber cinereum of the brain. The rest of the wall of Rathke's pouch forms the pars distalis or anterior lobe. The pars nervosa is intimately fused with the pars intermedia to form the posterior lobe and represents the thickened solid end of the infundibulum. The lumen of the infundibulum may extend down into the pars nervosa as in swine and carnivores, or it may end before reaching the hypophysis (Trautman and Fiebiger, 1952).

The anterior lobe is derived from the ectoderm of the stomodeum. It originates as a dorsal evagination of Rathke's pouch. The posterior lobe is derived from the neural ectoderm of the floor of the forebrain. This Rathke's pouch is destined to form the anterior lobe. It lies immediately ventral to the cephalic border of the stomodeal membrane, and it extends upward in front of the dorsal end of the notochord, in contact with the lower surface of the forebrain.

The surrounding mesoderm constricts to form a closed cavity. Epitheleal cells grow on each side and in the ventral wall of the cavity. The stroma of the anterior lobe develops from the mesenchyme.

Behind Rathke's pouch a hollow neural outgrowth extends down from the floor of the diencephalon. The neural process forms the infundibular process which becomes a solid body. The solid posterior lobe becomes surrounded by a dorsal extension of the anterior lobe on each side of the stem. The anterior lobe also grows two processes from its ventral wall which go along the infundibulum, constituting the pars tuberalis and fuse surrounding the upper end of the stalk. In the cleft which can been in sagittal sections are the remains of the original cavity of the stomodeal diverticulum. The posterior wall of the cleft remains thin and fuses with the adjoining part of the posterior lobe to constitute the pars intermedia. In a horizontal cut of an adult gland this can be shown by its content of colloid-full follicles. (Netter, 1965)

Blood supply of the pituitary gland

The blood supply of the pars anterior in most species of mammals and birds is provided by the portal vessels. This pattern of blood supply is of great and profound functional significance. Many workers have observed that the blood flows down the stalk and into the anterior lobe. Török in 1964 observed an upward flow in some of the sparse subendymal capillaries which run between the uppermost part of the pituitary stalk and the lower most part of the hypothalamus. Knigge (1967) stated that although the direction of the blood flow in the short segments of the portal vessels has not been thoroughly established, there is a vascular link between the pars nervosa and the sinusoids of the pars distalis which allows a direct access of posterior lobe peptides into the adenohypophysis. In the last 30 years it has become clear that the secretory activity of the cells of the anterior lobe is largely controlled by the central nervous system acting through the hypothalamus. However, there is no firm evidence that the cells of the anterior lobe are supplied by nerve fibers derived from the hypothalamus, but one link of the chain of control of the anterior lobe by the brain is provided by the portal vessels. This concept owes much to the work of Harris. Examination of the median eminence at the electron microscopic level has demonstrated the presence of nerve terminals in direct relationship to portal capillaries (Bargmann and Knoop, 1960). A great number of unmyelinated

nerve fibers terminated on the capillaries of the portal vessels in the outer layer of the median eminence (Rinne and Arstila, 1966).

The pituitary gland receives its arterial blood supply from two paired systems of vessels (Figure 5). From above come the right and left superior hypophyseal arteries (S.H.A.) and from below arise the right and left inferior hypophyseal arteries (I.H.A.). Each S.H.A. divides into two branches, the anterior and posterior hypophyseal arteries (A.H.A. and P.H.A.). These anterior and posterior (superior) hypophyseal arteries give branches to the hypophyseal stalk; some ramifications go to the optic chiasma and hypothalamus. Another branch of the anterior superior hypophyseal artery of each side gives the artery of the trabecula. This is an anastomotic artery which connects the superior and the inferior hypophyseal systems. It passes through the anterior lobe but does not supply epithelial cells. (The trabecula is a compact band of connective tissue and blood vessels lying within the pars distalis on either side of the midline.) This trabecular artery, after entering the gland when approaching the lower infundibular stem, gives off numerous straight parallel vessels to the superior portion of the area constituting the superior artery of the lower infundibular stem. Human and domestic animal reports indicate that the artery of the trabecula is of a large caliber throughout its course. It gives no branches to the

epithelial tissue through which it passes. It is very tortuous and is always surrounded by connective tissue. (Netter, 1965; Daniel, 1966; Ham, 1957; Bloom and Fawcett, 1957; Guyton, 1966)

The inferior hypophyseal arteries arise as a single branch from each internal carotid artery (Figure 5). It divides into medial and lateral inferior hypophyseal arteries. The neural lobe is surrounded by an arterial ring formed by the medial and lateral branches. From the arterial ring branches go to the posterior lobe and to the lower infundibular stem.

The epithelial tissue of the pars distalis receives no direct arterial blood; however, the rabbit does receive arterial blood as well as portal venous blood (Daniel and Prichard, 1966). The sinusoids of the anterior lobe receive their blood from the hypophyseal portal vessels which arise from the primary capillary bed in the median eminence and the upper and lower portions of the infundibular stem. In the zone between the pars tuberalis and the median eminence, anterior hypophyseal arteries form an anastomotic network of vessels constituting the primary capillary bed. The plexus is not restricted to the median eminence but to the contact surfaces of the p. tuberalis with the infundibulum and between the p. intermedia and p. nervosa (Knigge, 1967). Blood is carried from the first capillary bed through the hypophyseal portal veins to the epithelial tissue of the

anterior lobe, forming there the secondary capillary bed or secondary plexus of the pituitary portal system (Figure 5). The portal system is the vessel network which provides the blood supply to the anterior lobe. There are two groups of portal vessels, the long and the short ones, L.P.V. and S.P.V. (Figures 2 and 3). Most of the long portal vessels seen along the surface of the stalk leave the neural tissue and run through the pars distalis. The short hypophyseal portal veins are embedded within the tissue surrounding the lower infundibular stem (Daniel and Prichard, 1966).

The primary capillary bed is supplied with blood by the superior hypophyseal arteries whereas the secondary capillary bed is supplied by the inferior hypophyseal arteries. Vascular tufts comprising the primary capillary bed in the median eminence and stalk are closely related with the great mass of nerve fibers of the hypothalamo-hypophyseal tract running in this region. On exitation the neurons of the hypothalamic nuclei liberate through these nerve fibers into the portal vessels specific substances which are conveyed to the sinusoids of the pars distalis, acting as releasing factors for specific pituitary hormones (Adams et al., 1966; Nalbandov, 1964). The territory supplied by a particular portal vessel receives little or no blood from the portal vessels which supply adjacent areas. Therefore, in spite of free anastomosis of the sinusoids of the anterior lobe,

there is little if any mixing of blood conveyed by different portal vessels. This is important because some of the cells which are known to secrete a specific hormone tend to be concentrated in certain areas of the gland. Specific groups of neurons in the hypothalamic nuclei are linked, via the capillary loops draining into particular portal vessels, with specific groups of cells in the anterior lobe and thus control the secretion of those particular cells (Adams et al., 1964). Light microscopic studies on the development of the relationship between the neuro secretory pathway and the portal system in rats showed that neuro-secretory fibers made contact with the primary plexus after the sixth day of postnatal life and increased rapidly from then until about the tenth day of postnatal life (Daikoku et al., 1967). The posterior lobe function depends on having an intact connection with the hypothalamus. There is only a neural connection when the pituitary stalk is cut. The posterior lobe atrophies although its blood supply remains intact (Adams et al., 1963).

The venous drainage of the pituitary

The venous blood leaves the pituitary stalk by the portal vessels. The sinusoids of the anterior lobe drain into the cavernous sinuses which are lying around the anterior lobe; then the hormones go to the internal jugular veins. The capillaries of the posterior lobe drain into the

subhypophyseal sinus and then go to the cavernous sinus to pass to the jugular venous blood (Daniel, 1966).

Pituitary gland nerve supply

The nerve supply is derived from the cervical sympathetic carotid plexus, sphenopalatine ganglion or petrosal nerves, and the hypothalamo-hypophyseal tract. The most important one is the last one and it originates from the supra-optic nucleus of the hypothalamus and to some extent from the para-ventricular nucleus in the wall of the third ventricle. The nerves go down through the infundibular stalk and infundibular process. This is important since it has been discovered that the nerve hypophyseal hormones are formed in the nuclei of the hypothalamus and are transported along the nerve fibers from the hypothalamo-hypophyseal tract to the neuro hypophysis where they are stored. (Bloom and Fawcett, 1957)

Few small nerve fibers accompany the blood vessels in the adenohypophysis. It is generally believed that the secretion of the anterior lobe is not under direct control of nerve impulses but is mediated by neuro secretory products from the hypothalamus reaching the anterior lobe by way of the hypophyseo-portal system.

The nerve fibers of the pars nervosa arise from the diencephalon and reach the neuro hypophysis via the infundibulum. The vasomotor nerves originate at the carotid plexus. (Trautman and Fiebiger, 1952)

Hypophyseal Functional Histology

In general the pituitary gland cells have been traditionally grouped in

1) chromophils
   A) acidophils or alpha cells
   B) basophils or beta cells
2) chromophobes, C cells or chief cells.

This classification is set on the basis of their affinity or lack of affinity to the dyes. Chromophobes have very little affinity for dyes. The relative proportions of the three types of cells might be influenced by castration, thyroidectomy or other endocrine gland extirpations as well as age, species, breed, sex, stage of the estrus cycle and pregnancy (Ham, 1957; McEntee and Jubb, 1957, Allanson et al., 1966; and others). As is already known, in the adult hypophysis no mitotic divisions are usually found (Bloom and Fawcett, 1957). It has been concluded that the change of proportions among the three cell types are the result of the transformation of the chromophobes into alpha or beta cells, and under special conditions the chromophils are believed to discharge their granules and convert into chromophobes (Bloom and Fawcett, 1957). However, Bugnon (1963) found a significant phenomenon of hyperplasia with observable mitosis during the course of gestation. The staining procedures considered most useful for the study of the pituitary anterior lobe

cells are a combination of dyes. Basically the different types of cells which produce the different kinds of hormones, although they stain with the same color, have differences in their cytoplasmic shape, size, concentration of the cytoplasmic granules and amount of chromatin in the nucleus (McShan, 1965).

Acidophil cells (stain red with acid dyes)

They vary in shape, size and form. The spherical alpha granules vary in size even in the same cell. In some mammals such as man, dog, cat, and horse, they are coarse and in others such as guinea pig and mouse, they are fine. The amount of chromatin and the number of granules may vary too.

The acidophils can be further differentiated with azan stains into two specific cell types, depending on their affinity for an orange dye (orange G) or a red dye (azocarmine). The orange ones are called orangeophils or alpha acidophils with large numbers of dense granules. They are S.T.H. producers (somato tropin producers) and are small rounded cells. The red ones, called carminophils or epsilon acidophils (stain red with azocarmine), are prolactin producers. They are larger and coarsely granulated cells but with less number of dense granules. They appear in the pituitary of the female in early pregnancy and increase in number in late pregnancy and lactation (Bloom and Fawcett, 1957; Ham, 1957; Gurmeet and Prasad, 1965).

Basophil cells

These are oval, round and angular shaped cells. The cytoplasmic granules in basophils or beta cells are less dense and numerous than in the alpha cells. The size of the cytoplasmic granules varies from one species to another. They stain with aniline blue, resorcin, fuchsin, hematoxylin and musicarmine (basic dyes). The P.A.S. reaction demonstrates the basophils very positively stained and the acidophils negative or very weakly stained.

There are two kinds of basophils:

1) The beta basophils which are positive to P.A.S. and to aldehyde fuchsin too. They are polyhedral shaped cells with fine granules and are responsible for thyrotropin hormone production.

2) The delta basophils in man (Bloom and Fawcett, 1957) which are positive to P.A.S. but negative to aldehyde fuchsin. They are round or oval in shape with coarsely granulated cytoplasm and are responsible for gonadotropin production. They are more numerous in the male than in the female. However, Bugnon (1963) differentiated them among L.H. and F.S.H. producers and McShan (1965) differentiated them also by the size of their cytoplasmic granules seen by the electron microscope. In man four tinctorially different types have been described (Romeis),

but it is not known whether all of these are functionally distinct.

These basophil cells are larger in size and less in number than the acidophils. They can be found in clumps, more easily toward the peripheral zone than in any other part. When the sex glands are removed from an animal the gonadotroph basophils form large vacuoles in their cytoplasm; these have been called castration cells. A similar phenomenon may be observed among thyrotrophs when the thyroid gland is removed (Ham, 1957). It has been said that basophils constitute 25% of the chromophils.

Adams and Swettenham (1958) histochemically identified two types of basophils. Since the delta basophils clearly gave rise to the castration cells, it appeared that they were responsible for gonadotropin production. The beta basophils, on the other hand, elaborated thyrotropin and probably corticotropin. It is interesting to know that some serum proteins have the function of binding thyroxine for hormone transporting purposes in the blood (Rall, 1965).

A combined performic acid-Alcian blue-PAS-orange G method is described for the recognition of two types of basophil granules. The type S granule, which is stained by Alcian blue stain, is reported to be rich in the amino acid cystine. The type R cell is stained red by the P.A.S. stain because of the polysaccharide substance of the granules contained in the cytoplasm. The S cell is found in great

numbers in adrenal atrophy (Addison's disease) and in shock. Therefore, it is suggested that the cell is responsible for corticotrophin-synthesis while the R cell undergoes hyalinization in adrenal hyper function (Cushing disease) and after cortisone therapy (Adams and Pearse, 1959). Heath (1964), utilizing the performic acid-Alcian blue-PAS-orange G stain in pituitary glands of different species, found three different types of basophils: 1) the blue cells were correlated with thyrotropic activity, 2) the purple or violet cells were associated with F.S.H. activity, and 3) the rose or red cells were related with L.H. production. In pregnant rabbits the number of mucoid cells was below that of controls at day 9 of pregnancy. During the second half of pregnancy the percentages went up to values of 25% to 50% higher than normal and then declined to reach normal values during lactation. However, in pregnant and lactating sows F.S.H. did not decrease until day 80 of pregnancy but it rose again till the end of pregnancy. At the 14th day of lactation the F.S.H. level was still high. On the other hand, the L.H. increased until early pregnancy (18th day). Later on it decreased throughout pregnancy and lactation (Melampy et al., 1966). During the estrus cycle in the pig, pituitary gonadotropin content is low at estrus until day 4, increases from day 4 to day 10 and plateaus until day 18 of the cycle. Between day 18 and estrus a marked reduction occurs in F.S.H. and L.H. During pregnancy pituitary F.S.H. increases with advancing stages

of gestation while pituitary L.H. decreases in pigs (Anderson, 1966).

It has been suggested that L.H. gonadotrophs are located principally in the pars distalis and the F.S.H. producing cells in the zona tuberalis of the rabbit pituitary (Cameron et al., 1966). Gurmeet and Prasad (1965) found that in the squirrel pituitary gland the gonadotrophs are ovoid, coarsely granulated and mainly located on the sinusoidal borders of the lateral halves of the pars anterior, and that they are more numerous in the male than in the female. They are P.A.S. positive and aldehyde fuchsin (A.F.) negative. The thyrotrophs are larger, rounded or polyhedral, finely granulated, and are located at the antero-medial zone of the pars distalis. They are P.A.S. and A.F. positive. The basophils were differentiated by the performic acid-Alcian blue-PAS-orange G staining in which the gonadotrophs were stained purple and the thyrotrophs were red.

The chromophobes (C cells, the reserve cells or chief cells)

These are spherical, elongated or angular shaped cells which tend to occur in clumps toward the central part of the cords. The typical chromophobe is much smaller than the typical chromophil. Therefore, in a nest or group of chromophobes the nuclei are much closer together (this helps to identify them). Since chromophils are much larger than chromophobes there appear to be more chromophils than

chromophobes in the gland even though they are present in about the same numbers. This means that the chromophobes constitute the 50% of the cells in the pars anterior of the pituitary gland (Ham, 1957).

With electron microscopy all the adenohypophyseal chromophil cells appear to have granules. The difference between cells is established by the size of the granules which permits a more effective identification of the various cell types than by tinctorial affinity, but the size of the granules appears to vary with the state of activity of the cell and the state of maturity of the granules (Herlant, 1965). Farquhar and Rinehart in 1954 developed a classification of pituitary cells based on the size of the granules in the cells. However, A.C.T.H. has not been associated with a specific size of granule (McShan, 1965).

Differential centrifugation has permitted the isolation of different kinds of granules with different hormonal action (McShan, 1965). Of all the sources and methods used to approach the functional significance of the hypophyseal cells, the immuno-fluorescent technique seems to be the most specific and accurate. It holds the most promising results for future investigations (Herlant, 1965). The principle of this technique is based upon the antigen antibody reaction in order to localize a specific hormone.

Allanson et al. (1966) reported two types of acidophils

by using a modified Cleveland and Wolfe's staining procedure: one type of orange red staining coarse granules and another type of fine yellow staining granules. During the second part of pregnancy the orange cells increase in number. They undergo fast degranulation at the beginning of lactation. This suggests that prolactin is produced by the orange cells.

McEntee and Jubb (1957) described the cytological changes of the pituitary gland throughout the estrus cycle. They observed degranulation of the basophils two days following estrus and degranulation of acidophils during the middle of the luteal phase of the cycle.

Cell classification (general summary)

Chromophils

1) Acidophils (alpha cells)

A. Alpha acidophils or orangeophils: S.T.H. producers

Stained with orange G.

Small rounded cells with large number of dense granules.

B. Epsilon acidophils or carminophils: Prolactin producers

Stained red with azocarmine.

Smaller number of dense cytoplasmic granules.

Appear in the pituitary of females in early pregnancy and increase the number in late pregnancy and lactation (larger than the orangeophils).

2) Basophils (beta cells)

A. Beta basophils: — Thyrotropin producers

React positively to aldehyde fuchsin stain.

React positively to P.A.S. — Probably ACTH producers

Polyhedral shaped cells, finely granulated (bigger than delta cells).

B. Delta basophils: — Gonadotropin producers

React negatively to aldehyde fuchsin stain.

React positively to P.A.S.

Rounded or oval shaped cells, coarsely granulated, more numerous in males than in females (smaller than beta cells).

F.S.H. producers located in pars tuberalis. L.H. producers located in pars distalis.

When using the performic acid-Alcian blue-PAS-orange G stain:

purple cells = F.S.H. producers, blue cells = thyrotropin producers, red cells = L.H. producers.

Chromophobes

The Hypothalamus

The hypothalamus is a portion of the diencephalon. The diencephalon is the rostral most part of the brain which is embedded between the cerebral hemispheres (Bowsher, 1961). It is a coordinating center for the motor control of visceral activity among many other functions. Its location and relation to other nervous structures may be determined by its anterior limit which is the optic chiasma, the tuber cinereum in the middle and the mamillary body caudally, dorsally the thalamus and ventrally the pituitary gland. It is divided into halves by the third ventricle. In a coronal section through the middle part it may be seen that the anterior column of the fornix divides the hypothalamus into a lateral and medial part. The lateral hypothalamus consists of scattered cells. The medial hypothalamus consists of fairly large groups of nuclei, about twenty of them. Each has not been associated with a specific function (Krieg, 1955). Most anteriorly above the chiasma is the preoptic nucleus. The supra-optic nucleus lies dorsal and slightly lateral to the optic chiasma. The para-ventricular nucleus lies dorsal and caudally to the preoptic nuclei (Netter, 1958b). The tuber cinereum is a gray mass between the optic chiasma and the mamillary bodies. It ends ventrally in a tube shaped process, the infundibulum, by means of which the neurohypophysis is attached to the tuberal portion of the stalk (Miller et al., 1964).

The hypothalamus is composed of gray matter which is constituted largely of bodies of nerve cells; most of the fibers are nonmyelinated. The cells of the hypothalamus have been defined into nuclear groups. The significance of this system is that most of the blood destined for the pars distalis first passes through the median eminence (Gardner, 1964). The functions necessitating parasympathetic activity appear to be mediated by the posterior portion of the hypothalamus and the sympathetic by the anterior hypothalamus (Netter, 1958a). Finally the hypothalamus itself is activated in its hypothalamo-hypophyseal mechanism by a variety of impulses arriving from other parts of the nervous system.

In the lateral and anterior hypothalamic area the cell population is fairly uniform and consists of small neurons, except for a few large neurons scattered along a line between the para-ventricular and the supra-optic nuclei. The para-ventricular nuclei are slender wedge-shaped groups of cells near the third ventricle ventromedial to the fornices. The supra-optic nucleus overlays the beginning of the optic tract which separates it into two portions. The neurones of these two nuclei are large with dark staining Nissl substance located at the periphery. Both nuclei send fibers down to the infundibular stem into the infundibular process of the pituitary. However, not all the cells of the para-ventricular and supra-optic nuclei have that destination. It is a

question as to how many other connections are possessed by those nuclei. It must be mentioned that these neurones have certain cytologic characteristics which have given some evidence of neurosecretory activity. Afferent neural connections are not clearly understood.

Nerve fibers from poorly defined regions in the tuberal and posterior portions of the hypothalamus also enter the infundibular stem and many of these fibers may have neurosecretory characteristics.

Arterial supply of the hypothalamus

Lying at the base of the brain the arterial supply of the hypothalamus is derived from the circle of Willis. There are variations between different species but a general pattern can be established (Daniel, 1966). For these hypothalamic blood supply explanations the hypothalamus will be divided into anterior, middle and posterior parts.

The anterior part, consisting largely of the preoptic region, obtains its blood supply from branches of the anterior cerebral arteries which lie above the optic nerves and from the anterior communicating artery. The middle part of the hypothalamus, beneath which lie the two posterior communicating arteries, is irrigated mainly from the branches derived from these two arteries. The posterior part of the hypothalamus is supplied by branches coming from the bifurcation of the basilar artery and those regions of the

posterior cerebral arteries proximal to their junctions with the posterior communicating arteries.

The superior hypophyseal arteries leave the internal carotid arteries just after the latter have pierced the dura mater and these vessels run medially and dorsally to form part of the arterial ring around the upper extremity of the pituitary stalk. These superior hypophyseal arteries provide the afferent blood supply to the pituitary stalk which is the major part of the blood that indirectly and eventually reaches the anterior lobe. Foley, Kinney and Alexander in 1942 as cited in Daniel (1966) showed that many of these nuclei receive blood from more than one of the arteries mentioned above. It is also considered that each of the nuclei receive an arterial blood supply from more than one vessel.

The three hypothalamic nuclei which are most clearly defined histologically are the supra-optic, the para-ventricular and the mamillary nuclei. The supra-optic nucleus receives its blood supply from the communicating posterior cerebral arteries, anterior cerebral and internal carotid arteries. The para-ventricular nucleus is supplied by the anterior cerebral, internal carotid and posterior communicating arteries. The mamillary nucleus is supplied by the posterior communicating, posterior cerebral and basilar arteries.

Jewell and Verney in 1957 found similar results. However, in the supra-optic nucleus they found that the blood supply came from the anterior cerebral, middle cerebral, internal carotid and posterior communicating arteries. Prolo and Stilwell in 1962 stated that there was a constant branch, the para-ventricular ramus, which was a major source of supply to the para-ventricular nucleus in the rabbit.

The capillary bed

The richness of capillary vessels in some nuclei is very abundant. The best irrigated were the supra-optic and para-ventricular nuclei. The capillary counts gave figures of 2600 for the supra-optic nucleus and 1350 for the para-ventricular (Daniel, 1966). The supra-optic nucleus is supplied by the richest capillary bed of any nucleus or group of neurons in the central nervous system. Each individual nerve cell is surrounded by a network of capillaries, an arrangement seen nowhere else in the nervous system (Daniel, 1966).

Venous drainage

A venous circle lies at the base of the brain, very roughly outlining the middle part of the hypothalamus. It is situated above the arteries of the circle of Willis, between them and the cerebral substance. The venous circle drains into the basal vein which passes posteriorly around

the midbrain, lying in the cisterna ambiens, to enter the vein of Galen (Daniel, 1966).

Hypothalamo-Hypophyseal Relations

The hypothalamus influences the secretory functions of the pituitary. This does not mean that the pituitary is completely under hypothalamic control (Netter, 1958b). It is assumed that a great number of anterior lobe functions are autonomous in accordance with the variations in concentration of hormones in the blood. The hypothalamic influence is important, for instance, in the formation and releasing of L.H. in response to copulatory stimuli in some animals.

The number of nerve fibers entering the anterior lobe from the infundibular stem is too small to have any effect on the gland cells.

No hypophyseal hormone is released without a signal for such a release from the internal or peripheral environment. Those signals are intended for either the anterior or posterior lobe. If they are intended for the neural lobe, which is enervated, the demand of oxytocin or vasopressin is obeyed instantly. The neural lobe is only a reservoir for the hormones which are actually produced by the cells of the supra-optic and para-ventricular nuclei of the hypothalamus from which they descend along the supra-optic tract. If the tract is cut and blocked, neurosecretory granules will accumulate on the hypothalamic side and depletion will occur

in the neurohypophyseal side as the neural lobe is being emptied of its stored hormone (Nalbandov, 1964).

If the signals from the periphery are intended for the adenohypophysis the problem of transmitting the signal becomes more complicated because the gland is not enervated (Netter, 1958b). Instead it is connected to the median eminence and hypothalamic nuclei by the portal system. The afferent nerve endings from the periphery are known to end at the neurosecretory cells of the supra-optic and para-ventricular nuclei of the hypothalamus. The demand of a hormone, released by the adenohypophysis, is transmitted via the afferent nerve endings; the hypothalamic neurosecretory cells respond by elaborating neurosecretory material (releasing factors) which are transported and carried through the portal system directly to the epithelial cells of the anterior lobe, which responds with a release of the previously synthesized hormones.

The hypothalamo-hypophyseal neurosecretory pathway is known morphologically in all vertebrates. In all cases it consists of three segments found in every neurosecretory pathway, named the perikaryons of origin, the axons along which the neurosecretions travel and the storage organ in which it accumulates before discharge. Bratrachia and Drivry as cited in Gabe (1966) showed that secretory products accumulated in the perikaryons of the supra-optic and

para-ventricular nuclei. In man it contained no glycogen, no lipids staining with Sudan III, no mucins and no amyloid substances (Gabe, 1966).

Several releasing factors have been identified and separated out by function and they are known to be polypeptides. Whether some of these releasing factors (especially corticotropin releasing factor) are oxytocin or vasopressin is an open question. In the rat and chicken these hormones act as A.C.T.H. releasers but corticotropin releasing factor is chemically different from neurohypophyseal hormones (Nalbandov, 1964).

MATERIALS AND METHODS

Experimental Animals

Two main groups of animals were included in the experiment. The first group was made up of 24 gilts. Eighteen animals were medicated and six were controls (sow number 20 was not included in this experiment). The tissue collected for this work was pituitary gland only. The second group consisted of three medicated animals and three controls from which the pituitary gland and hypothalamus were collected.

All these animals were sexually mature. Before the experiment was performed the animals were checked for heat with the aid of a boar. Daily information was collected and certain numbers of cycles were observed in each gilt in order to know and assure the right functioning of the estrus cycle. This pretreatment history is given along with the number, breed, age and weight at the time of killing.

Sow no.	Breed	Age at necropsy in days	Weight in pounds	No. of recorded cycles	Length of cycles
		Synchronized Animals Group 1			
1	Poland China	253	278	2	19,21
2	Landrace	254	277	2	22,26
3	Yorkshire	277	294	3	23,20,22
4	Landrace	277	308	2	21,20
5	Yorkshire	259	271	2	23,20
6	Yorkshire	261	267	3	21,20,19
7	Yorkshire	283	315	2	28,21
8	Hampshire	293	306	2	23,20
9	Yorkshire	271	300	3	18,24,17
10	Yorkshire	268	311	1	20
11	Yorkshire	270	291	2	18,20

Sow no.	Breed	Age at necropsy in days	Weight in pounds	No. of recorded cycles	Length of cycles
		Synchronized Animals Group 1			
12	Landrace	271	285	2	20,22
13	Yorkshire	278	267	3	23,18,20
14	Poland China	275	304	2	23,21
15	Yorkshire	305	331	2	19,25
16	Poland China	285	294	3	21,20,20
17	Landrace	296	313	3	23,13,22
18	Yorkshire	301	309	2	26,21
		Control Animals Group 1			
19	Duroc	220	196	1	18
20	Duroc	253	252	2	20,20
21	Duroc	258	262	2	23,22
22	Poland China	276	275	3	21,19,20
23	Duroc	282	309	3	24,22,18
24	Duroc	274	296	2	25,21
		Synchronized Animals Group 2			
1*	Yorkshire	273	331	4	14,22.5,20,19.5
5*	Hampshire	295	310	1	21
8*	Yorkshire	257	292	1	20
		Control Animals Group 2			
2*	Yorkshire	295	308	3	20,19.5, 19
4*	Yorkshire	270	285	4	19,18,20.5,20
7*	Yorkshire	257	284	1	18

Experimental Design

There were 18 gilts treated with M.A.T.C.H. in Group 1 and 6 controls. The 18 medicated ones previously had manifested two definite standing heats. When medication was started the control animals had experienced only one definite standing heat.

A scheduled plan of slaughtering was arranged and each

animal had a random chance to be scheduled for slaughtering which was assigned in the following way. Six animals were killed during the period of medication (synchronization process). By the time synchronized estrus occurred (9 days after cessation of medication) six more animals were killed. The last six of the treated animals were artificially inseminated and sacrificed at days 4, 14 and 20 after the synchronized estrus occurred. The control animals were slaughtered at different stages of the estrus cycle for the purpose of providing results timed similarly to the treated animals (Chart 1).

One hundred eighty mg of M.A.T.C.H., the synchronizing drug of choice, was given to each sow per day during a period of 19 days as recommended. The drug was given in 1½ pounds of a commercial corn-soya feed ration each morning; later in the afternoon another 1½ pounds of feed with no drug in it was given to each animal. In some cases where anorexia became present as a side effect of the medication, only 1½ pounds of feed constituted the total daily feed intake per sow. Each animal was fed separately in order to assure the exact medicated feed intake.

Artificial insemination was utilized for breeding the animals which were synchronized. This procedure was performed every day during the heat period. The semen was provided by boars used as heat detectors. Previous to artificial insemination a microscopic evaluation of the semen

collected was performed. When it was necessary to make a dilution this was done with milk extender in order to obtain enough volume. Since estrus synchronization seemed to make the heat period last longer the synchronized animals were inseminated on four or more successive days, while controls were artificially bred only two to three times. The inseminating volume was of a 50 to 75 ml dose.

Posting, embalming, removal and preparation technique for obtaining the pituitary gland

In Group 1 the animals were killed by electrocution. As soon as possible the head was cut off and the pituitary gland was obtained, weighed and preserved in 10% formalin. The thyroid, adrenals, ovaries and genital tracts were removed and preserved for further studies.

In Group 2 electrocution was used also. Immediately the heads were cut off and saline solution of 8% concentration was slowly infused into the carotid arteries for rinsing purposes until the blood was completely washed out. Then 10% formalin solution was infused until the saline solution was washed out. At this moment the jugular veins were clamped as well as any other vessels in order to prevent loss of the formation solution in order to get the best possible fixation. The head was kept in an upside down position so the formalin would reach the brain more easily and hardening of the pituitary would be complete. This facilitates the

removal of the gland which is accomplished by lifting up the top of the cranium by sawing on a line extending from the superior part of the orbit to the top of the foramen magnum. When this is done on both sides the dorsal part of the brain and cranium is removed. When the olfactory lobes and the brain stem are lifted up the pituitary gland is exposed in the hypophyseal fossa. After removal from the cranial cavity the pituitary gland and the hypothalamic tissue were kept in new fresh 10% formalin for 72 hours and then they were changed to 70% alcohol for three days before the infiltration and embedding in paraffin. This technique was applied on the last six gilts (Group 2) which were not included in Group 1 of the experiment.

Staining Procedure

After trying various fixing fluids it was found that alcohol fixation was not satisfactory for the performic acid-Alcian blue-PAS-orange G stain used. The slides came out with such a pale color that it was not possible to identify any cellular elements of the tissue. Best results were obtained with ordinary 10% formalin solution. Formalin fixation allows a clear and sharp differential staining of the cells, and also produces practically no change in the weight or shape of the hypophysis (Rasmussen and Herrick, 1922). Therefore, it is possible to get the weight of the fresh organ from formalin fixed material without the need of

having delicate balances at the slaughtering time and without delaying fixation. After trying several stains, such as hematoxylin and eosin, and performic acid-Alcian blue for cystein (Adams and Pearse, 1959), the one chosen was performic acid-Alcian blue-periodic acid-Schiff (PAS)-orange G (Heath, 1964). It provided the facility to distinguish the different kinds of basophilic and acidophilic cells in the adenohypophysis. Heath (1964) used this technique on sections of pituitary glands from different domestic animals. He was able to distinguish three different types of basophils and two types of acidophilic cells. This staining method also provided in the present experiment the advantage of staining neurosecretory products in the pars nervosa and hypothalamus.

The fixed glands were embedded in paraffin and sagittally sectioned at 6-10 microns. The mounted slides were counted in order to estimate from a total and differential count of various cells in five slides from different levels of each pituitary according to the method of Rasmussen and Herrick (1922). This procedure was followed for animals No. 1 to No. 24 of Group 1.

Four of the cell types, dark blue, light blue, violet or purple and red, were considered basophils because of their relative affinity for P.A.S. and their relative lack of acidophilia. Heath (1964) found that the acidophils were

especially numerous in sections of pituitary gland from a sow as compared to other species with which he worked.

For Group 2 of the experimental animals (sow No. 1' to sow No. 8') the following modifications were done. The extraction, fixing, sectioning, paraffin embedding procedure as well as staining were performed with the pituitary gland attached to the hypothalamus so observations of their close relationships could be made. A total of approximately 7200 sections were obtained from these six animals. Four different stains were used and the best slides were selected for results and evaluation purposes. Hematoxylin and eosin, toluidine blue stain for the Nissl substance, performic acid-Alcian blue-periodic acid-Schiff (PAS)-orange G, and Bargmann's chrome hematoxylin for neurosecretory substances were the four staining procedures which were used progressively and continuously in sets of five slides throughout the glands. The fifth slide of each set remained unstained for further modifications of the staining procedure.

The pituitary gland counts and evaluation for Group 2 were done by following the same counting procedure and using the same staining method as for Group 1 except that the sectioning was done transversally. For hypothalamic evaluations slides stained with performic acid-Alcian blue-PAS-orange G and Bargmann's method were mainly utilized because of their neurosecretory material staining properties. However, toluidine blue and H.E. were of great assistance, especially

in estimating the intensity of discoloration and localization of the neural cells of the different hypothalamic nuclei.

Pituitary Gland Counting Procedure

The pituitary glands were sectioned; each slide contained several sections. In this way several slides from each gland were available for study. It has also been possible to study sections from a certain level of the gland. In the study of this material cell counts were made on five sections of each gland. Each section was picked from a different slide. For counting purposes the method used was the one introduced by Rasmussen and Herrick (1922). From each set of slides stained, representative sections from different levels of the anterior lobe were chosen for study and counting. The selection of the sections counted was arranged in such a way that there was no possibility of sections of the same cell appearing on more than one slide studied. Five sections of each gland and every fifth oil emersion filled in each section were counted in these glands (Figure 1).

RESULTS

Pituitary Gland General Observations

Pituitary gland weight observations (see Graph 1 and Table 1 of the Appendix)

The pituitary weights of experimental gilts in Group 1 showed a light weight which increased at day 6 of the treatment period. This was noted to increase progressively during the period of medication. Observations made two days after medication was suspended showed that the pituitary weight had continued to increase. The weight of the pituitary glands reached its peak when estrus synchronized animals came into heat. A slow decrease occurred at the onset of pregnancy. The control animals followed the same curve at a slightly lower level.

Pituitary gland histological observations

The general histological observation as well as the cell type study were accomplished by using the performic acid-Alcian blue-periodic acid-Schiff-orange G staining method (Heath, 1964). Several types of cells were found in the adenohypophysis of the pig. Three basic cell types, acidophils, basophils and chromophobes, were constantly found in the pituitary gland of each. Wide variations were present within each type of chromophil. These variations did not occur as a constant pattern in all of the glands observed.

The contrasting colors were observed as follows in the anterior pituitary structures. Magenta was seen in the outside membrane, encircling membrane of the cell cords and colloid droplets (Figure 15). Purple coloration as well as strong magenta were found in some colloidal material.

According to recent literature the colloidal substance among the cells is more often found in older animals (Trautman and Fiebiger, 1952). The colloid production is graded according to size and frequency of the droplets found. Five categories have been established by which estimates of the colloid production were determined:

1) very good
2) good production
3) fair production
4) poor production
5) very poor production.

The cytoplasm of the cells in the pars intermedia is pale magenta or light purple, frequently forming dense masses of cells. The colloid content in this portion of the gland is abundant most of the time. The erthrocytes appear yellow.

Acidophilic cells These cells take a coloration which varies from a deep dark reddish orange to the very light yellow color (Figure 11). Their size is quite variable also. They are mostly located toward the inside or internal part of the pars distalis near the region of the

pars intermedia. They are commonly found also in the inferior or ventral portion of the adenohypophysis and constitute the highest percentage of the total cell population. The following varieties of acidophils are observed:

1) Small dark and small light orange cells measuring an average size of 12 x 14 microns.
2) Small dark and small light yellow acidophils with average size of 13.5 x 14.5 microns.
3) Big orange cells with a cytoplasm rich in coarse granules and vacuoles (BOVCC) measuring an average size of 18.5 x 22 microns, generally located at the periphery of the pars anterior. They group in big clumps almost without mixing with other types of cells (Figures 9 and 10).

Basophilic cells Six different basic types of basophils were observed. Their size also varies a great deal.

1) Small deep blue stained cells which are widely spread over the tissue when present. These cells rarely appear in clumps and their size averages 13 x 19 microns (Figure 16).
2) Big blue vacuolated cytoplasm cells (BBVCC) which stain in light blue color and sometimes form clumps. This type of cell is scarcely found. Its average size is 15 x 21 microns (Figures 13 and 16).
3) Small purple or violet stained cells which are

located all over the basophilic area at the peripheral part of the gland; however, they are often found within the acidophilic area also. They are common in the pars tuberalis and their size averages 13 x 19.5 microns (Figure 14).

4) Medium sized purple cells which appear to be larger than the cells described before in number 3). They have a very well stained purple cytoplasm which sometimes looks vacuolated. These cells form clumps and average 19 x 21 microns (Figure 13).

5) Big purple vacuolated cytoplasm cells (BPVCC). They are the biggest cell type found. Their cytoplasm is greatly vacuolated and it is stained with a very light purple color. They occur in big clumps and measure 22.5 x 24 microns (Figure 14).

6) Big clumps of red stained cells were found; however, this type of cell did not occur very frequently. These cells have an average size of 12.5 x 16 microns.

Chromophobes Their location is mainly found toward the central middle part and anterior peripheral edges of the pars distalis. These are pale cells with no stainable material in their cytoplasm. However, their nuclei show some traces of acidophilic or basophilic coloration. They occur in big clumps as well as being individually mixed with

the other cell types. They sometimes form acini with a colloid droplet in the center, and happen to be the least numerous cell type in the adenohypophysis. The average size measurements were 8 x 8.5 microns. These are the smallest type of cell found. Their cell nuclei looked very well delimited but sometimes the cell membrane did not show up at all.

It may be possible that the cell membrane does not get stained at all, or that both cell membrane and nuclear membrane approach each other so much that they almost look like they are fused together once the cell empties its stainable cytoplasmic granules (Figure 12).

Cellular structure Within the same gland, acini and cord types of cellular arrangements are observed. The formation of cellular cords which are oval in shape give a tubulo-alveolar type of arrangement to the pars distalis of the pituitary gland. The medullary part has smaller and more compact acini and the cells stain more deeply than those of the cortex. The cells in the medullar and cortical part of the gland are polymorphic and the cell acini are surrounded by a connective tissue capsule.

Individual Pituitary Observations (Group 1)

The following histological observations are complemented with Graph 2, Chart 1 and Table 2 of the Appendix.

Sow No. 1:

1) There is a poor blood supply in the pars tuberalis.
2) The acidophils are low and posteriorly located in the anterior lobe. The chromophobes are central and peripherally located. The basophil location is at the upper central and peripheral part of the pars distalis.
3) The cell acini are very well shown.
4) The production of colloid is poor in the anterior lobe. However, the pars intermedia has a fair colloid production.
5) There is a wide variety of cell types, sizes and colors in this gland. The color in general is pale.
6) The peripheral part of the anterior lobe looks like a better quality tissue than the central part.

Sow No. 2:

1) There is a poor blood supply in the pars tuberalis.
2) An exact location of the different cell groups is not shown very clearly.
3) The colloid production is very poor. It is made up by few and small drops.
4) The tissue is very deeply stained.

Sow No. 3:

1) The pars tuberalis blood supply is constituted by large vessels but not many in number.

2) The acidophilic cell location is at the internal and posterior part of the anterior lobe. Big clumps of red basophils are located in the central part. The periphery is occupied by basophils and chromophobes.

3) The cell acini are very well formed and surrounded by a thin membrane.

4) There is good colloid production formed by large droplets. The pars intermedia also has a good amount of colloid.

5) The general appearance of the tissue is of a healthy and well functioning one.

Sow No. 4:

1) The pars tuberalis is irrigated by few large caliber vessels.

2) The acidophils are posteriorly located within the pars distalis. The basophils and chromophobes are quite mixed within the external part of the gland.

3) The cell acini are not very well delineated.

4) This specimen has a very poor colloid production; even in the pars intermedia the colloid production is poor.

5) A healthy and well functioning appearance is characteristic of this gland.

Sow No. 5:

1) The pars tuberalis shows some quite small vessels.

2) Most of the acidophils were on the bottom part of the anterior lobe (A.L.). The basophils occupy the top part of the A.L. and very few chromophobes seem to be present in the central and peripheral part.

3) The cell acini are very well shown.

4) There is poor colloid production. The droplets are of a large size but are very few in number.

5) The tissue has a dark coloration. This is a very light weight pituitary gland although the general appearance of the tissue is normal.

Sow No. 6:

1) The pars tuberalis shows a few but large sized vessels.

2) The acidophil cell location is ventral and lateral the pars intermedia. The basophils are widely spread in the center and periphery of the anterior lobe. The chromophobes are mostly located in the central part.

3) The cell acini are quite well seen.

4) There is a poor production of colloid constituted by few and small droplets. The pars intermedia has a few but good sized colloid drops.

5) In the periphery of this gland it is very noticeable how the acidophils increase in number and the basophils are diminished as well as the chromophobes.

6) The glandular tissue looks healthy and well functioning.

Note: The colloid drops have different intensity of color varying from magenta to dark purple. It appears as if there were no relationship between the color of the colloid and the cell type which produces it.

Sow No. 7:

1) The pars tuberalis is poorly irrigated.
2) The basophils tend to be located in the lower and peripheral part of the anterior lobe. The rest of the cell types are quite mixed.
3) The cell acini are very clearly seen.
4) There is a fair colloid production, especially close to the pars intermedia.
5) The peripheral area of the gland has a very pale staining color.

Sow No. 8:

1) The pars tuberalis is quite well irrigated.
2) The acidophils are located on the dorsal part of the anterior lobe, the basophils are in the ventral portion and the chromophobes are mostly gathered in the central area.
3) The acini are not very well formed.
4) This gland has a fair colloid production. The pars intermedia shows a good production of colloid.

5) The nearer it gets to the peripheral zone of the anterior lobe, the higher is the acidophil percentage.

6) The general appearance of the tissue is not very good. The stain did not work well in this gland, and the appearance given by the cells is not that of highly active tissue.

Sow No. 9:

1) The pars tuberalis is not very well irrigated.

2) The acidophil cell location is downward while the basophils and chromophpbes are located toward the upper part of the anterior lobe.

3) The cell acini are quite well formed.

4) This gland has quite a good colloid production. The droplets are of a good size and sometimes quite large. The colloid is especially located within the purple basophil area and also near the pars intermedia.

5) The appearance of the glandular tissue is of a well functioning and healthy one.

Sow No. 10:

1) The pars tuberalis shows a very good blood supply.

2) The acidophils are mainly located in the posterior and ventral portion of the anterior lobe. The basophils and chromophobes appear to be located toward the center and dorsal portion.

3) The cell acini are very well seen.

4) The colloid production is good. The drops are few in number but they are good sized. The pars tuberalis has many fairly large sized colloid droplets.

Sow No. 11:

1) Few blood vessels are supplying the pars tuberalis.

2) The inferior half of the anterior lobe is mainly occupied by the acidophil group, while the center is filled with chromophobes. The basophilic area is located in the upper half of the pars distalis.

3) The cellular disposition is arranged in cords.

4) There are some very large colloid droplets in the pars intermedia. Deep in the anterior lobe tissue close to the pars intermedia there are some regular sized colloid drops. The general colloid production is fair.

5) The cells have a good and healthy appearance even though the stain is rather pale.

Sow No. 12:

1) There is a poor blood supply in the pars tuberalis.

2) The acidophils are internal and ventrally located. The basophils and chromophobes are central and dorsally located.

3) The cell acini are perfectly formed.

4) The colloid content is poor and is mostly located

in the pars tuberalis. There are few but large colloid droplets in the pars intermedia.

5) The acidophils constitute the highest cell percentage in the periphery.

Sow No. 13:

1) A very good blood supply is present particularly in the pars tuberalis.
2) The acidophil area is ventral and posteriorly located. The central part is occupied by the chromophobes and the upper or dorsal part of the anterior lobe is occupied by the basophils.
3) The acini arrangement is perfectly shown.
4) This gland is one of the most outstanding in colloid production. Large and middle sized droplets are widely spread over the entire tissue with special occurrence in the pars tuberalis and intermedia.

Sow No. 14:

1) There is a very good blood supply espęcially in the pars tuberalis.
2) The acidophils are ventrally located while the basophils and chromophobes are dorsal and peripherally located.
3) Some of the acini are very large. Sometimes they are constituted by more than fifteen cells.
4) This gland is extremely poor in colloid. Very few

and small sized droplets are seen in the pars intermedia.

5) The general appearance is of a very good quality tissue.

Sow No. 15:

1) There is a fairly good blood supply in the pars tuberalis.
2) The acidophils are dorsally located within the anterior lobe, the chromophobes in the central portion and the basophils in the dorsal part of the pars distalis.
3) The cell acini type of arrangement is not very well formed.
4) The colloid production is somewhat poor. However, in the pars intermedia the production of colloid is better.
5) The acidophil percentage tends to increase quite considerably in the peripheral part of the anterior lobe.
6) Many cells appear to have vacuolated cytoplasm. The tissue does not look very functional.

Sow No. 16:

1) There is a good blood supply in the pars tuberalis.
2) The acidophils are posterior and dorsally located. The chromophobes are quite well spread in the

central portion. The basophils are dorsal and externally located within the anterior lobe.

3) A perfect acini arrangement is present in this pituitary gland.

4) The colloid production is poor in the pars distalis. In the pars intermedia the colloid production is better.

5) In the periphery the three cell types remain quite evenly mixed. The acidophils are not the predominating cell type as is the case most of the time.

6) The histological quality of the tissue is very good.

Sow No. 17:

1) There is a fairly good blood supply in the pars tuberalis.

2) The postero-inferior part of the anterior lobe is occupied by the acidophils, while the external and upper part is occupied by the basophils and chromophobes.

3) The acini are very well formed.

4) The colloid production is poor.

5) The quality of the tissue is of a well functioning gland.

Sow No. 18:

1) There is a poor blood supply in the pars tuberalis.

2) The acidophils are ventral and posteriorly located.

The chromophobes and basophils are central and peripherally located.

3) The acini and cord type of cellular arrangement are clear and easily seen.

4) There is good colloid production. Big droplets were found especially in the pars tuberalis.

5) The cells in the periphery are quite evenly mixed.

Sow No. 19 (control):

1) There is a fairly good blood supply in general.

2) The different cell types are quite well mixed within each other. There are big clumps of red basophils.

3) Well shown acini structures are seen in this gland.

4) The colloid is spread quite evenly in small drops. The gland has a fair colloid production.

5) The tissue in general looks well stained, healthy and functional.

6) The pars nervosa has quite a good neurosecretory substance production.

Sow No. 20: This sow is not included in this experiment.

Sow No. 21 (control):

1) There is a fairly good blood supply in general, especially in the pars tuberalis.

2) The acidophils are central and posteriorly located. The chromophobes are peripheral and centrally located. The basophils are mainly located in

the center but quite a few of them appear peripherally localized.

3) There are very well formed acinar groups of cells.
4) There is a very good colloid production.
5) Even though the tissue looks healthy and functional, the cells have a rather pale coloration.
6) The pars nervosa shows a good amount of neurosecretory substance (N.S.S.).

Sow No. 22 (control):

1) There is fair blood supply over all the tissue.
2) The alpha cell type location is posterior and inferiorly found in the anterior lobe. The chromophobes were central and peripherally located as well as the basophils.
3) Very well formed acini structures are seen.
4) There is a fair colloid production mainly constituted by small droplets.
5) The general appearance of the tissue is of a healthy and well functioning gland. However, the cells do not have a deep coloration.
6) The pars nervosa shows a fair production of neurosecretory substance.

Sow No. 23 (control):

1) There is a fairly poor blood supply.
2) The alpha cell type location is posterior and ventrally found. The chromophobe location is central

and peripherally found in the superior half of the adenohypophysis as well as the basophils.

3) The cell acinar type of structure is not shown very well.

4) There is a very good colloid production.

5) The general appearance of the tissue is not very good. The stain did not come out very well; the coloration is pale.

6) The pars nervosa does not store much neurosecretory substance (N.S.S.).

Sow No. 24 (control):

1) There is a poor blood supply in general.

2) The acidophil cells are posteriorly located. The chromophobes and basophils are central and peripherally localized.

3) The cell acini are not very well shown.

4) The colloid production is good.

5) The general appearance is of a healthy tissue although it is somewhat pale stained.

6) The neurohypophysis appears to be extremely poor in neurosecretory substance (N.S.S.).

Individual Pituitary Observations (Group 2)

Because of functional reproductive disturbances, sows No. 3' and No. 6' had to be eliminated from this experimental group.

The periodic acid-Alcian blue-PAS-orange G staining procedure did not turn out well on sow No. 1' and none of the slides came out well enough to permit any evaluation of this gland. This sow was killed on the 17th day of M.A.T.C.H. treatment.

The following histological observations were made on sows No. 2', 4', 5', 7' and 8'.

Sow No. 2' (control):

1) The blood supply in general is fairly good, especially in the pars tuberalis.
2) The absence of acidophils is observed at the very rostral part of the gland. The general distribution of the cell groups varies according to the depth in the pituitary gland tissue.
3) The cell acini structure is not very well seen.
4) Colloid production is good.
5) The most difficult cells to differentiate are located at the very peripheral part of the gland.

Sow No. 4' (control):

1) An extremely abundant blood supply irrigates the whole gland.
2) Cell type differentiation is difficult and inaccurate to some extent since this tissue did not come out well stained.
3) There are few well differentiated cells. The general appearance is that many chromophobes are

in the transitional stage to chromophils or vice versa.

4) The colloid production is poor.

Sow No. 5':

1) Poor general blood supply is observed.
2) The different cell types become more evenly distributed and mixed as the central part of the gland is approached.
3) The different cell types are clearly differentiated. Much staining material is contained in the cell cytoplasm.
4) Colloid production is very good.

Sow No. 7' (control):

1) The observations are limited because the specimen was poorly stained.
2) Poor colloid production and very scarce blood supply are present.

Sow No. 8':

1) A normal blood supply irrigated the gland in general.
2) The tissue is well colored. It has the appearance of a well functioning and healthy tissue.
3) Colloid production is poor; however, it is deep purple colorated.

Individual Histological Observations of the Hypothalamus and Neurohypophysis in Group 2 (see Graph 3, Chart 2 and Table 3)

Two staining procedures were mainly utilized for this purpose. The first was the performic acid-Alcian blue-periodic acid-Schiff (PAS)-orange G (Heath, 1964) which stained in green color all neurosecretory substances. The other staining procedure used, especially for neurosecretory substance evaluation, was Bargmann's chrome hematoxylin for neurosecretory substances (Pearse, 1961, page 819) in which the neurosecretory substances stain deep purple and the background pinkish-red. Magnification lenses of 35, 100, 420 and 950 were utilized for evaluation purposes. The different structures were examined and the information is recorded in the following summary.

Hypothalamic Observational Summary

Sow no.	Days of treatment	Nuclei		Neurosecretory substance (N.S.S.)
		Para-ventricular	Supra-opticus	
1'	16	Angular shaped cells well irrigated. 10 x 10.5 microns.	Round and angular shaped cells. 11 x 15 microns.	Located in M.E. and pars nerv. Axon fibers. Near nuclei. Around vessels.
2'	Control	Round cells. 16.5 x 20 microns.	Full shaped cells. Dark stained. 12 x 12.5 microns.	More concentrated where more axon intercrossing.
4'	Control	Light color. Angular cells. 15 x 17.5 microns.	Dark stained angular cells. 17.5 x 20 microns.	Only present in nervosa.

Sow no.	Days of treatment	Nuclei		Neurosecretory substance (N.S.S.)
		Para-ventricular	Supra-opticus	
5'	5	Well stained. Shrunk angular cells. 9 x 16 microns.	Angular cells lightly stained. Shrunken appearance. 8 x 14.5 microns.	Diffuse small granules around vessels. P. nervosa.
7'	Control	Not very sharply angled cells. 13.5 x 14 microns.	Well stained angular cells. 14 x 17.5 microns.	Great amounts found.
8'	17	Full cells. Deeply stained. Big size. 27.5 x 36 microns.	Round shaped cells. 17 x 20.5 microns.	Diffuse and clumped forms are found.

Hypothalamic Observational Summary (Continued)

Sow no.	Median eminence	Neural stalk	Neurohypophysis
1'	Some small clumps of granules.	Very scarce in N.S.S.	Mostly all N.S.S. is held here as Herring bodies. Around vessels. Central part location.
2'	Very little N.S.S. in clumps.	Very little N.S.S. in stalk M.E. junction.	N.S.S. clumped in central part. Well irrigated.
4'	No N.S.S.	No N.S.S.	N.S.S. abundantly present around blood vessels. Centrally located.
5'	Good accumulation of N.S.S.	Little N.S.S. Diffuse form.	N.S.S. located all over the tissue, especially toward center.
7'	Fairly good N.S.S.	Little N.S.S.	The whole P. nervosa is full of N.S.S.
8'	No N.S.S.	Very little N.S.S.	N.S.S. is stored toward the center.

A special observation was made in sow No. 5'. In the region of the supra-optic nucleus toward the anterior part of the brain at the level of the medium portion of the optic chiasma, there is a group of extremely large neurons which measured an average of 19 x 27 microns.

These cells have either round or angular shape and their cytoplasm is full of fine granules which stained purple with the Bargmann's stain. This suggests that these granules are neurosecretory products. These cells are not found in any other gland.

Some neurons looked very pale and they could hardly be differentiated among the abundant network of axon fibers stained with Bargmann's stain, so it was necessary to utilize the toluidine blue stain (Mallory, 1938, pages 220-222) which is specific for neuron bodies and Nissl substance.

DISCUSSION

Evaluation of Techniques and Methods

Two solutions were used for fixation of the pituitary glands from the experimental gilts. The pituitary glands were divided into two halves by mid-sagittal section. One-half was fixed in 10% neutral formalin and the other half was fixed in 80% alcohol. Both fixatives were satisfactory for hematoxylin-eosin staining but 10% formalin was the best for the performic acid-Alcian blue-PAS-orange G stain.

The method employed for infusion of the hypothalamus and pituitary in situ in the six gilts in Group 2 produced very good fixation of the hypothalamus, median eminence and pituitary stalk. It is a great help for histological work to have the pituitary gland attached to the hypothalamic structures. The hypothalamic tissue from Group 1 which was removed from the cranium and then placed in formalin was not satisfactory to work with.

For differentiation of the various types of cells in the pituitary glands the performic acid-Alcian blue-PAS-orange G staining method produced the best results. Neurosecretory substance was identified by the use of this staining technique as well. Although Knigge (1967) has reported that neurosecretory substance does not stain with the classical techniques of Gomori and Bargmann, it was found in this work that the neurosecretory substance was well demonstrated

utilizing the Bargmann's staining procedure. Clearly visible neurosecretory substance was not observed near the nuclei cells or within their cytoplasm. The evaluation of neurosecretory production was done by observations made from the median eminence, neural stalk and pars nervosa where it occurred in great quantities.

The number of animals in Group 1, 18 treated and 5 controls (sow No. 20 was excluded from this experiment), seemed to be sufficient to establish a pattern of pituitary cell percentages during different periods of treatment and withdrawal from M.A.T.C.H. The cell percentages of the 6 animals in Group 2 compare favorably with the findings in Group 1. The limited number of animals in Group 2 is not sufficient to arrive at any definite conclusion concerning neurosecretory substance. Any conclusion from this group of 3 treated and 3 control animals would be based only on subject evaluation based on the impression obtained from studying the slides.

Differences in Neurosecretory Substance Between Treated and Control Animals (Group 2)

The amount of neurosecretory substance observed in the median eminence, pituitary stalk and pars nervosa was essentially the same in both the treated and control groups. The general impression gained after observing the material was that the median eminence of the treated animals contained slightly more neurosecretory material than the controls.

This observation is based only on subjective evaluation. This might suggest that treated animals which have been affected by the action of the drug have a tendency to retain the neurosecretory substance in the median eminence region and thus the material does not reach the area in the pituitary stalk where it should be taken up for transport to the adenohypophysis. Therefore, pituitary gonadotropin releasing action would be inhibited.

Most of the neurosecretory material that was observed was stored in the neurohypophysis and probably was related to anti-diuretic hormone and oxytocin production from the pars nervosa. The neurosecretory substance had a very peculiar tendency for accumulation around an empty space observed in transverse sections which represented the lumen of the infundibulum.

Histological Observations and Cell Count Evaluations

Wide variations in color intensity of cells of the pituitary were observed. The granules within the cytoplasm of the cells constitute the stainable material. The cytoplasmic granules were stained more deeply and darker in almost all cases in animals killed during the period of medication than was found in the animals killed after medication was terminated.

This suggests the possibility that during treatment the hormones of the anterior pituitary were being formed but

not being removed or released, either because the releasing factors were not formed or because the effects of M.A.T.C.H. impede the release of the releasing factors themselves.

It was generally observed that the larger the cell size the lighter was the stain and the smaller the cell the darker the stain. Since all of the cell types were not found in every gland it seems probable that a variation of the staining technique might make one cell type resemble the form typically recognized as another type. After one develops experience in identifying and counting various cell types, confidence increases in the accuracy of obtaining a representative sampling of the specimen. All of the counts in this work were performed by the author.

Histological observations show that the chromophobes have a little affinity for dyes. This might be due to the fact that when a chromophobe has not been completely emptied of granules, the traces of stainable material that still remain in the cell retain some coloration according to the type of cell that it was before the transformation of chromophil into chromophobe and vice versa. The chromophobes have been reported to be the smallest and most numerous cell type in the human hypophysis (Ham, 1957). However, the following cell percentages demonstrate the opposite in the sow pituitary. These are cell percentages in swine females of an age ranging from 220 to 305 days.

Percent Averages

	Baso-phils	Chromo-phobes	Acido-phils
Medicated animals	37	17	45
Controls	37	20	42
Animals killed during medication	42	14	44
Medicated animals killed at estrus	33	21	45
Control animals killed at estrus	37	19	44

As shown in Graph 2 of the Appendix the basophils have a marked tendency to increase during the medication period. According to the recent literature (Bloom and Fawcett, 1957; Bugnon, 1963; McShan, 1965; and others) the gonadotropin producers are basophils. One could state that when the drug is fed these cells increase in percentage and possibly increase in hormone storage, if M.A.T.C.H. stops the production of gonadotropin releasing factor. This seems to be the case in sows No. 5 and 6; the gonadotropin producing cells would store up the F.S.H. and L.H. but would not release it. After termination of the medication period the basophils decrease in percentage during the post-treatment period, as is shown by sows No. 7 and 8 in Graph 2.

Possibly this occurs because the gonadotropin releasing factors are no longer suppressed by the action of the drug and the releasing factors act on the pituitary, causing the basophils to decrease in number since no demand for gonadotropins is present. The anterior hypophysis is a regulator of ovarian activity and appears to carry out a cyclic capacity to stimulate the ovary which would be reflected in variations

in the cell type percentages. This variation is correlated with the different phases of the estrus cycle.

The average basophil percentage in the pituitaries of medicated animals killed at estrus, sows No. 9, 10, 11 and 12 (Chart 1), show that gonadotropin levels are even lower than in control animals killed at estrus, sows No. 21, 2', 4' and 7' (Charts 1 and 2). This reinforces the concept stated by Anderson (1966) that gonadotropin levels at estrus are at the lowest level throughout the estrus cycle.

Cleveland and Wolfe (1933) found in their experiments with sows that in order to induce ovulation in a mature rabbit, it takes 10 mg of anterior lobe tissue of a sow killed at estrus or 1 mg of the same material of a sow killed in proestrus. In other words, anterior lobe tissue of a sow killed at proestrus has ten times as much biological activity as the same amount of anterior pituitary material collected at estrus. This proves the author's findings in relation to the low level of gonadotropins found during estrus.

In the early lutein phase sows No. 13 and 14, synchronized animals killed 3 days after estrus, show the lowest basophil cell count. Sow No. 22, a control animal killed 5 days after estrus, also follows the same basophil decreasing effect.

Sow No. 15, a synchronized animal killed at day 14 after estrus during late follicular stage, shows a noticeable augment on basophils. The maximum basophil level reached at

proestrus is shown by the cell counts made from sow No. 24, control animal killed before estrus on day 24 after previous heat.

Observations made on the acidophils show that during the period of medication the acidophil cell percentage did not change. It appears that the drug does not inhibit somatotropin and prolactin releasing factors so the acidophil levels are kept almost constant. Beyond this point, after the medication period, the acidophil percentages increase and show a pattern that is opposite to the one seen in the basophils.

As is already known, no mitotic divisions are usually found in the adult hypophysis (Bugnon, 1963) but during gestation there is a constant interchangeable process of substitution of chromophobes that become either basophils or acidophils and, reversely, the lower the count of the chromophils the higher the count of the chromophobes. At the very beginning the chromophobe percent is 21.5 (Graph 2). The percentage keeps decreasing until the period of medication is interrupted. The drug may have a diminishing action over the chromophobes, or perhaps while the basophils increase the chromophobes yield their own cells so the equilibrium is kept, or both actions may exist together.

During the post-treatment period and during the following period of estrus appearance the chromophobes keep increasing in number while the basophils decrease (sows No. 11 and

12, Graph 2). From that point on sows No. 13 and 14 as well as the controls No. 21 and 22 show a decrease of the chromophobes. This is not due directly to an augmentation of the basophils, which actually are diminishing, but it is due to the fast increase of the acidophils as is shown in Graph 2 by sows No. 13 and 14 and also by sow controls No. 21 and 22. Sows No. 15 and 16 show a slight increase of the chromophobes which is due to the decrease of the acidophils. Sows No. 17 and 18 show a decrease of the chromophobes which corresponds to the increase of beta and alpha cells. The controls follow quite closely the same pattern.

Pituitary Colloid

The term "colloid" has been generally used to describe the noncellular staining component of the pituitary tissue. This material is found particularly in the pars tuberalis, pars intermedia and pars nervosa. Within the pars distalis it appears interstitially located in empty spaces surrounded by parenchymal cells. Several authors have stated that the interstitial colloid might be dissolved before it is absorbed into the sinusoids, efferent veins and portal system. Since the blood direction is oriented from the stalk to the anterior lobe, the finding of colloid in these vessels has to do with the secretion of the pars tuberalis, median eminence and infundibular stalk and not with the secretions formed in the adenohypophysis (Rioch, 1936).

Hyperactive glands contained large amounts of colloid. Rasmussen as cited in Rioch (1936) found colloid in blood vessels in only two cases out of 100 autopsies of sudden death. Soyer and Rasmussen as cited in Rioch (1936) described and associated degeneration of cells with ruptured colloid cysts and direct discharge of colloid into the blood stream. However, generally all of the pituitary glands in which heavy colloid production was found did show a very healthy appearance, well functioning tissue characteristics and fairly good blood supply.

Most of the time the pars tuberalis was the anterior pituitary structure in which colloid was present. On the other hand, it can be stated that the pars tuberalis cytology always has a vacuolated blurry cytoplasm, as well as the pars intermedia in which colloid is always present in large amounts.

There was no apparent significant difference in colloid production between treated and nontreated animals observed in the animals in this study.

M.A.T.C.H. Synchronization: Purpose, Consequences and Effects

The main purpose of controlled breeding is closely related to artificial insemination but even in places where no artificial insemination can be accomplished, a control breeding program provides the swine producer with all the

advantages of breeding the animals at a proper and most convenient time. This leads the producer to higher productivity and increased financial return. On the other hand, when artificial insemination is possible it is important to be sure about the exact and proper time for insemination of the animals. This can be done with certain accuracy since 90% of the treated animals come in heat within a ten day period of time after administration of the drug.

Since all the endocrine system is closely related and one endocrine gland affects the other, the indirect effect of this drug in other organs, such as the thyroid, adrenal, pancreas and others, may also have important consequences. Even though drugs react differently from one species to another, further findings may also bring results which permit the use of this drug for human necessities.

Although the mechanism of M.A.T.C.H. is not fully understood the reproductive system is definitely suppressed. These effects were clearly seen when the ovaries of the animals necropsied during the treatment period indicated very little or no evidence of pituitary influence. The total weight of the reproductive tract decreases steadily during the treatment period but rapidly increases with the onset of the synchronized estrus (Marshall, 1967).

After withdrawal of M.A.T.C.H., normal reproductive function returned, bringing growth of follicles as well as ovulation and corpus luteum development. This temporary

inhibitory effect might consist of a total or partial suppressive action in the elaboration of neurosecretory substances or releasing factors in the supra-opticus and para-ventricular nuclei of the hypothalamus.

SUMMARY AND CONCLUSIONS

1) The pituitaries were studied from a total of 29 gilts in an effort to determine the effects of M.A.T.C.H. on the pituitary during its use in swine for estrus synchronization. Twenty-one individuals were treated and eight were used as controls.
2) The hypothalamus and pituitary stalk were studied from six gilts (Group 2) in an effort to determine differences in neurosecretory material in these structures between treated and control gilts.
3) Suppression of estrus during M.A.T.C.H. administration and synchronization of estrus within 4-7 days following withdrawal was very satisfactory.
4) The weight of the pituitary glands was lower during treatment than during the synchronized estrus.
5) There was a distinct decrease in the percentage of chromophobes in the pituitaries examined during treatment with M.A.T.C.H. The percentage of chromophobes increased during the synchronized estrus.
6) There was a distinct increase in the percentage of basophils (gonadotropin producing cells) during the treatment period. The percentage of basophils decreased when treatment was stopped and decreased even further when the post-treatment estrus occurred.

7) The percentage of acidophils remained constant during treatment and then increased during the synchronized estrus and the post estrus period.

8) No difference was observed in the amount of colloid in the pituitaries of medicated, synchronized or control animals.

9) Neurosecretory substance granules were not observed in the cytoplasm of neurons in the para-ventricular and supra-optic nuclei of any of the animals studied. Some extremely small dark stained granules were occasionally seen near some neurons. These small granules were found within axon fibers. Perhaps electron microscopic examination of these granules would yield additional information.

10) Despite a general impression that more neurosecretory substance was present in the median eminence of the treated animals than in controls, no quantitative difference could be demonstrated.

11) The amount of neurosecretory material in the pituitary stalk and the pars nervosa was essentially the same in both the treated and control groups.

12) The increase in percentage of basophils in pituitaries of animals treated with M.A.T.C.H. suggests the hypothesis that gonadotropins are stored but not released during suppression of estrus with M.A.T.C.H.

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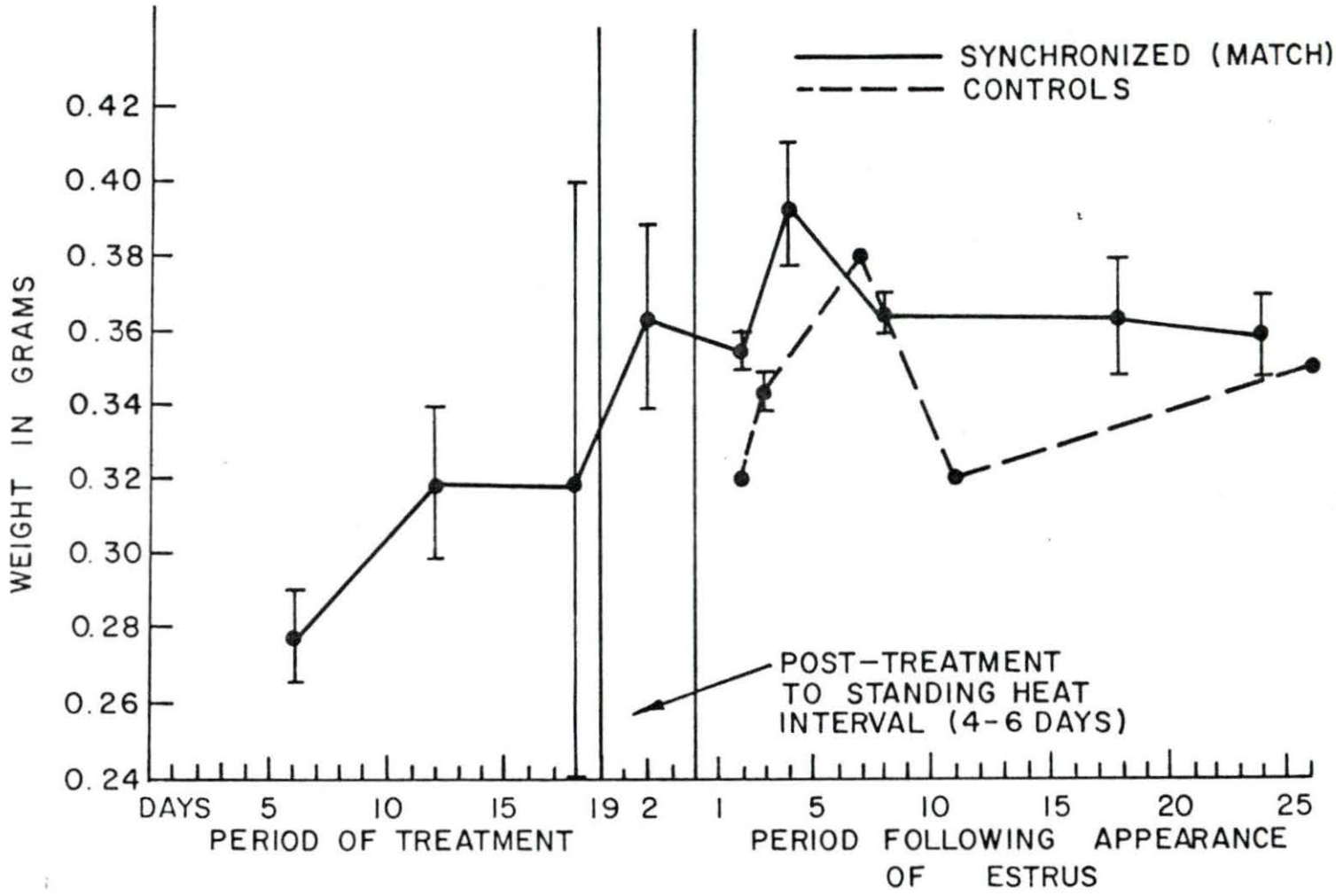
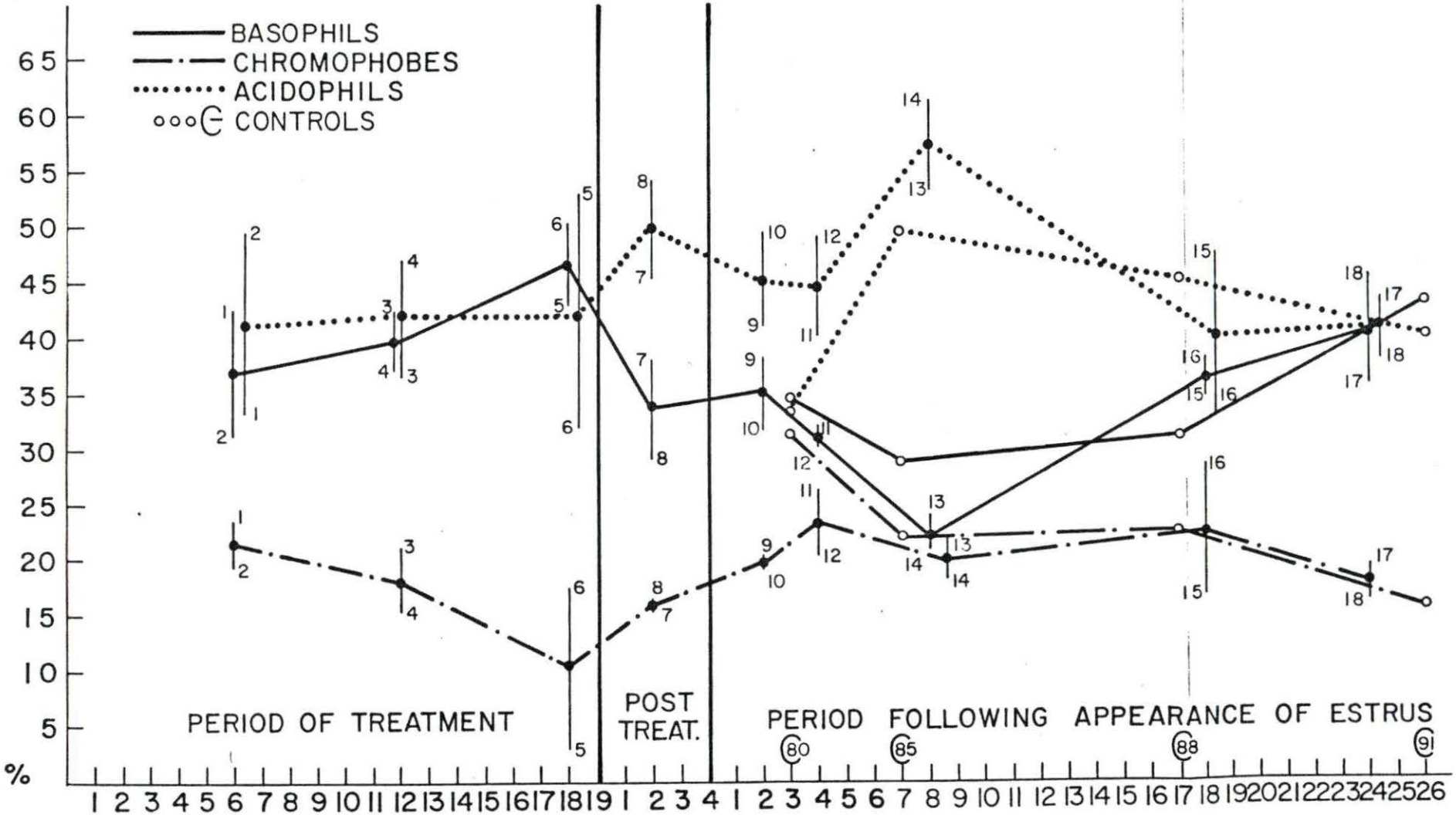
APPENDIX

Graph 1. Pituitary weights of experimental gilts group 1



PITUITARY WEIGHTS OF EXPERIMENTAL GILTS

Graph 2. Pituitary cell changes of experimental gilts group 1



GROUP NO. I. PITUITARY CELL CHANGES OF EXPERIMENTAL GILTS.

Graph 3. Pituitary cell changes of experimental gilts group 2

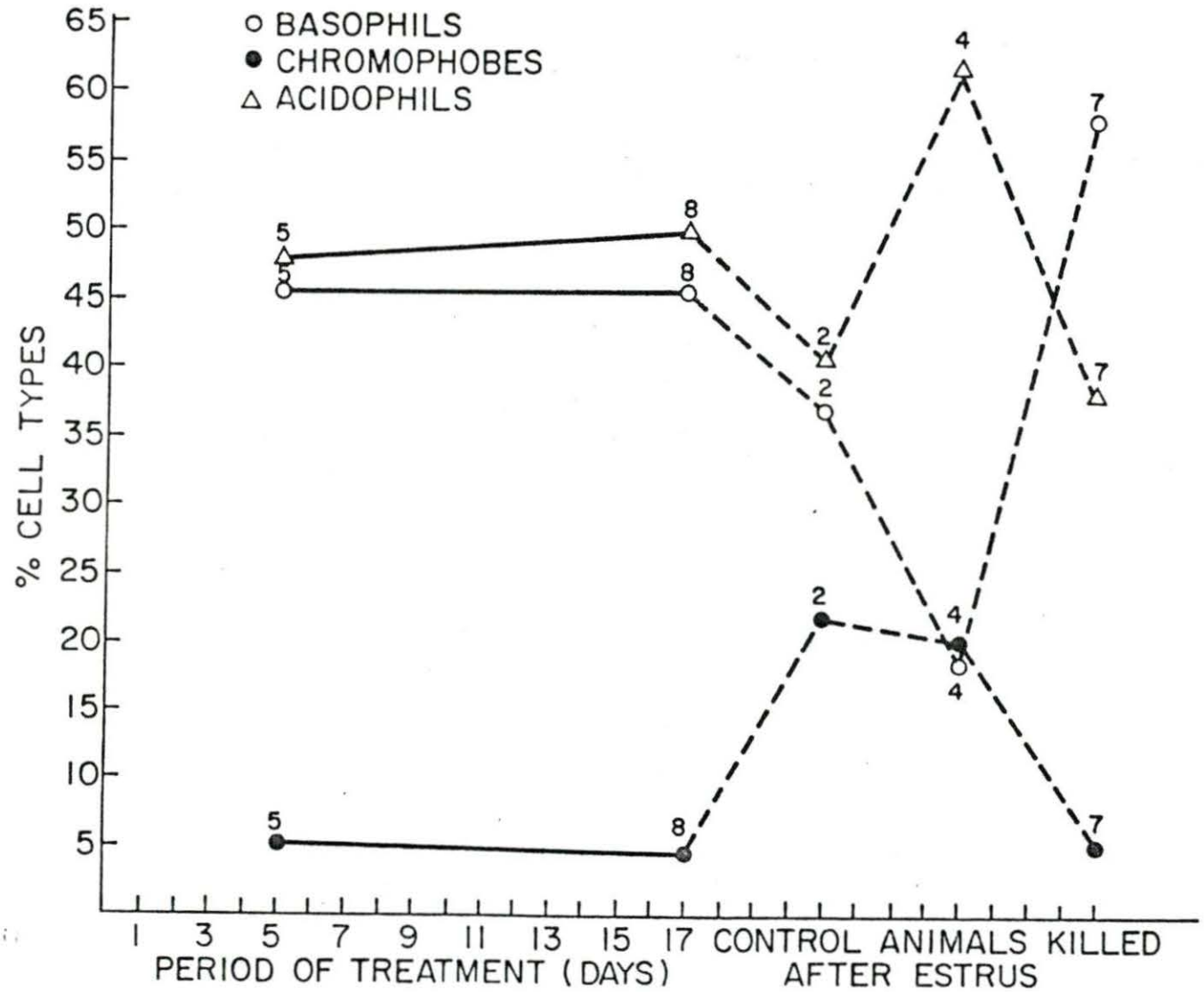

BASOPHILS
CHROMOPHOBES
ACIDOPHILS
% CELL TYPES
65
60
55
50
45
40
35
30
25
20
15
10
5
1 3 5 7 9 11 13 15 17 CONTROL ANIMALS KILLED
PERIOD OF TREATMENT (DAYS) AFTER ESTRUS

Chart 1. Daily record of estrus cycles of experimental gilts group 1

GROUP NO. 1 DAILY RECORD OF ESTRUS CYCLES OF EXPERIMENTAL GILTS.

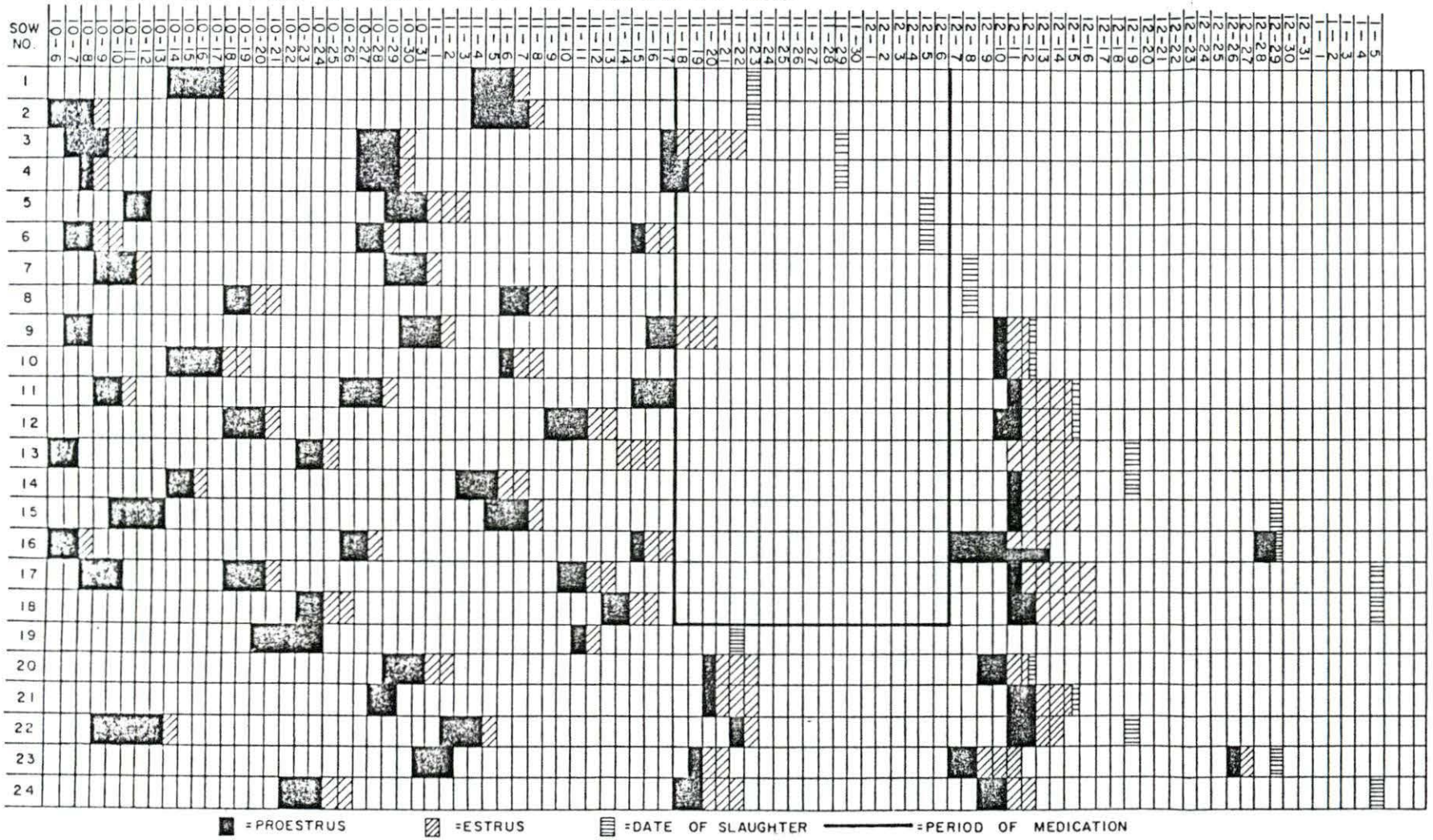
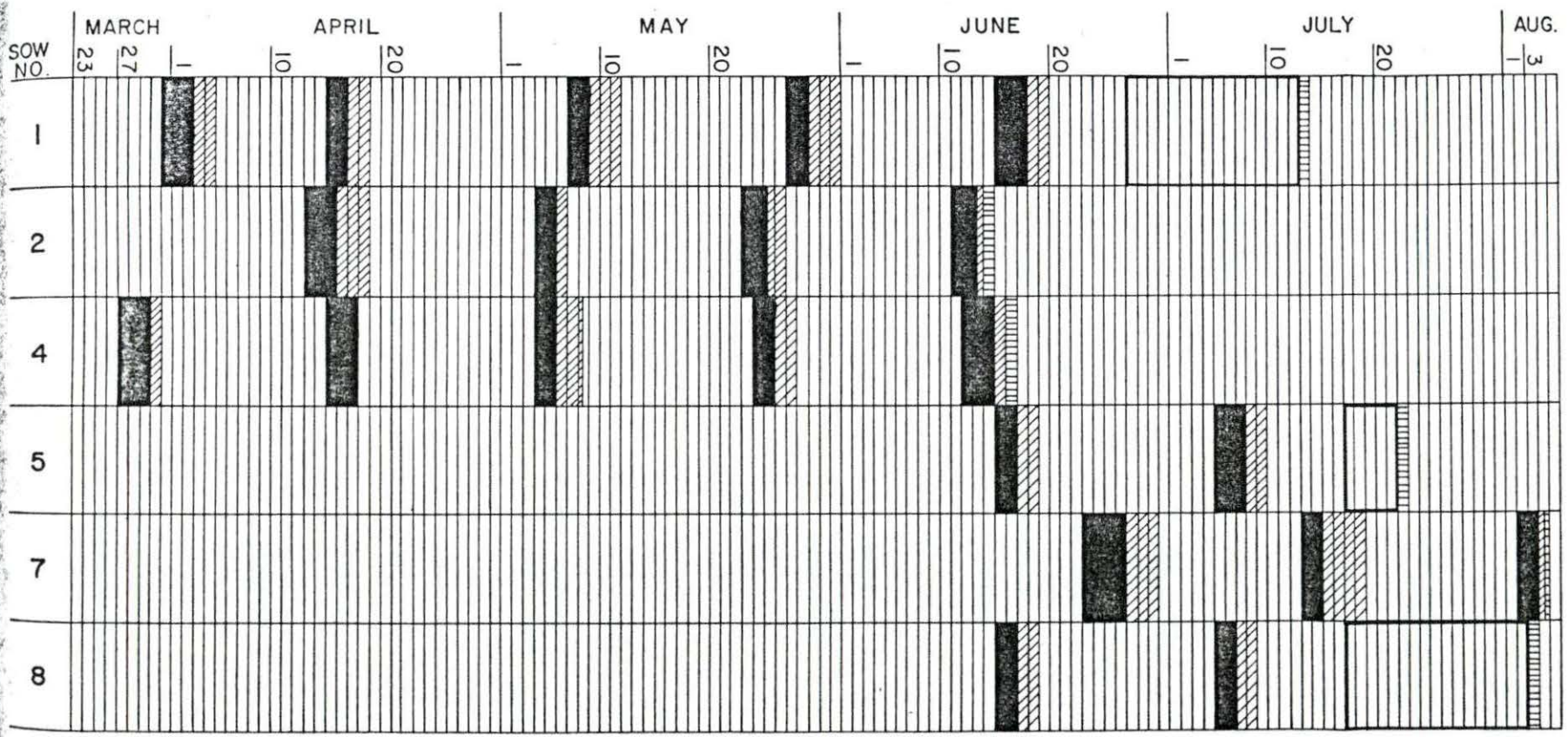


Chart 2. Daily record of estrus cycles of experimental gilts group 2



GROUP NO. 2. DAILY RECORD OF ESTRUS CYCLES OF EXPERIMENTAL GILTS

Table 1. Weight of pituitary glands of experimental gilts group 1

Sow number	Pituitary weight in grams
1	.27
2	.29
3	.34
4	.30
5	.24
6	.40
7	.34
8	.39
9	.35
10	.36
11	.38
12	.41
13	.36
14	.37
15	.35
16	.38
17	.35
18	.37
19	.32
20	.32
21	.34
22	.38
23	.35
24	.35

Table 2. Cell counts and percentages in pituitary glands of experimental gilts group 1

Pit. Gl. no.	Beta cells	Chromo-phobes	Alpha cells	BPVCC	BRVCC	BOVCC	BBVCC
1	385	442	354	224			
	405	318	335	144			
	223	227	642	117			
	515	268	413	151			69
	1528	1255	1744	636			69
2	50	5	156	2			
	106	45	204	12			
	167	105	213	26			
	241	203	271	34		70	
	344	306	772	58			
	908	664	1616	132		70	
3	668	366	429	63			
	676	495	510	177		14	1
	110	65	234	21			
	594	270	464	54		163	
	333	221	591	144			
	2381	1417	2228	459		177	1
4	105	64	293	105			
	78	76	282	26			
	250	173	306	251			
	210	166	494	108		2	
	197	111	448	101			
	840	590	1823	591		2	
5	202		251				
	412	52	541	34			
	614	52	792	34			
6	430	185	478	159			
	145	99	230	37			
	186	117	201	312			
	265	105	260	219		74	
	272	269	141	183		32	
	1298	775	1310	910		106	
7	232	212	522	182	3		15
	182	109	469	52	1		3
	287	198	625	227			19
	213	195	253	146	11	77	13
	103	69	226	133	1	23	39
	1017	783	2095	740	16	100	89

Total cell count	Total % beta	Total % chr.	Total % alpha	No. of fields	Colloid droplets
				39	12
				33	17
				32	16
				36	10
5232	42.67%	23.98%	33.33%	140	55
				6	1
				10	1
				15	3
				24	3
				52	10
3390	31.26%	19.58%	49.73%	107	18
				41	20
				57	17
				12	11
				56	60
				34	20
6633	42.63%	21.26%	36.09%	200	128
				14	4
				11	3
				26	7
				28	12
				25	13
3846	37.23%	15.35%	47.48%	104	39
				11	14
				20	12
1492	43.40%	3.40%	53.00%	31	26
				28	25
				15	7
				20	5
				23	3
				24	12
4399	50.21%	17.61%	32.20%	110	52
				25	23
				18	19
				29	10
				28	11
				21	5
4840	38.46%	16.17%	45.35%	121	68

Table 2 (Continued)

Pit. Gl. no.	Beta cells	Chromo-phobes	Alpha cells	BPVCC	BRVCC	BOVCC	BBVCC
8	243	177	468	3			
	223	112	477	16			
	217	179	629	82			
	164	52	216	69			
	115	82	287	5			
	962	602	2077	175			
9	537	309	474	202			2
	200	129	366	113	3	25	16
	351	422	739	180	22		2
	265	238	505	123	1	9	5
	164	98	335	72	2		8
	1517	1196	2419	690	28	34	33
10	37	111	516	151	5		31
	153	114	295	137	3		3
	107	195	383	171	6	19	20
	160	280	583	199	15	17	27
	8	93	344	104			41
	465	793	2121	762	29	36	122
11	82	443	376	379	19		4
	71	76	270	68			1
	47	381	398	479	11		21
	94	229	552	128			
	49	68	241				
	343	1197	1837	1054	30		26
12	120	157	278	329			1
	100	223	387	171		2	
	121	350	750	236			2
	171	220	614	71			24
	53	37	293	27			5
	565	987	2322	834		2	32
13	164	159	567	109	2	15	3
	123	135	470	114	18	1	3
	207	523	974	211	26		3
	69	236	488	144	6	5	
	46	98	286	36			
	609	1152	2785	614	52	21	9

Total cell count	Total % beta	Total % chr.	Total % alpha	No. of fields	Colloid droplets
				20	22
				24	20
				30	16
				16	6
				13	11
3816	29.35%	15.70%	54.60%	103	75
				38	36
				23	8
				45	31
				30	17
				18	4
5917	38.49%	20.21%	41.49%	154	96
				20	19
				16	5
				23	19
				29	25
				14	12
4328	31.83%	18.32%	49.84%	102	80
				27	24
				12	6
				31	20
				21	21
				9	17
4487	32.40%	26.70%	40.10%	100	88
				21	6
				24	14
				33	19
				26	17
				8	8
4742	30.20%	20.40%	49.40%	112	64
				24	29
				21	21
				50	75
				23	33
				12	25
5242	24.30%	22.00%	53.80%	130	183

Table 2 (Continued)

Pit. Gl. no.	Beta cells	Chromo-phobes	Alpha cells	BPVCC	BRVCC	BOVCC	BBVCC
14	171	188	638	38			4
	96	126	454	52			5
	109	122	375	12			
	376	436	1467	102			9
15	191	177	230	197	1		7
	190	221	460	198	2		
	113	61	243	25		1	2
	50	61	347	44			
	49	19	205	8	1		2
	593	539	1485	472	4	1	11
16	146	287	189	134	112		3
	122	275	397	97	13		16
	179	396	453	268	33		
	137	181	212	135	11	10	
	127	151	257	166	66	8	1
	711	1290	1508	800	235	18	20
17	196	230	590	98	1		
	138	76	401	25	6		3
	279	322	552	156	45		
	210	174	215	126	53		6
	228	106	317	58	32		
	1051	908	2075	463	147		9
18	274	182	374	162	85	3	19
	261	181	327	143	57		6
	189	126	158	108	66		45
	211	166	614	57	3		3
	43	69	178	187	51	14	
	978	724	1651	657	262	17	73
19	620	254	473				
	131	141	144				
	730	306	223				
	757	364	375				
	90	56	39				
	2328	1121	1254				

Total cell count	Total % beta	Total % chr.	Total % alpha	No. of fields	Colloid droplets
				22	14
				16	8
				16	8
2390	20.09%	18.30%	61.45%	54	30
				24	11
				25	18
				13	16
				16	13
				9	5
3105	34.80%	17.30%	47.80%	87	63
				28	22
				21	26
				26	27
				26	36
				25	28
4582	38.58%	28.09%	33.15%	126	139
				26	8
				16	8
				28	16
				21	16
				20	14
4653	35.88%	19.49%	44.57%	111	62
				22	19
				20	35
				17	27
				20	47
				21	27
4362	45.60%	16.60%	38.20%	100	155
				31	28
				9	7
				29	14
				39	18
				6	6
4703	49.40%	23.80%	26.60%	114	73

Table 2 (Continued)

Pit. Gl. no.	Beta cells	Chromo-phobes	Alpha cells	BPVCC	BRVCC	BOVCC	BBVCC
21	195	295	262	281	10		
	95	323	246	193	30		3
	19	217	221	107			5
	74	244	370	177	20		1
	88	124	186	33			
	471	1203	1285	791	60		9
22	92	162	435	45			2
	61	67	300	54			
	150	323	506	153	8		11
	58	141	439	42	7	2	1
	107	213	313	359	9		4
	468	906	1993	653	24	2	18
23	187	147	349	64	2		2
	54	138	269	135		10	
	48	168	267	229	4	4	1
	69	175	270	128	7	4	2
	54	149	362	102		36	
	412	777	1517	658	13	54	5
24	342	233	326	240	9		4
	209	141	371	120	1		
	276	111	361	68	9	8	
	401	202	474	115	20		
	102	43	323	78		5	
	1330	730	1855	621	39	13	4

Total cell count	Total % beta	Total % chr.	Total % alpha	No. of fields	Colloid droplets
				24	15
				23	22
				11	10
				17	21
				9	10
3819	34.85%	31.49%	33.64%	84	78
				17	26
				11	20
				26	29
				21	22
				29	39
4064	28.80%	22.27%	49.80%	104	136
				18	28
				17	8
				23	4
				16	15
				20	14
3436	31.41%	22.45%	45.24%	94	69
				25	21
				24	15
				20	20
				28	22
				17	9
4592	43.41%	15.89%	40.63%	114	87

Table 3. Cell counts and percentages in pituitary glands of experimental gilts group 2

Pit. Gl. no.	Beta cells	Chromo-phobes	Alpha cells	BPVCC	BRVCC	B0VCC	BBVCC
2'	91	193		70	38		4
	347	309	576	84	66	1	1
	244	146	424	19	17		2
	205	108	408	1	10		1
	255	102	207	25	3		
	1142	858	1615	199	134	1	8
4'	138	142	428				25
	149	210	542				100
	12	227	801	58	4	4	187
	21	191	318	44			
	13	24	376	4			
	333	794	2465	106	4	4	302
5'	262	42	533	30	3		
	252	43	516	108	21		1
	152	21	102	23	69	3	
	254	47	529	60	41		1
	218	30	33	13	146		
	1138	183	1713	234	280	3	2
7'	420	39	359	38	54		
	179	24	119	24	13		
	599	63	478	62	67		
8'	143	32	249	35	5		1
	350	44	703	164	3		6
	430	77	844	253	10		7
	269	26	552	93			
	345	27	182	115	64	4	
	1537	206	2530	660	82	4	14

Total cell count	Total % beta	Total % chr.	Total % alpha	No. of fields	Colloid droplets
				10	8
				35	34
				27	15
				26	20
				23	33
3957	37%	22%	41%	121	110
				22	11
				39	7
				41	8
				20	3
				16	5
4008	18%	20%	62%	138	34
				26	41
				39	44
				15	17
				38	46
				17	17
3553	47%	5%	48%	135	165
				30	6
				16	5
1269	57%	5%	38%	46	11
				15	2
				36	5
				42	16
				23	6
				20	5
5033	46%	4%	50%	136	34

Figure 1. Diagram showing how the field is explored in making the differential count of cells in the anterior pituitary. The solid circles represent the fields which were actually counted.

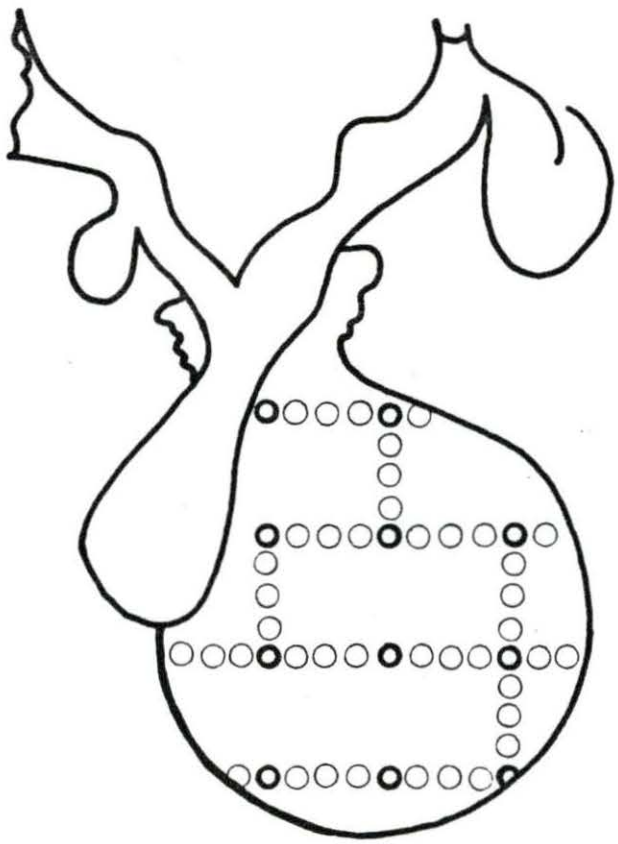


Figure 2. Diagram showing the suggested pathways by which individual nerve cells in the hypothalamus (H) transmit neurohumors through their axons into the primary capillary bed (P), and thence through the long (LPV) and short (SPV) portal vessels to control the output of hormone from cells (C) in a given area of the pars distalis. (Cap) capillary bed of infundibular process. (Adams, Daniel and Prichard, 1966)

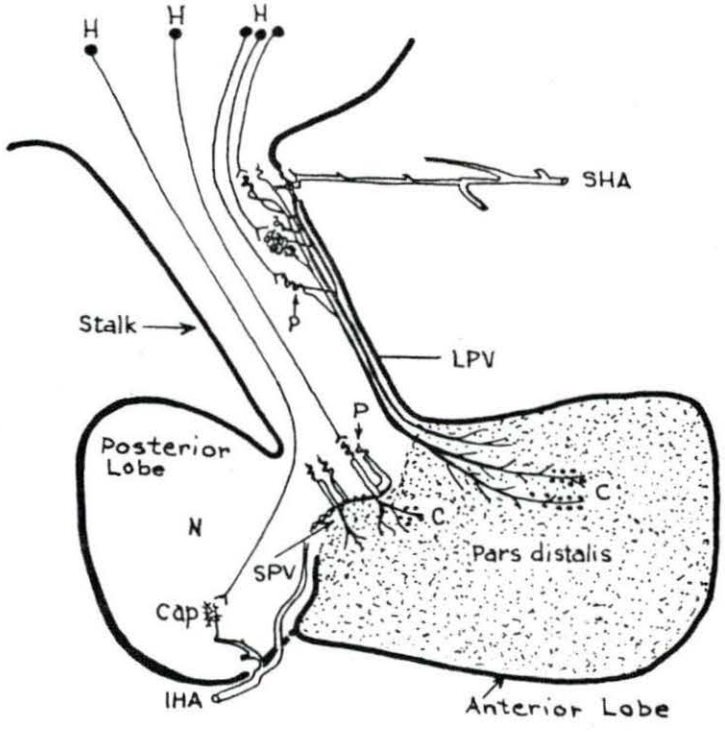

H
H
H
SHA
Stalk
P
LPV
P
Posterior
Lobe
N
C
C
Pars distalis
SPV
Cap
IHA
Anterior Lobe

Figure 3. Diagram of a human pituitary gland, in sagittal plane showing its blood supply. Note the particular long portal vessels (LPV) and the short portal vessels (SPV); both provide the blood supply of the pars distalis. The artery of the trabecula (AT) runs down without supplying the parenchymal cells. (SHA) superior hypophyseal artery; (IHA) inferior hypophyseal artery; (V) venous sinus. (Adams, Daniel and Prichard, 1966)

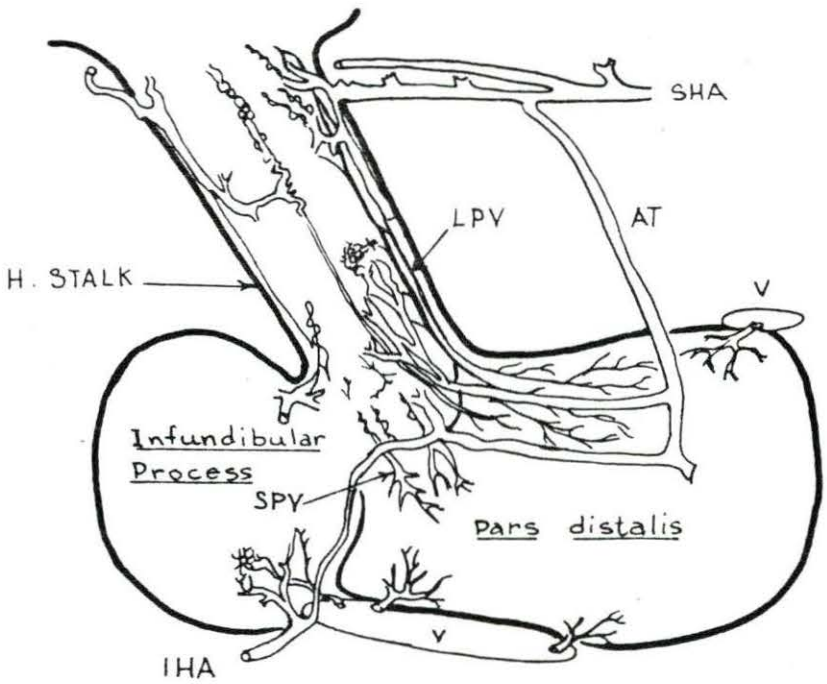

SHA
LPV
AT
H. STALK
V
Infundibular
Process
SPV
Pars distalis
V
IHA

Figure 4. Divisions of the pituitary gland and relationship with the hypothalamus (Netter, 1965)

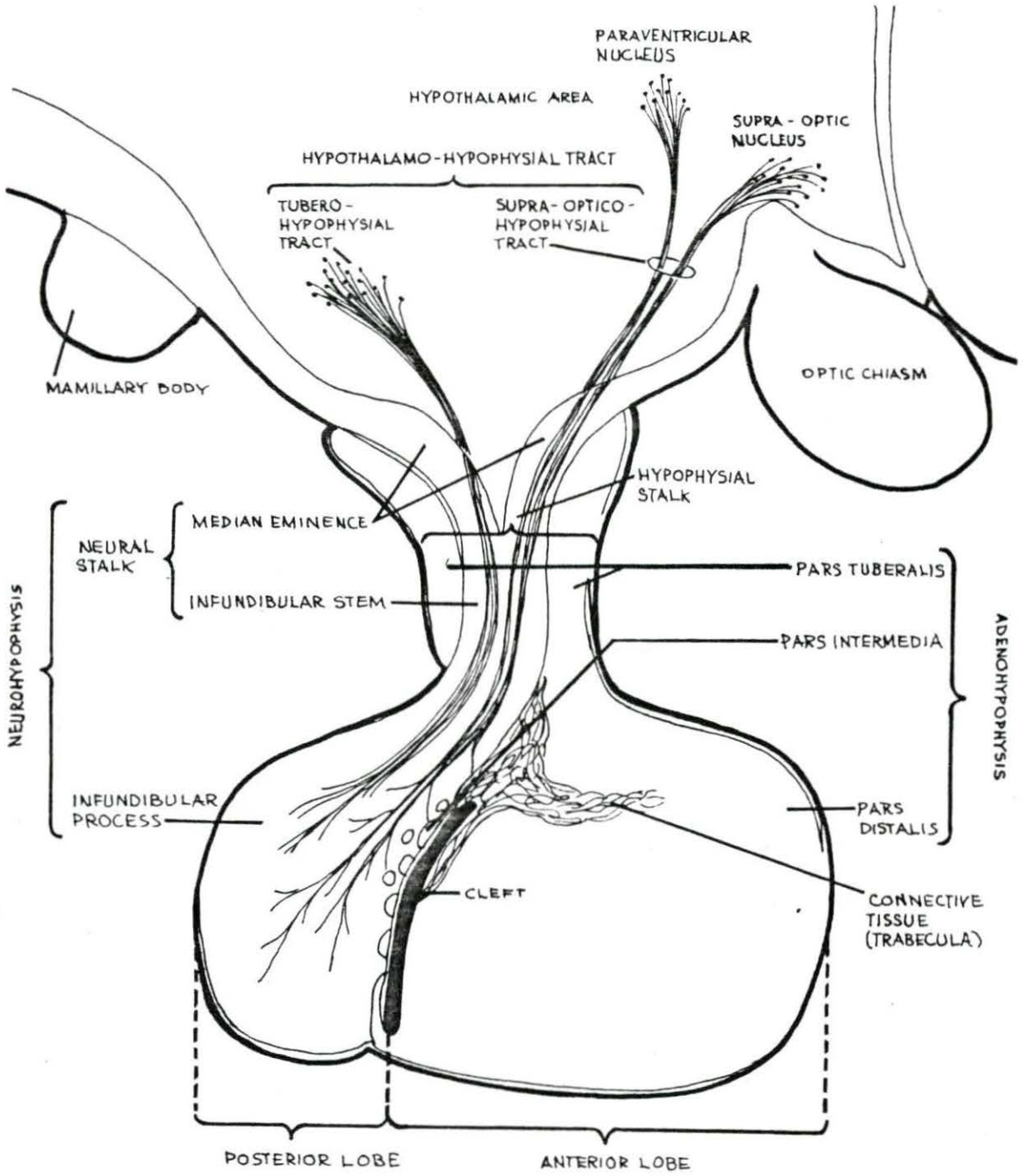

PARAVENTRICULAR NUCLEUS
HYPOTHALAMIC AREA
SUPRA - OPTIC NUCLEUS
HYPOTHALAMO-HYPOPHYSIAL TRACT
TUBERO-HYPOPHYSIAL TRACT
SUPRA-OPTICO-HYPOPHYSIAL TRACT
MAMILLARY BODY
OPTIC CHIASM
HYPOPHYSIAL STALK
NEURAL STALK
MEDIAN EMINENCE
INFUNDIBULAR STEM
PARS TUBERALIS
PARS INTERMEDIA
NEUROHYPOPHYSIS
ADENOHYPOPHYSIS
INFUNDIBULAR PROCESS
PARS DISTALIS
CLEFT
CONNECTIVE TISSUE (TRABECULA)
POSTERIOR LOBE
ANTERIOR LOBE

Figure 5. Blood supply of the pituitary gland (Netter, 1965)

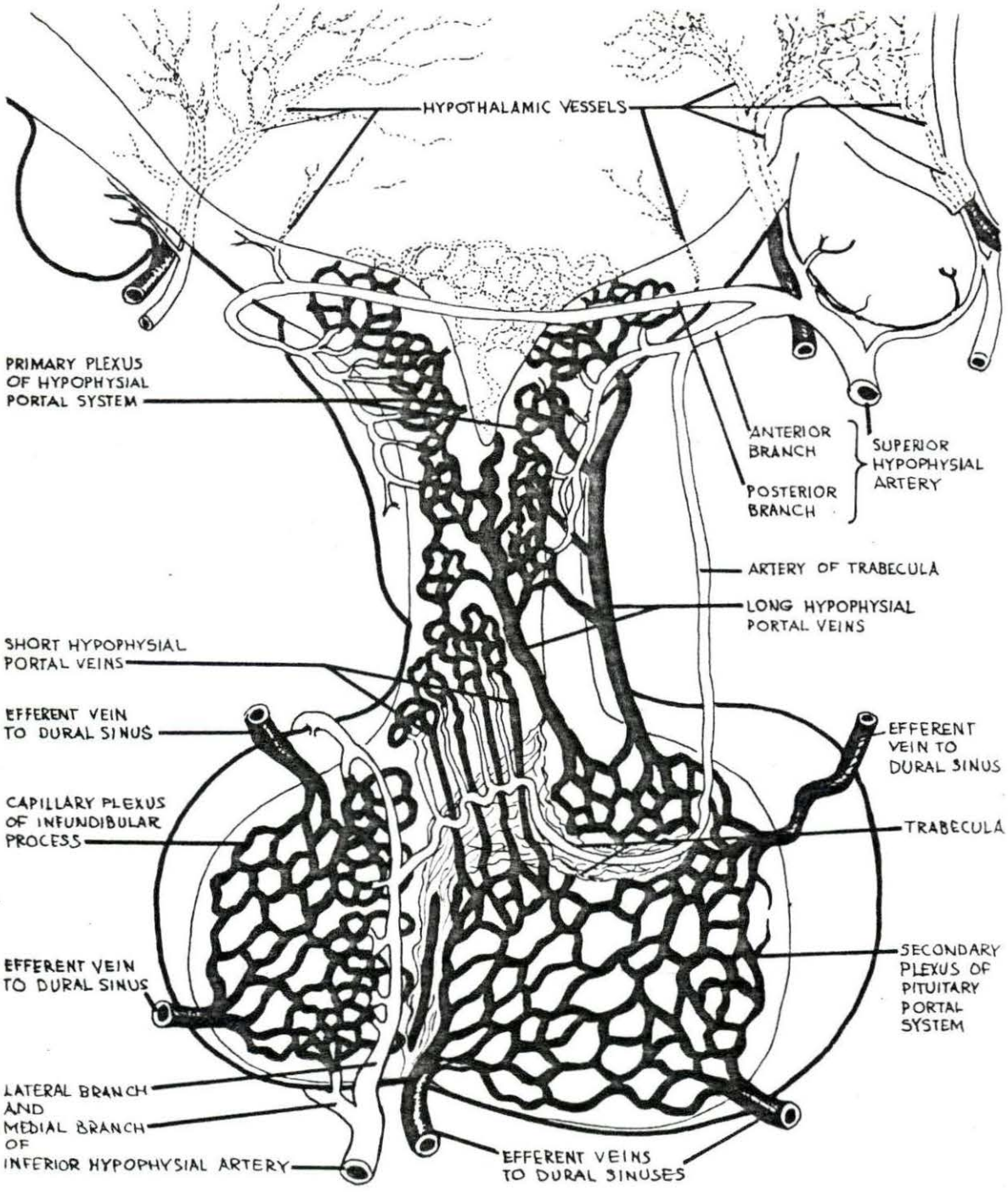

HYPOTHALAMIC VESSELS
PRIMARY PLEXUS OF HYPOPHYSIAL PORTAL SYSTEM
ANTERIOR BRANCH
POSTERIOR BRANCH
SUPERIOR HYPOPHYSIAL ARTERY
ARTERY OF TRABECULA
LONG HYPOPHYSIAL PORTAL VEINS
SHORT HYPOPHYSIAL PORTAL VEINS
EFFERENT VEIN TO DURAL SINUS
EFFERENT VEIN TO DURAL SINUS
CAPILLARY PLEXUS OF INFUNDIBULAR PROCESS
TRABECULA
EFFERENT VEIN TO DURAL SINUS
SECONDARY PLEXUS OF PITUITARY PORTAL SYSTEM
LATERAL BRANCH AND MEDIAL BRANCH OF INFERIOR HYPOPHYSIAL ARTERY
EFFERENT VEINS TO DURAL SINUSES

Figure 6. Hypothalamic structures (Netter, 1958b)

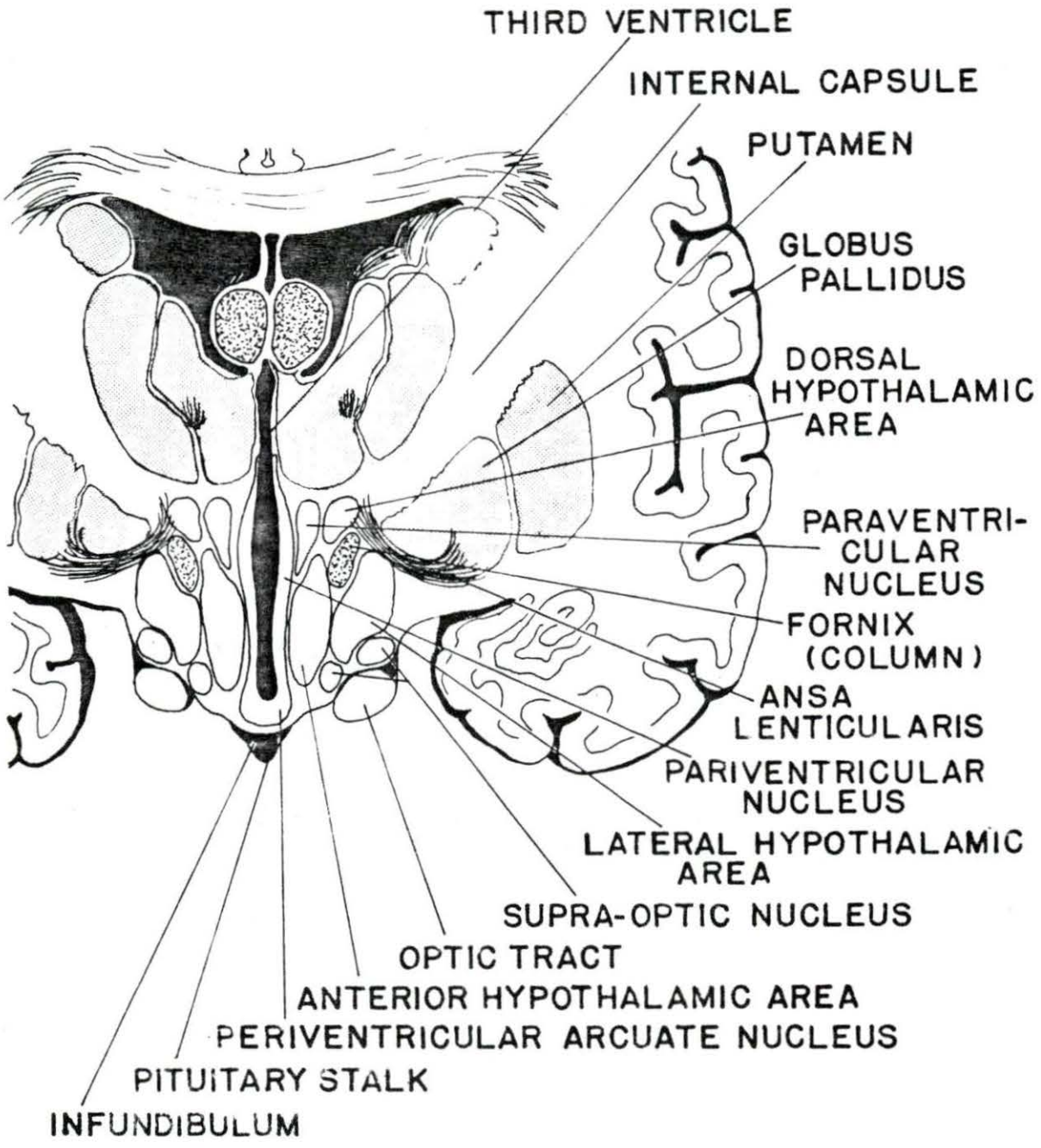

THIRD VENTRICLE
INTERNAL CAPSULE
PUTAMEN
GLOBUS PALLIDUS
DORSAL HYPOTHALAMIC AREA
PARAVENTRI-CULAR NUCLEUS
FORNIX (COLUMN)
ANSA LENTICULARIS
PARIVENTRICULAR NUCLEUS
LATERAL HYPOTHALAMIC AREA
SUPRA-OPTIC NUCLEUS
OPTIC TRACT
ANTERIOR HYPOTHALAMIC AREA
PERIVENTRICULAR ARCUATE NUCLEUS
PITUITARY STALK
INFUNDIBULUM

Figure 7. Pituitary gland structures in sow No. 10

Upper right, pars nervosa, watch for N.S.S.

Central part, pars intermedia, see the colloid droplets.

Lower part, pars distalis.

Stained with performic acid-Alcian blue-PAS-orange G method. 100X

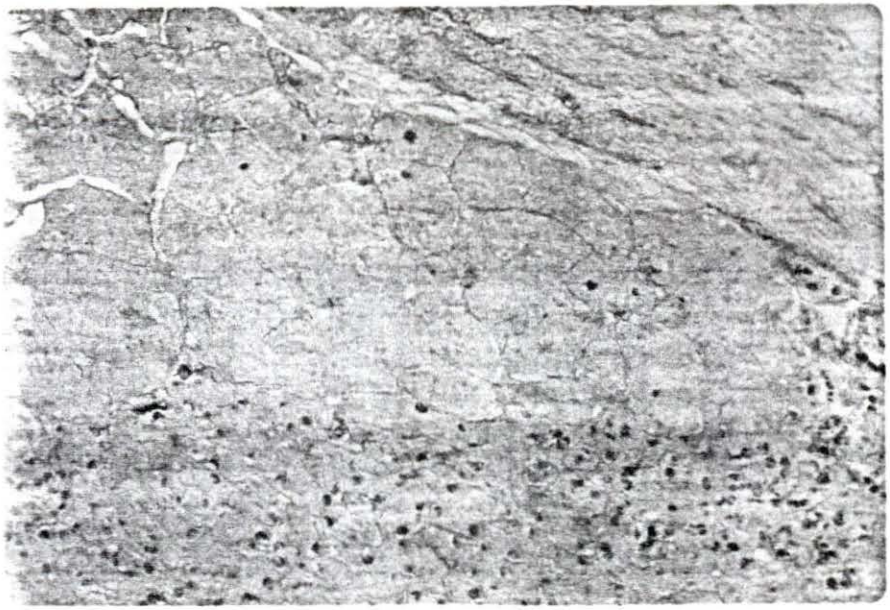
Figure 8. Region of pars tuberalis in sow No. 10

The pars tuberalis may be observed in the upper right corner.

The pars nervosa is in the upper left corner.

The rest of the anterior lobe is in the lower part of the picture.

Stained with performic acid-Alcian blue-PAS-orange G method. 100X



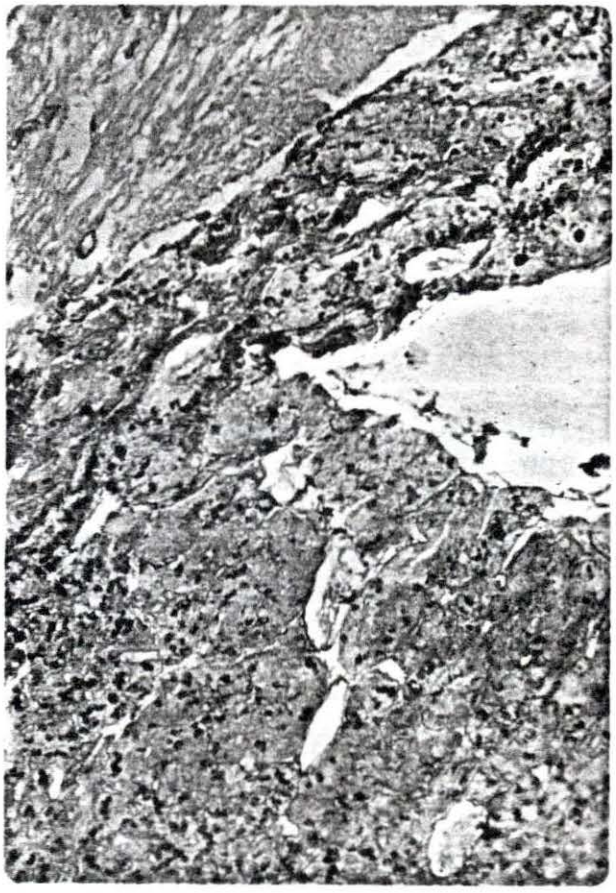
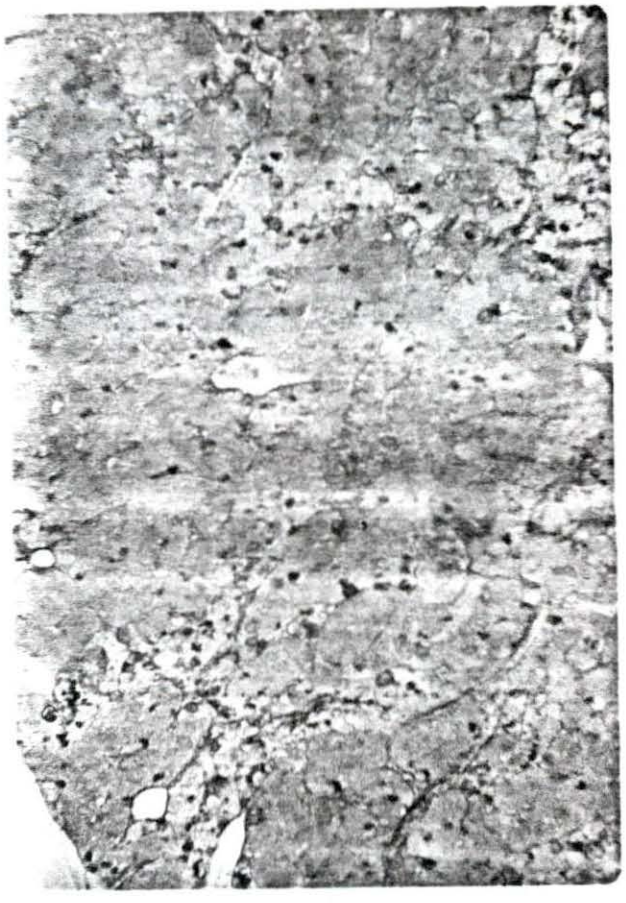


Figure 9. Anterior lobe of the pituitary in sow No. 10. Basophils and chromophobes occupy the peripheral and central part of the pars anterior. This is observed in the upper right corner of the picture. In the central part of the picture, big orange vacuolated cytoplasm cells (BOVCC) appear located in the acidophilic area. The lower left corner of the picture is occupied by the pars intermedia. Stained with performic acid-Alcian blue-PAS-orange G method. 100X

Figure 10. Big orange vacuolated cytoplasm cells (BOVCC) in sow No. 2. In the center of the picture a clump of BOVCC can be observed. These cells measured 18.5 x 22 microns on an eyepiece micrometer. Stained with performic acid-Alcian blue-PAS-orange G method. 950X



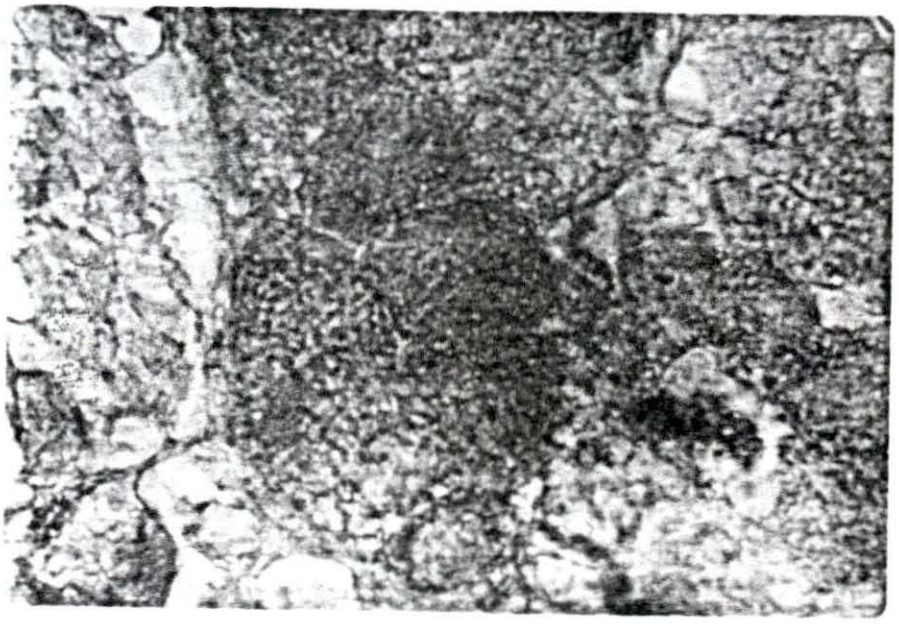


Figure 11. Variations in color observed in acidophilic cells in sow No. 10. In the upper left part of the picture is a very light yellow acidophil. In the lower right there is a dark reddish orange acidophil. These cells measured an average of 12 x 14 microns on an eyepiece micrometer. Stained with performic acid-Alcian blue-PAS-orange G method. 950X

Figure 12. Clumps of chromophobes in sow No. 21. In the central part of the picture a large clump of chromophobes may be observed. The staining is not definite but it is mainly the nucleus that can be observed. Notice how closely they are packed together. Compare the size of these cells with those illustrated previously. These cells measured an average of 8 x 8.5 microns on an eyepiece micrometer. Stained with performic acid-Alcian blue-PAS-orange G method. 950X

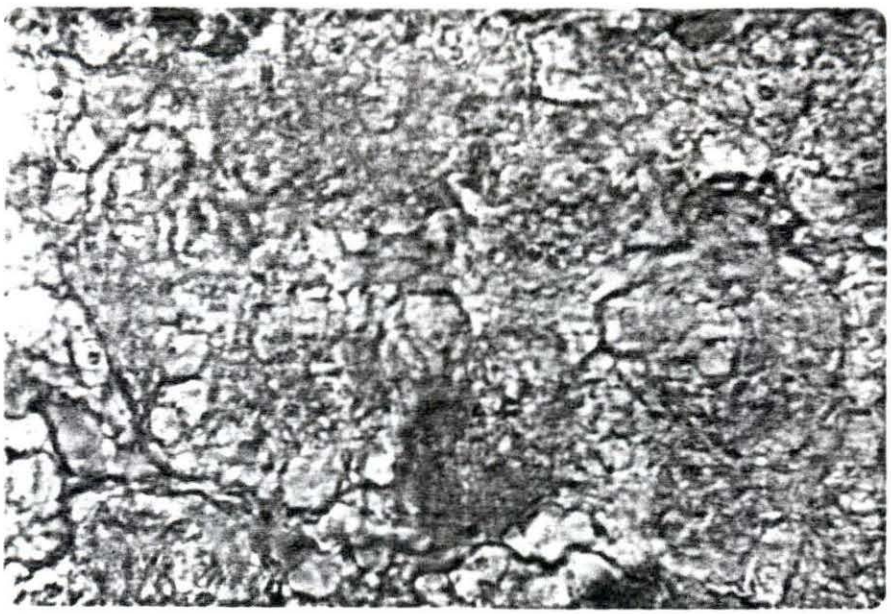
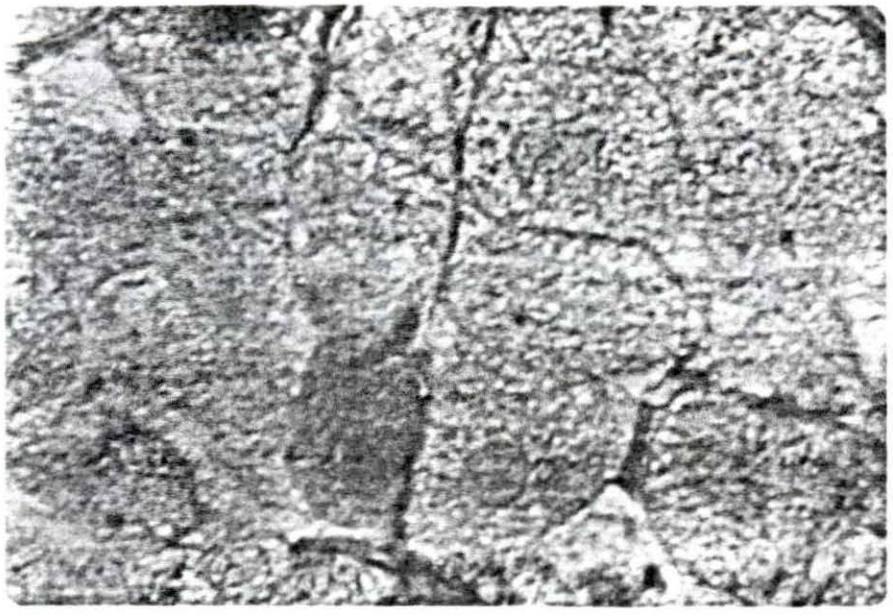


Figure 13. Various types and color staining was observed in basophils in sow No. 1. Upper center of picture shows big blue vacuolated cytoplasm basophil (15 x 21 microns). Right side of picture contains medium sized light purple vacuolated cytoplasm basophils (18 x 21 microns). Lower part of picture just to the left of center contains a medium sized deep purple vacuolated cytoplasm basophil (15.5 x 21 microns). Stained with performic acid-Alcian blue-PAS-orange G method. 950X

Figure 14. Variation in size and color of pituitary basophils in sow number 4. Upper center contains big purple vacuolated cytoplasm cells (BPVCC) that measured an average of 22.5 x 24 microns on an eyepiece micrometer. The left lower corner contains several small purple cells which measured an average of 13 x 19.5 microns on an eyepiece micrometer. Stained with performic acid-Alcian blue-PAS-orange G method. 950X



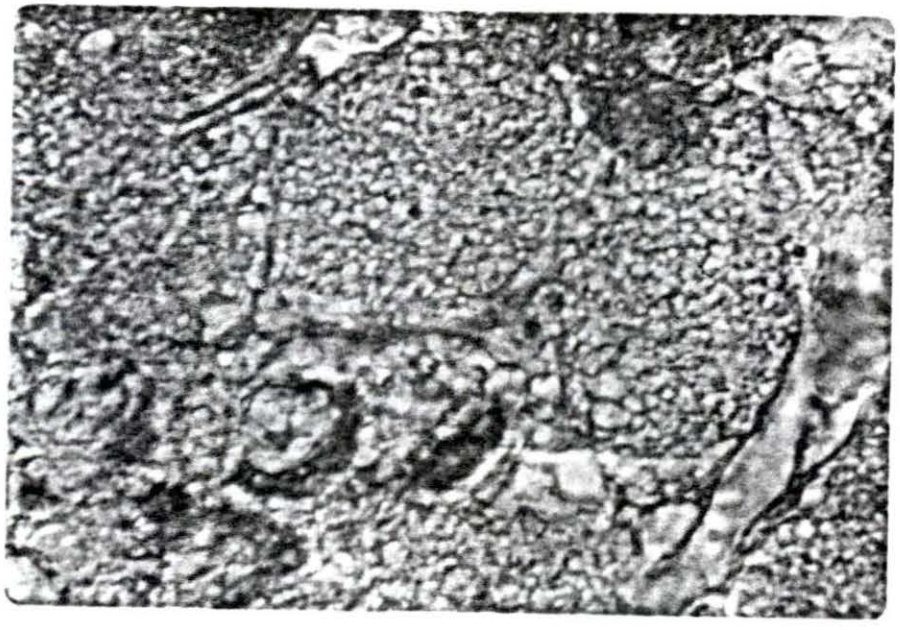
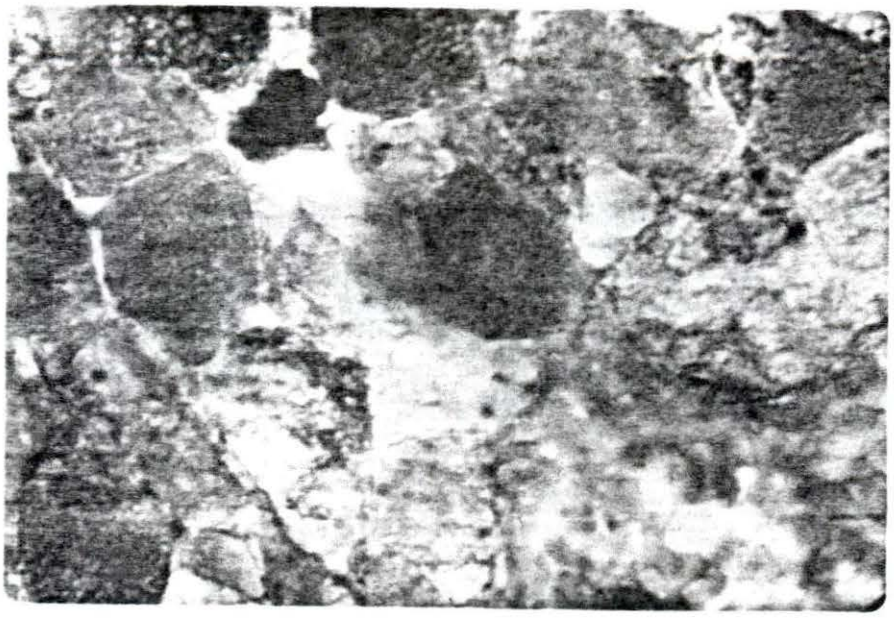


Figure 15. Big red vacuolated cytoplasm basophil in sow No. 19. Central part of picture contains a BRVCC that measured an average of 12.5 x 16 microns on an eyepiece micrometer. A small granule of colloid can be observed in the upper left. Stained with performic acid-Alcian blue-PAS-orange G method. 950X

Figure 16. Contrast between small deep blue stained basophil which measured an average of 13 x 19 microns on an eyepiece micrometer, and the big vacuolated cytoplasm basophil which measured an average of 15 x 21 microns on an eyepiece micrometer. At the upper right corner a small deep blue stained cell may be observed. The cells contained within the centrally located acinus are of the big blue vacuolated cytoplasm type. Stained with performic acid-Alcian blue-PAS-orange G method. 950X



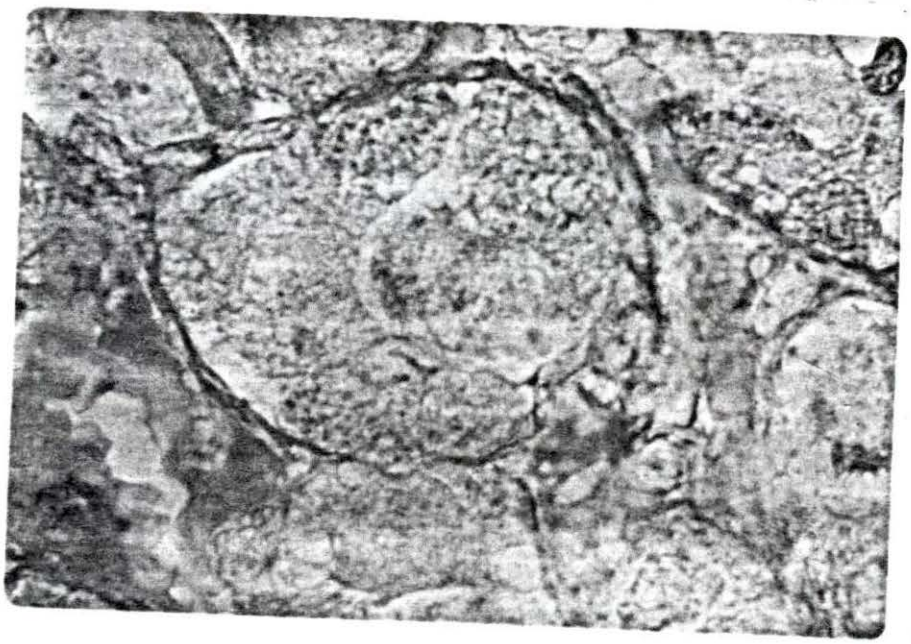
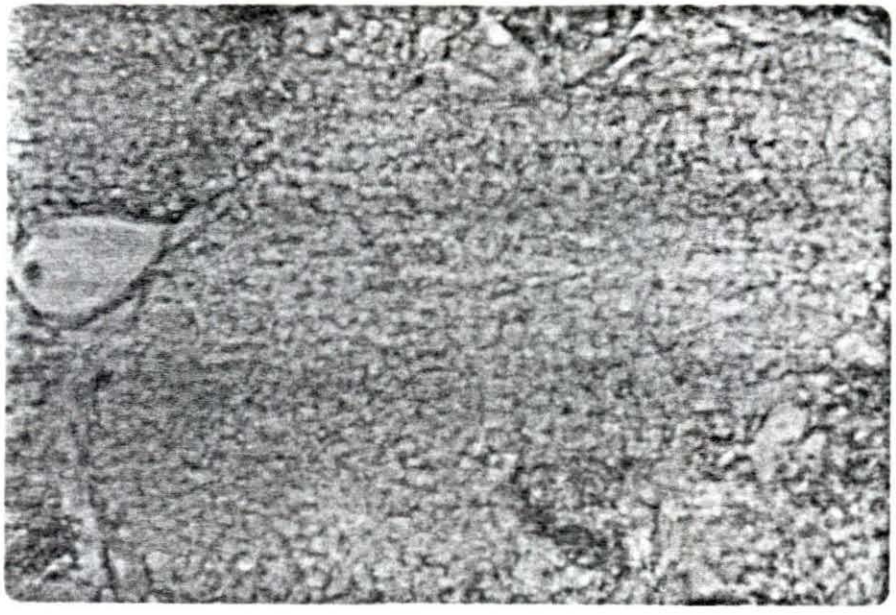


Figure 17. Big purple vacuolated cytoplasm basophil type in sow No. 4. Observe the degree of vacuolization of the cytoplasm of this cell type. The size is quite large. The cell membrane within each cell is not well shown. Stained with performic acid-Alcian blue-PAS-orange G method. 950X

Figure 18. Neurosecretory substance in close proximity to vessel in the pars nervosa in sow No. 1'. Note the dark blue staining material around this small blood vessel. Stained with Bargmann's chrome hematoxylin for neurosecretory substance (N.S.S.). 950X



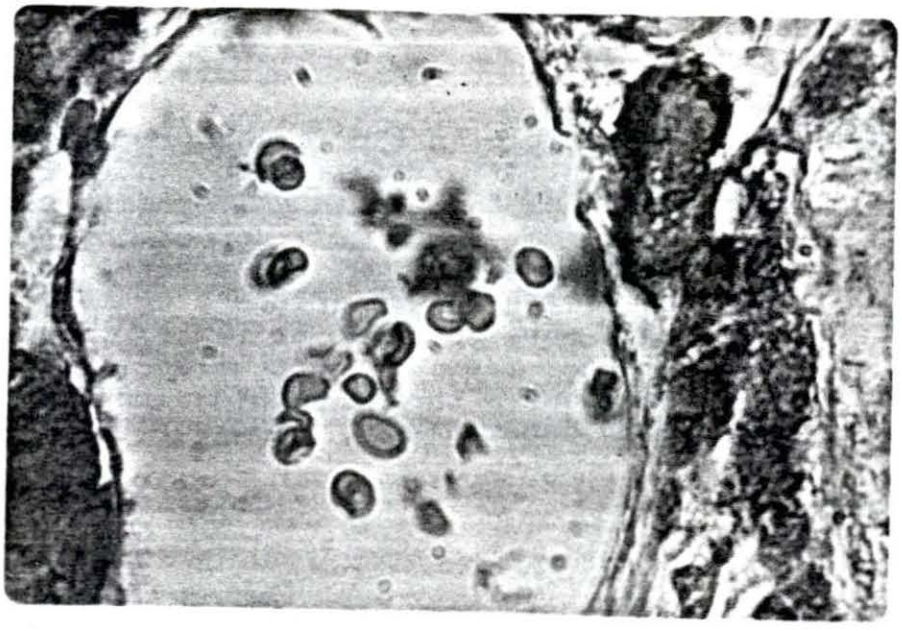


Figure 19. Network of axon fibers in the median eminence in sow No. 2'. Note the neurosecretory substance accumulations contained within the axon fibers. Some granules are larger than others. N.S.S. was observed in this location but not in the cell bodies of the supra-optic and para-ventricular nuclei. Stained with Bargmann's chrome hematoxylin for neuro-secretory substance. 950X.

Figure 20. Third ventricle in the region of the attachment of the pituitary stalk to the hypothalamus in the region of the median eminence in sow No. 5'. At the extreme upper left corner the third ventricle is shown. At the central part of the picture is the median eminence containing neuro-secretory substance and at the extreme upper right corner the pars tuberalis is seen. Stained with Bargmann's chrome hematoxylin for neuro-secretory substance. 100X