TISSUE CULTURE STUDIES OF THE PITUITARY

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A. CYTOLOGICAL STUDY

B. TRANSPLANTATION

C. PRODUCTION OF HORMONES IN VITRO

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1. INTRODUCTION

In regard to terminology, Hypophysis cerebri and Glandula pituitaria cerebri are used in this thesis without distinction. Basically, it is difficult to decide which term is preferable. The term hypophysis has been considered as the more correct since it denotes only an anatomical position. Even so, the word has its disadvantages, for it means "to grow under"; and since two of the three parts of the hypophysis cerebri grow upwards, its use is obviously inexact. No less inexact is the term pituitary, but it has been sanctioned by long usage and it has the flavour in it of the historical conceptions of the function of the glandula pituitaria cerebri. Cicero meant by pituita mucus, slime or phlegm. This conception was no doubt a hang-over from the days of Galen when the brain was thought to void mucus and excrementitious matter via the infundibular stalk and from there to filter down into the nose and upper pharynx. (As Hyrtle points out, the word sentina was used at times in place of pituita. Sentina was the term for the filthy water that collects in the bottom of ships - i.e. bilge water. This term was also applied to the lowest, most degraded of people, the rabble of the state.) The chief criticism, then, of the term pituitary is that it connotes a gland of external excretion. In their own ways, both terms have their faults; it becomes a matter of personal taste as to which is employed.

The following terminology will be adhered to in the ensuing pages; use of the synonyms referred to in this introduction will be avoided as far as possible.

I. Pars buccalis (derived from Rathke's pouch)

Pars anterior
Pars intermedia (synonym: juxta-neural epithelium)
Pars tuberalis
Parahypophysis

The cells making up the pars anterior are the chromophobe (synonym: Hauptzell), the acidophil (synonyms: eosinophil, cell with alpha granules) and the basophil (synonyms: cyanophil, cell with beta granules).

II. Pars neuralis (derived from the floor of the third ventricle)

Infundibulum (synonym: stalk)
Pars nervosa (synonym: neurohypophysis)

The space between the pars anterior and the posterior lobe will be referred to as the intraglandular cleft.

In the section on Transplantation, the anterior and posterior lobes are referred to. The former term is used synonymously with pars anterior; the latter term includes both pars intermedia and pars nervosa.

2. DEVELOPMENT OF THE PITUITARY

To Herring (1) is assigned much of the credit for working out the development of the pituitary. A brief resumé of his contribution follows: Rathke's pouch, seen at a very early age just in front of the bucco-pharyngeal septum, grows upwards to be met by another invagination from the neural ectoderm -- an invagination of what will afterwards become the floor of the third ventricle. As development proceeds, the upper half of the buccal invagination expands into a considerable cavity which retains its connection with the mucous membrane of the mouth by a small hollow stalk. The stalk presently becomes solid, thereby separating the communication between the buccal cavity and the pouch which evaginated from it. In exceptional cases, traces of the stalk persist - even in adult life - as detached strands of cells anywhere along the previous course of the stalk. Another vestigial structure, the parahypophysis, may be found between the two layers of the dura lining the floor of the sella immediately below the centre of the hypophysis.

With further development of the animal, there occurs an enlargement of Rathke's invagination with a considerable multiplication of cells of its anterior wall. In this way is formed the pars anterior. The posterior wall of the invagination thickens to a lesser degree. It becomes firmly adherent to and eventually in continuity with the pars nervosa; it forms the pars intermedia. What remains of the original cavity of Rathke's invagination becomes the intraglandular cleft.

The pars tuberalis is developed relatively late from two lateral secondary diverticula of the main pituitary vesicle; they ultimately fuse together in the mid-line around the infundibulum and spread over the under surface of the tuber cinereum. The cavities of these diverticula become obliterated and the cells of their walls form the substance of the pars tuberalis. These cells are often disposed in vesicles which contain a colloid material.

The pars nervosa is developed from the hollow process of neural ectoderm which comes to lie in contact with the posterior wall of the buccal invagination. At the point of contact, the two eventually come into complete continuity. In practically all animals the hollow neural process becomes obliterated.

The development of the pituitary of the rat has been described by Schwindt (2). The salient points are that at fifteen days the pars anterior cells break up into epithelial cords and masses; at fifteen days and seven hours vessels begin to grow into the pars anterior.

The pars intermedia begins to take shape at the end of twelve days and six hours.

The pars tuberalis appears at thirteen days and four hours of life as two thickenings of the epithelium just above the opening of Rathke's pouch. At thirteen days and twelve hours these lateral lobular structures look like buds just above where the pouch is being constricted off from the roof of the mouth. At fourteen days, they are larger, are connected across the median line by a low ridge and are separated from

the anterior lobe by a ridge filled with mesenchyme. At fifteen days, the lateral lobes have their expanded buds pressed against the floor of the diencephalon.

The pars neuralis makes its appearance at twelve days and six hours as a trigger-shaped process from the floor of the diencephalon. It then grows rapidly and at thirteen days and twelve hours its cells have become arranged in cords between which are capillaries and a small amount of connective tissue.

3. COMPARATIVE ANATOMY OF THE PITUITARY

In the adult amphioxus (De Beer, 3, 3a) the hypophysis is represented by the ciliated organ of Müller (a development from the walls of the preoral pit) whose chief function apparently is to create a current of water.

Later, phylogenetically, the organ sank beneath the surface, retaining its communication with the exterior by means of a duct. Still later, it became associated with the pars neuralis of the brain and communication with the exterior was obliterated.

In cyclostomes, Tilney (4) finds a definite pars anterior, the cells being arranged in parallel columns set perpendicularly to the base of the brain. The pars intermedia cells are arranged in convoluted cords and are attached to a small infundibular process. No intraglandular cleft is seen at this stage.

The nearest approach to anything resembling the posterior lobe has been found by Gentes (5) in the elasmobranch fishes. This 'posterior lobe' is, however, not constantly present in fishes. An interesting feature of the anterior lobe of the skate (raia batis) is that it possesses acini which have blood vessels in their lumina: here is strong evidence of a secretion passing directly into the blood stream. According to Herring (1) no chromophils are yet to be seen. In the teleost fish, the cod, Herring notes the very close connection of pars intermedia cells with those of the pars nervosa. This latter is a branching structure which is deeply penetrated by the chromophobe cells of the pars intermedia. Furthermore, the

intraglandular cleft is continuous with the third ventricle. The eel (anguilla vulgaris) shows an interesting stage in evolution in that the pars anterior is divided into a central mass made up of basophilic cells and two lateral masses composed of acidophilic cells (Tilney, 4); these latter are arranged in columns with intervening blood spaces. This is perhaps the lowest stage in which basophils appear.

In amphibia the pars nervosa is characterized by its relative smallness; it is a thickened indentation of the wall of the pars neuralis abutting upon the pars anterior (Gentes, 5). Its blood supply is abundant. The pars anterior is made up entirely of acidophils which are closely related to its internal blood supply (Tilney, 4).

In the reptiles the pituitary is fairly similar to that in amphibia. In the alligator mississippiens, Tilney (4) notes that the acidophils are centrally placed in the anterior lobe whereas the basophils occupy the periphery. The intraglandular cleft is usually present.

In the Gallus domesticus the anterior lobe is lined on its intraglandular cleft aspect by basophils. The remainder of this lobe is composed of acidophils (Tilney, 4). Some of these latter stain lightly, others darkly. Bell (6) remarks that what most investigators would consider chromophobes in gallus domesticus have been regarded by Tilney as "basophil". The foregoing must then be considered in that light. In the fowl, the poorly formed pars intermedia is present for the most part about the neck of the gland. The pars nervosa is

convoluted and hollow and its cavity opens into the third ventricle.

In the lowest stage of mammals (monotremes) a prominent interdigitation of pars intermedia and pars nervosa cells is to be seen. In fact, Bell (6) points out that the cells of the pars intermedia of the ornithorhynchus entirely surround the pars nervosa in a thick layer; and from this capsule of intermedia cells columns of cells pass deeply down and even completely through the pars nervosa. Varying degrees of this same phenomenon appear in the bony fishes and in the reptiles. This feature of pars intermedia invasion of the pars nervosa apparently disappears in higher stages of evolution except in the cat and lemur. It may be said, in general, that the pars intermedia decreases in size as the evolutionary stage advances so much so that in monkeys and man it is considered (Plant, 7) a rudimentary organ. Rasmussen (8) points out that the human pars intermedia represents a little more than 1% of the total epithelial portion of the gland, while in the rat it represents 5 to 6%, and in frogs 25%. The pars intermedia is entirely absent in the porpoise and in some birds.

Apparently the only mammals possessing a communication between the cavity of the pars nervosa and the third ventricle are the opossum, the dog and the cat.

Of mammals, the cat and the dog possess the greatest abundance of pars intermedia cells. In the lower animals, such as the dog, cat and ox (Bucy, 9), the pars intermedia cells take on a definite alveolar structure. It is thought that many of the alveoli in the intermedia are incomplete due to

the small amount of supporting connective tissue in that part of the gland. In the cat the pars intermedia cells completely surround the pars nervosa and the neck (pars tuberalis ? W.H.). and in some places the cells form wedge-shaped masses projecting inwards (Herring, 1). In the ox, the pars intermedia surrounds the pars nervosa in a layer many cells thick. There is no dipping down of these cells into the pars nervosa. In the monkey (macacus rhesus), according to Herring (1), the pars intermedia forms an investment varying in depth; it is usually thin over the anterior and lateral aspects of the pars neuralis. These cells have been described as having either neutrophilic or lightly basophilic affinities.

In general, it may be said that the anterior lobe of the mammal contains both acidophil and basophil cells. Bell describes them in the monotremes, Tilney in the opossum, sheep and rabbit, Herring in the cat and dog, and Bell in the hedgehog. Neither Herring nor Tilney were able to find basophils in the anterior lobe of the macacus rhesus. The chromophobe is present throughout the mammalian series.

One of the most striking impressions one gets in pituitary development, according to Hogben and De Beer (10), is the identity of the pars tuberalis. This component, first recognized by Tilney (11) in 1913, is present in all classes of land animals with the possible exception of some lizards and snakes. An invariable feature in the development of the pars tuberalis is its paired origin (Atwell, 12). In the rabbit, from the thickened epithelium which lies in front of the early

Rathke's pouch, two thickened ridges are formed. These are the anlagen which fuse and form the pars tuberalis -- a thin lamina surrounding the infundibular neck and spreading out under the tuber cinereum. In rana pipiens (Atwell, 13) the two thickened ridges develop into two small bodies lying in the pia in close relation to the brain. It does not appear to invade the neural tissue after the manner so characteristic of the pars intermedia. In reptiles, according to Baumgartner (14), thin bands of pars tuberalis tissue are formed about the periphery of the anterior lobe. It appears to be an accessory of the thin plate of pars tuberalis tissue spread out under the brain floor. The cells of the pars tuberalis are, in most mammals, of acinar arrangement, the acini containing colloid.

Trautmann (15) states, in reference to the intraglandular cleft, that it is present in the goat, hog, dog and cat and in sheep and cattle.

4. PARS ANTERIOR OF THE PITUITARY

(a) Cytology.

Hannover (16) in 1844 first called attention to the occurrence of more than one type of cell in the pars anterior in frog and Man. In 1881 Flesch (17), and independently of him Dostojewsky (18) named these two types of cells chromophobes and chromophils. To Lothringer (19) is due the credit of showing the staining affinities of these cells. Schönemann (20) pointed out in 1892 that chromophils are divided into two types, namely, acidophils and basophils. These three types of cells, whose staining characteristics depend upon the nature of their differing granules, remain today the well-established cell types of the normal gland. It is generally thought that none of these cell types contains more than one type of granule.

In Nukariya's excellent review (21) is recorded the numerical incidence in the anterior lobe of these three types of cells. In male rats ranging from five weeks to eighteen months, the chromophobes represent 54.5%, the acidophils, 31.4% and the basophils, 14.1% of the cellular content. In female rats the figures are very similar: chromophobes, 52.9%, acidophils, 31.6% and basophils, 15.5%. Rasmussen's (22, 23) figures are essentially the same for the human pituitary. In the male, chromophobes are present in the relative proportion of 52%, acidophils, 37% and basophils, 11%. The figures vary somewhat in the non-pregnant female -- chromophobes, 49.5%, acidophils, 43.5% and basophils, 7%.

Addison (24) states that in the newborn rat most of the cells are of the primitive undifferentiated type (he appears

to be the only writer who does not call them 'chromophobes' at this stage). From these cells, chromophobes develop and are to be seen scattered through the gland in the form of cords. The acidophils and basophils, derived from the primitive, undifferentiated cells, come to lie upon or near the margin of the blood stream.

Nukariya points out the well-accepted regional distribution of the various cells in the pars anterior of the rat. Chromophobes are found in groups quite regularly distributed throughout the gland. Acidophils are most plentiful in the central portions of the gland and adjacent to the intraglandular cleft. Basophils have a more peripheral distribution and are especially numerous at the lateral junctions of the pars intermedia and pars anterior.

Severinghaus (25) describes in excellent detail the three types of pars anterior cells. The chromophobes, when stained with hematoxylin and eosin, are neither acidophilic nor basophilic. The slightly chromatic nucleus with one or two intensely staining nucleoli is surrounded by a slender margin of cytoplasm. The clear cytoplasm, devoid of granules except for a few granular mitochondria, takes only a faint basophilic stain even after the rest of the tissue is much overstained. Chromophobes with increased amounts of cytoplasm are also to be found: these not infrequently show an indistinct granular cytoplasm. Such cells are interpreted as illustrating a phase of the transition from chromophobe and chromophil. The Golgi apparatus of the chromophobe is either a narrow and elongated network closely applied to one side of the nucleus or a compact spherical mass lying free in the cytoplasm.

The acidophils constitute the most conspicuous cell type of the anterior pituitary. In the rat the largest acidophils are about 10 μ in greatest dimension. They are often round or ovate, but not infrequently angular or elongated, especially when lying grouped along a blood capillary. The nucleus is central with a prominent acidophilic nucleolus and usually a second nucleolus of basophilic character. The granular cytoplasm may appear homogenous and non-granular after certain fixatives or when heavily stained. The mitochondria are masked by acidophilic granules unless especially differentiated. Under these conditions they stand out as large brilliant fuchsin granules among the finer orange-red granules of the cytoplasm. They are never abundant and are polarized in the meshwork of the Golgi. The Golgi apparatus consists of a basket-like network closely applied to one side of the nucleus. It is typically restricted as a cap to one side of the nucleus. The Golgi is almost invariably polarized away from the capillary.

The basophils, which are the largest of the anterior pituitary cells, sometimes measure as much as 25 μ in diameter. They are round or polyhedral depending upon how compactly they are massed together. The nucleus is always markedly excentric and its ground substance always stains palely basophilic. The nucleus differs little from that previously described in the chromophobes and acidophils. The cytoplasm shows a wide variation in structure. It may be either finely granular or have granules of a coarse, irregular or flocculent type: at times granules are entirely absent. When that is the case, one sees

a vacuolization of the cytoplasm which gives the cell an alveolar appearance. Such alveolar structure is not to be confused with vacuolization in the formation of the so-called 'castration cells'. The Golgi of the rat's basophil is a constant cytoplasmic structure which definitely identifies it regardless of cytoplasmic variations. It is somewhat spherical in shape and lies in the cytoplasm separated from the eccentrically-placed nucleus. Both in form and position it is sharply contrasted with the acidophilic Golgi. In the smaller basophils the Golgi appears as a hollow sphere with one or two invaginations. (Cushing (26) cites the vital staining of pituitary adenomas by Eisenhardt as confirmatory evidence of Severinghaus' Golgi studies. Eisenhardt was able to identify the circular spot which represents the position of the Golgi which had formerly been looked upon as a vacuolated area.) The Golgi body increases in size with the enlargement of the cell and this expansion frequently results in the separation of the wall into a system of anastomosing strands giving to it the appearance of a fenestrated membrane. One or more clear vesicles are normally associated with the Golgi, and in negative image preparations, one gets the impression that in the walls of the sphere are canals opening into these vesicles. The mitochondria are identical in appearance with those of the other cell types. They appear as brilliant fuchsin granules which vary in size from minute granules to spheres as large as the nucleoli. The smaller mitochondria are frequently massed about the Golgi region which often appears as a centre from which they radiate into the cytoplasm. Mitochondria are much

more numerous in basophils than in acidophils. There is no evidence at present in Severinghaus' studies to show what part, if any, mitochondria play in the actual elaboration of the cell's secretion.

(b) Relationship between the Various Cell Types in the Pars Anterior.

Ever since the chromophobes, acidophils and basophils were first described, the relationship each bears to the other has been a matter of continual disagreement. Attempts have been made to correlate function with a specific cell type, as, for example, growth with the acidophil. What is the source of these various cells? Are these three distinct types of cells each a morphologic and functional unit unto itself, or do they represent different stages in activity of the same cell type?

The second alternative seems offhand to be the more logical when based upon observations of mitotic activity in the adult gland. Even when the pituitary is undergoing marked cellular changes in response to a physiological demand, mitotic figures are rarely seen. Jackson (27) points out that in the new-born rat mitoses are very numerous, the average being sixty-two in each section of the pars anterior. Mitotic activity decreases markedly as the rat grows older -- eighteen at one week of age, seven at three weeks, and two at ten weeks. In the adult, mitoses are very rare. This observation is suggestive, as Severinghaus (25) points out, of some interchange between the three types of cells.

The earlier investigators (Flesch, 17; Lothringer, 19; and Schönemann, 20) regarded these three pars anterior cells as distinct types. Saint Remy (28) was the first to express the opinion that these cells are various stages of a single cell type in which the mother cell is the acidophil. He found that by staining his sections by Altmann's method acidophilic granules were present in all cells. Bailey (29) has commented that no doubt Saint Remy's acidophilic granules were nothing more than mitochondria. Saint Remy expressed the opinion that through loss of granules the acidophil became a chromophobe. Basophilic cells were thought to be derived from acidophils because of the presence in them of acidophilic granules. (See Figure 1 (page 17) for illustration of the various conceptions of anterior cell relationships.)

Benda (30), too, thought the cells were different forms or functional stages of the same cell, the mother cell being the chromophobe. This cell gradually acquired more and more acidophilic granules until it became a frank acidophil. He describes all transition stages between chromophobes and acidophils and between acidophils and basophils. The end stage is the large basophil cell which Schönemann had named the cyanophil. Because of the vacuoles it possessed, Benda suspected that it was a degenerating form of cell.

The unitarian view of Collin (31, 32) has recently received a great deal of attention. On purely anatomical grounds he presents evidence that the chromophobe (the mother cell) represents the primitive stage of activity. In the process of development, the chromophobe acquires an

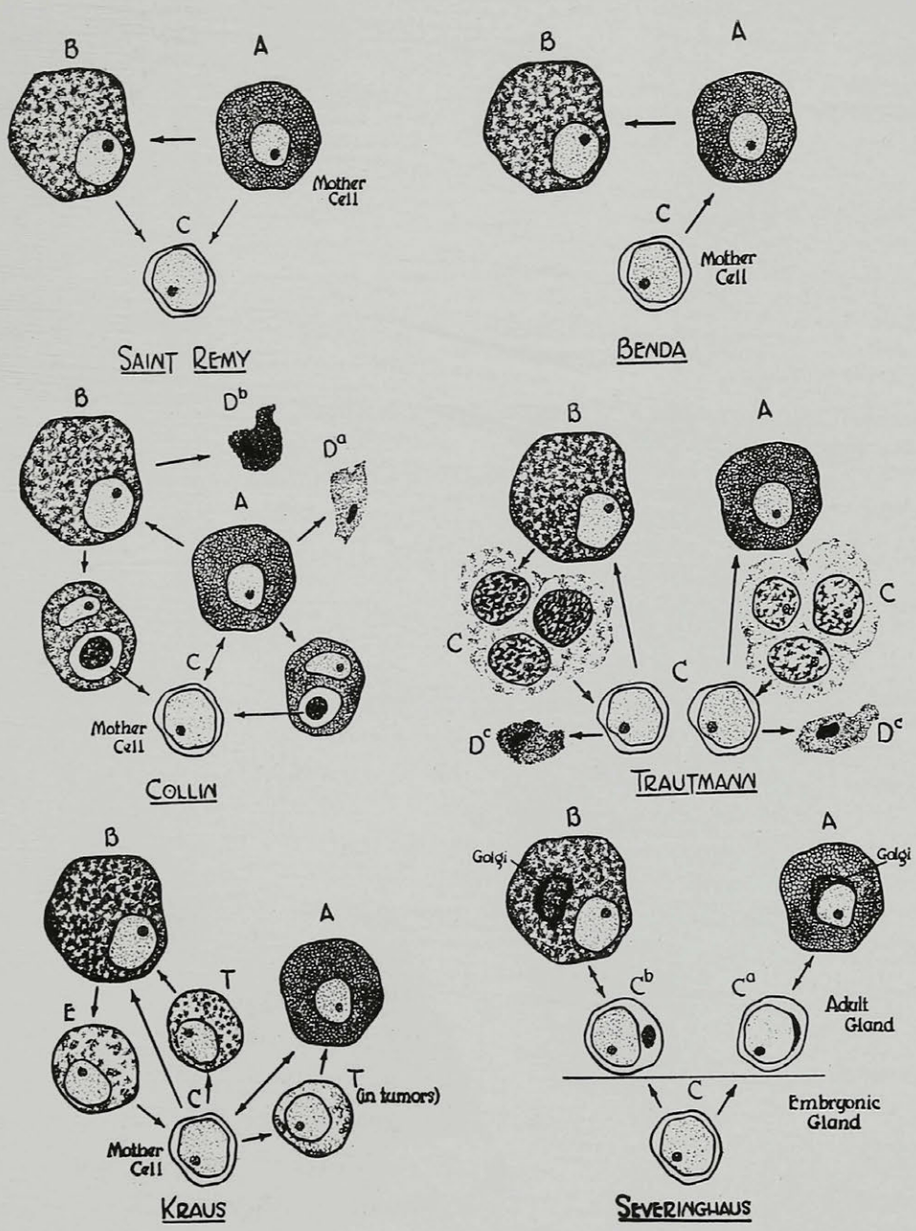

Fig. 3 Diagrams to illustrate various ideas of relationships among the cells of the anterior pituitary.

Figure Ia Photograph from Severinghaus (25).

acidophilic granular cytoplasm which may in turn become basophilic. The acidophil, which represents the secretory stage, may discharge its granular cytoplasm and degenerate, or it may, by this cytoplasmic loss, become a chromophobe and repeat the cycle. The same applies to the basophil. Both cells, upon discharging their granules, are transformed into free nuclei with a scarcely visible cell membrane: these nuclei multiply by amitosis so that here and there large clumps of them may be seen. They ultimately return to the chromophobe cell stage. Another, and a most unusual, process of cell multiplication is described by Collin as follows: The nucleus of the pituitary cell divides into two secondary nuclei, slightly unequal in size, so that the cell becomes binucleated. About one of the nuclei there develops a thin, almost transparent cytoplasmic zone sharply defined from the surrounding cytoplasm and limited by a membrane. It increases in size in the cytoplasm of the host and soon it herniates and separates en masse from the host cell (which goes on to degeneration). In this way a new chromophobe is formed, a cell which differs in no way from the chromophobe developing from the "free nuclei". He names this process endocellular cytogenesis.

Severinghaus (25) casts doubt on the conception of Collin that both acidophils and basophils may give rise by endocytic budding to new chromophobes. He thinks that Collin's endogenous cells are, in fact, other cells which are superimposed upon the pituitary cell. He has found frequently in the monkey a chromophil cupped upon a chromophobe and only a careful study of serial sections revealed that the apparently

enclosed cell was actually superimposed.

Two of the chief advocates of the opinion that basophils and acidophils are distinct types were Erdheim (34) and Trautmann (15). Trautmann was opposed to the ideatthat there was any direct interchange or transition between basophils and acidophils. He felt that the basophil, upon discharging its secretory granules, in a few cases degenerates; in other cases it goes on to form another basophil; and in still other cases, again becomes a chromophobe. Acidophils, too, pass through a series of stages. Neither of these investigators could distinguish one chromophil from another after they had lost their granules.

The next theory to be given consideration is that of Kraus (35). He describes granular and non-granular cells. The granular cells include basophils, acidophils and pregnancy cells. His non-granular cells include the chromophobe, the transitional cell (Uebergangzell) present in the pars intermedia, and, lastly, the degranulated cell (chromophils from which granules have been discharged). According to Kraus, chromophobe cells give rise directly to acidophils. In the case of basophils, chromophobes give rise to them through an intermediate form. The chromophils, through a reverse process, again become chromophobes. Kraus does not know for how many generations this reversal process may occur. He goes on to apply his theory to adenomataof the hypophysis and arrives at the classification which follows: typical and atypical chromophobic adenomata and transitional, acidophilic, basophilic and

pregnancy cell adenomata. The presence of pure adenomas, he feels, is good evidence that the cells of the pituitary are distinct types. Bailey (36) finds fault with Kraus' use of the term 'transitional' since one is apt to interpret it as the stage between acidophils and basophils, a stage which Kraus was never able to establish.

The last theory to be discussed is that which Severinghaus (25) proposes -- a theory which he bases on his study of differences in the Golgi present in the various cell types. He views the chromophobes as the progenitors of the granular cells. Certain cells are destined to become acidophils, others basophils. This writer believes, from studies of pituitaries of castrated rats, that chromophils revert back into chromophobes. He has, furthermore, ably demonstrated, on the basis of the Golgi, that chromophobes may be divided into two types, those with acidophilic Golgi and those with basophilic Golgi. He comes to the conclusion that already in the chromophobe stage certain cells are destined to become acidophils, others basophils. "It seems definitely established in the rat that direct transitions between the basophil and the acidophil do not take place, for it is unlikely that a complete change in the morphology of so definite a cytoplasmic component as the Golgi apparatus would occur."

Study of pituitary adenomata has contributed little in determining relationship of cell types. Cushing (26) states that in the chromophobe adenomata(a tumor composed of ontogenetically young cells) there is seldom any mitosis;

in the acidophilic and basophilic adenomata, on the other hand, there are numerous mitotic figures. Cushing refers to an adenoma of mixed cellular type, predominantly chromophobe, in some chromophobes of which is to be seen a peripheral disposition of acidophilic granules "suggesting the functional retrogression of previously mature acidophil elements; and since these cells resemble the hypo-acidophil stage of development as described by Collin, the observation might be construed as an argument favouring his views".

(c) Modus Operandi of Secretion of the Anterior Lobe Cells and Discharge into the Circulation.

Bailey (36) has pointed out that the pars anterior cells lining vascular channels are larger, are more or less columnar-shaped and are usually filled with granules, whereas those cells at a distance from vessels are smaller, polygonal and more or less free of granules. In the pars anterior of the adult, one often finds cords composed of small, undifferentiated, non-granular cells. About such regions are to be found areas of degeneration filled with a mixture of hyaline- and colloid-like bodies mixed with a débris of degenerated cells. Bailey has further pointed out that a number of intracellular structures have been regarded as secretion products of the cells of the pars anterior. They are, namely, acidophilic granules, basophilic granules, various hyaline and lipoid collections and the lipoids.

The granule theory: From cytological observations, numerous investigators, among them Collin (32,33), have noted a thinning out of the granules of the basophils and acidophils: this they interpreted as extrusion through the cell membrane of secretory products. Analogy can easily be drawn between this and other secretory glands. Cushing (26) points out that histologists have shown that the various secretory cells discharge in different ways. They may (I) discharge their granules without any particular change in form, as in gastric secretion; they may (II) wholly disgorge their ripened cytoplasm, as in mammary secretion; or (III) the entire cell may be cast off, as in sebacious secretion -- and this latter is apparently what takes place with some of the pars intermedia cells.

Urasov (37) has attempted to correlate, in the basophil, secretion with mitochondrial changes. He finds that mitochondria increase during growth of the cell and subsequently decrease in number during the late secretory phase of the cell. He therefore concludes that the secretion is elaborated at the expense of the mitochondria under the influence of the Golgi apparatus. Severinghaus (25) has found in the basophils of the castrate, a cell presumably in a phase of increased activity, that mitochondria actually appear diminished in number. He therefore cannot agree with Urasov's conclusion. Nor can he come to any definite conclusion as to the rôle the Golgi plays in secretion in the basophil ..... "the compact, finely granular character of the cytoplasm within the confines of the Golgi body, in contrast to the remaining cytoplasm, might be looked upon as indicating that this region is the site of granular as

well as mitochondrial elaboration and growth, and in this respect might be suspected of having a relation to the elaboration of secretory product". Since the largest cells appear to correspond to stages of highest secretory activity, and the Golgi increases proportionately, he comes to the conclusion that the Golgi increase is related to the heightened secretory activity.

It has been found by Urasov (37) in the mouse pituitary that in the acidophils which border a capillary, the Golgi is polarized toward the capillary lumen. He joins with many others in pointing to this position as indicating that the Golgi is concerned with the discharge of secretory products from the cell into the capillary. This, however, does not apply in the rat, according to Severinghaus (25). He finds, on the contrary, that the Golgi is almost invariably polarized away from the capillary. He further points out that polarization of the Golgi, as an indication of the portal of secretion, is losing significance in light of the work showing that if the portal of excretion is reversed, as it is in pituitary implants, the Golgi is not altered from its original position.

The colloid and hyaline theory: (discussed in relationship with Physiology of the Posterior Lobe).

The lipoid theory: Launois (38) proposed the theory that one of the secretory products of the hypophysis is a lipoid substance which enters the blood stream where it is taken up by leucocytes and spread about the body. This lipoid substance is present in all the cells of the pituitary, particularly the chromophobes (Caselli 39; Kraus, 35).

The Degeneration Theory: In the histological picture of the hypophysis one encounters many cells which appear to be in a state of degeneration. On the subject of cell destruction and secretion, Stendell (40) states that the two go hand in hand in the hypophysis and cannot be separated from one another.

In the pars tuberalis, Atwell (41) has shown that colloid first appears as small droplets in the central ends of the groups of cells comprising the cords. The intracellular vesicles coalesce and the colloid collection is discharged into a common lumen. This cannot be regarded as a degenerative process since the nuclei of the cells elaborating the colloid do not die but remain attached to the wall of the vesicle. In addition, the Golgi bodies and mitochondria of these cells remain intact. Incidentally, there are far more colloid vesicles in the pars tuberalis than in the pars intermedia and pars anterior.

The Intraglandular Cleft and Colloid Discharge into the Circulation: In the rat the intraglandular cleft is usually filled with colloid. Brander (42) concludes from his observations that the colloid may well be discharged into the vascular space present at the lower end of the intraglandular cleft, where there is a patent opening. In the blood spaces below the pituitary, Brander reports having observed masses of colloid in Man and in the ox. Rasmussen (56) could find no evidence to support these views.

Vascular Supply of the Pituitary: The vascular arrangement differs in the two lobes. Each lobe is supplied by a

different branch of the internal carotid, each of which is accompanied by fine amylinated nerve fibers(Dandy and Goetsch, 214; Herring, 1). There is, however, a connection of the veins of the two lobes, the veins coursing from the anterior lobe and uniting with those of the posterior lobe. In the pars anterior, the small vessels are sinusoidal in arrangement, an arrangement entirely dissimilar to that in the posterior lobe which closely simulates that in the brain.

Popa and Fielding (137) have described a now widely-quoted system of veins which take origin from the sinusoids of the buccal portion of the hypophysis and from the capillaries of the neural portion. They ascend through the stalk to the region of the floor of the third ventricle. Here they break up into a secondary capillary net. As they ascend, they run first in the substance of the pars tuberalis, mostly in front of the stalk, and then at various levels they penetrate into the neural portion of the stalk and ascend within "glial sleeves" towards the floor of the infundibular recess, where they lose their glial wrapping and break up into a secondary net. The authors feel that the direction of blood flow is certainly hypophyseo-hypothalamic, thus drawing the implication that pars anterior hormones are transported to the tuberal and possibly to other hypothalamic nuclei. Sometimes globules occur within the lumina of these vessels, sometimes in the intervascular tissue of the neural portion of the stalk and sometimes in the vicinity of the secondary net. Pretsch (215) has independently made similar observations.

(d) Time at which Hormones first appear in the Pars Anterior.

In order to determine at what state in the developing foetus the first detectable amounts of hormones are present, Smith and Dortzbach (43) made implants of the pituitaries of young pig foetuses into immature mice. They found that these implanted pituitaries first produce a gonad-stimulating hormone when they are taken from pigs which have reached a rump-crown length of 17-18 cms. In hypophysectomized mice it was found that regrowth took place when implants of pig foetus anterior pituitary of 10 cms. or more were done. These results indicate the existence of a time difference in the attainment of threshold values of these two functions. Nelson (44) was interested in knowing whether there were histological changes in the pituitary of pig foetuses between the 10 and 18 cm. stages; i.e. the stages at which growth and gonadotropic hormones respectively occurred -- changes which could be correlated with the onset of function. Accordingly, they sectioned the anterior lobes of a graded series of foetal pigs and found that the histological picture characteristic of the 10 cm. stage is decidedly a basophilic one (basophilic or chromophobic ? W.H.). By the 18 cm. stage many acidophils were in evidence. It would seem that at this early stage (10 cm.) the chromophobes had some influence upon growth and that at a later foetal stage (18 cm.) acidophils were in some way connected with the onset of gonadotropic function. There are so many other factors involved in these two physiological functions that it seems unwise to attempt to draw any positive conclusions.

(e) Various Cell Types in Pituitary Adenomas and the Syndromes they call forth.

Observation of the type cell growing in adenomas, whether it be the acidophil, the basophil or the chromophobe cell, supplies a lead in ascribing function to these various cells. The subject is well covered by Cameron (45, 88); a summary of his review follows: Chromophobe cell adenomata lead to syndromes such as those of Frohlich and Lorain. The symptoms of pituitary hyposecretion accompanying them are thought to be caused by local pituitary pressure effects ..... the pressure being upon the basophil, which is no longer capable of elaborating gonadotropic hormone. As a consequence amenorrhea and other gonadal deficiencies result.

In pituitary adenomata accompanied by gigantism and acromegaly, it is well known that the type cell is the acidophil, a cell accepted as in large measure controlling growth. Overgrowth takes place as the result of hypersecretion of acidophils. Gigantism is also associated with non-adenomatous acidophilic cell hyperplasia.

An adenoma of the basophil elements of the anterior pituitary calls forth a polyglandular syndrome (pituitary basophilism - Cushing's Disease) characterized in its full-blown state by adiposity, genital dystrophy, by osteoporosis, and so on. There is a question as to what the obesity is due to. Cameron thinks it due either to pressure upon the posterior pituitary lobe (depression or occlusion of secretion) or to secondary involvement of other endocrine glands. If I am correct, Cameron does not mention possible pressure upon the tuber cinereum as a cause.

(f) Physiology of the Pars Anterior.

When this lobe becomes deranged either spontaneously or experimentally, a profound alteration takes place in other of the endocrine glands. The physiological changes and the alterations in the histological picture of these other glands are, after pituitary ablation, in most instances, well-defined ones. While great strides have been made in the demonstration of hormonal effects, it is well to keep in mind that conceptions of responses elicited by various hormones will be changing until pure hormones are produced. In this connection, P.E. Smith (46) (1935) emphasizes that the pituitary hormones thus far produced are extremely crude from a chemical point of view and usually impure physiologically.

Changes in the animal following experimental hypophysectomy and replacement therapy will be described in the ensuing pages together with a discussion of hormones where it seems pertinent.

I. THE GROWTH HORMONE

Evidence that the anterior pituitary produces a growth principle was earliest set forth (1886) by Pierre Marie (47) and again in 1900 by Benda (30). In their descriptions of acromegaly they noted that the pituitary was enlarged. P.E. Smith (48), and many after him, showed in the mammal that with complete pituitary ablation, growth stops. There is difference of opinion, however, as to whether this applies in the case of very young animals. Collip (49, 50) and Collip, Selye and Thomson (51) found that rats of about three weeks of age do not stop growing after such an operation: those

weighing approximately 30 grams at the time of operation continued to grow until they had reached the weight of 60 grams. Evans (52), on the other hand, on the basis of his own work, feels that if hypophysectomy is complete in these young rats, growth ceases. Injury to the hypothalamus during the operation could easily result in gain in weight on the basis of ensuing adiposity.

If hypophysectomized rats are allowed to go on for some time until they become cachectic, and are then given extracts prepared from the anterior lobe, pronounced restorative effects are obtained. This has been described by Evans and Long (53) and by P.E. Smith (54) and many others. When these rats are treated with the "Q extract", an extract containing growth hormone prepared by Collip (50), rats gain 2-3 grams per day for 35 days. Growth gradually subsides and in the course of some weeks the rats cease to grow and may actually lose weight in spite of continuous treatment with Q extract.

As convincing a bit of evidence as has been found to link the hypophysis with growth has been supplied by Evans and Long (53). They produced gigantism in normal rats by injecting beef pituitary extract over a protracted period starting at the fourteenth day. Invariably these rats underwent changes in their post-puberal growth so that they became on an average of 80% heavier than the controls. Putnam, Benedict and Teel (55) went a step further in producing a condition of overgrowth in a dog in all respects comparable to that of acromegaly.

II. THE GONADOTROPIC HORMONE

The genital organs atrophy after hypophysectomy has been performed, as was shown by Cushing and co-workers (58) as far back as 1910. Germinal epithelium of the testis atrophies and sperm cell formation stops (P.E. Smith, 46). In the ovary, follicle maturation and corpus luteum formation is inhibited and the animals become continuously dioestric. The thecal cells, which ordinarily have a spindle-shaped nucleus and a finely granular structure, are altered in that the nuclei become circular and their chromatin aggregates in large dark lumps peripherally arranged, leaving white, chromatin-free spaces in between, so that the nucleus comes to present a somewhat wheel-like appearance. Cells of this sort have been named by Collip 'theca deficiency cells'.

In pregnancy, the hypophysis probably plays an important role. Hypophysectomy during the latter half of pregnancy does not, however, interfere with the maintenance of gestation, but does prolong the gestation period (Pencharz and Long, 59). Hypophysectomy done in the early stages of pregnancy leads, as Teel (60) has shown in the rat, to the resorption of the embryos.

Evans (61), in 1924, found that injection of pituitary extracts into mature rats brought about a complete cessation of oestrus. The explanation of the phenomenon was that the extract had speeded up corpora lutea formation from the Graafian follicle stage to such an extent that few or none remained to bring about the changes characterizing oestrus. In 1927, Smith and Engel (62) in America, and

and Zondek and Aschheim (63) in Germany discovered quite independently that the anterior lobe secreted a substance which was essential to the functioning of the gonads. Since then it has been demonstrated (Evans, 64) that this gonadotropic substance is composed of two hormones, one which produces follicle growth in the ovary (the 'follicle-stimulating' hormone) and growth of the germinal epithelium of the testis, and the other which acts upon the lutein cells in the ovary (the 'luteinizing' hormone) and the interstitial cells of the testis. The former stimulates the follicle without transforming it into a corpus luteum; the latter does not stimulate the growth of the follicle but transforms granulosa cells into corpus luteum cells. The gonadotropic principle differs from the anterior-pituitary-like hormone (A.P.L.) prepared from pregnancy urine or placenta (probably from chorionic villi) -- it differs in that the latter produces no follicle formation and no corpora lutea when injected into the hypophysectomized rat (Collip, Selye and Thomson, 65). A.P.L. does produce, on the other hand, lutein changes in the theca cells of the ovary and hypertrophy of the interstitial cells of the testis.

The experiments of Fee and Parkes (66) show what an important rôle the pituitary plays in the rabbit immediately after copulation. During coitus the anterior pituitary is stimulated into producing a hormone which induces in the ovary the changes associated with ovulation. If the pituitary is removed more than an hour after copulation, ovulation takes place normally, although the subsequent development of the

corpora lutea appears to be abnormal. If, on the other hand, hypophysectomy is done immediately after copulation, ovulation does not take place.

Furthermore, when a fresh suspension of pituitary extract is injected into normal adult female rats, an abnormally large number of ova ripen and oestrus sets in, as Evans (64) has noted. Ultimately, a large number of corpora lutea are formed, or, if pregnancy is established, an abnormally large number of embryos are produced.

After castration, the anterior pituitary (presumably the castration cells) contains a larger amount of gonadotropic hormone. Engle (67) found that the ovarian response of immature mice and rats after being implanted daily with fresh anterior lobe taken from gonadectomized rats is significantly greater than the response to anterior lobe implants taken from normal animals.

In summary, Robson (68) states, in a review of the literature, that the pituitary can bring about in the ovary: I, follicular maturation, ovulation and oestrin secretion; and II, formation of luteal tissue followed by secretion of the corpus luteum hormone.

III. THE LACTOGENIC HORMONE

Selye, Collip and Thomson (69) report that mammary gland development is not interfered with in rats and mice hypophysectomized in the latter half of gestation. In such animals, milk secretion starts normally at parturition: it lasts but a few hours and then definitely stops. Similarly, if hypophysectomy is performed during the course of an already

established lactation, the milk secretion stops within a few hours (Collip, Selye and Thomson, 70).

Opinions differ as to exactly how much of a rôle the anterior pituitary lactogenic hormone (named 'prolactin' by Riddle and 'galactin' by Turner) has in lactation. In 1929 Grüter and Stricker (71) reported the initiation of secretion of milk in suitable rabbits, dogs, pigs and cows by the injection of anterior pituitary extracts: such stimulation was not produced by injection of corpus luteum. In 1932 Riddle and his associates (72) isolated a lactogenic factor separate from the growth, the gonadotropic and the thyreotropic hormones. They found that there are two phases inmmammary gland development, I, unfolding of the duct system through the action of an ovarian estrogenic substance, and II, stimulation of milk secretion by prolactin. That this prolactin is responsible for the initiation of lactation is doubted by Selye, Collip and Thomson (73) who found that a brief lactation takes place at parturition in rats that have been hypophysectomized for some days. However, it seems generally conceded that the lactogenic hormone causes lactation, but only when the mammary gland has been previously subjected to the action of the estrogenic hormones.

IV. THE ADRENOTROPIC HORMONE

Definite atrophy of the adrenal cortex follows removal of the hypophysis in rats. Histologically, this cortical atrophy is characterized by an intense hyperemia at the cortico-medullary junction. This acute reaction is followed by a progressively narrowing of the two inner zones of the

cortex, namely, the fasciculata and the reticularis. Numerous cells containing yellowish-green pigment appear in the zona reticularis (Collip, 49).

Attempts at replacement therapy to offset the effects of hypophysectomy upon the adrenal have been most successful. When administered after hypophysectomy, extracts containing the adrenotropic hormone (but free of growth and thyreotropic hormone) prevent atrophy of the adrenals (Evans, 61; Collip, Anderson and Thomson, 74; and P.E. Smith, 46). Collip (49) reports that the atrophic zones of the adrenal are restored in the course of one week. On the other hand, injection of the hormone into normal rats does not produce any marked changes in the size or histological appearance of the adrenals.

Kraus (75) has pointed out that a relationship exists between the human adrenal cortex and the basophil count of the pituitary. With hyperplasia of the adrenal cortex, basophils are increased. This applies particularly in certain cases of hypertension. The converse is also true, namely, that in cortical hypoplasia, basophils are diminished.

V. THE THYREOTROPIC HORMONE

Cushing (76), in 1912 observed in the dog that following a transient hyperplasia of the thyroid gland occurring twenty-four hours after extirpation, involution of the gland ultimately resulted. Smith (77) noted a similar result in the rat. Flattening of the vesicular epithelium takes place together with an increased collection of intra-alveolar colloid. In addition, the metabolic rate is lowered to 74% of the normal (Anderson and Collip, 78).

The thyroid, too, responds to replacement therapy in the hypophysectomized rat. It was in 1927 that P.E. Smith (48) first demonstrated this in mammals. The work of Anderson and Collip (78) shows that the hypophysectomized animal is very sensitive (ten times more than the normal) to minute doses of the thyreotropic hormone. The basal metabolic rate was shown to be brought back from the hypophysectomy level of 74% to the normal level within an average of three to four days. If, on the other hand, normal rats are treated with thyreotropic hormone, the rates are elevated to 35% above normal. The peak is reached in a week or ten days and is followed by a decline. Three or four weeks later the metabolic rate has fallen to subnormal. With continued treatment, metabolism falls to the hypophysectomy level. Anderson also notes that this decline takes place in hypophysectomized rats after they have been treated over long periods of time with thyreotropic hormone. Their metabolic curves in general run 25 points less than those of normal rats.

In 1929, Leo Loeb (79) in the United States and Aron (80) in France succeeded, quite independently of each other, in producing hyperplasia of the thyroid gland of the guinea pig by the ingestion of anterior lobe extracts; it resulted in a picture closely resembling that seen in patients with Graves' disease. This work suggests that the thyreotropic hormone may in some way act as the etiological agent in Graves' disease. It has been shown by Verzar and Wahl (81) and others that such hyperthyroidism cannot be produced in animals from which the thyroid had been previously removed.

Conversely, the thyroid is essential for hypophyseal normality, as Rogowitsch (82) pointed out in 1889. Dwarfism in cretins is regarded by Evans (61) as a deprivation on the part of the hypophysis of thyreotropic principle.

As to which cell or cells of the anterior pituitary secretes the thyreotropic hormone, nothing entirely conclusive has been offered. P.E. and I.P. Smith (83), taking advantage of the regional distribution of basophils and acidophils in the bovine anterior pituitary, separated the two components fairly completely and injected each of them into hypophysectomized tadpoles. They found that the chromophobe and basophil cells produced hyperplasia of the thyroid and that the chromophobes and acidophils were responsible for growth (this latter, a finding that is well conceded). Unfortunately, these authors did not mention gonadal or adrenal effects in their tadpoles.

VI. PARATHYROID AND THUMUS GLANDS IN HYPOPHYSECTOMIZED ANIMALS

The association of parathyroid adenomas with tumours of the pituitary gland suggests a relationship between these two glands (Hertz and Kranes, 84). Several investigators, including Hoffman and Anselmino (85) and Anselmino, Hoffman and Herold (86) report proliferation of the parathyroid glands upon administration of anterior pituitary extracts. There occurred active mitotic division and vacuolization of the parathyroid cells.

Results following hypophysectomy have not been consistent. Collip (87) has been unable to detect degenerative changes in the parathyroid following hypophysectomy. Smith (77),

on the other hand, reports parathyroid atrophy following pituitary ablation. No consistent results have been obtained upon blood calcium changes after the injection of pituitary extracts.

In regard to the thymic changes following hypophysectomy, Smith (89) records that the thymi of rats cease to grow immediately after ablation and that they continue to regress.

VII. THE DIABETOGENIC AND KETOGENIC PRINCIPLES.

Another action of the pituitary is that upon carbohydrate metabolism. In 1930, Houssay and Biasotti (90, 91) reported the remarkable finding that symptoms of diabetes in dogs after pancreatectomy were cured or alleviated by hypophysectomy. The dogs were able to go 6-9 months without insulin, whereas the depancreatized dog lived only a few days. These dogs could, however, be rendered markedly diabetic again by the injection of anterior lobe extracts, but not by posterior lobe extracts. These actions of the anterior lobe extract upon carbohydrate metabolism go hand in hand with what has been observed by others; namely, that hypophysectomy increases sugar tolerance (Cushing, 93), that hypophysectomy lowers the blood sugar (D'Amour and Keller, 94), and, lastly, that hyperglycemia and glycosuria may be produced in normal rats by injecting anterior lobe extracts (Baumann and Marine, 95). Black, Collip and Thomson (96) studied the ketogenic action of the various extracts of the anterior lobe and came to the conclusion, which would tend to support the view of Magistris (97), that the ketogenic substance is distinct from

the thyreotropic hormone. Acetone bodies in the blood of rabbits increase with the injection of a pituitary preparation (Hoffman and Anselmino, 98; Magistris, 97).

This at least tells a large part of the story of pituitary influence upon carbohydrate metabolism. Discussion of the further rôle played by the posterior pituitary and the hypothalamus will be discussed under the subtitle Physiology of the Posterior Lobe.

VIII. ANTIHORMONES

It has been observed that in many animals a state of increased resistence or of actual non-responsiveness may gradually become manifested in those animals that have been treated for a long period of time with some glandular extract. Friedgood (99) and Siebert and Smith (100) noted that thyroid stimulation is not maintained after continuous administration of the thyreotropic hormone. Animals into which thyreotropic hormone had been injected twice daily over a long period of time not only failed to give a positive metabolic response but also showed a lowering of the metabolism to the level of hypophysectomized animals. Upon these observations has developed one of the recent contributions in the field of endocrinology, namely, the antihormone. Anderson and Collip (101, 102) demonstrated that the serum from a horse which had been injected with thyreotropic hormone for two months contains a substance which has an inhibitory effect upon the thyreotropic hormone. This antithyreotropic hormone (or substance) is capable not only of inhibiting the action of a dose of thyreotropic hormone in the normal rat which is five times the minimum effective

dose, but at the same time of inhibiting the thyreotropic hormone of the animal's own pituitary by lowering the metabolic rate to the hypophysectomized level. There is evidence, as Anderson points out, that this antithyreotropic hormone is not produced in the hypophysis because hypophysectomized animals are also capable of producing the antihormone.

IX. SECRETORY CAPACITY OF ANTERIOR LOBE AFTER PARTIAL ABLATION

Smith (103) performed twenty partial hypophysectomies in rats in order to determine the amount of anterior hypophysis requisite for carrying on normal physiological function. His data show that this gland has a wide margin of safety. Fragments of anterior hypophysis recovered at autopsy varied from 10 to 90 per cent of that of respective littermate controls. If 30 per cent or more by weight of the anterior hypophysis was present, general body growth, thyroids, adrenals and gonads were normal, as were also sex cycles, mating and reproduction. When distinctly less amounts were present there was dwarfing, and the above mentioned glands were below the weight of the controls; however, the histological picture of the thyroids, adrenals and gonads were unaltered. With as little as 10 per cent remaining in the sella, there was loss in weight and a partial development of the disabilities invariably appearing in total ablations. In these rats there was no alteration in the histological picture of the thyroids and adrenals; the ovaries, on the other hand, showed a decrease in the number of follicles and corpora lutea in the more complete ablations, and the sex cycles were irregular in that there were unusually long dioestrous periods.

This large margin of safety in the rat may not obtain in man and larger mammals, Smith concludes, in that the rat possesses so much more anterior hypophysis per unit of body weight. In the rat, after two-thirds of the anterior hypophysis has been removed, there remains three times more anterior hypophysis per unit of body weight than in Man, and thirteen times as much as in the ox.

The question of regeneration of the anterior hypophysis after partial ablation (one which is definitely apropos in transplantation attempts) is not discussed in this study of Smith's.

(g) Changes in the Anterior Pituitary following Castration.

Earlier investigators found changes occurring in the acidophils of animals after castration had been done. Fichera (104) in 1905 noted in castrated male and female chickens, rabbits, guinea pigs and steers a relative increase of acidophils. Jutaka (105) and Trautmann (15) in 1909, and Kolde (106) in 1913 also found acidophil increase.

With Biedl's (107) work the conception of cell alteration in the anterior pituitary after castration took on a different aspect. He found in the pars anterior of castrated rats and dogs large vacuolated cells with bluish-red cytoplasm and a lightly staining nucleus. These, he felt, were not true acidophils but rather cells which had developed from acidophils, these latter cell elements being reduced at their expense. Schleidt (108) went on to stress the marked degree of vacuolization of these cells.

It seems that Addison (109) was the first to point out the conception that prevails at the present time: he felt that these so-called 'castration cells' were vacuolated basophils. At first acidophils remained unchanged in number and appearance whereas basophils become definitely enlarged and more granular. After several months basophils developed large vacuoles filled with a colloid-like substance. He traced their origin from chromophobes on the basis of their decrease in the number of the latter. It would appear that these changes in the rat pars anterior are not in accord with those in other mammals. Schenk (110) found after castration an increase of acidophils in man, rabbit, guinea pig, chicken, cat, dog, ass and horse; of chromophobes in rabbits; and the appearance of so-called castration cells in capons, rats and dogs. Stein (111) could find no posterior lobe changes after castration.

Severinghaus (25) shows quite conclusively in the rat that the castration cell is a transformed basophil: this he bases on the observation that the Golgi apparatus is identical in the basophil and castration cell (as did Addison, 112). Furthermore, acidophils progressively decrease in size so that at the forty-sixth day following castration the acidophil is about one-half the volume of the normal cell. Chromophobes show a marked reduction in number, they being reduced from 50% to 20% of the pars anterior cellular constituents (Nukariya, 21).

It seems that evidence is at least presumptive that the basophils elaborate gonadotropic hormone. This is based on observations that basophils increase after castration, and, furthermore, that extracts of the pituitary gland of castrates have a greater gonad-stimulating power than those of the normal gland.

This latter is thought to be due to storage of endocrine principle in 'castration cells'. Evidence associating the gonadotropic principle with the basophil is strong, but not final.

(h) Rôle of the Anterior Pituitary Cells in Oestrus and in Pregnancy.

Changes have been recorded in the cells of the anterior pituitary during oestrus. It is difficult to determine from a review of the literature as to whether changes taking place during oestrus affect the pituitary cells primarily, causing changes in them, or whether the pituitary cells as they reach a certain developmental stage produce primarily, in the natural course of events, the changes characterizing the oestral cycle. Another alternative is that the cells of the two organs are reciprocal in their functional activities.

In the normal animal, Wolfe (113) and Wolfe and Cleveland (114) observed the histological picture of the pituitary during the various phases of the oestral cycle. Studying the serial sections of one hundred and forty-three female rats, they found only a qualitative alteration. During oestrus, the acidophil is well packed with eosinophilic granules which stain intensely with Orange G. During dioestrus, on the other hand, the granules in these cells are less packed. In the transition stage between prooestrus and oestrus the basophil cells are most tightly packed with granules; in the oestrus and metoestrus stages the basophils exhibit a marked loss of granulation.

Significant observations by Charripper and Haterius (115) differ in regard to the basophil changes during oestrus. They noted that oestrus is characterized by a pronounced basophilia. These changes they corroborated by experimentally inducing continuous oestrus. During the dioestrus phase of the cycle, the structure became essentially acidophilic.

Phillip (116) employed quite a different approach in attempting to correlate the various anterior pituitary cell types with the changes characterizing the oestral cycle. To attain this end he implanted human pituitaries of various ages into infantile mice and observed what their effect would be upon the ovaries. He found that with implantation of foetal and new-born human pituitaries, which were made up for the most part by chromophobes, the mice showed only slight swelling of the ovarian follicles and of the uterus; with the pituitaries of the one-year human these reactions were more prominent. Human pituitaries of from three to nine years, which possessed large numbers of acidophils, produced a marked reaction upon the mouse follicles but did not cause any corpora lutea to form: there was also pronounced uterine enlargement. With implantation of adult pituitaries at a stage when acidophils are less relatively abundant, there occurred an increase in numbers of follicles and of luteinization. Phillip concludes that the acidophil has the function of ripening the follicle; he feels that the chromophobe has no effect upon the ovary but that it does produce a principle causing growth of the pregnant uterus and its contents. He denies the acidophil the function of producing a growth-promoting hormone, an opinion entirely at

variance with what seems well established, namely, that the growth-promoting hormone is elaborated by the acidophil. This opinion is also at variance with the prevalent view that chromophobes have no secretory function.

During pregnancy and pseudo-pregnancy a preponderance of acidophil cells in the pars anterior has been observed by Charripper and Haterius (115). In regard to the so-called 'pregnancy cells' of the pars anterior, Rasmussen (22) states that he could not identify any such specific cells. He feels that the enlargement of the pituitary during pregnancy is not due to hyperplasia of any one of the three types of cells. There has been no experimental evidence, according to Evans and Simpson (117), to support the view that so-called 'pregnancy cells' store endocrine principle in a like manner as the 'castration cell' probably does.

5. CYTOLOGY OF THE PARS TUBERALIS OF THE PITUITARY

It has been pointed out that the pars intermedia and the pars tuberalis are closely associated in that they are both developed from the pars buccalis. In some animals the border line between the pars anterior and the pars intermedia and tuberalis is difficult to make out.

In the cat (Herring, 1) the epithelium of the pars tuberalis is distinctly tubular. The cells lie about the central lumen which often contains colloid bodies. Between these small lumina there runs a rich network of vessels. The cells are small and in their cytoplasm are found fine granules. Atwell (118, 119, 120) points out in the cat that the cells are somewhat smaller in size than in the pars anterior and the pars intermedia and that they are arranged in cords supported by trabeculae which are considerably more abundant than in the pars intermedia. A Golgi apparatus is present and is usually located between the nucleus and the colloid; in cell cords not adjacent to colloid, the Golgi seems to have no regular position, as may be said also for the mitochondria. Colloid collections, such as are seen in the posterior portion of the pars anterior, are frequent. Atwell describes small droplets appearing in the cells which eventually coalesce. As the material accumulates, the cell breaks down to liberate its contents. This makes it appear as though the colloid is formed through a degenerative process rather than through a secretory one, there being no definite lining about the colloid collections. Vascular channels, similar to those present in the pars anterior, are to be seen. Bailey (121) states that there are no chromophils present in the pars tuberalis.

6. PARS NEURALIS OF THE PITUITARY

(a) Cytology.

The pars nervosa of the posterior lobe is continuous with the floor of the third ventricle by means of the infundibulum which joins the two.

The characteristic cell of the pars nervosa, the pituicyte, (which makes up its major bulk) was described by Berkley (122) and Retzius (123) in 1894. More recently, with an improvement of staining technique previous descriptions have been amplified by Bailey (121) and by Bucy (9, 124). The latter states that in pituicytes of both cattle and Man there are two characteristics in common: (I) they are impregnated by Penfield's silver carbonate technique, and, (II) each has one or more processes which terminate upon connective tissue fibres -- either that of the wall of a blood vessel, or upon the connective tissue septa of the gland or upon the fibres of the connective tissue capsule. There is a tendency for these cells to arrange themselves parallel to the long axis of the pars nervosa probably due to a similar arrangement of the nerve fibres. At the periphery of the infundibulum the cells are arranged at right angles to the axis of the pars nervosa.

The pituicytes, which are not very numerous, vary in size and shape. Their rather small nuclei are round and oval and contain a moderate amount of chromatin. Nucleoli are rare. The cytoplasm has no constant shape and is always finely granular. On the whole, its processes are considerably longer than those of glial cells seen elsewhere. Many of the cells are bipolar: in the infundibulum, unipolar forms are prominent. In

the pars nervosa, the processes are for the most part multipolar and vary greatly in shape. They may divide dichotomously or give off several smaller branches.

Throughout the pars nervosa can be seen cells which have small pigmented granules in their cytoplasm. In unstained preparations these are a light greenish-brown or yellow. This pigment, which stains with neutral red and toluidin blue in fresh tissue, and with methyl green in formalin-fixed tissue, is undoubtedly in the pituicytes, but it is also found in the cells of the connective tissue meshes, cells which, according to Stumpf (125) might easily be wandering phagocytes. Kohn (126) states that the pigment is neither fat, nor iron, nor melanin, nor lipochrome and is of the opinion that it represents a degeneration product. On the other hand, Vogel (127) concludes that they are products of metabolism. Both Stendell (40) and Schönig (128) interpreted the pigment as a secretory product of the basophilic intermedia cells interdigitating the pars nervosa. Bucy (9) denies this latter interpretation on the basis that such pigment occurs in the pars nervosa of cattle in which there is an absence of pars intermedia invasion of the nervosa.

Both Bailey and Bucy remark that pituicytes have no analogue in the rest of the nervous system. The cell which it resembles most closely is the astroblast: even these two are only superficially similar and they possess entirely different staining characteristics. Bailey (36) likens them to the glial cells growing in tumours, being protoplasm-rich and process-poor.

As to other cell types in the pars neuralis, there are no cells which resemble astrocytes, microglia or oligodendroglia. Nor are there any cells present which may be interpreted as neurocytes. Nissl bodies are entirely absent.

A number of investigators, among them Greving (129, 130) and Pines (131), have studied the arborization of nerve fibres in the pars neuralis first described in the mouse by Cajal (132) in 1894. The large number of unmyelinated fibres are grouped together in dense bundles; here and there among the fibres are seen the nuclei of pituicytes. As the fibres reach the pars nervosa they spread out in a fan-like manner. Bucy states that as they proceed into the pars nervosa the bundles become smaller and the individual fibres are seen to separate from the others and form a network about the cells. Not infrequently, a fibre may be seen to be divided into two or three small branches which surround an individual cell. Some nerve fibres terminate in the connective tissue capsule of the gland some proceed to the cells of the pars intermedia where they wind about the epithelial cells and end as large end bulbs. Tello (133), confirming Cajal's work, has pointed out in the human with what great frequency the nerve fibres entering the pars neuralis end in large end bulbs. These end bulbs, which have a dense, reticulated appearance not unlike a ball of very fine fibres, are seen in both the pars nervosa and infundibulum. Tello's opinion, which finds confirmation in Bucy's work, is that these end bulbs upon degenerating form the 'hyaline' bodies described by Herring and known generally as 'Herring bodies'. Herring, it will be remembered, considered these bodies as a secretion of the cells of the pars intermedia.

(b) Physiology of the Pars Neuralis.

In the discussion of the posterior lobe several structures must be taken into consideration -- the pars intermedia and tuberalis derived from the pouch of Rathke and the pars nervosa and infundibulum which are of nervous origin. These structures are closely bound together in the integration of 'posterior lobe' function. Bailey (36) points out that in consideration of the pars neuralis it should be kept in mind that this body is an undeveloped part of the brain and in its blood supply and in its histological structure is not glandular. Furthermore, the pars tuberalis and intermedia are embryologically undeveloped parts of the pars buccalis. The pars intermedia slowly decreases in relative amount as the vertebrate rises in the scale and is inclined to undergo degeneration probably because of a poor blood supply.

It is not surprising to note the confusion in allocation of function of the pituitary among those earlier investigators who based conclusions upon the premise that in their experimental hypophysectomies, the removal of hormone-bearing tissue was complete. It has been shown by Smith (48) in the rat and by Abel (134) in the dog that in the established 'complete' hypophysectomy, the upper part of the infundibular stalk, a structure elaborating hormones, is left intact.

I. ORIGIN AND PATH OF ESCAPE OF THE SECRETION

There are those (Vincent, 135) who have maintained that the cells of the pars nervosa are incapable of secreting a hormone. Vincent finds himself "strongly inclined to adopt the view of Houssay and others that the substance or substances

are secreted in the cells of the pars intermedia and then collected and concentrated or changed into some active form in the nervous system proper". Cushing (186) in 1933 and Biggart (136) in 1935 maintain, as Vincent did, that the so-called posterior lobe secretion is manufactured in the epithelial covering of pars intermedia and pars tuberalis cells. This conclusion has not met acceptance with some recent investigators. Geiling (57), in 1935, states that the simplest explanation of posterior lobe secretion, and not at all inconsistent with the facts is that the secretion is elaborated by the intrinsic elements of the lobe itself. The secretion then enters the circulation (capillaries of Popa and Fielding, 137) whence it takes an hypophysis-hypothalamic direction. Whether the secretion actually takes this venous route or proceeds along the interstitial network has not been clarified.

The origin of pituitrin has been studied in two vertebrates in which the pars nervosa is easily separated from the pars intermedia: these are the chicken and the whale. De Lawder, Tarr and Geiling (138) found that in the chicken the pars nervosa extracts possess pressor, oxytocic and antidiuretic properties and that the pars anterior and tuberalis possess slight oxytocic and pressor effects. In the chicken the melanophore component was absent in the pars nervosa and the pars tuberalis but it was found in the anterior part of the pars anterior. In the whale, Valso (139) showed that only the pars intermedia contains the melanophoric principle, intermedin. These observations exclude the pars nervosa from taking part in intermedin production, but implicates in some

way the pars anterior. It may be that intermedin seeps over into the pars anterior: this point has not as yet been entirely clarified.

Identification of the so-called Herring bodies as a secretory product in the pars nervosa was first made by Herring (140) in 1908. They are acellular structures of variable size and shape, distributed indiscriminately and showing a variable staining capacity. These bodies were regarded as secretion antecedents migrating from the cells of the pars intermedia on their way to enter the third ventricle. A few observers (Bailey, 121; DeBeer, 141) accepted Herring's conclusion wholly or in part. Chief among them has been Cushing who maintained in 1926 (142) and who reiterated in 1933 (142a), that the pars intermedia cells ripen into basophils, invade the pars nervosa, are cast off and degenerate; they are transformed into hyaline bodies which pass up to the third ventricle. Geiling (57), in experiments still unpublished, states that these bodies are artifacts produced by a clumping together as the result of fixation. Nevertheless, a number of investigators believe on indirect evidence that the colloid and hyaline material which collects in the posterior lobe is a secretory product (Cushing, 142).

There seems to be some doubt as to whether the posterior lobe secretion which finds its way up the stalk enters into the cerebro-spinal fluid. There is strong evidence that secretions travel up the stalk: Cushing (142) states that when the stalk becomes mechanically obstructed, the secretory products become dammed back so that the lobe becomes rigid with

hyalin. He is of the opinion that the posterior lobe secretion comes into direct contact with the cells of the tuber, and that a part of the secretion enters the cerebrospinal fluid (1926). That the pars intermedia hormone, intermedin, travels up the stalk has been shown by Zondek and Krohn (143a) and by Zondek (143) who demonstrated this melanophoric principle in the walls of the third ventricle, and small amounts in the fluid of the third ventricle, but not in the cerebrospinal fluid elsewhere.

It would seem that if the cerebrospinal fluid were found to contain more hormone in a greater concentration than that in the blood, it would be good presumptive evidence that the secretion escapes directly into the cerebrospinal fluid. The opposite condition of affairs has been found by Krough (144) who demonstrated that the oxytocic and melanophore principle in jugular serum is considerably higher than in the saphenous serum and in the cerebrospinal fluid. What significance to assign to this finding is difficult in the light of Van Dyke, Bailey and Bucy's (145) work on cerebrospinal fluid analysis for the oxytocic substance. For them the oxytocic substance found in the cerebrospinal fluid is calcium and not the true posterior lobe oxytocic substance. They found that when they reduced the calcium to a concentration equal to that of artificial 'cerebrospinal fluid', a process which would not remove any of the true posterior lobe oxytocic principle which may be there, and bathed isolated uterine muscle in this artificial solution, there were no contractions. This experiment rendered scarcely tenable the view that the oxytocic

substance of cerebrospinal fluid is identical with or analogous to the hypophyseal oxytocic principle. Similarly, Houssay (146) obtained from the injection into test animals of concentrated spinal fluid a definite rise in blood pressure simulating that obtained with pituitary extract. If the spinal fluid were heated with lead acetate, it no longer caused a rise in blood pressure, whereas pituitary extract under the same conditions continued to raise arterial pressure.

Karplus and Peczenik (184) have demonstrated a pressor substance in the cisternal fluid only after electrical stimulation of the tuber cinereum: this pressor substance was not found in the spinal fluid. This finding indicates a tuberal mechanism in the introduction of the pressor hormone into the ventricular cavity.

II. CHEMISTRY OF POSTERIOR LOBE EXTRACT

Herring (147, 148) came to the conclusion that there were two different principles elaborated by the posterior lobe, the pressor substance present only in the pars nervosa and the oxytocic substance found for the most part in the pars intermedia and to a lesser extent also in the pars neuralis. Hogben and de Beer (10) confirmed the latter part of this observation. Abel (134, 149) held out in his opinion that there was but one specific hormone elaborated by the posterior hypophysis. He isolated a very potent tartrate which possessed diuretic, antidiuretic, pressor and oxytocic functions ("A" substance of Abel and Nagayama). Besides this there were two other fractions, one, "B", a blood pressure lowering and a mildly oxytocic

substance, differing from histamine in that it resembles a proteose, and, two, "C", histamine. Abel felt, as did Atwell and Marinus (150) that properly made extracts of the pars intermedia, the neural portion of the hypophyseal stalk and the posterior lobe are practically all of equal potency and they each exhibit all the characteristic reactions of posterior lobe extract.

Most of the recent investigators of this subject (Dudley, 151; Führner, 152; Dale, 153) have separated from pituitary extracts the two fractions, pitressin (beta-hypophamine) and pitocin (alpha-hypophamine). Pitressin has pressor and antidiuretic properties; pitocin has oxytocic function.

III. PHYSIOLOGICAL EFFECTS OF POSTERIOR LOBE EXTRACTS

Geiling (57) summarizes the effects of injecting pitressin into Man. It produces no significant rise in blood pressure in spite of the pallor which would lead one to infer that arterial tension is elevated. There is a prolonged rise in pulse rate, increase in cardiac putput and increase in oxygen consumption, a concerted action which is preceded in each case by a brief fall. This preliminary depressor effect is probably due to the constriction of the coronaries. The action of pitressin on the respiratory centre is that of quickening the respiratory rate.

With therapeutic doses of pitressin, Geiling states that there is a marked antidiuresis. [Melville and Holman (156) noted no such effects after the administration of pitocin.] Oehme and Oehme's (157) explanation of the antidiuretic

properties of pituitrin (pitressin) is that it functions as a depressant upon the renal epithelium, a view which has been accepted by Bailey (36). Wright (158), on the other hand, states that pituitrin stimulates the renal tubules to absorb more water and thus concentrate the urine. A rather different idea is expressed by Stehle (159), who found that he could bring about diuresis with doses of pitressin far too small to affect vessels. From this he concluded that the function of this hormone must be that of regulating water balance, rather than a primary effect upon the vascular system. There has been a great deal of confusion, still encountered today regarding the nature of diuresis resulting from the injection of pituitrin into animals under anaesthesia. Stehle (160) says that it is well accepted that pituitrin exerts only an antidiuretic function: the diuresis taking place as the result of anaesthesia is temporary and is the result of altered pressor effects upon the renal capillaries. This view cannot be considered as definitely proven in the light of Cushing's (161) statement that the posterior lobe extracts may "have diuretic as well as antidiuretic effects, the former conceivably being the property of the epithelial investment, pars tuberalis in particular".

As regards pitocin, Geiling goes on to say that its reaction upon uterine muscle varies according to the nature of the ovarian, placental or anterior pituitary hormone whose influence is preponderant at the time of injection. During the early stages of pregnancy the human uterus does not react

to pitocin, probably because of the inhibitory effect of luteal secretion. Later in the gestation period the reactivity of pitocin returns, and during parturition the uterus is very reactive to this substance. In puerperium, however, pitocin evokes little response.

In spite of the huge amount of work on posterior lobe secretion, Geiling remarks, one cannot assign with certainty any specific role in the animal economy to this potent autopharmacological agent (pitocin). Possibly the pressor component acts as an efficient regulator of the exchange of metabolites between the blood and tissues (capillary hormone) and exercises a renal function. The oxytocic function may during parturition to quicken and render more effective the uterine contractions.

IV. RELATIONSHIP OF PARS NEURALIS AND TUBER CINEREUM TO WATER BALANCE WITH DISCUSSION OF ETIOLOGY OF DIABETES INSIPIDUS

Conceptions of the pathogenesis of diabetes insipidus have undergone progressive changes since the time when Bernard (1860) (162) produced a transient polyuria in a rabbit by puncturing the floor of the fourth ventricle. Eckhard (163), Kahler (164) and Dresel and Lewy (165) went further to show that injection of silver nitrate into the medulla resulted in a permanent polyuria. These experimentors came to the conclusion that the area concerned with diabetes insipidus lay somewhere in the floor of the fourth ventricle.

With the work of Magnus and Schäfer (166) and Herring (1) interest shifted to the posterior pituitary as the site concerned with diabetes insipidus. The assumption that diabetes

insipidus could be explained as an overactivity on the part of the posterior lobe cells was based on the belief that the posterior lobe principle produced diuresis. This post-hypophyseal location was furthered by Cushing (161) when he demonstrated that the syndrome of diabetes insipidus could be induced by hypophysectomy. When in 1913, Farini (167) and Van Der Velden (168) showed that the polyuria of diabetes insipidus could be controlled by posterior lobe extracts the belief was fostered that diabetes insipidus was associated with hypo rather than hyperfunction of the posterior lobe.

The prevailing conception of the pathogenesis of diabetes insipidus may be said to have begun with the work of Camus and Roussy (169, 170), who, amplifying the work of Aschner (171) showed that the hypophysis in itself did not produce polyuria: in animals which developed the syndrome, lesions were found in the floor of the third ventricle. By puncturing the grey matter of the tuber cinereum in the hypophysectomized animal they produced a prompt polyuria. Shortly afterwards Bailey and Bremer (172) confirmed these results by producing polyuria by injuring the infundibulo-tuberal region. Polyuria occurred in all his thirteen dogs starting at the second day and lasting six to eight or an indefinite number of days. In these experiments they eliminated the possibility of a nervous or vasomotor mechanism controlling renal excretion by denervating the kidneys. Richter (173) produced in the cat a persistent and marked polyuria (but no adiposity) by puncture of the hypothalamus through the base of the skull anterior to the tuber cinereum, as did Smith (48) a considerable time after

the pituitary had been ablated. Smith was able to separate the anterior pituitary deficiency syndrome following hypophysectomy from the adiposo-genital and diabetes insipidus syndromes occurring after injury of the tuber cinereum. He characterized the tuberal syndrome by extreme obesity (as much as three times the normal weight), by occasional atrophy of the genital system, and by frequent polyuria. No thyroid or adrenal cortical atrophy occurred. The genital atrophy following tuberal injury in the hypophysectomized rat was not nearly so profound. Furthermore, injection of anterior pituitary extract cleared up the symptoms produced by pituitary ablation but it had no effect upon the diuresis or upon the adiposo-genital symptoms produced by tuberal injury.

There have been various attempts to explain the rationale of polyuria following puncture of the tuber cinereum. Abel (134) reasoned that polyuria did not take place after simple hypophysectomy because there remained sufficient pituitrin-producing tissue in the pars tuberalis and tuber cinereum to offset any deficiency that would tend to occur with loss of the posterior lobe. Puncture, he felt, destroyed this last bit of functioning tissue. He demonstrated that from the part left after hypophysectomy (pars tuberalis and tuber cinereum) an extract may be made which has pressor-oxytocic-antidiuretic properties. Camus and Roussy (169) also demonstrated that hypophysectomy produced polyuria only when the tuber had been injured or removed. But they explained this phenomenon in a different way: it was, namely, that the puncture injured the nervous centre in the tuber cinereum which controls water balance.

That lesions of the posterior lobe have little or nothing to do with diabetes insipidus seems to be well borne out clinically. Hann (174), Larson, Weir and Rountree (175) have pointed out that in the majority of cases pituitary disease there is no appearance of diabetes insipidus and that most cases of diabetes insipidus show no pituitary changes. Furthermore, what is now considered the true nature of the lesion has been pointed out by Kahler (176) and Leschke (177, 178) who isolated the lesion at autopsy in the tuber cinereum. Biggart (136) describes three cases of diabetes insipidus in each of which there was a hypothalamic lesion -- one neoplastic, one a syphilitic meningo-encephalitis, and the other an epidemic encephalitis. Two of these cases had a concomitant lesion of the hypophysis. Fink (179) analyzing one hundred and seven cases of diabetes insipidus found various lesions at the base of the brain: neoplasms, syphilitic processes, non-specific inflammatory lesions, tuberculosis and those of trauma.

Greving (129, 130) in his excellent reviews of the causation of diabetes insipidus names three basis hypotheses which have been advanced to explain the etiology of diabetes insipidus. The first is that there is a nervous centre in the tuber cinereum and adjoining hypothalamus, which, when stimulated by hormones reaching it through the blood stream, sends impulses through nerve tracts to the kidneys where it influences the water excreting power of the renal epithelium. This, <u>die nervose Theorie</u>, he holds untenable in view of Bailey and Bremer's demonstration that polyuria occurs with puncture of the tuber even after the kidney has been completely denervated.

The second proposed explanation is that the hormone of the pars nervosa work directly upon the water excretory power of the kidney. This, the innersekretorische Theorie has little or no evidence to support it, according to Greving. Those who support the idea maintain that with hypophysectomy the pars tuberalis and the tuber cinereum take over the posterior lobe function, and secrete sufficient compensatory hypophyseal hormones. Greving attacks this idea on what seems rather precarious grounds -- the indirect evidence of embryology -- for he declares that the pars tuberalis, being derived from Rathke's pouch, is not made up of the sort of tissue which elaborates posterior lobe hormone. The third hypothesis embodies a combination of the two preceding ones - a nervous-hormonal mechanism, whereby hormonal production is controlled by a regulating centre in the tuber cinereum. This third, die nervos-hormonal Theorie, finds Greving's support. He has demonstrated a tract, the Tractus supraoptico-hypophyseus, composed of unmyelinated fibres leading from the nucleus supraopticus in the tuber cinereum down to the pars nervosa via the stalk. In the lower animals the tract innervates the pars intermedia. With further development (at least in man) this tract innervates the pars neuralis (and according to Cajal (180) the pars tuberalis). It is the centre that controls posterior lobe hormone production. Puncture of the tuber interrupts this very small tract going to the hormone-producing cells and then they can no longer control these cells in their production of the antidiuretic hormone. Regulation, then, of water balance depends upon an intact tuberal-hypophyseal nerve system.

Presumptive evidence that it is the posterior lobe which is accountable for the antidiuretic pituitary hormone has been produced by Verney (181). He found that the polyuria of an isolated heart-lung-fed kidney was checked if the blood was circulated through a head: this did not occur if the pituitary had first been ablated.

It has been concluded by Cameron (45) that diabetes insipidus cannot properly be attributed to any specific lesion. Cushing (161) in 1930 summed up his opinion as to the causation of diabetes insipidus by stating that "this disorder can be produced by nuclear degeneration from disease, by surgical injuries of the supraoptic region in operations about the chiasma, by interruption of the nerve tracts in course, whether from tuber tumours or punctures, by the experimental placement of a compressing clip on the infundibulum, and probably also (if this could be accomplished) by complete removal of the epithelial investment which apparently elaborates the posterior lobe secretion -- all of which indicates a diencephalo-hypophyseal mechanism which can be broken at any one of three principle points, nucleus, fibre tract and pars intermedia et tuberalis".

V. CARBOHYDRATE METABOLISM AND ITS RELATIONSHIP WITH THE TUBER CINEREUM

Aschner (182, 183) produced a glycosuria of 4% in animals in which he stimulated the hypothalamus with faradic current. Puncture of the tuber cinereum also produced a glycosuria as was demonstrated by Bailey and Bremer (172) and by Leschke (178). Weed, Cushing and Jacobson (185) produced a

glycosuria by faradic stimulation of the superior ganglion (rabbit, dog, cat) -- that is, when there was an abundance of glycogen stored in the liver. If the posterior lobe had been previously removed this stimulation failed to produce glycosuria. Greving (129) comments that without doubt the centres in the tuber have the function of regulating the sugar content of the blood and to maintain the osmotic pressure of the blood at a constant level. He states that this centre has been traced by Camus, Gournay and LeGrand to the nucleus paraventricularis in the tuber cinereum. Glycosuria was experimentally produced in fourteen guinea pigs: in each case the nucleus paraventricularis in the tuber cinereum was found injured.

VI. FAT METABOLISM AND ITS RELATIONSHIP TO THE TUBER CINEREUM

Bailey and Bremer (172) and Camus and Roussy (170) have denied any importance of the hypophysis for physiological regulation of fat metabolism and for the development of obesity. A long series of experiments by Raab (187, 188) show that pituitrin regularly exerts a definite effect upon the fat content of the blood. When large doses of pitressin or pitocin are injected subcutaneously, the blood lipoid phosphorus and neutral fat decreases. Under certain conditions, however, pituitrin will not affect the neutral fat content of the blood. If there has been pharmacological paralysis of the centres of the tuber cinereum or a mechanical obstruction of the tuber or transection of the spinal cord, the pituitrin has no effect upon blood fat. From these facts, Raab concludes that there

is a nervous pathway starting in the centre in the tuber cinereum, running through the cervical spinal cord and the abdominal splanchnics to the liver, and furthermore, that this nervous mechanism is necessary to promote the absorption and destruction of circulating fat by the liver. If Raab's conclusion is correct then any disturbance along this tract would lead to the retention of fat with resultant obesity.

The adiposity frequently associated with tumours of the anterior lobe of the pituitary is probably associated, as Cameron (45) remarks, with an interference with discharge of secretory products by pressure upon the posterior lobe. If a diminution of the circulating pituitrin leads to obesity, then theoretically, increased amounts of it should tend towards emaciation. Both Cushing (161) and Raab (188) show that this happens when animals are given prolonged treatment with pituitrin.

7. PARS INTERMEDIA OF THE PITUITARY

(a) Cytology.

The pars intermedia contains four types of epithelial tissue: 1, the parenchymal cell; 2, basophilic cells invading the pars nervosa; 3, evaginations of the intraglandular cleft; and 4, tubulo-racemose glands. Of the evaginations of the intraglandular cleft it may be said (Guizetti, 193) that they are quite shallow, that they are lined by a cuboidal epithelium and that they contain a secretion, mucin, produced by these cells.

It has been pointed out elsewhere (page 6) that in lower animals the parenchymal cells form central alveoli which are incomplete due to the small amount of connective tissue supporting them. Such incomplete alveolar arrangement does not appear to have been described in the human pars intermedia.

The human pars intermedia, according to Rasmussen (189) is represented by a thin sheet of cells frequently only a single layer in thickness. Their cytoplasm stains faintly basophilic. A number of these cells appear to project into the posterior lobe. The pars intermedia may be entirely unrelated to colloid. On the other hand, there may be a number of colloid vesicles presenting a variety of forms and staining reactions which penetrate the pars neuralis from almost any part of the pars intermedia. These vesicles are usually lined by a single layer of cells which not infrequently include ciliated and mucus-secreting cells. Such colloid-containing

structures, as Halliburton, Candler and Sikes (190) point out, are more numerous in the pars intermedia than in any other part of the pituitary.

The cells forming the pars intermedia of the rat, as described by Nukariya (21) are made up of a cell layer of from seven to sixteen cells in thickness. The cells present a massed, compact appearance. Nevertheless, the cells have interwoven among them sparse connective fibres and a fine network of blood vessels. The cells are rather large, seem to have a structureless cytoplasm which stains poorly. Only where the intermedia cells are in contact with the anterior lobe cells on the lateral border of the pars anterior is the cytoplasm more deeply staining and the cell border more distinct. The cells lining the cleft, which is incidentally filled with colloid, are flattened and have deeply-staining nuclei.

In regard to the staining affinities of the intermedia cells, Stendell (191) describes in all vertebrates fine basophilic-staining granules. Acidophilic granules are seldom found. Apparently only in Man do acidophilic and basophilic cells supplement the many chromophobic cells (Lewis and Lee, 192).

The rate of mitosis in the rat's pars intermedia at various stages has been described by Jackson (27). In the new-born rat, nine mitoses are seen in the pars intermedia for every section examined (as compared to sixty-two in each section of anterior lobe and seven in the case of the pars nervosa). At the end of three weeks only one mitosis is seen to each section.

The tubulo-racemose glands course across the pars intermedia tc invade the pars nervosa. These glands, most prominent in the human in the first four years of life, communicate by ducts with the intraglandular cleft. They are made up of acini lined by a single layer of pyramidal cells. According to Guisetti (193) these glands secrete a sero-albuminous material. Rasmussen (203) has found that the secretion contains mucin -- another factor supporting the belief of Lewis and Lee (192) that these glands are comparable to salivary glands. This seems plausible when one considers that the intraglandular cleft is the remains of an invagination of the buccal mucosa. According to these authors and Bucy (9) the route of excretion of these glands is entirely into the intraglandular cleft; neither they nor their derivatives, the invading basophil cells, are responsible for the colloid material found in the pars neuralis, the latter a view denied by Cushing (186) and others. There seems to be a great deal of confusion regarding the origin of the colloid cysts in and near the pars intermedia. On indirect evidence, Bucy (9) derives such cysts from degenerating tubulo-racemose glands, but this seems rather unlikely in view of the absence of mucinous material characteristic of these glands.

The basophil cells invading the pars nervosa have been the object of a great deal of study. Lewis and Lee and Guizetti are of the opinion that these basophil cells develop from the tubulo-racemose glands and as cords invade the pars nervosa even to the extent of piercing the posterior capsule. It seems that in the human they first appear in abundance as the glands

begin to disappear at the fourth year. Apparently the only difference between the basophil cells of the posterior and anterior lobes is that the latter are a bit larger. Cushing (186) (1933), on the other hand, thinks that in the process of ripening, the pars intermedia cells invariably become transformed into basophilic elements indistinguishable from pars anterior basophils. These basophils at times disgorge their cytoplasm in apocrine fashion but more often the whole cell body is cast off in a manner of a holocrine secretion. The highly vacuolated elements thus discharged become 'hyalinized' in their passage through the pars nervosa and for a long time retain the ghost of the swollen nucleus.

(b) Physiology of the Pars Intermedia.

That the pars intermedia elaborates a specific hormone seems now fairly well established. Knaus, Dreher and Clark (194) in 1925 and Stehle (195) in 1934 showed that the melanophore dilator principle is distinct from the oxytocic and pressor principles. Dreher and Clark (196) had come to that conclusion two years earlier when they showed that the principle containing the oxytocic and pressor principles differed from the melanophore principle. More recently, Zondek (197), who quotes Swingle, Allen and Atwell, states that all authors agree that the chromatophorotropic substance is produced in the pars intermedia. Analyzing the hypophyses of cattle, Zondek and his associates found that the pars intermedia contains forty times as much of this hormone (intermedin) as the pars anterior per gram of tissue, and eight times as much as the pars nervosa. Similarly,

Valso (139), studying the secretion of intermedin in the whale, in which the pars nervosa and pars intermedia are separate, demonstrates intermedin in the pars intermedia but not in the pars nervosa.

It has been mentioned immediately above that intermedin is found in the pars anterior. Whether it arises there or somehow seeps into that lobe from the intermediate is still open to some question.

8. OCCURRENCE OF CILIATED EPITHELIUM IN THE INTRAGLANDULAR CLEFT OF THE HYPOPHYSIS

The earliest reference in the literature to ciliated cells in the intraglandular cleft was made, apparently, by Paremchko (198). In 1867, he stated that occasionally in Man the intraglandular cleft is lined by a ciliated epithelium. A few investigators (among them Joris, 199, Erdheim, 200 and Kiyono, 202), since that time, have made the same observation. (It may be mentioned at this point that it has been stated that the intraglandular cleft disappears in the adult human pituitary. According to Brander (42) this statement is misleading. It is true that the anterior and posterior walls fuse over considerable areas leaving what appears to be isolated cysts containing colloid. Serial sections show that most, if not all, of these cysts retain communication with one another. Furthermore, Bailey (36) states that pathological retention will reestablish the cleft at any age. Cysts form by a degenerative change taking place in the cells lining the intraglandular cleft, a process which may be followed in all its stages.) As to location in the cleft of the human of ciliated cells, they have been found on the pars intermedia aspect by Henle (201), by Bailey (121) and by Kiyono (202). Rasmussen (203) found ciliated cells in the intraglandular cleft of the human pituitary quite by accident and then examined with great care a hundred more pituitary glands and was unable to find them. Of his two human cases, one was a twenty-eight year old man. The cilia were present over the posterior and lateral portion

of the pars anterior. The other was that of a seventy-one year old man: cilia, about 15μ in length, were present for the most part on the posterior aspect of the pars anterior at the pole furthest removed from the infundibular attachment. A few cilia were found also on the wall adjoining the pars intermedia.

Of those who have made a point of looking for cilia in the intraglandular cleft, Boyce and Beadles (204) were unable to find cilia after careful search in over a hundred pituitaries. Guizetti (204a) found cilia in the intraglandular cleft in only three of fifty-four human pituitaries. Bryant (205 appears to have been more successful: he states that he has found a ciliated epithelium in the intraglandular cleft of every human pituitary (but he does not state how many!) in which he has looked for them except in those in which the parenchyma was almost completely replaced by connective tissue. He describes a lining of tall, ciliated, columnar epithelium present for the most part over the posterior aspect of the cleft but also over the anterior aspect. These 'sensory' ciliated cells are interspersed with bipolar cells which have their nuclei towards the periphery; whereas in the ciliated cells, the nuclei are near the base. He likens this epithelium to the sensory elements of the <u>maculae acousticae</u>. [It may be mentioned, by way of digression, that non-ciliated sensory stratified cylinder epithelium has been found in cats and dogs (Gentes, 206), in the rat (adjacent to the pars intermedia) (Cajal, 207), and in the guinea pig (Gemelli, 208).]

Ciliated cells have been found in the pars intermedia of the rabbit by Lothringer (19) and in the guinea pig by Vanderburgh (209). The latter found them on the side of the cleft adjacent to the pars anterior but never on the opposite side. Most authors have described ciliated cells in cysts (in or near the pars intermedia) which are presumably outgrowths of the intraglandular cleft. Since the lumina of these cysts are invariably found to be continuous with the lumen of the cleft, Vanderburgh concludes that the cysts and the cleft have a common origin. The contents of both cysts and cleft appeared to be a mucus-like material. Collin (210), describing ciliated-cell-lined cysts in the chromophilic part of the fowl pituitary, states that the contents react like mucin and not like colloid. Likewise, in rats and one marsupial (la Gamba), Martin (211) found ciliated cysts whose cells were excreting a mucus-like material.

There is one account in the literature of the growth of ciliated cells from the pituitary in tissue culture. Lewis and MacNeal (212), in growing the glandular portion of the pituitary of various fishes (particularly the dogfish and the skate), observed that epithelial cells, often ciliated, grew out. The cilia continued to beat for eight to ten days.

The significance of these ciliated cells is not clear. Rasmussen (203) could not agree to Bryant's (205) view that they represent a sensory epithelium. Tello (213), in his study of the epithelial cells lining the residual cleft in the human pituitary, shows that nerve cells ramify among the basal ends of the cells lining the residual lumen and the associated

vesicles and interprets his findings as suggesting a differentiated sensory mechanism of some unknown function. He makes no mention of ciliated cells. Rasmussen (203) suggests that ciliated cells of the hypophyseal cleft represent abnormal migration early in development of embryonal elements from the naso-pharynx, where ciliated and mucus-secreting cells are normally present.

9. TRANSPLANTATION*

(a) Reactions to Various Types of Transplantation.

Loeb (216) describes in detail how connective tissue, blood vessels and lymphocytes of the host behave, each in its own specific way, towards auto-, syngenesio-, homoio-, race or variety, and heterotransplants. Defining his terms, he means by autotransplantation, the transfer of a tissue to a different place in the same individual; by syngenesiotransplantation, the transfer into a closely related individual; by homoiotransplantation, the transfer into another, not directly related, individual of the same species (also called isotransplantation); and by heterotransplantation, the transfer into an individual belonging to a different species or class of animal. These various types of transplantation were done by Loeb on guinea pigs and rats; as a routine, thyroid and cartilage were transplanted simultaneously from donor to host. His results follow.

Autotransplantation: After transplantation of the thyroid gland, the peripheral part remains alive, while the insufficiently nourished central portion becomes necrotic. A supply of blood vessels soon develops in the living peripheral portion as well as in the adjoining portion of the central

* The attempt has been made to furnish a complete bibliography on the subject of transplantation of tissue cultures. On the other hand, subject matter of transplantation of ulcultured-in-vitro tissues will be drawn from Loeb's excellent review(of his own work and that of others, brought up to 1930. Original sources have been consulted where elucidation and further detail seem pertinent.

necrotic zone. This latter is absorbed by the vessels in about three weeks. Fibroblasts grow into the graft only in moderate amount. More and more the transplant assumes the character of the normal thyroid and lives permanently.

Homoiotransplantation: In this case, the fibroblasts grow in greater numbers into the central necrotic part where it forms a dense mass of fibrous tissue. In addition, the fibroblasts grow around the living acini of the peripheral zone, forming a dense fibrous tissue around them which presses on the acini and causes a gradual obliteration of their lumina. At about the seventh day a characteristic change takes place: there develops a remarkable infiltration of the transplant by lymphocytes which surround and invade the living tissue, often overwhelming it, and thus helping to destroy it. In autotransplants, on the contrary, there are found only here and there a few lymphocytes which tend to disappear. In this type of graft, vascularization is less than in autotransplants. Usually the homoiograft is destroyed in twenty to thirty days following transplantation, but in some instances, in rats, it survived this critical period and was at least partly preserved after about two months; presumably this occurred in related animals. Homoiografts of cartilage behaved much the same way as did the thyroid, the former being more resistant to invading cells.

Transplantation into Different Varieties: If instead of grafting thyroid from, say, one white rat to another such grafting is done from a white rat to a hooded rat or to a certain inbred strain of yellow rat, very much the same result

is obtained as after homoiotransplantation except that the reactions between different varieties are more accentuated. After twenty days such tissue is completely destroyed.

Syngenesiotransplantation: In the first period following transplantation between brothers and sisters or between parents and children the reaction is similar to that towards an autotransplant. After about forty days disharmonies between the transplant and host develop and it is now especially the lymphocytic reaction which manifests itself. The lymphocytes first enter the septa between the acini and then invade the acini themselves and in the end overwhelm them. At last the transplant may almost resemble a lymph node, so densely is the tissue invaded with lymphocytes.

Heterotransplantation: In transplanting tissues into another species the results differ in various respects from those obtained after homoiotransplantation. In the case of tissues not very resistant, such as thyroid, kidney, bone marrow and even skin, the direct injury to the transplants, inflicted evidently through the body fluids of the host, is so great that they show marked degenerative changes at seven days. This happens before connective tissue and the lymphocytic reaction have had a chance fully to develop. Moreover, polymorphs, not seen in other types of transplantation, appear in company with the lymphocytes in the later stages. In the majority of tissues mentioned, the injurious effects of heterotransplantation are always so intense that there is little chance for the observation of such differences in reaction as may be graded in accordance with the nature of relationship between donor and

host. It may be stated here that killed homoiografts do not elicit a lymphoid and connective tissue response on the part of the host, whereas killed heterografts do.

Multiple Transplantations: If different kinds of tissue are transplanted simultaneously from one donor into the same host, all the pieces behave in a corresponding way; they show the reaction characteristic of the relationship between host and donor. On the other hand, if there are transplanted into the same host, at the same time, pieces from different donors, the reaction against the tissue of each donor, on the part of the host, is in accordance with the specific relationship between the particular donor and the host; it is a matter of dealing with localized reactions, which differ around each piece, in accordance with the character of the individual donor.

(b) Basis of the Bodily Reaction against Transplants.

Loeb raises the question as to whether one is dealing with primary substances (syngenesio-, homoio-, or heterotoxins) given off by the graft, or whether such substances act as antigens and call forth secondary (immune) substances, which are in turn responsible for the reactions. Experimental work indicates that a direct toxic reaction does very probably take place in homoiografting, and that, in addition, it calls forth the production of secondary immune substances, which work in the same direction as the primary substances and intensify the effect of the latter. Loeb approached the problem of what constitutes the background of the organismal differential* by

*Erdmann(239) has defined the organismal differential (or individual differentiation) as the sum of all differences of a chemical or physical or other nature, in short the sum of all structural differences in an individual on comparison to another of the same species.

means of successive transplantations of the same kind of tissue (thyroid) into the same host. If one were dealing with secondary (immune) reactions, it should be expected that the homoioreaction following the second transplantation, at a time when the homoioreaction against the first graft is already manifest, would appear much more promptly, because in this case the immune substances are already on hand as the result of the first transplantation. Loeb could find no acceleration of the reaction against the second transplant. He arrives at the conclusion that the primary type of reaction is an essential factor in the fate of the homoiotransplant but was not prepared to say what influence the secondary type of reaction plays in grafting. Fichera (217) found in transplanting homoio-embryonic tissue at intervals in the same rat that an immunity could be produced. In twenty-six rats he planted successively five homoio-embryonic grafts (he does not mention, so far as I am aware, what type of embryonic tissue he used), each graft being transplanted after the previous one had disappeared. The first graft, which sometimes took as long as six months to be absorbed, represented the vaccinated material which it was thought brought about an altered response on the part of the body to subsequent transplantation. With each new transplantation the grafts survived a lessened time so that when the fifth grafting was done it was completely resorbed in an average of three weeks. He believes, on the basis of this work, that an immunity is developed against homoiografts. Fleischer (218) found that in immunized animals, the host connective tissue invades the

transplant less actively than in non-immunized animals. In the immunized animal the graft seemed to be more slowly absorbed despite the increase over the immunized animal of 'leucocytic' infiltration. The parenchymal cells of the transplant (kidney epithelium) are little or not at all affected in immunized, as compared to the non-immunized animals. Fleischer immunized the mouse against mouse kidney by four intraperitoneal injections of freshly removed and ground kidney given at intervals of two days. The pieces of kidney were implanted twelve to fourteen days after the last injection.

It is conceded that the lymphoid cell is associated with the resistance mechanism. It seems off hand that the lymphoid cell assumes its protective function at about the same time that organismal differentials assert themselves. No doubt, the success of grafting upon embryonic structures is due to a lack of development of a differential on the part of the embryonic tissue. In such embryonic transplantations no lymphocytic reaction is elicited. When rat tumour graft, for example, is transplanted into chicken embryos the cellular reaction is much delayed and does not become evident until the nineteenth day of incubation (Murphy, 219). It is significant that the cellular reaction about and subsequent disintegration of the graft are hastened by the implantation into the embryo of a fragment of spleen from an adult chicken. These findings indicate unmistakeably, according to Murphy (220), that the lymphoid cell reactions are associated with the resistance mechanism; the importance of this association is emphasized

by the fact that adult animals deprived of their lymphoid tissue by repeated exposure to suitable small doses of x-rays fail, like the embryo, to destroy foreign tissue grafts. These latter not only continue to grow actively, but may be transferred repeatedly to other individuals which have been prepared for their reception by x-ray treatment.

(c) Transplantation of the Pituitary Gland.

There does not appear to be any record in the literature concerning transplantation of pituitary tissue cultures. Comparatively few have grafted fresh pituitary tissue. Numerous workers in endocrinology (among them Smith and Engle, 221, Evans and Simpson, 222, Emanuel, 223, and Guyénot and Pouse, 224) have implanted fresh pituitary into the subcutaneous areolar tissue of rats and mice for purposes other than that of grafting. They found that single implants produced little or no physiological effect. The necessity for repeated implantation indicates that the implants survived but a short time. It is safe to assume that the effect was comparable to that following administration of an amount of extract equivalent to that contained in the implant.

Crowe, Cushing and Homans (225) transplanted pituitary gland for a different purpose, namely, as a therapeutic measure to compensate for pituitary deficiency in dogs which had had their pituitaries ablated. As a rule, untreated hypophysectomized dogs died three days following the operation. Grafting of pituitary into these dogs did not produce a cure but it did postpone the fatal issue. The cerebral

cortex proved to be the most satisfactory site for transplantation. Grafts grown in the brain were examined a month after transplantation when the animals were sacrificed. Invasion of the graft by connective tissue was not pronounced and there was histological evidence in both anterior and posterior lobes of retained physiological activity. (When pituitary gland was transplanted into the bone marrow and the rectus muscle the dogs' lives were prolonged to about twelve days.) With cortical transplantation of the entire gland a persistent polyuria developed. A graft of both the anterior and posterior lobes seems to be necessary in the production of polyuria, for it does not occur after cortical grafting of the posterior lobe alone. To prove the point that it was the pituitary graft which was responsible for the polyuria, Crowe and his associates succeeded in stopping the polyuria by the surgical removal of the graft.

It is difficult to determine how much importance to attach to Crowe, Cushing and Homans' results. The weak point is in the shortness of life of the dogs after hypophysectomy. Since that time hypophysectomized dogs have been shown to live for a period of months. Could it not possibly be that the dogs which lived longer (presumably as the result of transplantation) were not subjected to so much operative trauma and thus survived longer? Cushing (76) reports a case of pituitary transplantation into the brain in Man which might support his work in dogs. This patient had had an hypophyseal cyst removed and was thereafter treated for the ensuing profound torpor by consecutive daily injections of "boiled whole gland

extract in a dosage representing two grains of the dessicated preparation". Owing to the increasing soreness from the injections, however, they could not have been continued for a much longer period, and finally the hypophysis from a new born child was grafted in the subcortex of the temporal lobe at the site of original decompression, and it was found possible to discontinue the injections without the patient's relapsing into his former somnolent state. Cushing does not state how long such a transplantation was efficacious. The fact that the extract was boiled, a procedure which it is recognized destroys thyreotropic, ketogenic and growth principles, leaves one in a quandary as to how much importance to attach to the pituitary grafting.

In order to study early embryonic differentiation of the chick hypophysis Stein (226) did chorio-allantoic grafts of chick pituitary. She isolated the hypophyseal ectoderm at a time when there was no morphological indication of the existence of the pituitary and transplanted it into the chorio-allantoic membrane. The donors ranged in age from the pre-somite stage to a stage five days after incubation. When transplanted in such a fashion, the pituitary anlage in three of fifteen cases differentiated in a normal manner as fairly compact and branching anastomozing cords in a manner similar to that of the normal embryo of the same age. Differentiation into acidophils and basophils did not take place in chorio-allantoic pituitary grafts -- a result quite to be expected, since such differentiation did not take place in the gland of the chick of the same age. Stein thinks that the

differentiation in these three cases was due to the retention in the anlagen of fairly normal tissue relationships -- and that the lack of differentiation in the other twelve cases was due to incomplete removal of all the tissue which goes to influence such anlagen. The main conclusion at which she arrived, but which she herself questions, is that 'hypophyseal' tissue does not differentiate in the absence of brain tissue. In other words, the anterior lobe depends upon the latter for its differentiation.

A recent work (1935) by Gardner and Hill (227) presents evidence that the most successful site for transplantation of pituitary gland is the testis. Their syngenesiografts of a single adult pituitary into the testis of twenty-two to fifty day old mice proved superior to transplantation into the brain. The mice employed were from highly inbred strains, littermate brothers and sisters being used as donors. Of the thirteen animals transplanted in this way, eleven showed healthy-appearing pituitary grafts three to twelve weeks following transplantation when the autopsies were done. The anterior lobe was found well vascularized and appeared normal in arrangement. The pars intermedia persisted in most of the grafts but not as well as did the anterior lobe. The pars nervosa consistently degenerated. It remains to be seen whether these results in syngenesiografting into the testicle can be duplicated by homoiografting into that site.

(d) Rôle of Preexisting Deficiency in Grafting.

In 1909 Halstead (228) found that autografts of parathyroid grew only when there was a deficiency on the part of the host. Autografts of parathyroid planted into the thyroid and under the rectus sheath were successful in 61% of the cases in which a deficiency greater than one-half had been created. Furthermore, parathyroid transplanted in excess of what was urgently required did not live. This theory of the necessity of a preexisting deficiency has since become a working hypothesis in grafting. His homoictransplantations were entirely unsuccessful. It would seem that autografting does not present the most convincing method of testing out this theory of deficiency, for in autografting it seems unlikely that one could transplant more than is needed. Moreover in Halstead's cases, tetany was not produced so that he had no way of gauging the amount of functional deficiency. His law does not necessarily hold for syngenesiografting. This has been demonstrated by Gardner and Hill (227) who successfully grafted pituitary gland into the testis of non-hypophysectomized mice.

(e) Blood Groups and Transplantation.

According to Loeb (216) the vast amount of opinion rejects a connection between blood groups of host and donor in homoiografting of skin. This he explains on the grounds that the individuality differential is determined by all or at least by a large number of the genes of the individual, while the blood groups depend upon only a few genes. At best, he feels, these genes may contribute in a small way to the result of

transplantation, but they cannot be a decisive factor. It would seem that these theoretical considerations do not hold in practise in regard to skin grafting. Shawan (229) applied the principle of blood grouping to skin grafting in twenty-six human cases and came to the conclusion that skin grafting obeys the principle of blood grouping, in the same manner that it does in blood transfusion. Homoiografts obtained from donors of the same group or from Group IV donors became permanent takes. Other successful takes were in those of Group I who grew permanent skin from donors of all of the four groups. Other combinations of host and donor were unsuccessful in homoio skin grafting. Dobrzaniecki (230) was not as specific, but stated that the life of the homoiotransplant of skin can be prolonged by paying attention to blood groups in selecting donors.

(f) Site of Transplantation.

Experimental evidence indicates that the various tissues of the same body each have their individual differentials. The ocular lens seems to possess the same differential throughout different species. Fleischer (231) has shown that with homoiotransplantation of the iris and cornea the characteristic homoioreaction of lymphocytes and connective tissue takes place in the host. But the homoiotransplanted lens is not reacted upon any more than the autotransplanted lens. It appears probable that this difference in reaction is due to a biological identity of the lens substance in all animals of a species just as there exists a biological identity of lens substance in different species.

The brain seems to possess a less organized differential than most other tissues of the body since homoiotransplantation there of various tissues is, according to most observations, more successful than elsewhere. The main reason lies in the paucity of lymphocytic infiltration into the brain. A number of investigators have done transplantations into the brain. Shirai (232) employed the brain as the locus of heteroplastic tumour transplants, in which situation, according to him, grafted tissue grows as readily in an alien as in an homologous host. Murphy and Sturm (233) found that transplantable mouse tumours grew actively when inoculated into the brains of rats, guinea pigs, and pigeons, whereas subcutaneous or intramuscular grafts in the same animal failed. The growth takes place only when the grafted material lies entirely in the brain tissue; if it comes into contact with the ventricle a cellular reaction takes place with resultant destruction of the graft. And, too, if autologous spleen is planted in the brain with the homoiograft, the growth of the latter is entirely inhibited. Mice which are highly immune to subcutaneous transplantation of mouse cancer show no resistance to such tumours when the inoculation is made into the brain.

The work of Shirai, Murphy and Sturm, above described, was done with hetero-tissue. Siebert (234) pursued transplantation of homoio-tissue into the brain. He compares the effects of cortical transplantation of auto- and homoio-thyroid gland. Employing the guinea pig, he found at autopsy done at periods of from twenty to one hundred and twenty days after transplantation that homoiotransplants differed from autotransplants in

that the acini of the former were more irregular in size and shape and that fewer acini contained colloid. Lymphoid cell reaction in the brain was not marked; it was present to a much less degree than after transplantation into subcutaneous tissue. A marked increase of glial tissue about the homoiotransplant was noted. In fact, there seemed to be as much of a connective tissue response on the part of the brain as is called forth by subcutaneous connective tissue grafting.

(g) Adaptation on the Part of the Host to Homoiografts.

According to Erdmann (235) many authors have found that in lower animals there is a greater healing capacity after homoiotransplantation than after autotransplantation; this applies to such animals as the hydra and the earth worm. The absence of an individuality differential in insects is shown, according to Erdmann, in the experiments of Koppanyi, Meisenheimer and Kopec-Kopec who were able to exchange eyes, heads and ovaries from insect to insect without producing a functional disturbance.

It has been stated that the individuality differential is more marked the higher the animal is in the scale. Even in the guinea pig and in the rat there is evidence, according to Loeb (216), that the host may gradually adapt itself to certain homoiotransplants. He has observed that "after homoiotransplantation of cartilage, not only no accumulative effect, in the reaction of the host against the transplant, takes place with the increase of time during which the transplant is kept in the host, possessing an individual

differential which differs from that of the transplanted cartilage, but, on the contrary, in the course of time a diminution in the number of lymphocytes may be found around the graft. It appears as if a gradual adaptation occurred between the transplanted tissue and the host".

On the whole, such spontaneous adaptations between host and transplant are unusual. However, it appears that grafts can be modified by being subjected to heat before transplantation or by growing them in vitro for some time before transplanting them into the host. It appears that after such treatment, the homoiograft is altered so that it is adapted either temporarily or perhaps permanently for growth in the new host.

As to modification by heat, Siebert (236) has shown that if the homoiogenous cartilage and perichondrium are subjected to the temperature of 47°C. for thirty minutes (a temperature which does not injure the perichondrium but kills the cartilage) the graft survives for a greater length of time than does the unheated cartilage and perichondrium. (Incidentally, the thermal death point of large mononuclear cells and sarcoma cells is 44°C. for thirty minutes (Friedgood, 237)). Lymphocytic and connective tissue reaction in the host to the tissue heated to 44°C. were absent. Cartilage grafts subjected to lower degrees of heat before transplantation called forth the usual homoioreaction.

In 1912 Cushing (76) wrote, "In order to insure the greatest probability of a successful implantation, it would

seem that the best plan of procedure would be, after the method of Harrison, Carrel and Burrows, to secure a growth in vitro of the tissues to be implanted. When a gland is finally secured which can be cultivated in the plasma of the prospective host*, the growing fragments may then be injected into the most favourable tissue -- with a probability of 'taking'". This proposed procedure was first carried out, as far as I can determine, by Rhoda Erdmann (238). In 1918 she reported having cultivated heart muscle of a twelve day old chick embryo in a medium of chicken plasma for a period of three days, and then transplanted the cultures subcutaneously into the chicken. She does not compare its survival with that of non-cultivated embryonic heart, but states that those grafts about which she injected growth-promoting extract appeared larger at autopsy than those not so treated. The cellular reaction about the graft was that usually found in an uncultured homoiograft. Again in 1922 (235) and in a more complete report in 1927 (239), she carried the work further by the homoiotransplanting of skin in frogs. At the same time she demonstrated that skin grown in vitro is more resistant to bodily defenses than that not so explanted. Skin grown in homologous plasma was successfully transplanted from R. esculenta to R. temporaria, from R. arvalis to R. esculenta, and from R. esculenta to Bufo communis. She was of the opinion that the further the individuality differentials were between donor and host, the longer it was required to grow the donor's tissue in vitro before adaptation could take place.

*Underlined by W. H.

Gassul, a pupil of Erdmann's, repeated in substance in 1922 (240) and in 1923 (241) the work of Erdmann. The skin of the donor was grown in various media. Of the plasmata employed, namely frog, rat, chicken and human, only those grown in frog plasma were definite 'takes'. After a 'certain time' the skin fragments were removed from the plasma clot and placed directly upon the wound surface; in ten to twenty days they had fixed themselves. In three to five days they had healed. Gassul raises the question as to whether the success was due in the case of the frog to a condition of individuality differential which does not happen to be as prominent in the frog as in other animals, or whether the tissue had actually been modified by growth in vitro. He favours the second since there was no 'take' when the skin had previously been grown in other than frog plasma. In this connection, Taube (242) showed in 1921 that Rana do not tolerate the usual homoiotransplantations, but that, on the other hand, the Triton do. Such differences in the individuality differential are also seen in the Urodels and the Anuren, the latter being the more outspoken as may be demonstrated by the reaction of each to homoiotransplantation of skin.

Stone, Owings and Gey (243, 244, 245, 246, 247) were the first who successfully adapted homoiografts to mammalian hosts by subjecting the tissue to growth in vitro in the host's plasma and serum. Using the dog first, and then Man, they grew homoio-thyroid and parathyroid tissue over a period averaging ten days and up to thirty, in a medium composed of

the plasma and serum of the host, a balanced salt solution and beef embryonic extract. From the tissue cultures, sheets of cells were removed and transplanted into various locations in the host - in the adrenal, spleen, under the rectus sheath, in bone cavities, in subcutaneous tissue and so on. They found that the loose areolar subcutaneous tissue, where there was a proximity to a good blood supply, offered the best advantages for growth, since there was no pressure upon the graft as presumably occurred when the graft was placed under the rectus sheath and there was no hemorrhage to stifle the graft as occurred after transplantation into a vascular organ.

The first year and a half of thyroid transplantation in the dog by this method resulted in but one success out of more than a hundred attempts. Then a new series was started and of eleven dogs, five showed definite, unquestionable, long-standing 'takes'. Stone shows photomicrographs of thyroid homoiotransplants removed from the animal as long as one hundred and forty-eight days after grafting (244) and also those of parathyroid grafts (247). In Dog. P.O. 2 (244) the right thyroid was removed in order to create a deficiency and the animal was transplanted with a homoiograft which had been grown in autoplasma for a period of thirty days. One transplant was made into the left groin and the other under the sheath of the rectus. The dog did not show any signs of thyroid deficiency; no laboratory tests were done. Eighty days after transplantation the grafts were removed for examination. Sections of the tissue showed no persistence of the thyroid graft in the muscle, but in that taken from the

groin a definite mass of thyroid tissue, five to six centimeters in diameter, was found. The histological picture varied in places from that of small, almost adenomatous-like alveoli to large, irregular alveoli. The donor and the host were not matched or grouped and nothing was known of their relationship. Stone comments that he cannot therefore be sure that it was not an accidental successful 'take' due to relationship, as sometimes apparently occurs.

Stone and his collaborators (244) report grafting of thyroid and parathyroid in ten human cases, five thyroid and five parathyroid. Case I was a woman who had tetany following a thyroid operation. Blood calcium averaged about 5.6 milligrams per 100 cubic centimeters of blood. Eighteen months after operation she was transplanted with human parathyroid tissue cultures. Her blood calcium rose gradually to 9.8 milligrams. A year after transplantation her blood calcium was still up and she showed no signs of tetany. Another parathyroid case, spontaneous in origin, was to all appearances cured. The patients grafted with thyroid had not gone a sufficient length of time to warrant conclusions.

Does growth in tissue culture modify the individual differential of the donor cells and if so how is it brought about? No satisfactory answer can be furnished at present. Loeb (216) cites the work of A. Fischer which indicates that no real change in the differential of the cells takes place by prolonged growth in tissue culture. Fischer found that rat fibroblasts which had grown previously for a period of two and a half years in chicken plasma showed the same

sensitiveness toward immune cytotoxin produced against rat tissue as did normal rat tissue. The rat cells had therefore retained their own species differential, notwithstanding their long continued growth in chicken plasma. It would be interesting to know whether these findings would apply also to cells grown in homoioplasma and tested for the possible alterations of its homoiotoxins.

One might expect at first glance that the principles of homoio- and heterotransplantation *in vivo* would apply to tissues grown *in vitro*. Such is obviously not the case. It is well recognized that tissues grow equally well in homoio- and heteroplasma as they do in autoplasma (Fischer, 248). Loeb (216) suggests as reasons for this phenomenon the absence of host tissue and the lack of a continuous current of the host's circulating blood -- the means by which homoiotoxins are constantly being renewed *in vivo*.

10. TRANSPLANTATION OF RAT PITUITARY TISSUE CULTURE GRAFTS*

(a) Experimental.

The pituitaries were removed from hooded rats of from one to sixty-five days of age, and were placed in a Petri dish containing isotonic salt solution. By means of the dissecting microscope anterior lobe was separated from the posterior. The anterior lobes were cut into fragments about 0.5 square millimeters in size and were planted within a short space of time upon cover slips or into Carrel D-3 flasks. In a large number of cases the entire gland was cut into fragments and explanted.

The tissue culture technique was altered from time to time in the attempt to find a medium in which mature pituitary would readily grow, the medium, at the same time, approaching, as closely as theoretically possible, the conditions appertaining in the rat's body. It was borne in mind, particularly in regard to making up the salt solution, that adaptation to the body tissues was of more importance than an adjustment to the conditions of the blood stream alone.

Chicken plasma and rat homoioplasma were used, each independently and combined, as a basis of the culture media. Sixteen rats were grafted with anterior pituitary cultured in this manner. These plasmata were then supplanted by autogenous plasma. The blood of the rat to be grafted was obtained by

* This transplantation work was done in collaboration with Dr. Evelyn Anderson of the Dept. of Biochemistry, McGill University.

cardiac puncture four to ten days before transplantation. Immediately preceding the operation the rat was given an intraperitoneal transfusion of about three cubic centimetres of blood. The blood from which the plasma was obtained was diluted 1:6,000 to 1:10,000 with heparin. The serum was heparinized or used unaltered. The other two constituents of the culture medium were isotonic salt solution and embryonic extract. Stone's balanced salt solution* was used in about half of the experiments and a modification of that, which Van Slyke** (249) suggested, was employed in the remainder. According to Van Slyke, this solution has an osmotic pressure sufficiently close to that of plasma to obviate shock from osmotic change when the tissues are transplanted into the body. Furthermore, it gives a physiological pH when in contact with air containing 6% of CO_2. It may be stated here that the most satisfactory growths were obtained in media containing the modified Van Slyke solution. To this solution was added phenol red indicator in the final concentration of

	* Stone's Balanced Salt	** Modified Van Slyke's
Na Cl	8.000 Gm.	6.800 Gm.
KGl	0.372	0.372
$NaHCO_3$	0.500	2.000
$CaCl_3$ Anhyd.	0.203	0.203
$MgCl_2.6H_2O$	0.209	0.209
$Na_2HPO_4.2H_2O$	0.143	0.143
KH_2PO_4	0.052	0.052
Glucose-d	1.000	1.000
Dist. H_2O	1000.	1000.

It is necessary to filter these solutions through a Berkfeld filter.

0.005% so that the pH could be adjusted to 7.4. This latter was done by allowing a mixture of 5% CO_2, 21% O_2 and 74% N to flow through the solution until the desired pH was obtained. Rat embryonic extract* was employed in contrast to the beef embryonic extract used by Stone.

The proportions of the various constituents of the medium which was found to be most satisfactory were as follows: for cover slips, in terms of cubic centimetres, plasma, 0.04; serum, 0.015; salt solution, 0.02; and embryonic extract, 0.03, and for Carrel flasks, plasma, 0.5; serum, 0.2; salt solution 0.3 and embryonic extract, 0.3. To the Carrel flasks was added a supernatant fluid containing 0.1 cubic centimetre of serum and 0.3 cubic centimetre of salt solution which was then adjusted to a pH of 7.4. It will be noted that the total amount of salt solution was considerably less than employed by Stone, Owings and Gey in their medium.

The cultures were grown on an average of from five to seven days and were transferred two or three times, or more if liquefaction of the clot was unusually rapid. In the Carrel flasks the supernatant fluid was changed daily after a twenty minute washing of the clot. They were incubated at a temperature of 38.5° C. Sheets of epithelial cells interspersed

*Rat embryos, preferably 15 mm. in length, are finely divided by crushing through a record syringe. The material is placed in a test tube to which two times its quantity of salt solution (Stone's or Van Slyke's modified) is added. The mixture is allowed to stand at room temperature for one hour and then centrifuged for thirty minutes. Supernatant fluid is pipetted off and frozen twice; it is then ready for use during the course of seven days.

with connective tissue cells began to grow out in six to eight hours from pituitary fragments in rats under seven days or so of age. At the end of thirty-six hours, growth was, as a rule, abundant. Pituitaries removed from older rats grew well but only after two or three transfers. Growth was best in those fragments planted close to the edge of the clot, where, no doubt, the oxygen supply was better.

The pituitary cultures were grafted into hypophysectomized rats. Male rats, three months of age, were used for this purpose. Practically all of them were albino rats of the Wister strain. In some of the rats it is not known precisely how pure the strain is because of the interbreeding with hooded rats. A few rats of the hooded variety were used as hosts. As sites of transplantation, the axilla and groin and supra-thyroid areolar tissue were selected. A small incision was made under light ether anaesthesia and into the areolar tissue a variable number of fragments and epithelial sheets (from six to some two hundred) were either pipetted into the wound in salt solution or planted singly. In ten of the eighty-nine rats grafted there were two successive transplantations; in two rats there were three. Special care was taken to remove all the clot surrounding the fragments before they were transplanted; otherwise the cells would be smothered. In a few cases the autogenous plasma was obtained and the pituitary cultures started before the recipient had been hypophysectomized, so that the tissues were ready to be grafted as early as two days after hypophysectomy. At this stage the

animals had only begun to suffer from the disabilities associated with pituitary loss. This factor may be of importance since the state of body nutrition may determine the chances of the graft to 'take'.

Frequent weighings were made in the animals during the course of the experiment. At the end of the observation periods varying from forty-five to one hundred and nine days the animals were autopsied and the testes, thyroid and adrenals examined. The sella in every case was studied by means of serial section for remaining pituitary tissue. Only those animals in which pituitary tissue was not found in the sella are included in this series. Serial sections of the areolar tissue into which the transplants had been made were examined for pituitary grafts. This serially sectioned material was fixed in Susa and stained by the Cleveland and Wolfe method in which hematoxylin, acid fuchsin, aniline blue and orange G were used.

(b) Results.

The data covering the effects of transplantation of pituitary-tissue-culture grafts will be considered in the light of histological and physiological changes in the transplanted animals as compared with such changes in the untreated hypophysectomized controls. It is to be expected that a successful graft of pituitary tissue will repair the disabilities which occur after the pituitary has been removed. The evidence of repair will be presented under the following headings: I, changes in body weight, and II, changes in the

histological picture of the adrenals, testes and thyroid. Furthermore, in order to determine the importance of the factor of growth *in vitro* prior to transplantation, a comparison will be drawn between the effects of transplantation of fresh pituitary tissue in contrast with that grown previously in tissue culture.

A group of eighty-nine rats were transplanted. Of these only thirty-one could be considered satisfactorily under the conditions of the experiment because of casualties. None of the rats grafted with pituitary grown in chicken plasma showed histological or physiological restoration. Seventeen rats of this group of thirty-one showed definite improvement over the untreated hypophysectomized controls. It was felt that results could be significant only after a period of forty days following transplantation, a time at which non-cultured homoiotransplants no longer survive. (Loeb, 216, states that usually the homoiograft is completely destroyed twenty to thirty days after transplantation.) Consequently, the fortieth day was chosen as the arbitrary point at which effects could be assigned to survival or growth of the graft. The animals were allowed to live from forty-five to one hundred and five days after transplantation before autopsy was done.

I. Effect of Grafting of Pituitary Cultures upon Body Weight.

A tendency to maintenance of body weight after grafting of pituitary cultures into hypophysectomized rats has been taken as evidence of replacement of pituitary function. The data showing changes in body weight in the grafted rats as compared with the untreated controls is shown in Table 1. The

loss of weight between the time of hypophysectomy and transplantation was the same in both experimental animals and the ten controls. It should be pointed out that the maintenance of transplanted weight was felt to be significant only in animals transplanted before 16% of original body weight had been lost, since untreated hypophysectomized controls also maintain their weight for many weeks after losing 25 or 30% of body weight. The rats were transplanted at various times from the sixth to the twenty-seventh day after hypophysectomy. After the tenth day the untreated hypophysectomized rats continued to lose weight rapidly whereas the transplanted animals showed a less pronounced loss of weight.

Forty-five days after transplantation the thirty-one grafted rats had lost on an average of 24% of their original pre-hypophysectomy weights while the controls had lost 32% (Figure 1). When the ten best animals were selected and a comparison made to the ten controls the difference is more striking (Figure 1), the transplanted rats having lost 18% of pre-hypophysectomy weight as compared to 32% loss in the controls. Collectively, then, after transplantation there was a diminution in the amount of weight loss occurring in the hypophysectomized rat. In certain individual cases this checking of weight loss was rather striking. Rat No.37 was the outstanding example of weight maintenance after transplantation. This animal was grafted with two day old anterior lobe cultures twenty-five days following hypophysectomy. At that time it had lost 16% of its body weight. It maintained its transplanted weight for sixty days thereafter and then

gradually gained weight so that at autopsy, eighty-four days after transplantation, it had gained 6% above its transplanted weight. Over a similar period of eighty-four days, rat No.35 lost only 2% of its transplanted weight. Over a period of forty-five days after transplantation rat No.96 and rats Nos. 75 and 81 lost 5 and 7% respectively, the loss of weight in the controls over a corresponding period being 22%.

Not all the animals in which there was a tendency to weight maintenance after transplantation showed repair of the atrophy occurring in the endocrine organs following hypophysectomy. It will be pointed out that animals which showed repair of some of the endocrine organs did not necessarily show a tendency to weight maintenance.

A control group of ten hypophysectomized rats were transplanted with pituitary fragments not cultivated in vitro. Various numbers of pituitary fragments (eight to eighty) taken from rats two to sixty-five days of age were transplanted into the areolar tissue of the axilla. The loss of body weight in these animals corresponded to that of the untreated hypophysectomized controls.

II. Effect of Grafting of Pituitary Cultures upon Endocrine Organs.

Repair in the transplanted rat of the atrophy which occurs in thyroid, adrenal cortex and gonads after hypophysectomy has also been taken as evidence of a successful pituitary graft. The data showing these changes are given in Table 1 and in Plates 1 to 5 inclusive. In the ten untreated hypophysectomized controls the average weights of

the organs were as follows: thyroid 10 mgms.; adrenals, 12 mgms.; and testes, 380 mgms. Upon histological examination of these organs, marked atrophic changes are to be seen. The epithelial cells of the alveoli of the thyroid are transformed from a cuboidal to a flat type of cell (Plate 1, Figures 2 and 6). The amount of colloid remains unchanged. In the adrenal there occurs atrophy of the zona reticularis and the zona fasciculata, with a resultant narrowing of the cortex as a whole (Plate 3, Figure 5). In the testes, the most striking finding is a degenerative change in the germinal epithelium; atrophy of the interstitial cells also occurs (Plate 2, Figures 4 and 6).

Ten of the animals transplanted with pituitary cultures showed some evidence of repair of one or two of the endocrine organs. In no animal could repair of all three of these endocrine organs be demonstrated. The weights of the organs of the animals are recorded in Table 1. (In a few cases, e.g. rat No.88, the thyroid sections were poorly cut so that conclusions could not be drawn.) It will be noted that several of the animals show organ weights considerably higher than the average weights listed above for the controls.

In the thyroid of rat No.92 there were several circumscribed areas in which the alveoli were lined with a cuboidal epithelium (Plate 1, Figure 3). The rest of the gland showed the involutional atrophy seen in the untreated hypophysectomized rats. Similar findings in the thyroid were seen in rats Nos.19 and 42. It will be noted that there are various degrees or types of repair. The thyroid of rat No.42 (Plate 1, Figure 4) shows localized proliferation not unlike that found in foetal adenomas. One can see several minute

vesicles and larger ones varying in size. Rats Nos. 92 and 19 (Plate 1) show a difference in the height of their cuboidal epithelium.

The adrenal cortex exhibited varying degrees of repair in eight of the grafted rats (Table 1). Photomicrographs of these are shown in Plates 3, 4, and 5. When the adrenal cortex of these animals is compared with that of untreated controls which has been hypophysectomized for the same length of time it will be seen that there is a widening of the cortex approximating that of the normal rat. In some cases the zona fasciculata alone has been restored; in other cases both zona fasciculata and reticularis have been so affected. The outstanding repair is that in rat No. 107. Here the adrenal cortex is almost as wide as in the normal (Plate 3, Figure 4). In three of the grafted animals (rats Nos. 73, 88 and 107) the seminiferous tubules of the testes contained spermatozoa. This is evidence of excellent, if not complete, repair of this organ (Plate 2). The testes of other rats showed an increase in number of spermatocytes or even spermatids.

In those hypophysectomized rats which were transplanted with non-cultivated pituitary tissue, the thyroid, adrenals and testes after forty-five days showed the atrophy characteristic in untreated hypophysectomized animals.

III. Recovery of Grafted Fragments.

Pituitary grafts could not be identified with any degree of certainty. In a few instances there were large epithelial cells massed together in the areolar tissue which

were suggestive of pituitary cells. Some of the structures were heavily invaded by lymphoid cells and others by connective tissue and giant cells.

(c) Discussion.

There are three criteria which might be used for judging the success of a pituitary graft. The first criterion is that of histological repair in the thyroid, adrenals and testes in hypophysectomized animals, the second is the checking of the weight loss in the adult rat with gradual return to the pre-hypophysectomy weight level, and the third is the recovery of healthy pituitary grafts in the implanted area. The first criterion has been satisfied in a few cases; the second has been partially complied with. The third criterion, on the other hand, as has been mentioned above, has not been fulfilled.

In an analysis of the data presented it is evident that definite repair of the disabilities which follow hypophysectomy in the rat was obtained in a number of the animals. In no transplanted animal was there evidence of repair in all the organs which atrophy after hypophysectomy, nor was there complete restoration of pre-hypophysectomy weight. In some of the animals the thyroid and adrenal alone showed repair; in other animals in which the testes were repaired to the point of producing spermatozoa and in which the adrenal cortex showed a corresponding degree of repair, the thyroid remained atrophic and the loss of body weight was as severe as in the untreated hypophysectomized control.

It is felt that the lack of complete restoration of pituitary function might well be due to an insufficient amount of pituitary tissue grafted. P. E. Smith (103) has found that when less than 10% of the pituitary remains in the sella after partial hypophysectomy the animal loses weight without showing any alterations in the histological appearance of the thyroid and adrenal and only slight changes in the gonads. The amount of pituitary tissue actually grafted was probably equivalent to 0.01 to 0.02 of the animal's own pituitary so that unless there was marked compensatory hypertrophy of the grafted tissue the animal would still be considerably deficient in pituitary hormones.

It is difficult to interpret the findings since apparently the same type of tissue was transplanted into the various animals. Since the types of epithelial cells would not be recognized in tissue culture (Plates 6 and 7), one cannot correlate in this study cell type with specific function. On speculative grounds it may be suggested that certain types of cells, e.g. acidophils, were in preponderance. The age of the grafted pituitary appeared to be of little or no importance; the four most successful rats were grafted with pituitaries of rats five days or less of age.

That the various effects were due to hormone content of the transplanted fragments is inconceivable in the light of past experience with pituitary implantation. Furthermore, the control group which received an amount of fresh pituitary equivalent to that grafted into rats after growth <u>in vitro</u> did not show any of these specific effects forty-five days after implantation.

It would seem then that pituitary tissue was adapted to homoiotransplantation by being grown in autoplasma and autoserum.

(d) Summary and Conclusion.

1. As the result of transplantation of anterior pituitary tissue cultures into hypophysectomized rats, three (Nos. 73, 88 and 107) showed a restoration of testes to the point of producing spermatozoa.

2. Three rats (Nos. 19, 42 and 92) under the same condition of experiment showed circumscribed areas in the thyroid which were made up of alveoli lined with cuboidal epithelium.

3. Eight rats (Nos. 40, 43, 49, 55, 73, 88, 92 and 107) were found to have suggestive or definite repair of the adrenal cortex.

4. One rat (No. 37) gained over 6% over its transplanted weight. Four other rats came within 2 to 7% of maintaining their transplanted weights. In numerous other rats the loss of weight was considerably less than in the untreated hypophysectomized controls.

5. On the basis of these observations, the conclusion is drawn that pituitary tissue is adapted for homoiotransplantation by being cultivated in autoplasma and autoserum prior to grafting.

I wish to emphasize that the transplantation work recorded in this thesis is a record of a joint piece of work carried on with Dr. Evelyn Anderson. Without her very generous collaboration the work could not have been done.

11. CYTOLOGICAL STUDY OF PITUITARY CELLS GROWN IN VITRO

(a) Literature.

There appear to be only two articles in the literature dealing with growth of pituitary *in vitro*. Kasahara (261) of Tokio published in 1931 the first work on this subject. More recently (1935) Margaret Lewis and P. S. MacNeal (212) of Baltimore published the second account of cultivation of pituitary cells. Kasahara employed the hypophysis of the rabbit of from one to several months of age. From the explanted pars anterior there grew out (1) epithelial cells, (2) endothelial cells and histiocytes, and (3) fibroblasts. In many cases there was a pure growth of epithelial cells. They took on the form of tongue-like, membranous or island-like structures. Protoplasm was thick, transparent or finely granular. Giant cells with two or more nuclei appeared. Direct division and various stages of mitosis were seen. Kasahara could not trace the origin of the various epithelial cells. He states, however, that there were "epithelial cells which contained colloidal or hyaline metamorphotic products of the acidophilic granules".

Pars intermedia cells grew out in a fashion similar to pars anterior cells. They could not be differentiated. From the posterior lobe there was growth of epithelial cells (pars intermedia cells) and growth of nerve fibres, not to mention the endothelial cells, histiocytes and fibroblasts. All that is stated in regard to the growth of nerve fibres is that "there occurred emigration of the nerve cells and the development of nerve fibres". He successfully subcultivated the tissue for five generations in thirty days.

Lewis and MacNeal in a preliminary report summarized the salient points in the growth in vitro of the pituitary gland of certain fishes. They used the dogfish and skate and also the flounder, sculpin and angler. The most extensive outgrowths were obtained by cutting up the tissue in a salt solution as isotonic as possible with the tissue in question and explanting it into hanging drops composed of one part of chicken plasma to two or three parts of autoplasma. From the pars nervosa there grew networks of long delicate fibrils which rose within twelve to twenty-four hours. From the glandular portions there grew epithelial membranes usually only one or two cells in thickness which continued to proliferate for two or three weeks. The cells were often ciliated and continued to beat for eight to ten days. Patches of granular cells were present here and there throughout the epithelial membrane. In the skate, granular cells grew from the neuro-intermediate lobe.

(b) Introduction.

Tissue culture offers a means perhaps of clarifying some of the riddles of pituitary cytology and physiology. It may be used as an approach for the determination of the nature of the cyclic changes which are said to occur in the pituitary. If acidophils or basophils can be got into pure cultures it will be of importance to see whether they give rise to chromophobes or to daughter chromophils. Furthermore, if such cultures can be obtained, the functions of these various cells can be determined by injection into hypophysectomized rats of

the secretory products they elaborate in vitro. It is possible that basophilic changes may be brought about in vitro by growing them in a coagulum of the blood plasma of castrated animals.

The present cytological study is but the first step in the attempt to work out some of these problems. In this study it is seen in what manner the cells of the pars anterior, pars intermedia and pars nervosa grow during the first four or five days after fragments of anterior and posterior lobes are explanted. A method for silver staining of tissue cultures has been devised in collaboration with Sánchez-Pérez (250) and will be reported under Methods in this study. The imminent problem of securing a dependable differential stain for the various pituitary cells within the coagulum has not as yet found complete solution.

(c) Materials and Methods.

The pituitary of the eight day old rat and 11 centimetre pig foetus were selected for most of the studies. With the aid of the dissecting microscope, posterior lobe was readily separated from the anterior so that cellular contamination could easily be obviated. The respective lobes were cut into fragments of about 0.5 square millimetres in size and planted on cover slips or in Carrel flasks. The technique will not be gone into since it was described at length under the chapter headed Transplantation. Suffice it to say that rat plasma, chicken embryonic extract and Stone's balanced salt solution were employed in making up the clot. Fragments

were planted at the periphery of the clot on cover slips so that they could be more easily stained. After four or five days of growth in vitro at a temperature of 38.5°C. they were fixed and stained.

The procedure for staining with silver devised by Sánchez-Pérez and the writer is as follows:

1. Fix for 24 hours in equal amounts of 10% neutral formalin and normal salt solution, after removing all paraffin and vaseline from the cover slip.

2. Wash in 30 cc. of distilled water to which has been added 6 drops of ammonium hydroxide -- 5 minutes for thin clots on cover slips and up to 15 minutes for thicker clots in Carrel flasks. (Petri dishes used throughout. It is necessary to use doubly distilled water.)

3. Wash in distilled water.

4. Place into the following mixture: 30 cc. of a 2% silver nitrate (reagent), 50 drops of 95% alcohol, 25 drops of pyradine, 5 drops of ammonium hydroxide. This is heated slowly up to 40°C. until the characteristic yellow colour develops. (About 12 minutes)

5. Wash in distilled water.

6. Place in the following mixture: 30 cc. of silver carbonate, 25 drops of 95% alcohol, 15 drops of pyradine, and heat slowly up to 40°C. until the fragments take on a brown colour. (About 9 minutes)
   The silver carbonate is made by adding to 5 cc. of a 10% silver nitrate (reagent) 20 cc. of a 5% sodium carbonate. A white precipitate is formed which is dissolved by the addition of ammonium hydroxide drop by drop, being careful not to add an excess. It is then made up to 75 cc. with distilled water.

7. Wash in distilled water.

8. Reduce in 1% formalin.

9. Wash in distilled water.

10. Place in gold chloride 1-500. Heat very slowly up to 40°C. until the characteristic violet colour develops. (About 10 minutes)

11. Place in 1% hyposulphite for a few minutes.

12. Run through the alcohols, up through absolute, place in xylol and mount in balsam.

For differential staining the Cleveland-Wolfe method was tried and found unsatisfactory since the coagulum was so deeply stained that the cells were obscured. Wolbach's Modification of the Giemsa Stain (77) was next tried and was found partially successful. It was found that if two of the steps advocated by Wolbach were omitted the result was more satisfactory. Thus, the use of sodium hyposulphite as one of the steps preliminary to staining, and the use of colophonium as a clearing agent were omitted. The best results were obtained with Giemsa after immersing the preparations for six or seven days in the stain. The Unna-Pappenheim methyl-green-pyronin stain (251) was employed in the attempt to stain granules in the pars nervosa cells. Results were not constant.

(d) Results.

I. Cell Types Obtained from Cultures of the Anterior Lobe of Pituitary of Eight Day Old Rat.

About eight hours after explantation cells were seen to project from the periphery of the fragment. After twenty-four hours, proliferation is pronounced, the colonies, in some cases, doubling their diameters. In most of the cultures the first cells to appear are epithelial cells which have the tendency to spread out in sheets. The growth is often so prolific that fibroblasts, which are undoubtedly present, do not proliferate. The form which the advancing epithelial takes depends largely upon the extent and position of liquefaction of the coagulum. Characteristically, they stretch across the liquefied

zone as broad cords of cells and then spread out in the coagulum, assuming many shapes. The numerous empty hiatuses left between the cords of cells are invaded on the second or third day and are soon filled by epithelial cells; they here assume the form of a pavement epithelium. Intercellular spaces give the cells the appearance of a mosaic (Plate 7; Plate 11, Figure 3; Plate 9, Figure 3).

The epithelial cells, invariably flat, vary in size and shape, their contour apparently being determined by contact with the neighbouring cells. Most of them are of polygonal shape, some are triangular, many are elongated. The cells are three or four times larger than any of the chromophobes or chromophils in the original fragment. When stained with Wolbach's Giemsa the nuclei become markedly granular (Plate 10, Figure 3); when impregnated with silver (Plate 11, Figure 3) one can see a lightly-staining linin-like network. One to four deeply staining nucleoli are present in each nucleus. The cytoplasm is uniformly very finely granular in the silver preparations. With the Giemsa stain, the cytoplasm takes on the stain of chromophobes. (It is quite probable that the staining is at fault here since chromophils, undoubtedly present in the fragment, fail to stain differentially.)

The cells on the periphery of the advancing zone of epithelium form interweaving branching processes two or three layers in thickness which give to the rim of the colony the appearance of a corona (Plate 10, Figure 1; Plate 16, Figure 4; Plate 17, Figure 2). These cells seem to be joined together end to end in the form of a syncytium. At least the

fibrillar-like structure passes continuously from the cytoplasm of one cell to that of another and, in addition, no intercellular membrane is seen.

Numerous mitoses are present throughout the epithelial sheets. All stages from the early prophase to telophase are to be seen. In Plate 10, Figure 3 is depicted a cell with chromosomes gathered about its equatorial zone. In this Figure is another cell undergoing mitosis and also a large binucleate epithelial cell (P). This may be an incompleted amitotic division. No structures suggesting endocellular cytogenesis are seen.

Cells other than epithelial are infrequent in cultures of the pars anterior. In Figure 1 of Plate 10 are seen macrophages (M) and fibroblasts. Giant cells (Plates 6, 13 and 14), one measuring 360 micra in diameter, were cultured from the anterior lobe of the pituitary of a twenty-three day old rat.

Subcultures of epithelial cells in pure state were carried through four passages for three weeks. At this time they were destroyed by bacterial contamination.

II. Cell Types Obtained from Cultures of the Anterior Lobe of the Eleven Centimetre Pig Foetus.

The anterior lobe cells of the pig foetus upon growth <u>in vitro</u> present quite a different picture from those of the cultivated rat pars anterior cells. The cultivated cells did not increase in size as will be seen by comparing the cells pictured in Figures 1 and 2 of Plate 8. In the pig cells grown <u>in vitro</u> (Plate 8, Figure 2) there are both

chromophobes and chromophils. Cells such as Cell A with their darkly staining zone of cytoplasm are highly suggestive of acidophils. It is highly suggestive that Cell B with its excentric nucleus and wide zone of cytoplasm is a basophil. A curious circular hollow structure with minute cytoplasmic projections invaginated into its lumen is seen in the cytoplasm of this cell. This is identical with the Golgi apparatus described by Severinghaus and by Eisenhardt as characteristic of the basophil.

The pig foetus anterior cells grew rapidly and were closely packed together. In Figure 3 of Plate 8 is shown a pure culture of pig foetus anterior cells stained with silver.

III. Cell Types Obtained from Cultures of the Posterior Lobe of the Eight Day Old Rat.

Invariably the epithelial cells of the intermediate lobe grew with rapidity and apparently more readily than the epithelial cells of the pars anterior. After five days of growth in Carrel flasks with daily washing, and patching with fresh plasma, the anterior lobe colonies attained the diameter of about two millimetres whereas those of the posterior lobe measured three millimetres. No cytological differences could be detected between anterior and intermediate lobe cells when stained with silver (Plate 15, Figure 2). There was one noticeable difference though in that the peripheral zone of the advancing intermediate cells were crowded with phagocytes (or microglial cells). Some of these latter cells are shown in Plate 15, Figure 3 and a number of the Figures in Plates 16, 17 and 18.

Cells which could be said to arise from the elements of the pars nervosa were sparse. Such cells are shown in Plates 16, 17 and 18. If one is justified in using the term 'pituicytes', some of these cells can be so designated. Cells having similarity with astrocytes and oligodendroglia are seen as are also cells resembling in shape the monopolar and bipolar spongioblasts. It seems striking that most of the cells possessed granules in their cytoplasm. An attempt was made to determine whether such granules could be stained by Unna-Pappenheim methyl-green-pyronin. A conclusion could not be reached because of paucity of 'pituicytes' in those cultures, but here and there was a green-stained cell with a few dark purplish-green coarse granules in its cytoplasm.

IV. Ciliated Cells in Pituitary Cultures.

In pituitary cultures of eight, thirteen, twenty-three and sixty-five day old rats, ciliated cells were found. Located in mass formation along the border of the explant, the cilia were seen waving at a very rapid speed, the rate of which could not be ascertained by the human eye. There were two distinct tempos, one which may be described as waving, and the other, a wave-like undulation which swept at frequent intervals down across the zone of cilia. These ciliated areas appeared invariably in the periphery of the explant, the cilia waving free in the liquefied zone. They were seen to multiply during the five or six days that they persisted. There must have been hundreds of these ciliated cells lining the periphery of these fragments, judging from the number of beating cilia on one single cell which was seen to break out of file and

wander into the liquefied zone. The beating cilia created a current which brought numerous foreign particles toward them. In Plate 15, Figure 1 is seen a small round cell caught and held by cilia which rendered the latter immobile. The divided current created by the waving cilia on either side sent the immobilized cell into the most bizarre shaking movement which continued for hours. The ciliated cells were found in cultures of both anterior and posterior lobes, thus indicating that these cells were present on both anterior and posterior aspects of the intraglandular cleft.

(e) Discussion.

In culture of the pars anterior of the pig foetus, the cells have undergone differentiation as judged by the presence of both chromophobes and chromophils which have not deviated from the size and shape of the cells of the explant from which they grew. On the other hand, the cells proliferating from fragments of eight day old rat anterior pituitary are two or three times the size of those not grown in vitro. In addition there is no evidence of differentiation into chromophils. Thus it would appear that in the latter case there had been a reversion to a more primitive type of epithelial cell. This in itself is not an indication of dedifferentiation. After several passates over a period of twenty-one days the cells retained their epithelial appearance and, as in the first day of growth, varied in the number and size of their intracellular globules (in the unstained preparations).

A criticism was brought forward by Champy in the early days of tissue culture that epithelial cells dedifferentiated when grown in vitro. Champy (252) states that epithelial cells migrating from fragments of kidney take on an indifferent aspect, assuming the appearance of connective tissue cells. He furthermore comes to the conclusion (253) that dedifferentiation is certain in epithelial cells if they are cultivated in vitro without connective tissue. On the other hand, Ebeling (254) claims that growth in vitro does not cause a cell to dedifferentiate and states that Champy, in arriving at his conclusion, was employing the early technique and that his cultures were impure. Ebeling was able to bring about differentiation in vitro of the thyroid of the chick. Cells which grew on the surface of the clot assumed the appearance of pavement epithelium but they did not dedifferentiate; after five or six passages those deeper in the coagulum took on a glandular structure. After some four months of growth there was colloid secretion morphologically similar to that from a freshly extirpated thyroid gland. Differentiation was seen to occur in the pituitary of the pig foetus by the writer during the first four days of growth. Definite acidophils were also seen in one well-stained preparation of the anterior lobe cultivated cells of the two day old rat. In case of the eight day old rat, the cells seemed to revert to a more primitive type (pavement epithelium composed of enlarged cells). That this does not represent a functional dedifferentiation has been shown by the injection of the washings of these cells into hypophysectomized rats. Such washings produced a rather

remarkable restoration of gonad, thyroid and adrenal cortical repair. (This work is not in a sufficiently complete state to report in this thesis.)

It will take further study before it can be stated definitely whether the epithelial cells of the outer invading zone forms a syncytium. Evidence at present points toward it. Bauer (255) and Levi and Meyer (256) have clearly pointed out a syncytial arrangement of the large indifferent neuro-epithelial cells in cultures of chick embryo brain, so it will not be surprising if such an arrangement of cells can be demonstrated as occurring in pituitary tissue cultures.

The presence of giant cells in the cultures (Plates 6, 13 and 14) have previously been reported as occurring in tissue cultures of such organs as the spleen, lymph nodes, bone marrow and blood and buffy coat (Lewis, 257). They arise from large mononuclears or some of their modifications and not as a form of specific pituitary cell. The giant cells arise in several ways. They may be formed by a fusion of mononuclear-epithelial cells with a small giant cell. Apparently such fusion takes place under peculiar circumstances for other mononuclear cells in close contact with the giant cell retain their complete independence. Giant cells may fuse together; for a while after fusion takes place the nuclei are irregularly scattered, but when the rearrangement is complete all the nuclei are in a circle about a single large central area. Lewis (257) has observed the amitotic division of the nucleus without division of the cytoplasm in the mononuclears in the same cultures where giant cells were also forming by fusion. In this division by amitosis Macklin (258) concurs. In practically all cases these

giant cells are derived from the large mononuclears or monocytes or from modified mononuclears (epithelioid cells and clasmatocytes). There may be two or three or a hundred or more nuclei. The nuclei may be arranged about the periphery of a central area, or may be more or less scattered throughout the mass, singly or in clumps.

The giant cells found in the pituitary are thought by inference to have been formed in one of two ways, either by amitosis of their nuclei or by a fusion of giant cells. It is likely that cell H was formed by the process of amitosis and that cell G was formed by a fusion of giant cells. There was no suggestion that these cells were formed by the fusion of mononuclears to an enlarging giant cell as is unmistakable when once seen.

There is especial interest in the finding in tissue cultures of the posterior lobe of various types of glia-like elements with cytoplasmic granules. Could these be the secretory granules by means of which pituitrin is elaborated? These granular cells possess monopolar, bipolar and multipolar processes. Cells simulating the astrocyte (Plate 17, Figure 2) is an unusual finding in cultures of the normal gland. While they have been cultured from astrocytic tumours they have never been shown to grow as such from the brain (Lazarenko, 259; Grigorjeff, 260).

12. PRODUCTION OF MELANOPHORE-EXPANDING PRINCIPLE IN VITRO

(a) Introduction.

The purpose of these experiments is to determine whether the pars intermedia cells growing *in vitro* continue to elaborate the melanophore-expanding principle which is present in the non-cultivated cells of the rat pars intermedia. Evidence of the production of the melanophore-expanding principle will be proof that the epithelial cells have not dedifferentiated with growth *in vitro*. It has been shown in a preliminary experiment that the saline washings from the cultures contain very much less melanophore-expanding function than does the saline extract of the non-cultivated posterior lobe. There are two possible explanations for this result: the first is that the colonies are buried so deeply in the plasma that they are not washed, and the second, which is more likely, is that the melanophore-expanding principle is retained in the cell. A stimulus not provided in tissue culture may be necessary to cause an extrusion of secretory principles. To determine whether the cultivated pars intermedia cell has stored up a greater amount of melanophore-expanding principle than the non-cultivated cell because there has been no physiological demand for secretion, is a problem that cannot at present be settled. If it can be determined that the fragments planted *in vitro* multiply their volumes, say by ten times, one would not necessarily expect the hormone to be correspondingly increased by ten times since it is not known what proportion of the cells in the central portion of the explanted fragment have died (as those in the central

portion of the fragment usually do) during growth in vitro. And, too, it is still undetermined whether death of those cells leads to concomitant destruction of the melanophore-expanding principle contained within them. The melanophore-expanding principle remains unaltered at a temperature of 35° to 39°F. when mixed with 0.25% acetic acid, but it might be destroyed in the dead fragments by remaining several days at the incubation temperature of 99.4°F. Taking these various factors into consideration the following experiments were run in order to determine whether those pars intermedia cells which have grown in vitro retain the property to produce melanophore-expanding principle.

(b) Material and Methods.

The cultural technique previously described was used. The posterior pituitaries of ten eight day old rats were grown in a single Carrel flask for a period of six days. (Eight day old rats were used exclusively in these experiments.) The colonies were washed daily with salt solution, and fresh plasma and embryonic juice was added to take the place of the liquefied plasma about the growing fragments. Phenol red was not added to the cultures since it was found in preliminary control experiments that the injection of phenol red into the frog causes a coloration of the skin which, though temporary, confuses the interpretation of melanophore-expanding effects. On the sixth day, the colonies, which had grown considerably (each on an average of two or three times the diameter of the explanted fragment), had added to them acetic acid in 0.5% concentration; this was then added to the washings. The

combined material was placed in a water bath and heated to a boiling point for a period of one minute and it was then ready for injection into frogs. Ten posterior pituitaries were removed, and without being cultivated were placed in a like manner in 0.5% acetic acid and brought to a boil in a water bath. Both cultivated and non-cultivated pituitary were ground thoroughly in sand before heating on the water bath so as to liberate all intracellular hormones.

Four series of frogs were injected with material obtained in this way. In the first series, large doses were used (material containing the equivalent of ¼ to 2 posterior pituitaries). In the other series, very much smaller doses were used (material containing the equivalent of 1/10 to 1/320 of a single posterior lobe). In all the series there was included an untreated control frog and also one injected with 0.05 units of Frosst pituitrin. This latter contains a minute amount of melanophore-expanding principle and thus serves as a standard for comparison. (One cubic centimetre of Frosst pituitrin contains twenty International Units.) The frogs were allowed to remain in intense light for an hour before being injected, the purpose being to contract equally the melanophores of all the frogs.

(c) Results.

In the first series of injections (Table 2, Plate 18), the doses of cultivated and non-cultivated material (both of Lot 40) equivalent to ¼, ½ and 2 posterior lobes resulted in a pronounced melanophore-expansion. The doses were obviously too

large to permit a calculation of the relative expansion-effect of cultivated and non-cultivated pituitaries. Hence smaller doses were injected into frogs in the succeeding three series of experiments.

In the second series of injections in which doses of from 1/10 to 1/80 of a posterior pituitary, both cultivated and non-cultivated, were used, the difference in the potency of these two is clearly visible (Table 3, Plate 19). In all four dilutions (1/10, 1/20, 1/40 and 1/80) the material grown in vitro gave a definite melanophore-expanding response. On the other hand, injection of the non-cultivated pituitaries produce a definite effect only in the dilutions of 1/10 and 1/20. Thus, the minimum effective dose of non-cultivated posterior pituitary capable of producing melanophore-expansion in an average-sized frog (thirty-six grams) is 1/20 of the eight day old posterior pituitary. Injections of greater dilutions were done in order to determine the minimum effective dose of the pituitaries grown in vitro (Table 4); 1/80 of an eight day old pituitary was the minimum effective dose. Therefore, in the case of this Lot 40, the pituitaries grown in vitro possessed approximately four times the amount of melanophore-expanding hormone (intermedin) as did those not so grown in vitro.

The experiments were repeated with Lot 42, which consisted of ten other posterior lobes of eight day old rats grown under the same conditions for the same length of time. Ten non-cultivated pituitaries formed the controls for Lot 42.

It will be seen in an examination of Table 5 that with 1/10, 1/20, 1/40 and 1/80 dilutions, the pituitaries grown *in vitro* have a greater melanophore-expanding effect than do those not so grown *in vitro*. The effect is increased approximately four fold by proliferation in tissue culture.

(d) Conclusion.

Posterior pituitary gland grown *in vitro* for a period of six days, during which time it proliferates to a marked degree, increases its melanophore-expanding content by approximately four times, as ascertained by injection of the material into frogs. It is perhaps more exact to state that the minimum effective dose of cultivated pituitary which brings about melanophore-expansion in the frog is $\frac{1}{4}$ of that of posterior pituitaries not so cultivated. The intermediate cells of the pituitary, upon multiplication *in vitro*, retain their specific function of elaborating melanophore-expanding hormone.

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Figure I. Graph showing the percentage loss of weight of ten (IO) grafted and ten (IO) untreated hypophysectomized rats. The ten of the thirty-one grafted rats which showed the most pronouced tendency to weight maintenance are used in the above figure. They are Nos. 35, 37, 42, 64, 75, 8I, 88, 96 and I04. It will be noted that the loss of weight was the same in the grafted and in the control animals at the time of transplantation (TR.). The grafted animals tended to maintain their transplanted weight.

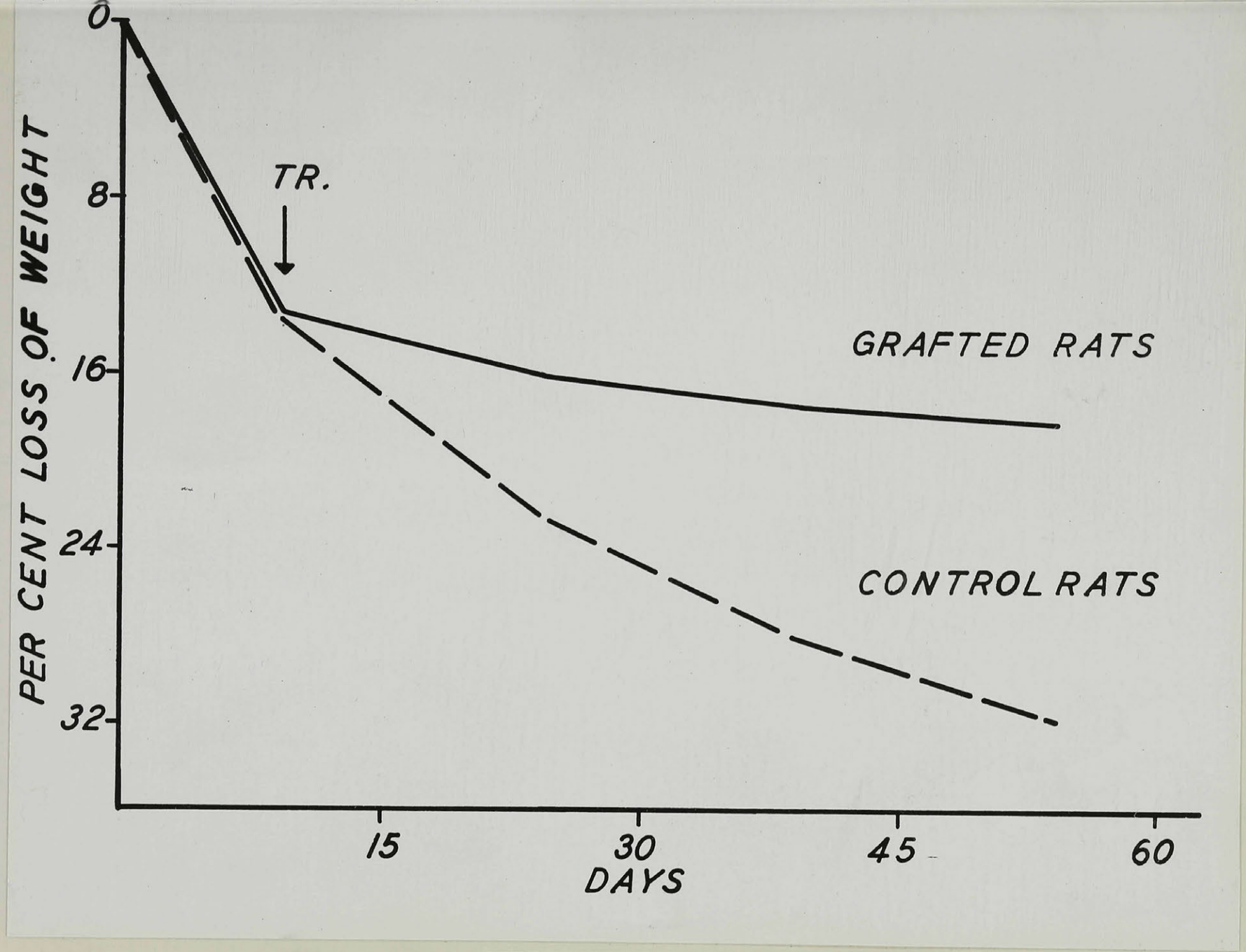

PER CENT LOSS OF WEIGHT
0
8
16
24
32
TR.
GRAFTED RATS
CONTROL RATS
15
30
45
60
DAYS

Figure I

Table 1. Percentage weight differences in transplanted animals between time of transplantation and forty-five (45) days later as compared to ten (10) untreated hypophysectomized controls -- With autopsy data.

TABLE OF WEIGHT PERCENTAGES											AUTOPSY DATA						
Animal No.	No. days between hypophysectomy and transplantation.	% loss original wt. at transplantation.	Compos. %. wt. loss controls same period.	% loss original wt. 15 days fol.transpl.	Compos. %. wt. loss controls same period	% loss original wt. 30 days fol.transpl.	Same in controls	% loss original wt. 45 days fol.transpl. a.	Same in controls b.	DIFFERENCE IN % WT. OF CONTROLS & EXP'IS. 45 DAYS FOL. TRANSPL. b-a.	No. days between hypophysectomy and autopsy.	% loss original wt. at autopsy. c.	Compos. %. wt. loss controls same period. d.	DIFFERENCE IN % WT. CONTROLS & EXP'IS. AT AUTOPSY d-c.	Wt. of adrenals (mgm.)	Wt. of thyroids (mgm.)	Wt. of testes (mgm.)	Histological Data **	Age in days of donor's pituitary
19*	27	18	19	22	28	30	30	31	32	1	132	28	34	6	11	12	380	Thyroid +	1†
35	25	14	18	16	24	14	29	16	33	17	109	20	33	13	13	13	320		2†
37	25	16	18	13	24	16	29	16	33	17	109	14	33	19	12	12	312		2†
40	15	15	15	24	23	28	29	25	32	7	75	32	32	0	20	10	530	Adrenals++	26†
42	10	10	10	13	21	14	27	13	31	18	85	19	32	13	12	11	300	Thyroid +	26†
43	13	20	13	23	22	26	28	29	32	3	75	28	32	4	17	10	230	Adrenals +	26†
47	14	24	14	27	23	27	29	32	32	0	88	33	33	0	18	10	230		24†
49	9	9	9	21	20	27	26	30	31	1	83	34	32	- 2	14	10	250	Adrenals+	22†
53	13	15	13	22	22	24	28	27	32	5	76	29	32	3	19	9	390	Adrenals++	65†
55	13	17	13	24	22	22	28	27	32	5	76	27	32	5	12	11	230		30
62	13	10	13	11	22	13	28	-	-	-	52	35	30	- 5	-	-	-		18
64	9	20	9	21	20	21	26	21	31	10	73	23	32	9	14	13	280		18
68	15	15	15	18	23	25	29	26	32	6	86	25	32	7	12	9	260		23
69	7	13	7	18	19	23	26	32	30	- 2	64	31	32	1	13	12	450		13†
71	13	10	13	17	22	20	29	23	32	9	68	22	32	10	11	8	340		13
73*	9	11	9	20	20	24	26	28	31	3	64	29	32	3	20	12	1440	Testes ++ Adrenals +	23
75	15	10	15	17	23	16	29	16	32	16	68	16	32	16	12	11	490		23†
77	15	9	15	13	23	16	29	28	32	4	67	35	32	- 3	-	-	-		10
78	15	13	15	14	23	18	29	20	32	12	67	26	32	6	10	10	300		10
79	12	10	12	15	21	19	27	23	32	9	57	25	30	5	13	9	380		11
80	13	18	13	19	22	21	28	23	32	9	62	25	32	7	12	10	480		7
81	13	13	13	16	22	18	27	19	32	13	62	20	32	12	13	10	350		11
85	13	8	13	13	22	19	27	30	32	2	64	30	32	2	-	-	-		7
88	15	9	15	13	23	16	29	19	32	13	60	19	32	13	22	10	2100	Testes ++ Adrenals++	5
92	15	15	15	22	23	25	29	27	32	5	60	27	31	4	15	14	384	Thyroid + Adrenals ++	5
96	15	17	15	20	23	24	29	22	32	10	60	20	32	12	15	13	410		8
101	15	17	15	21	23	24	29	24	32	8	60	23	32	9	12	9	320		8
104	15	10	13	17	22	18	27	19	32	13	58	19	30	11	11	8	450		2
107	6	8	6	16	18	21	26	22	30	8	51	22	30	8	16	10	1515	Testes ++ Adrenals++	2
110	16	16	16	18	24	24	28	23	32	9	61	23	32	9	-	-	-		2
112	16	12	16	18	24	24	28	26	32	6	61	23	31	8	14	12	310		2
AVERAGES:	9.6	13.6	13.4	18.1	22.3	21.2	28	23.8	31.8			25.1	31.8						

* Pituitary tissue grown in homoioplasma instead of autoplasma.

** Suggestive repair + ; definite repair + + (see photographs)

† Anterior lobe only; remaining cases were planted with cultures of both lobes of pituitary.

PLATE I

Figure I. Photomicrograph of normal rat thyroid. Hematoxylin and eosin; x 520.

Figure 2. Photomicrograph of thyroid of untreated hypophysectomized rat. Hypophysectomized 60 days. It shows flattening of the vesicular epithelium. Hematoxylin and Van Gieson; x 520.

Figure 3. Photomicrograph of thyroid of Rat 92 autopsied 45 days after grafting. Hypophysectomized 60 days. The thyroid showed circumscribed areas of vesicles lined with cuboidal epithelium as that pictured in this figure. The rest of the thyroid resembled that of the untreated hypophysectomized rat. Hematoxylin and Van Gieson; x 520.

Figure 4. Photomicrograph of thyroid of Rat 42 autopsied 75 days after grafting. Hypophysectomized 85 days. Note the presence of small round vesicles lined with cuboidal epithelium. Hematoxylin and Van Gieson; x 520.

Figure 5. Photomicrograph of thyroid of Rat I9 autopsied I05 days after grafting. Hypophysectomized I32 days. Here again the thyroid showed circumscribed areas of regeneration. Hematoxylin and Van Gieson; x 520.

Figure 6. Photomicrograph of thyroid of untreated hypophysectomized rat. Hypophysectomized II0 days. This forms an adequate control for Rat I9. Hematoxylin and Van Gieson; x 520.

PLATE I

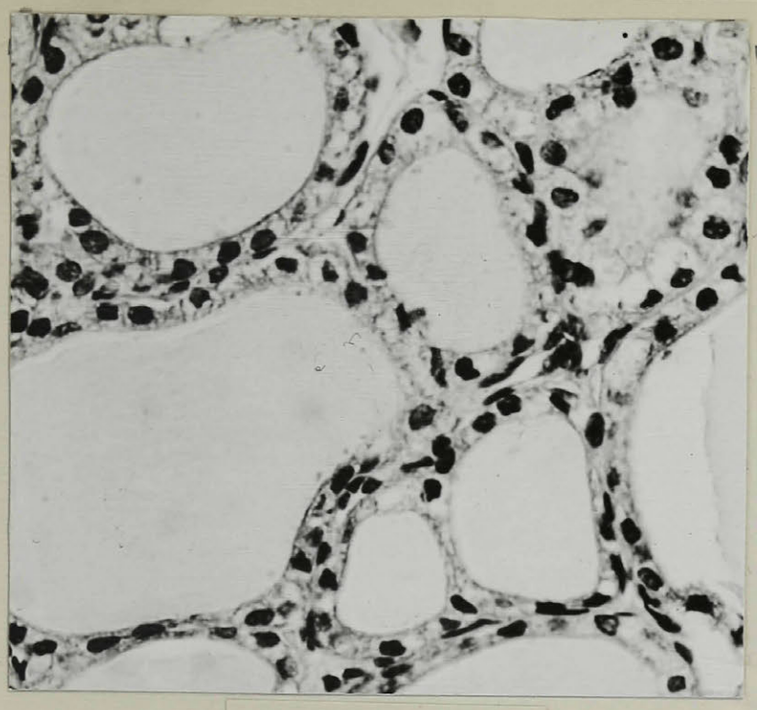


Figure I

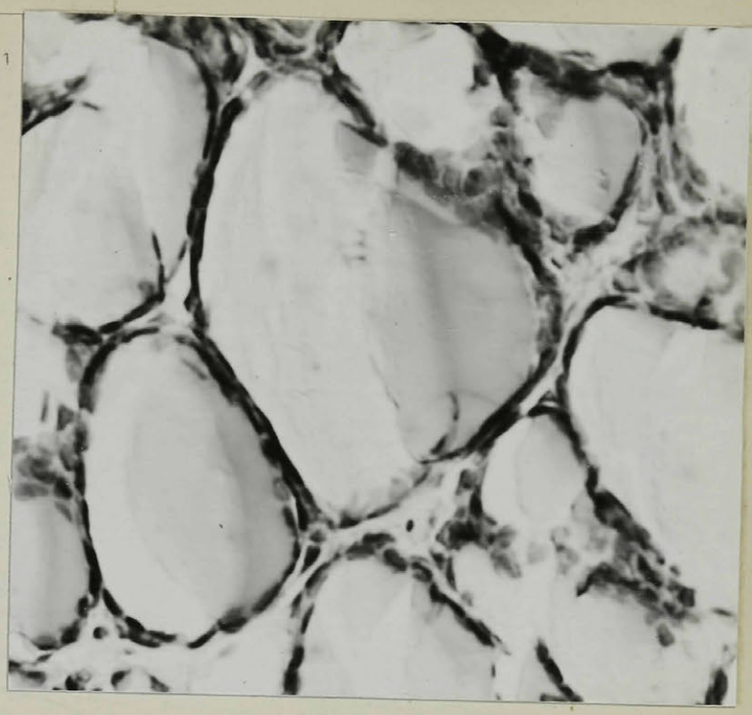


Figure 2

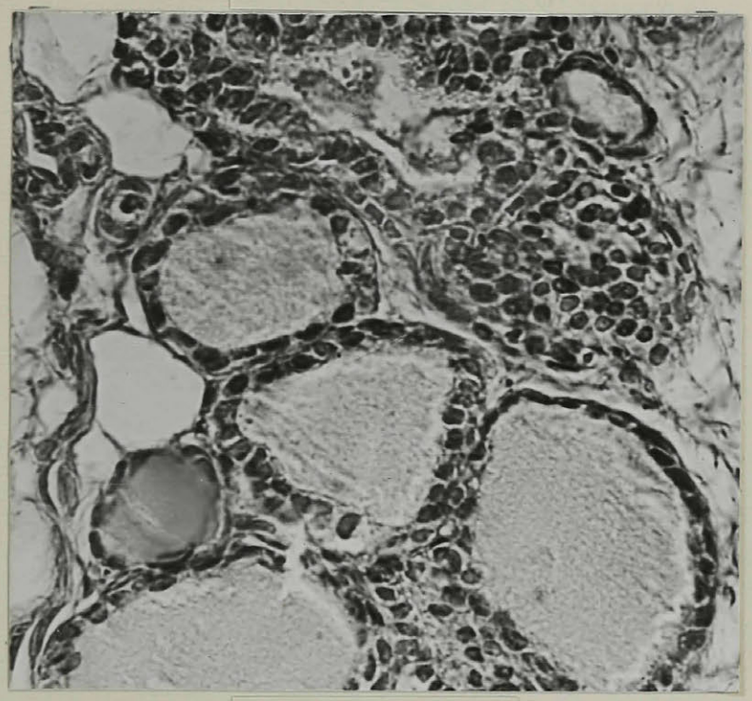


Figure 3

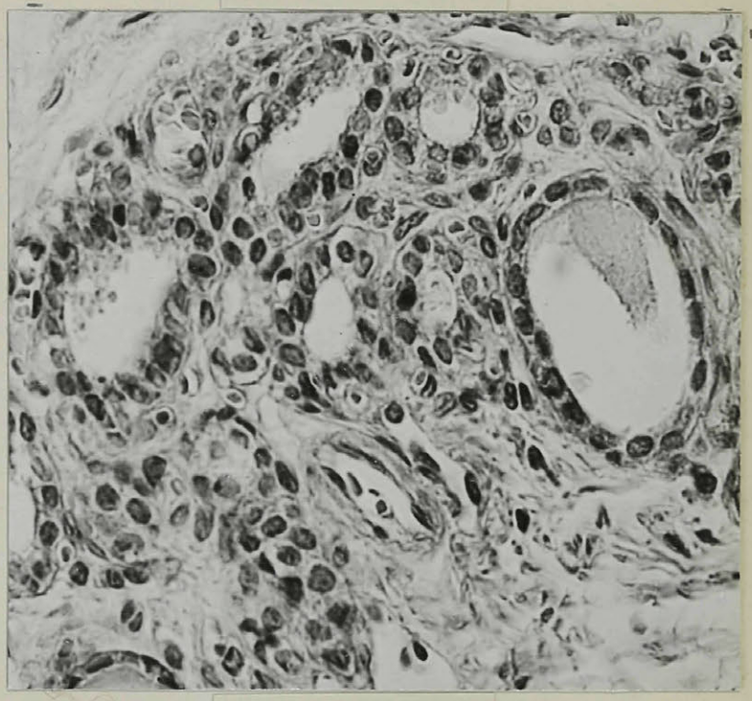


Figure 4

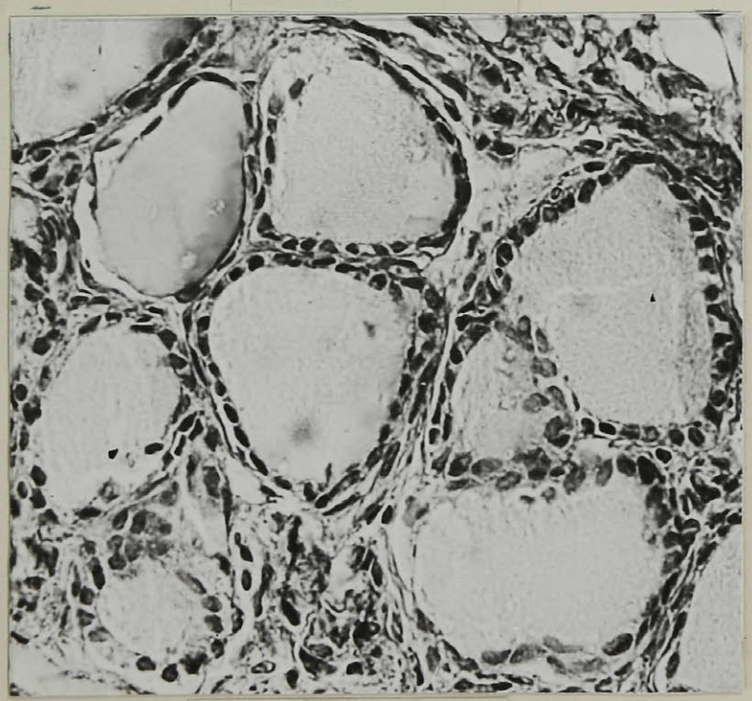


Figure 5

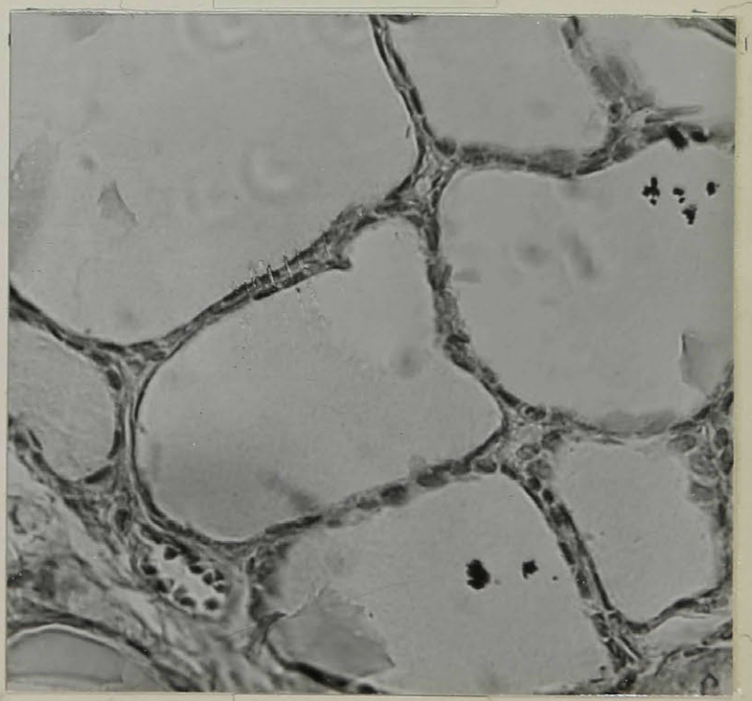


Figure 6

PLATE 2

Figure I. Photomicrograph of normal rat testis. Hematoxylin and Van Gieson; x 230.

Figure 2. Photomicrograph of testis of Rat73 autopsied 55 days after grafting. Note regeneration to the point of spermatocyte and spermatid proliferation. Hematoxylin and Van Gieson; x 230.

Figure 3. Photomicrograph of testis of Rat 88 autopsied 45 days after grafting. Hypophysectomized 60 days. Note in the presence of spermatozoa what appears to be a complete regeneration. Hematoxylin and Van Gieson; x 230.

Figure 4. Photomicrograph of testis of untreated rat hypophysectomized 60 days. Testis shows severe atrophy of the germinal epithelium and interstitial cells. Hematoxylin and Van Gieson; x 230.

Figure 5. Photomicrograph of testis of Rat I07 autopsied 45 days after grafting. Hypophysectomized 5I days. In this testis seminiferous tubules contained spermatozoa. Hematoxylin and Van Gieson; x 230.

Figure 6. Photomicrograph of testis of untreated rat hypophysectomized 68 days. This is an adequate control for Rat 73. Atrophy is severe. Hematoxylin and Van Gieson; x 230.

PLATE 2

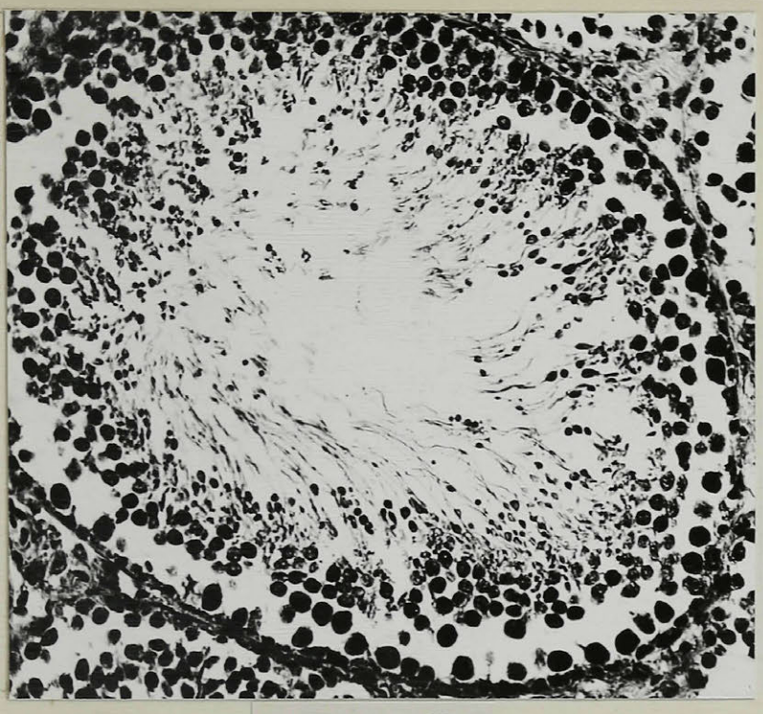


Figure I

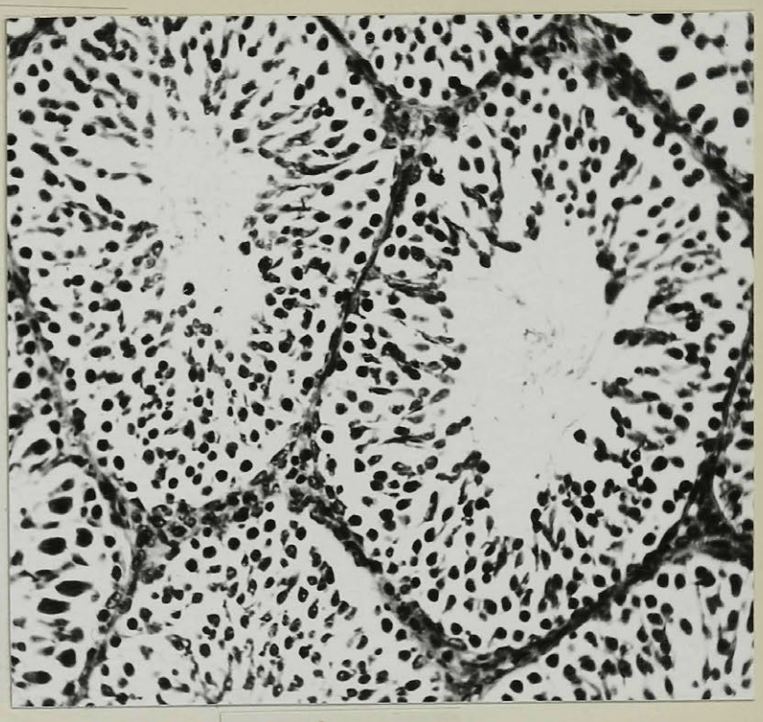


Figure 2

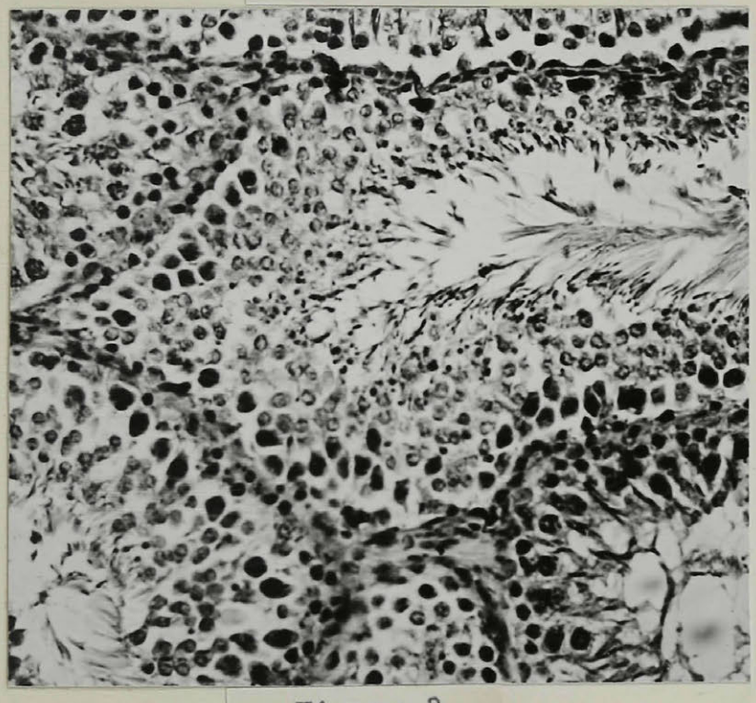


Figure 3

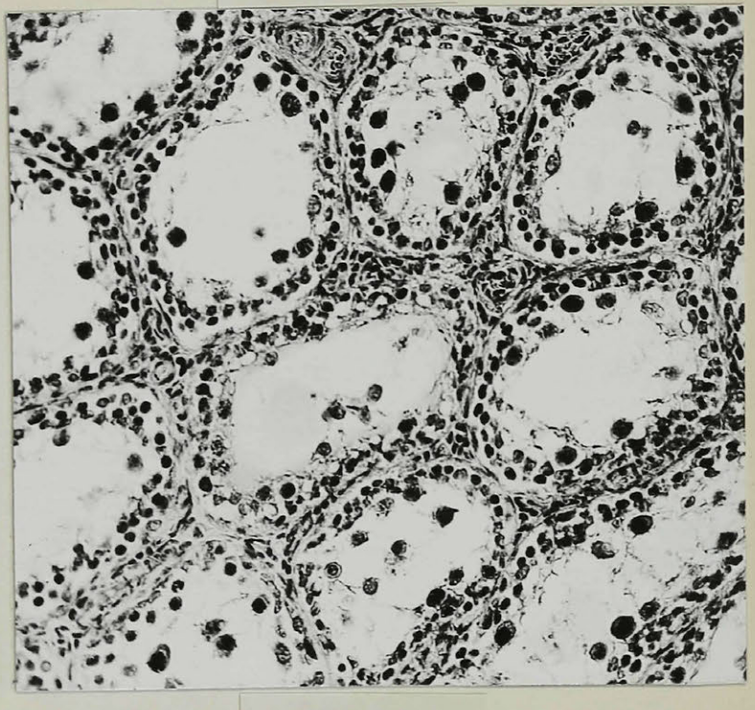


Figure 4

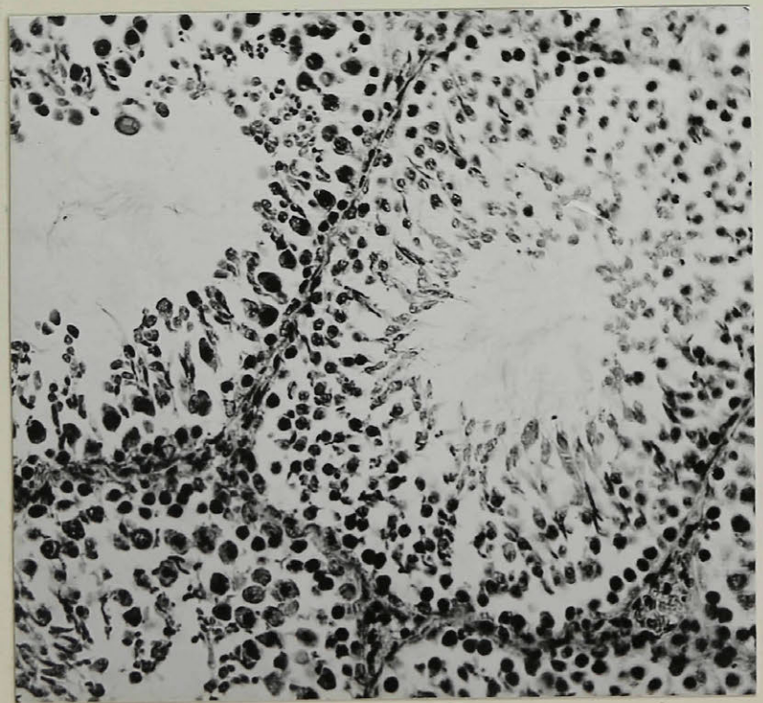


Figure 5

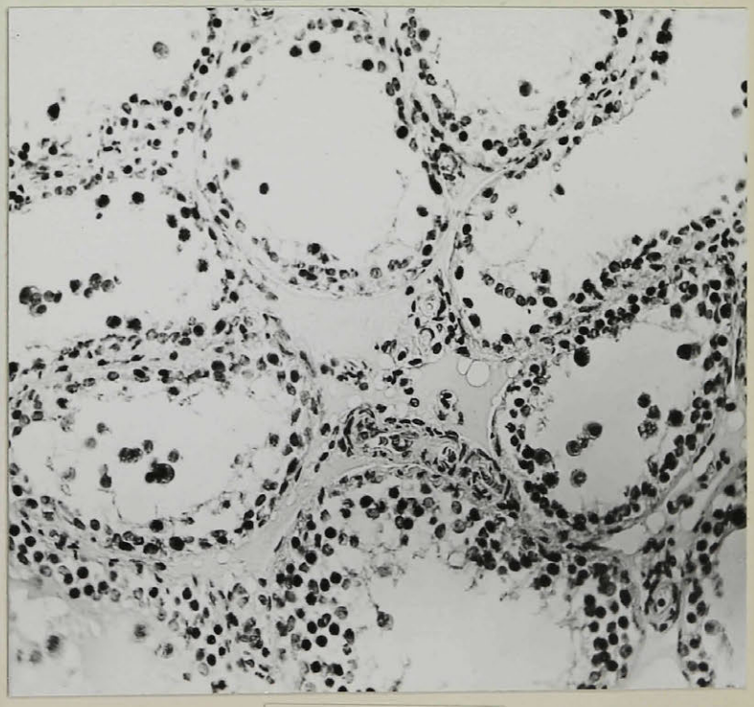


Figure 6

PLATE 3

Figure I. Photomicrograph of normal rat adrenal. Hematoxylin and Van Gieson; x I4.

Figure 2. Photomicrograph of cortex of normal adrenal shown in Figure I. Hematoxylin and Van Gieson; x 230.

Figure 3. Photomicrograph of adrenal of Rat I07 autopsied 45 days after grafting. Hypophysectomized 5I days. Hematoxylin and V. G.; x I4.

Figure 4. Photomicrograph of adrenal of Rat I07 under higher magnification. As compared to its control (Figure 5) there may be seen a marked repair of the two inner zones of the cortex (Zona fasciculata and Zona reticularis). (For the histological picture of the testis of this animal see Plate 2, Figure 5) Hematoxylin and Van Gieson; x 230.

Figure 5. Photomicrograph of cortex and neighboring medulla of untreated hypophysectomized rat. Hypophysectomized 48 days. There is extreme atrophy of the zona reticularis and zona fasciculata. Note the unusual sharp demarcation of the three zones. Hematoxylin and Van Gieson; x 230.

PLATE 3

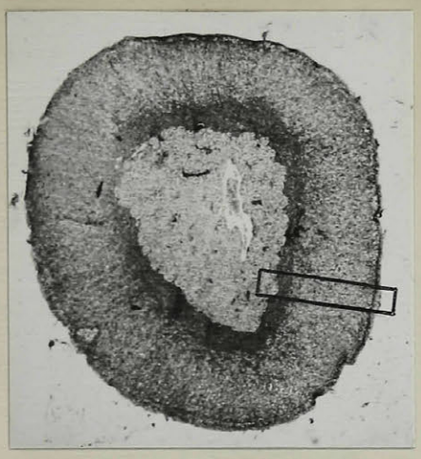


Figure I

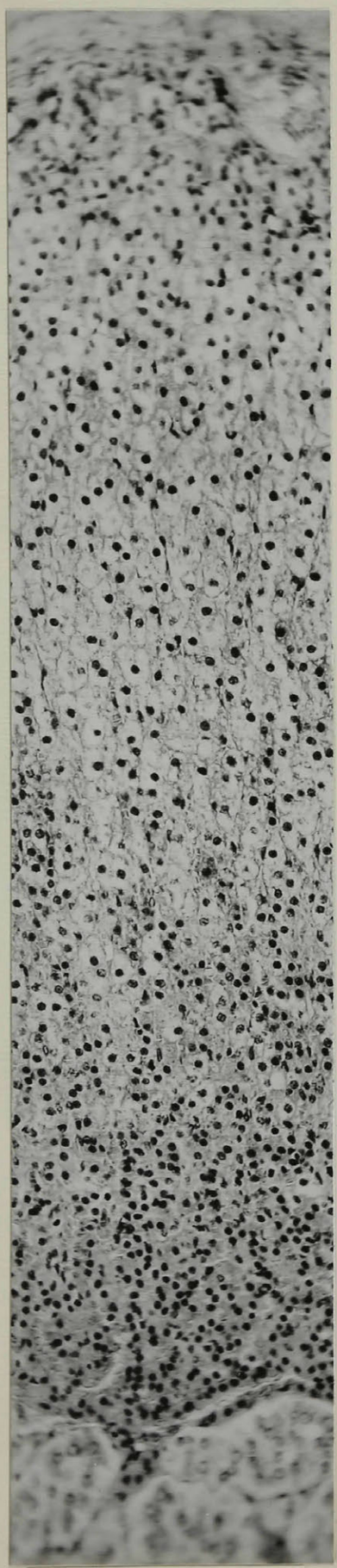


Figure 2

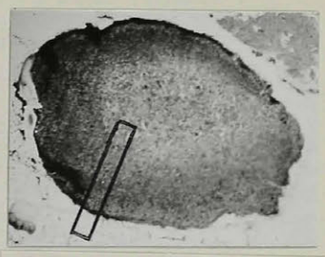


Figure 3

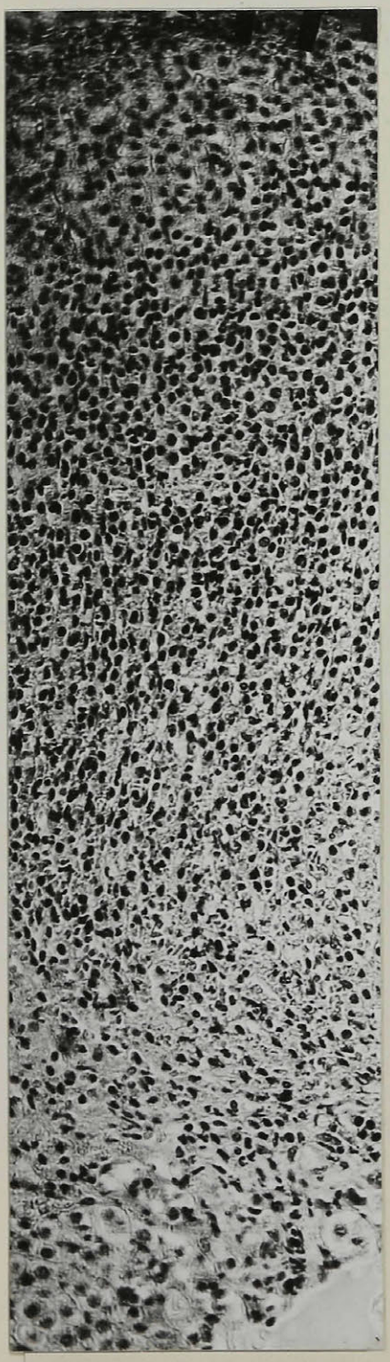


Figure 4

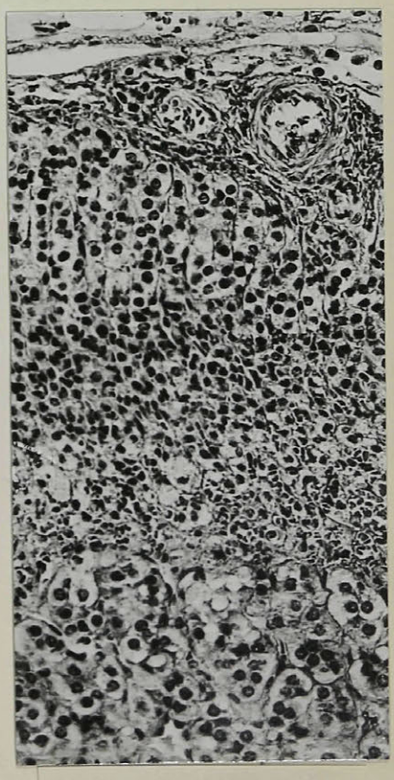


Figure 5

PLATE 4

Figure 6. Photomicrograph of adrenal of Rat 92 autopsied 45 days after transplantation. Hypophysectomized 60 days. Note the width of the cortex as compared to that in Figure I2 which illustrates the adrenal of an untreated control. Hematoxylin and Van Gieson; x I4.

Figure 7. Photomicrograph of adrenal of Rat 92 at higher magnification. Repair is most evident in the zona fasciculata. H. and V.G.; x 230.

Figure 8. Photomicrograph of adrenal of Rat 40 autopsied 60 days after grafting. Hypophysectomized 75 days. The cortex shows a degree of widening similar to that in Rat 92. Hematoxylin and Van Gieson; x I4.

Figure 9. Photomicrograph of adrenal cortex of Rat 40 at higher magnification. Here there is good repair of the zona reticularis. Hematoxylin and Van Gieson; x 230.

Figure I0. Photomicrograph of adrenal of Rat 43 autopsied 62 days after grafting. Hypophysectomized 75 days. Moderate widening of the cortex is to be seen. Hematoxylin and Van Gieson; x I4.

Figure II. Photomicrograph of adrenal of Rat 43 at a higher magnification. There is good evidence of repair of the zona fasciculata. Hematoxylin and Van Gieson; x 230.

Figure I2. Photomicrograph of adrenal of Rat hypophysectomized for 43 days before autopsy. Untreated control. Hematoxylin and V.G., x I4.

Figure I3. Photomicrograph of adrenal cortex of the above control rat at a higher magnification. Note the marked atrophy of the two inner zones. This animal was more than an adequate control for the above three animals since its span of life was shorter than that of the transplanted animals. Hematoxylin and Van Gieson; x 230.

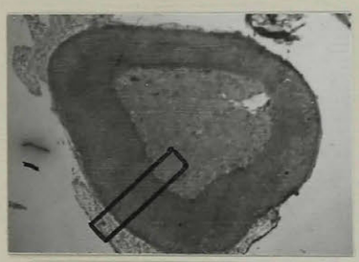


Figure 6

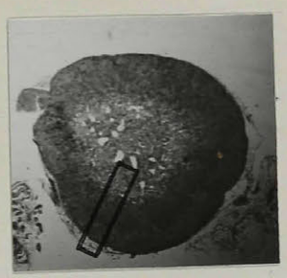


Figure 8

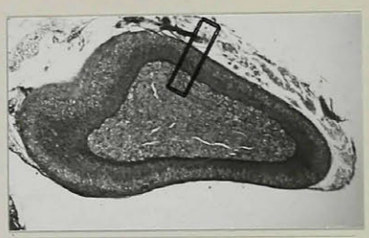


Figure IO

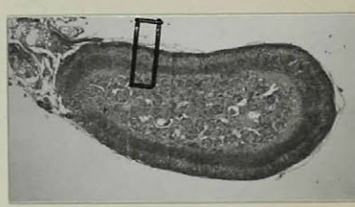


Figure I2

Figure 7

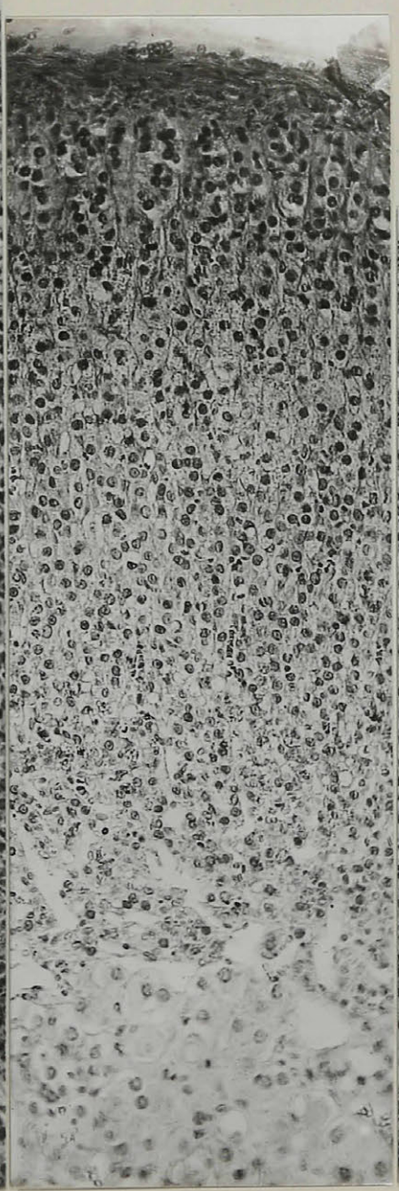


Figure 9

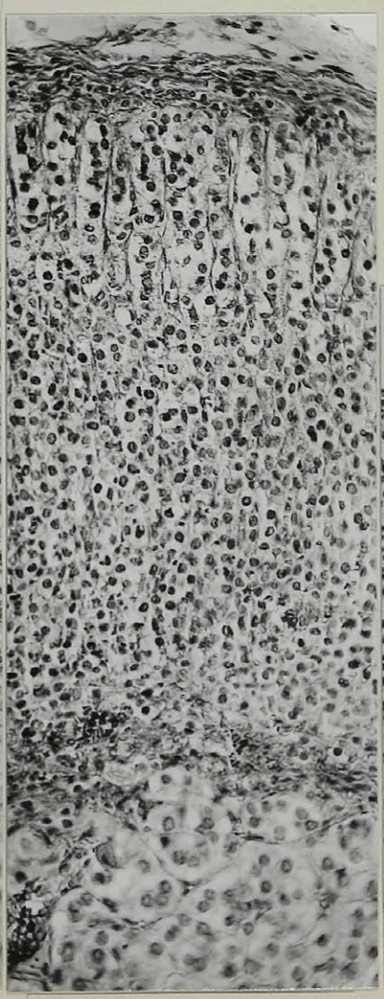


Figure II

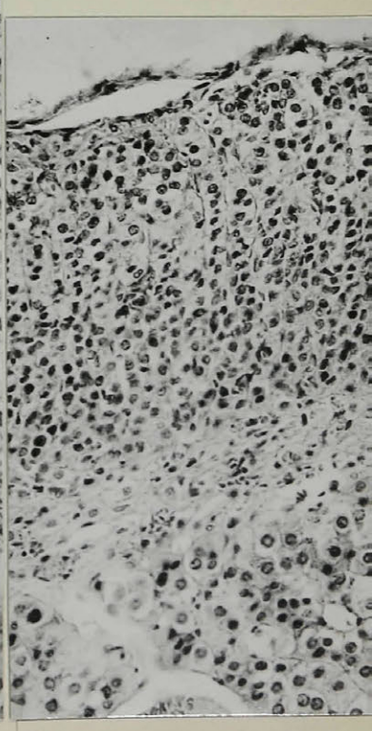


Figure I3

PLATE 5

Figure I4. Photomicrograph of adrenal of Rat 49 autopsied 74 days after grafting. Hypophysectomized 83 days. The cortex is quite wide. Greater magnification of the cortex was not done. Hematoxylin and Van Gieson; x I4.

Figure I5. Photomicrograph of adrenal of Rat 88 autopsied 45 days after grafting. Hypophysectomized 60 days. The cortex is somewhat widened. Hematoxylin and Van Gieson; x I4.

Figure I6. Photomicrograph of cortex of adrenal of Rat 88 taken under higher power. There is a moderate widening of the cortex and a considerable alteration in the cells of the innermost zone (Zona reticularis) so that the cells are fairly plump with round or oval, non-pyknotic nuclei. Compare the repair of the inner zone in this adrenal with that of the control in Figure I9. Hematoxylin and Van Gieson; x 230.

Figure I7. Photomicrograph of adrenal of Rat 53 autopsied 63 days after grafting. Hypophysectomized 76 days. Its cortex is fairly wide. Hematoxylin and Van Gieson; x I4.

Figure I8. Photomicrograph of adrenal cortex and adjoining medulla of Rat 53. There is a moderate degree of repair of the two inner zones. Hematoxylin and Van Gieson; x 230.

Figure I9. Photomicrograph of adrenal of untreated hypophysectomized rat. Note the marked cortical atrophy. Hypophysectomized 54 days. Hematoxylinn and Van Gieson; x 230.

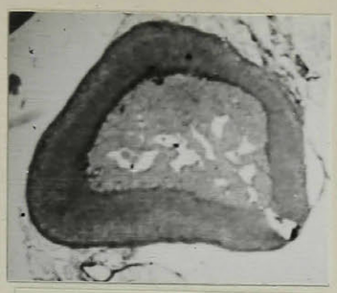


Figure I4

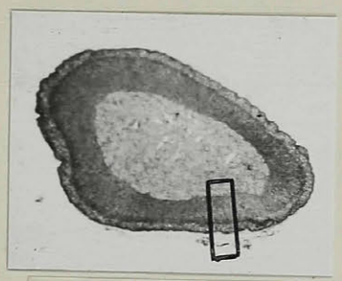


Figure I5

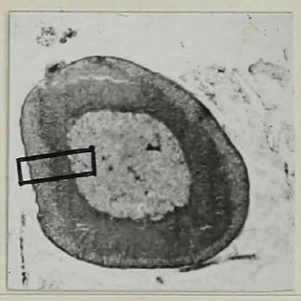


Figure I7

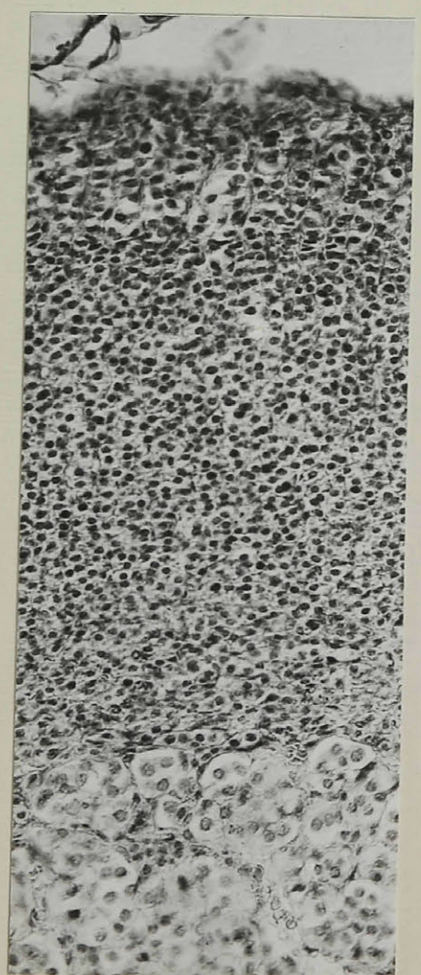


Figure I6

Figure I8

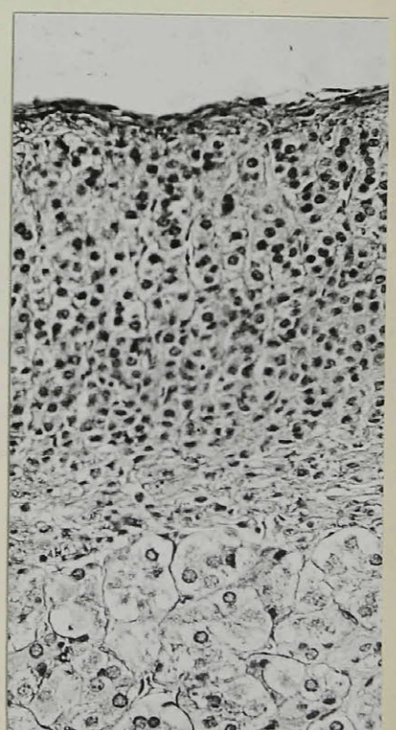


Figure I9

Plate 6

Photomicrograph of tissue culture of fragment of anterior pituitary explanted from a 23 day old rat. This culture was grafted into Rat 76. It is fairly representative of the type of culture grafted into the other animals. This fragment planted on a cover slip began to grow in about seven hours, epithelial sheets advancing en masse into the liquefied zone which in this preparation is extensive. The sheets are interpreted as parenchymal cells. It will be noted that the cytoplasm of these cells stain more or less deeply ostensibly beaause of the degree of globulation. Whether this difference in the refractibility in the globules may be laid to acidophilia or basophilia can in such an unstained preparation only be surmised. There are also to be seen a number of histiocytes or macrophages scattered about indiscriminately. Giant cells are also to be seen. The largest measured 360 micra in size. Further on in the thesis these giant cells will be described in greater detail. They are probably formed from monocytes.
Unstained preparation. (Higher magnification of some of parenchymal cells are to be seen in Plate 7.)

PLATE 6

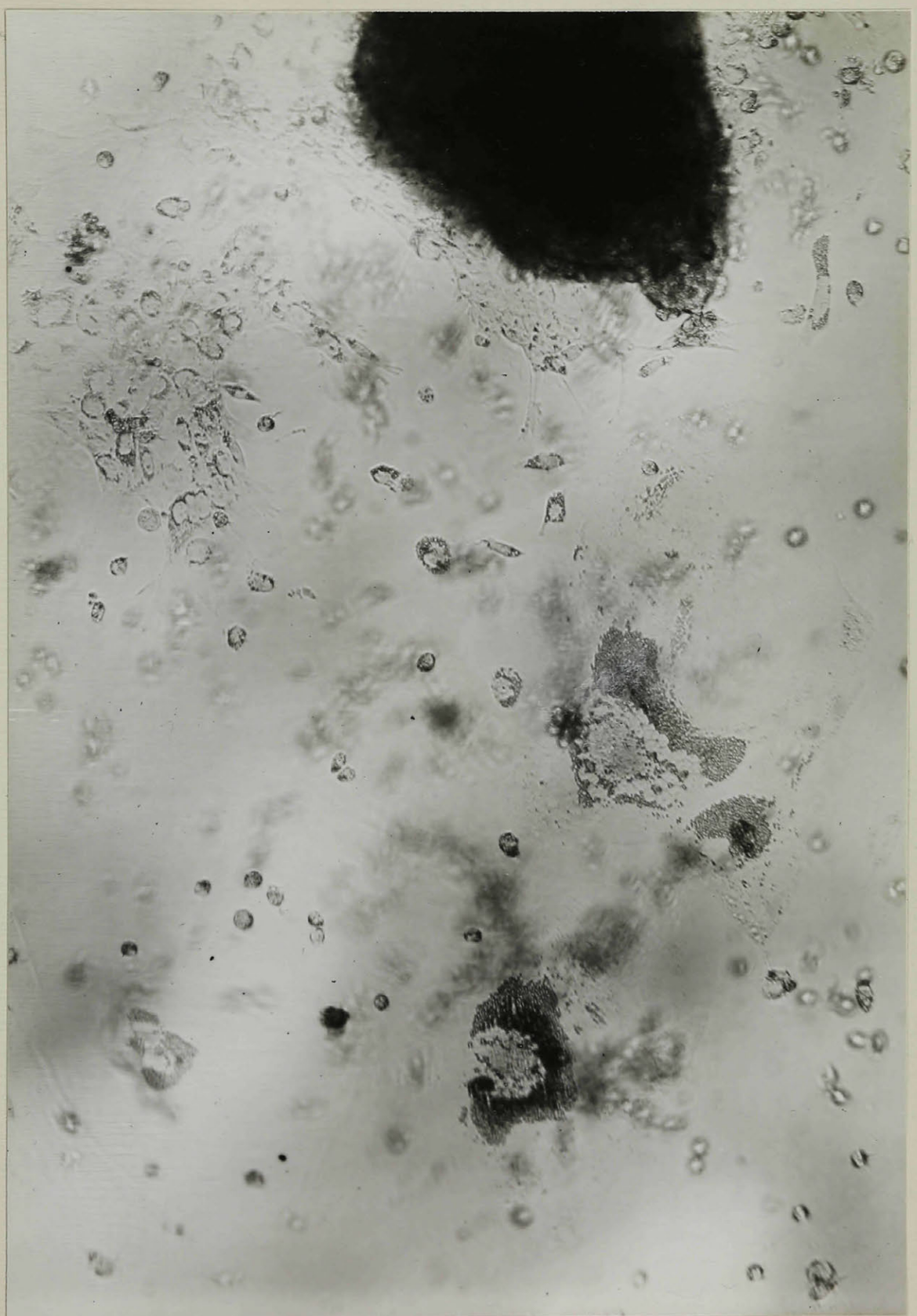


PLATE 7

Photomicrograph of a sheet of parenchymal cells which have grown out from a fragment of anterior pituitary taken from a 23 day old rat. It is a higher magnification of a portion of photograph in Plate 6. This culture has grown for six days in all and has been transferred once. Note the varying amount of cytoplasm, the variable number and size of globules and also the intranuclear bodies which are probably nucleoli. Unstained fresh specimen.

PLATE 7

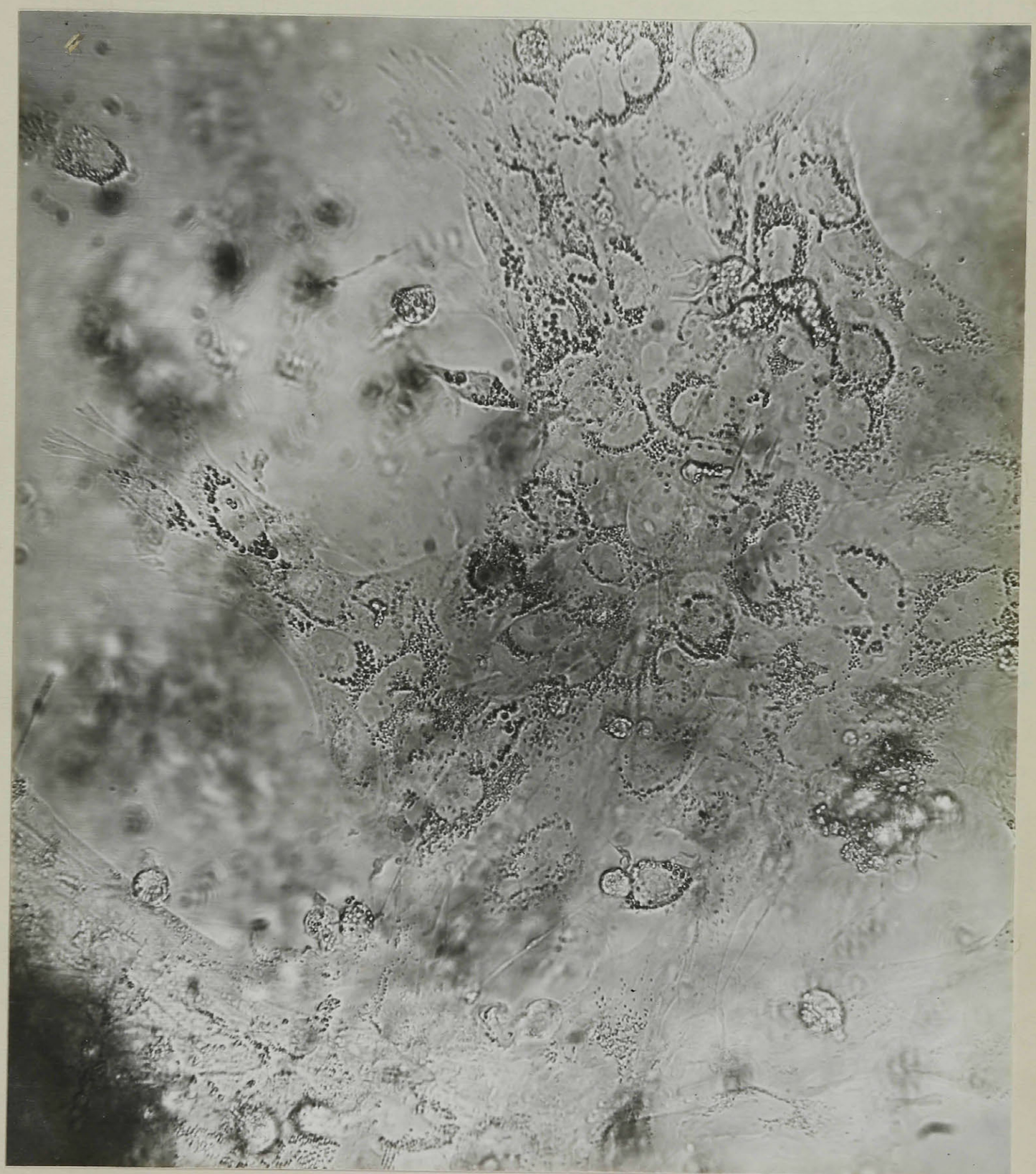


PLATE 8

Figure I. Photomicrograph of anterior lobe of 33 mm. rat foetus. Note the preponderance of chromophobe cells with here and there the more deeply staining acidophils bordering the large sinusoids. Cleveland and Wolfe stain; x 260.

Figure 2. Photomicrograph of anterior lobe of II cm. pig foetus grown in vitro for 4 days. The more deeply staining to the left is part of the fragment from which the cells have grown. Most of the cells are chromophobes with large nuclei such as cell C. A few cells (A) with smaller nuclei are suggestive of acidophils. Cell B with an eccentric nucleus looks like a basophil. The lighter staining circular area in the cytoplasm of cell B is suggestive of the Golgi apparatus described by Severinghaus as characteristic of that of the basophil. Mitotic figures are present.
Adaptation of Wolbach's Modification of Giemsa Stain; x260.

Figure 3. Photomicrograph of pure culture of anterior lobe cells of II cm. pig foetus grown 3 days. The fragment from which the cells have grown is to the right. The dark line to the left is the border of the liquefied zone. In many cultures of this type no wandering cells or fibroblasts were seen.
Adaptation of Hortega's Double Silver Impregnation; xI50.

PLATE 8

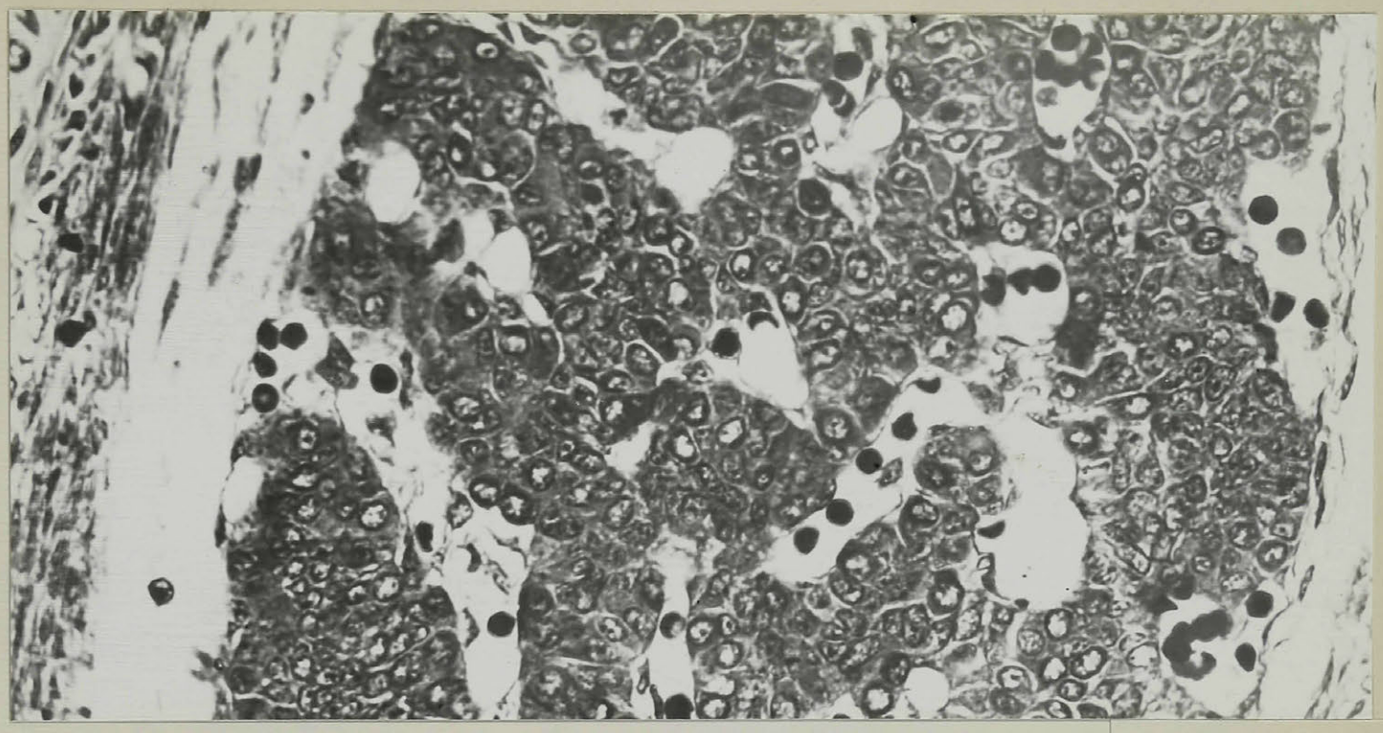


Figure I

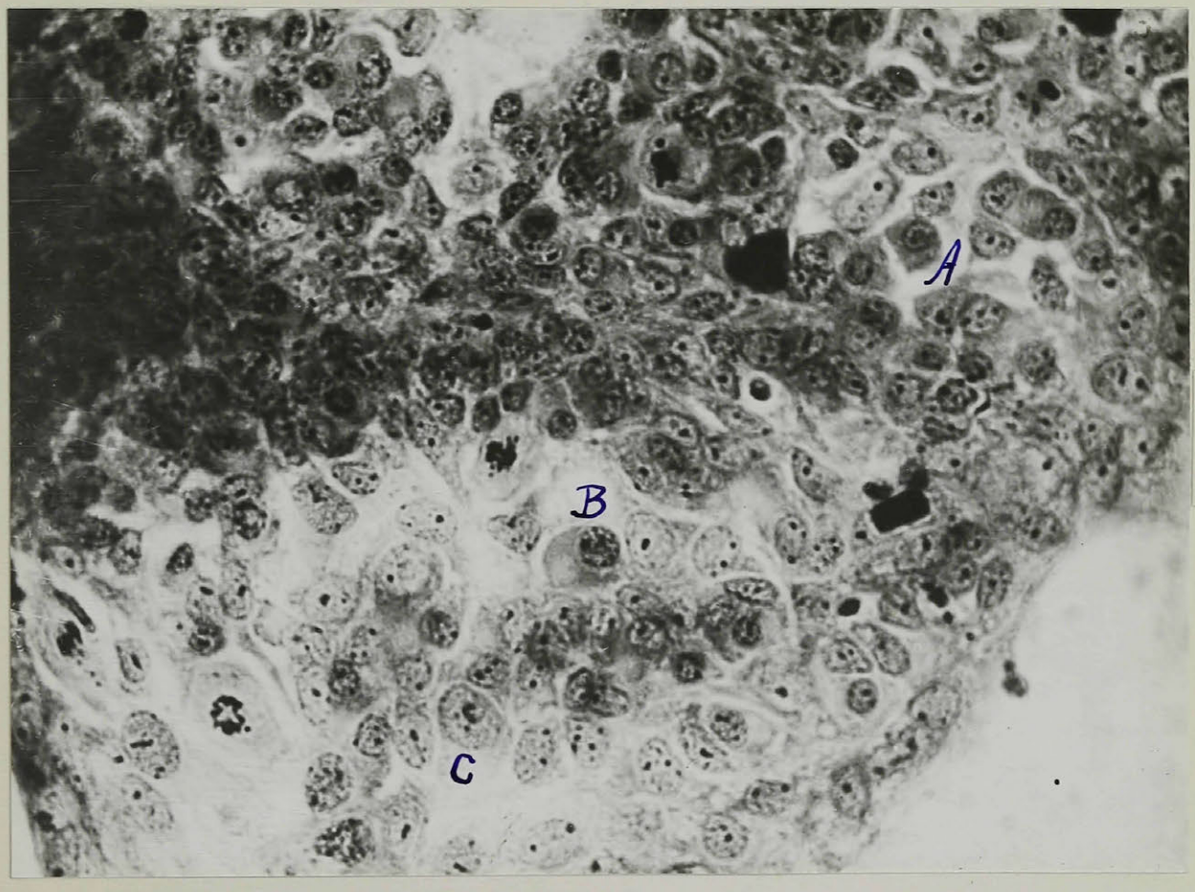


Figure 2

Figure 3

PLATE 9

Figure I. Photomicrograph of I day old rat anterior pituitary. The space at the top is the intraglandular cleft. Chromophobes predominate. Scattered throughout the section are acidophils (A). A few large cells with excentrically placed nucleus are seen here and there; these are probably basophils (B). Sinusoids are much less prominent than in the foetal pituitary.
Cleveland and Wolfe stain; x 260.

Figure 2. Photomicrograph of anterior pituitary of 5 day old rat. Note increase in number of acidophils and basophils over those in the I day anterior pituitary. A large clump of basophils are present in the center of the section (B).
Cleveland and Wolfe stain; x 260.

Figure 3. Photomicrograph of anterior pituitary of 8 day old rat cultivated <u>in vitro</u> for 4 days. The growing parenchymal cells have taken on the form of a pavement epithelium. The cytoplasmic contour varies considerably in different parts of the growth. In other cultures in which there is not a uniform liquefaction of the clot about the fragment the cells instead of forming a membrane grow out in cords or in finger-like projections or grow as isolated islands. Note an occasional mitosis (M). Also a phagocyte in the liquefied zone(P).
Adaptation of Hortega's Double Silver Impregnation; x I50.

PLATE 9

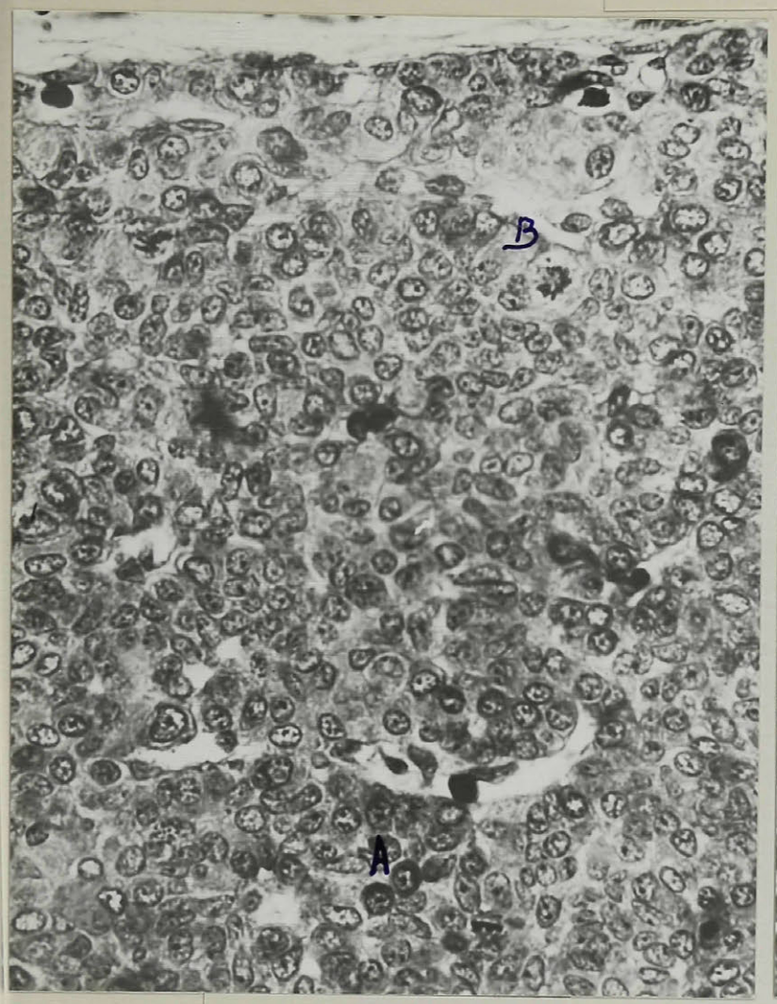


Figure I

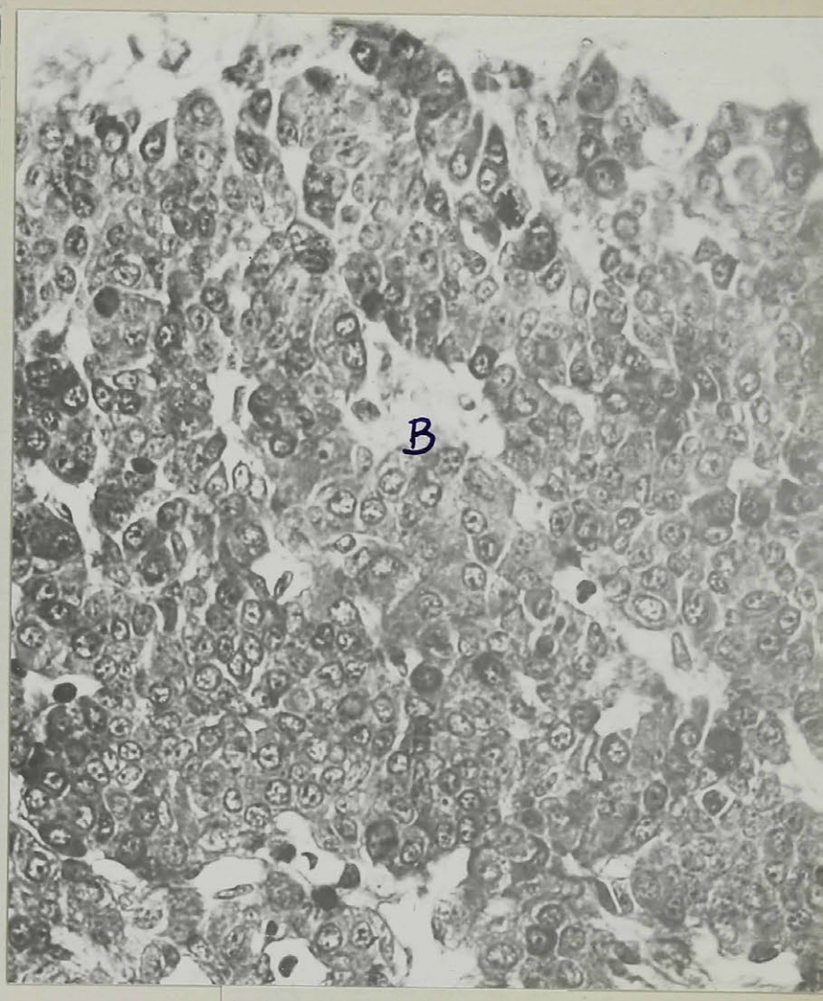


Figure 2

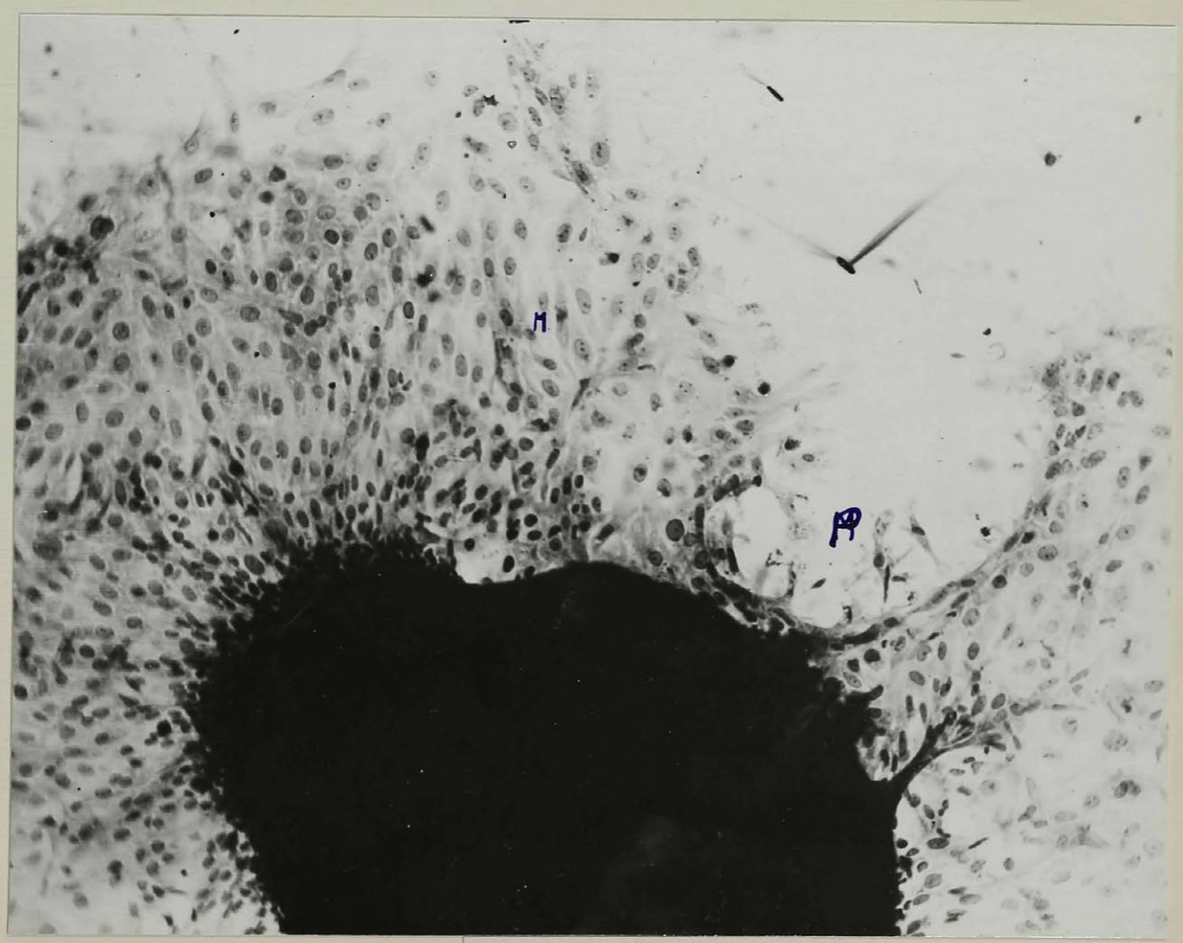


Figure 3

PLATE IO

Figure I. Photomicrograph of tissue culture of anterior pituitary of an 8 day old rat. This is shown in order to demonstrate the various types of cells whcih are to be found in anterior lobe cultures. The round cells with the large or oval nuclei are epithelial parenchmal cells (E). The elongated cells are fibroblasts (F). The smaller free wandering cells may be designated as polyblasts or macrophages (M).
Adaptation of Wolbach's Modification of Giemsa's Stain; x 260.

Figure 2. Photomicrograph of anterior and intermediate lobe cells of the pituitary of a ten day old rat. The intraglandular cleft (I.C.) separates the two types of cells. In the anterior lobe (A) are to be seen numerous acidophils in contrast to the intermediate lobe which is made up of chromophobe cells. At X the intermediate cells are joined to the anterior lobe.
Cleveland and Wolfe stain; x 260.

Figure 3. Photomicrograph of tissue culture of anterior pituitary of an 8 day old rat. Note how much larger the parenchymal cells are than in the section (Fig. 2) of a corresponding age. Mitotic figures (M) are present as is also a large binucleate epithelial cell (P).
Adaptation of Wolbach's Modification of Giemsa's Stain; x 260.

PLATE IO

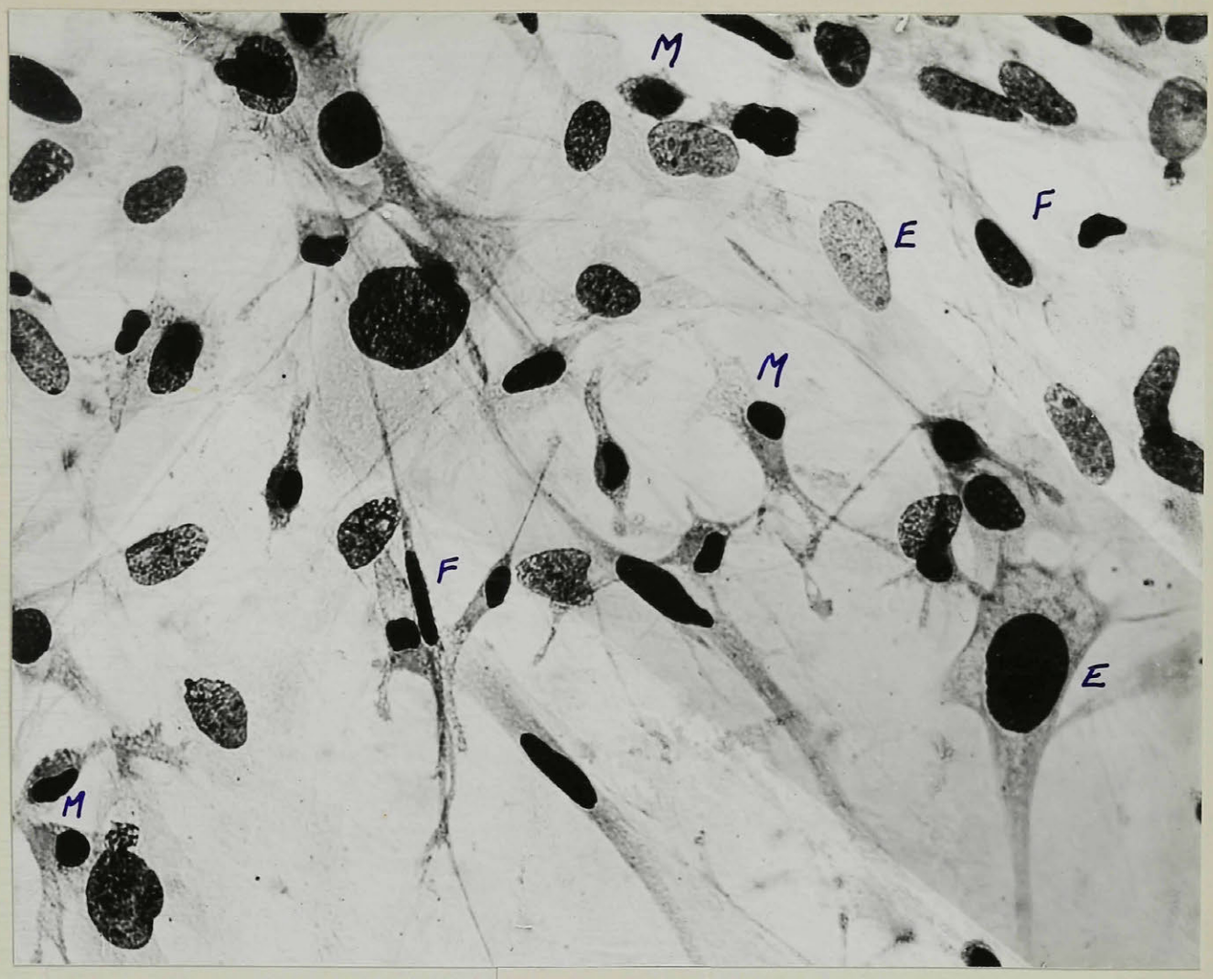


Figure I

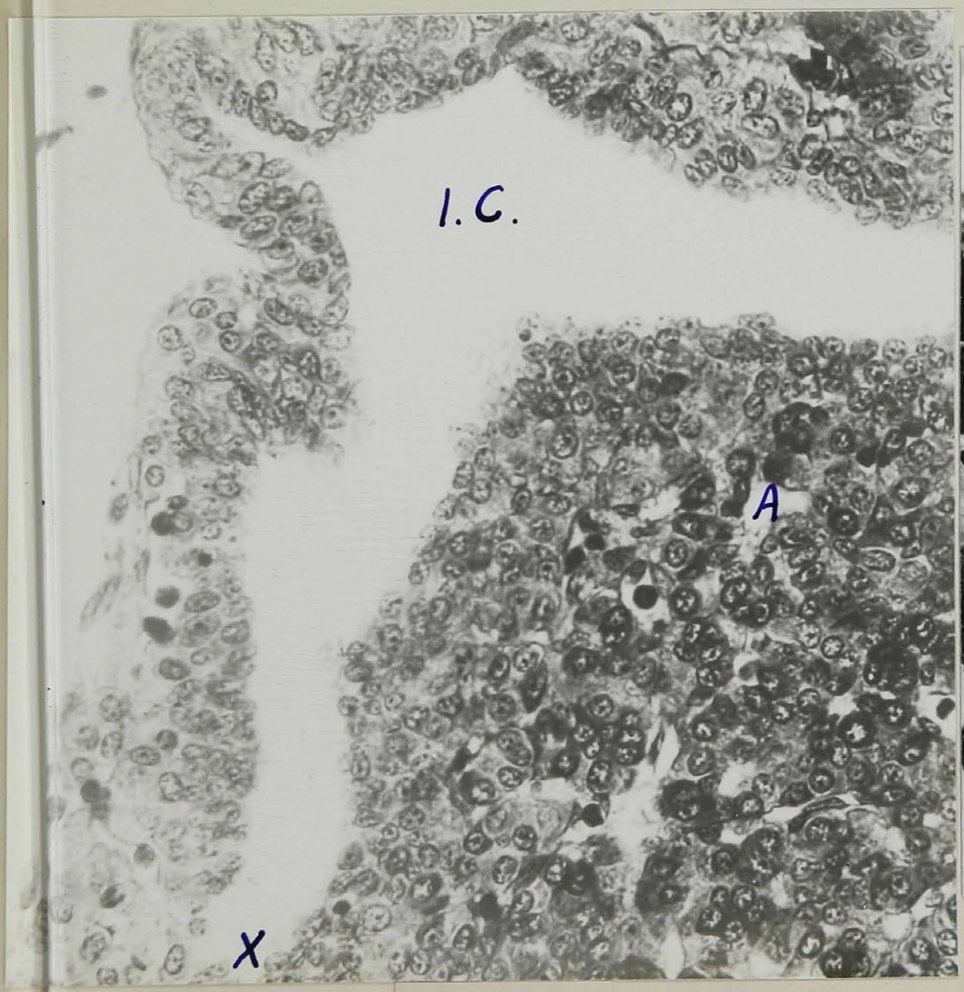


Figure 2

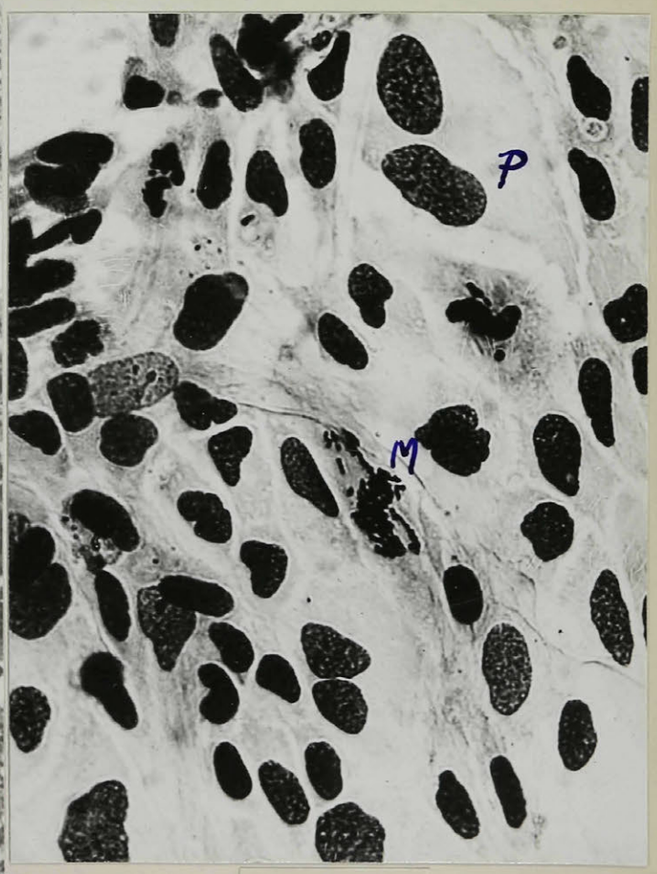


Figure 3

PLATE II

Figure I. Photomicrograph of anterior and intermedia cells of the pituitary of a 28 day old rat. A greater relative proportion of eosinophils is seen than in the preceding stages of the anterior lobe (A). It may be noted here that acidophils border directly upon the intraglandular cleft (I.C.). Acidophil A is less deeply stained than most others which may suggest that it is an early stage. The cells of the intermediate lobe all stain with the same intensity (P.I.). They are joined directly to the pars nervosa (P.N.). In the present study of the sections interdigitation of the intermediate cells into the pars nervosa is seldom seen.
Cleveland Wolfe stain; x 260.

Figure 2. Photomicrograph of anterior and intermediate lobe cells of the pituitary of a 59 day old rat. The intraglandular cleft (I.C.) contains homogeneously staining material which is probably colloid (C).
Cleveland Wolfe stain; x 260.

Figure 3. Photomicrograph of tissue culture of anterior lobe cells cultivated from an 8 day old rat pituitary. The cytoplasmic borders vary in shape to conform to the neighboring cells. Their size varies markedly being much larger than the cells from which they sprang. Could this mean that if in the sella there was more scope for spreading out that pituitary cells would attain this size or have they become a more indifferentiated type? Note that the nuclei possess one to four deeply staining nucleoli.
Adaptation of Hortega's Double Impregnation Silver stain; x 260.

Figure 4. Photomicrograph of anterior lobe cells of the pituitary of a IO month old rat. Note clumps of acidophils (A) and of basophils (B). Granules have disappeared from some of the basophils (ba).
Cleveland Wolfe stain; x 260.

PLATE II

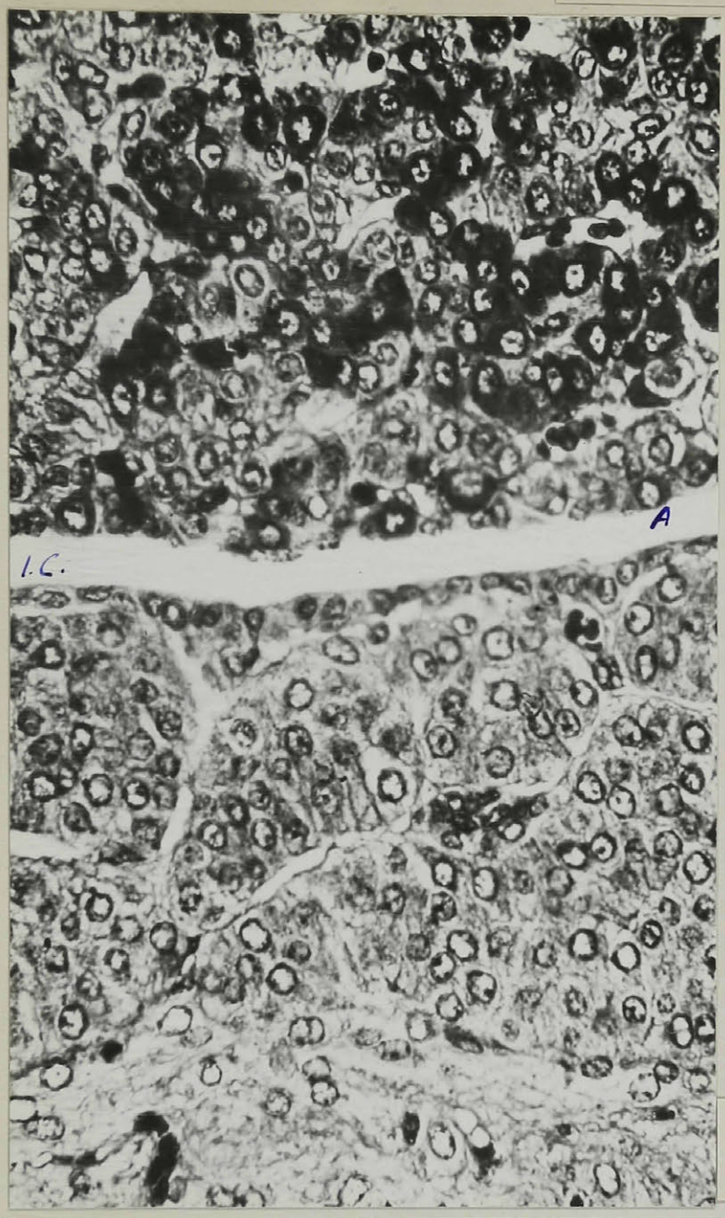


Figure I

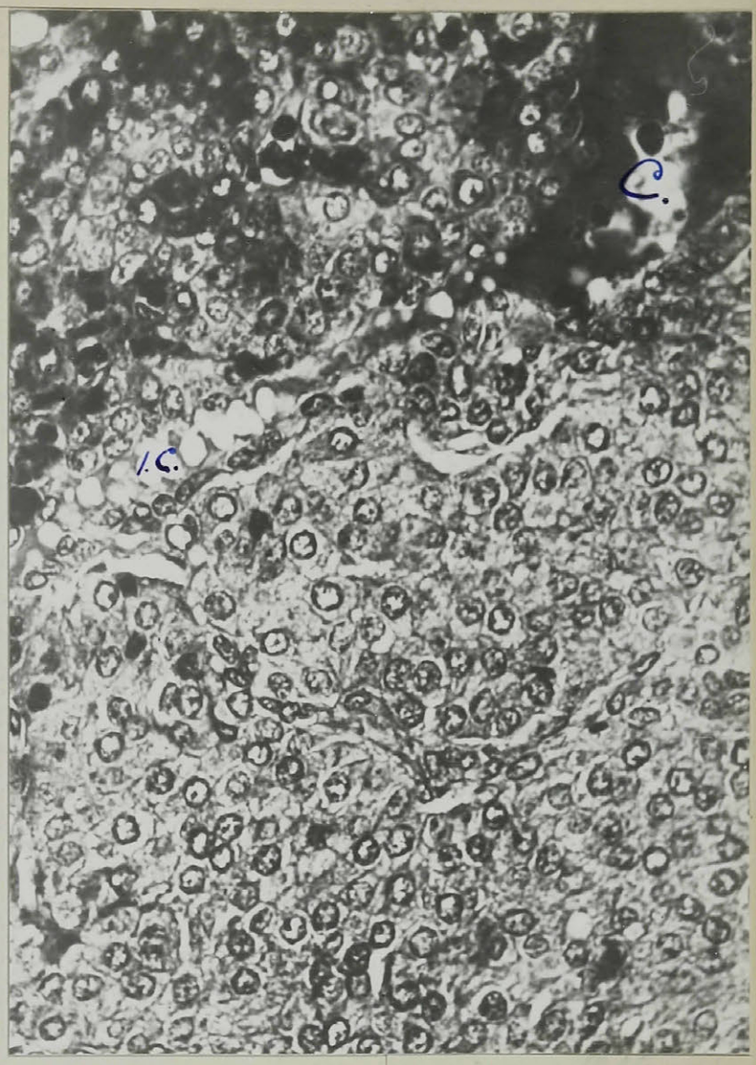


Figure 2

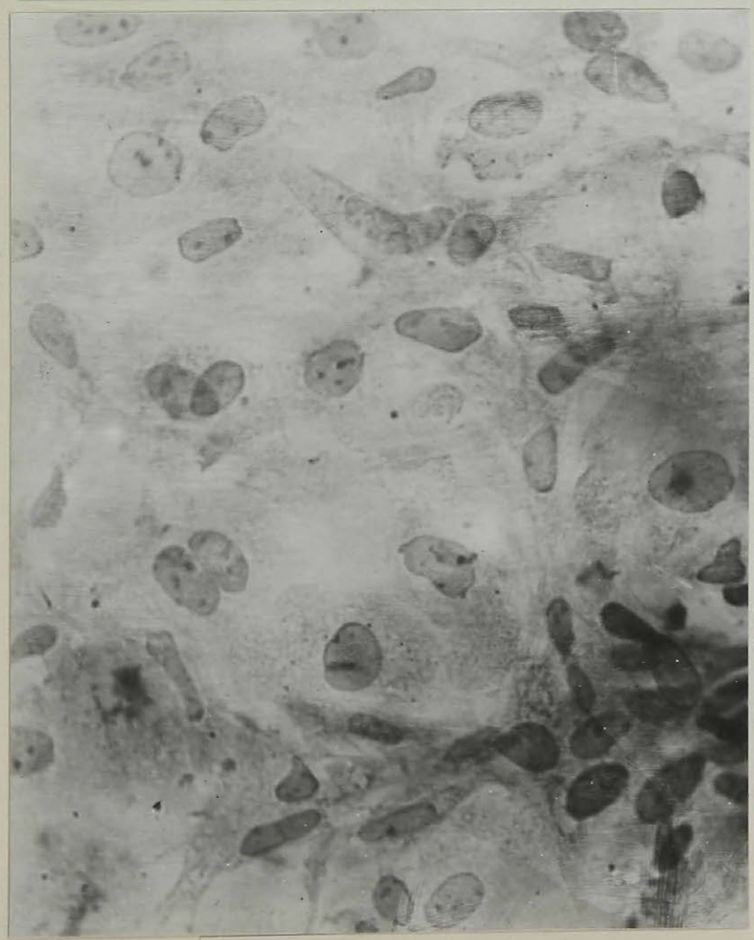


Figure 3

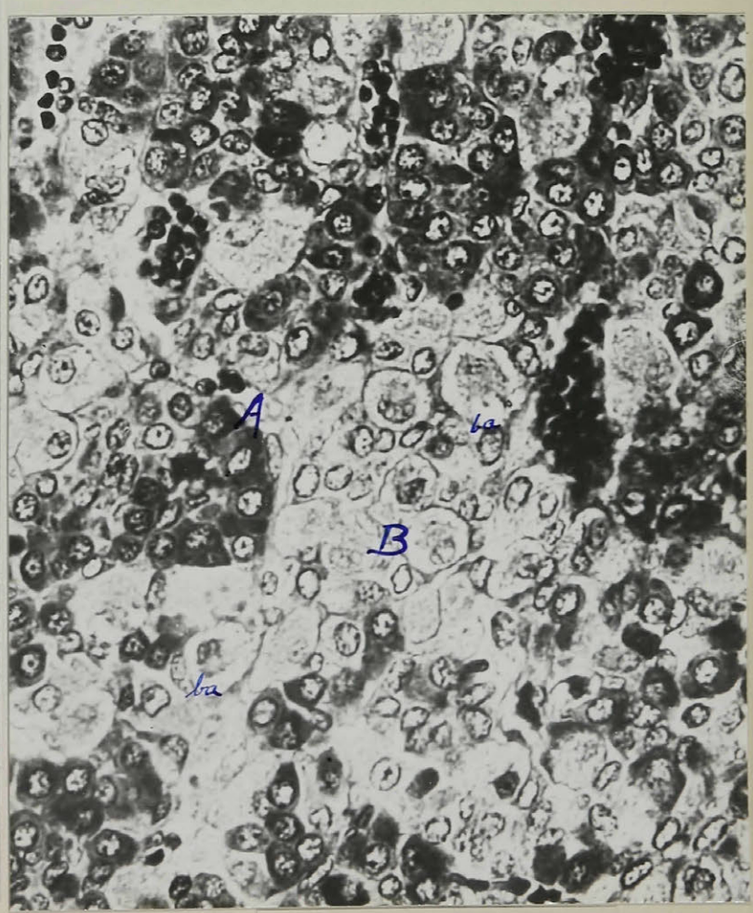


Figure 4

PLATE I3

Photomicrograph of two large giant cells which have migrated into the liquefied zone surrounding the fragment. These were formed in a culture of the anterior pituitary of a 23 day old rat. They were seen after 24 hours following explantation of the fragment and were photographed on the third day. Note the great number of nuclei containing nucleoli. Globules are present for the most part about the mass of nuclei. The cytoplasmic borders are outlined with arrows. One of the cells has a protoplasmic extension which appears to be joined at X with a degenerating cell. A few smaller cells whcih are monocytes or polyblasts are to be seen. Cell C is seen at a higher magnification in the next plate.

Unstained preparation.

PLATE I3

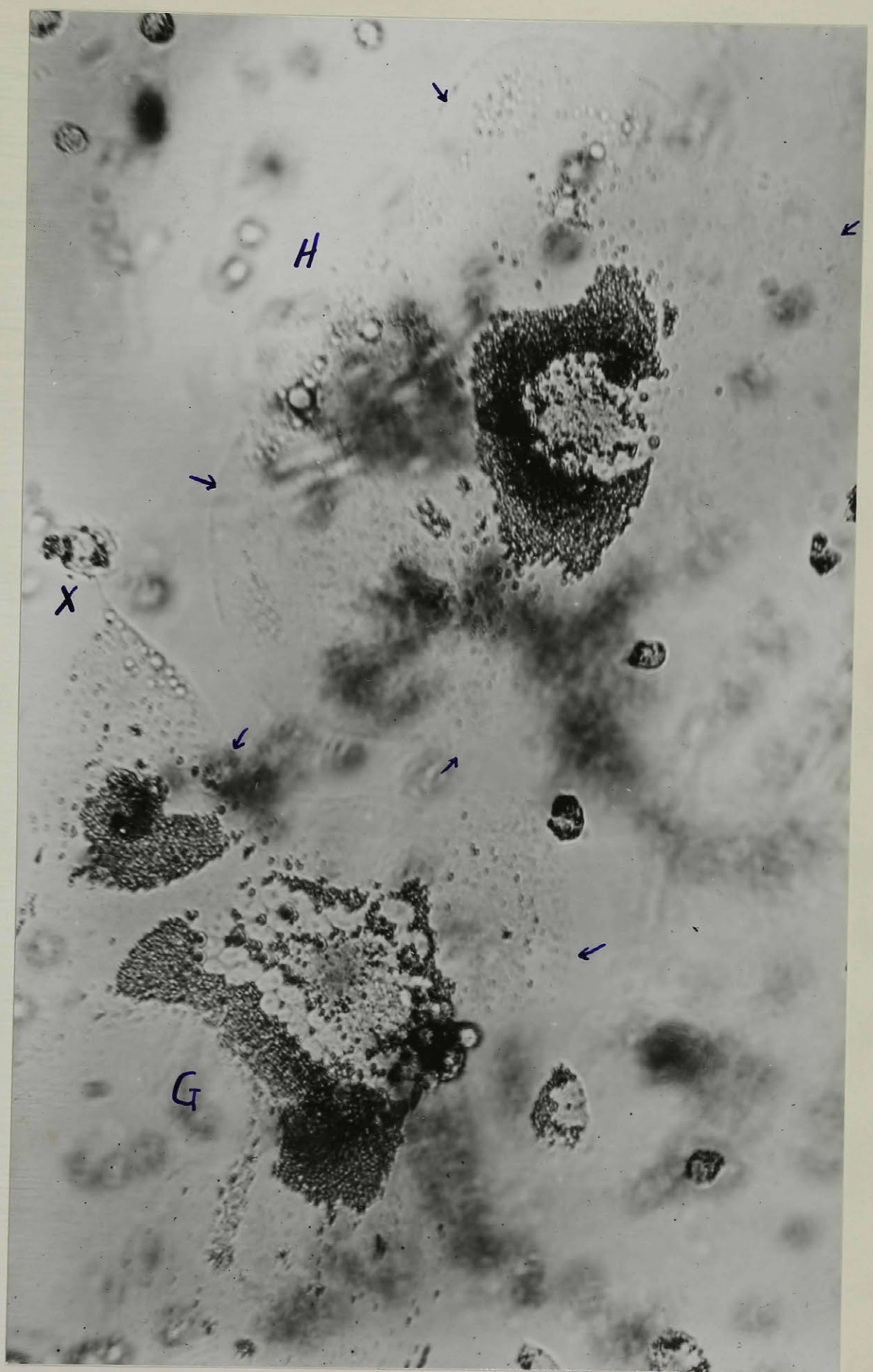


PLATE I4

Photomicrograph of Cell G in Plate I3. The size of the cell varied from hour to hour; on the second day it measured 360 micra in diameter. The border is pointed out by arrows. Nuclei and nucleoli are to be seen. Three wandering cells, probably monocytes are seen.

Unstained preparation.

PLATE I4

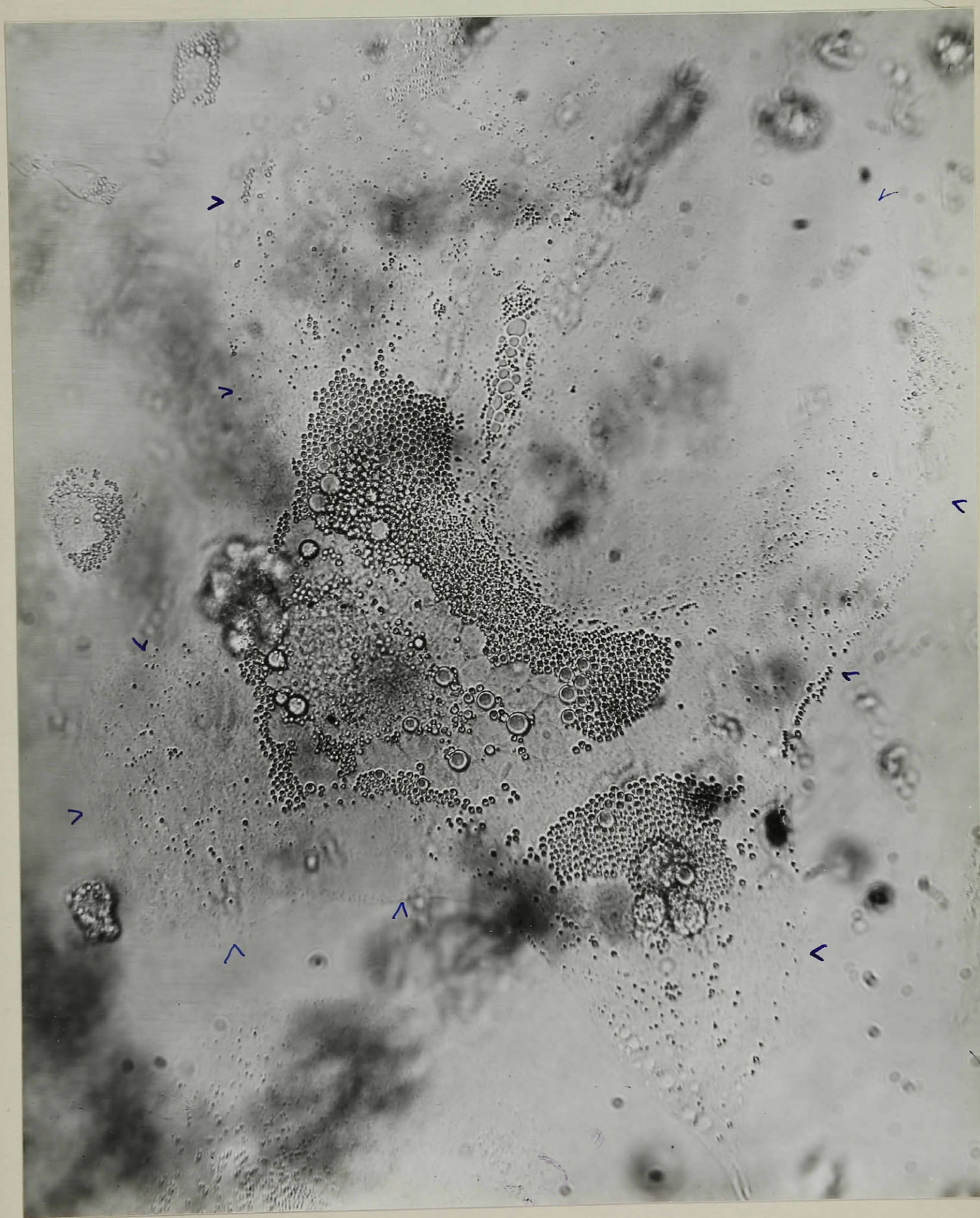


PLATE I5

Figure I. Camera lucida drawing partly diagramatic of a patch of ciliated cells appearing on a border of a fragment of anterior pituitary grown in vitro. The cilia were beating at a tempo far too rapid to count. The stream of the current was directed toward the cilia so that free cells such as that pictured in the drawing were drawn to the cilia. The cilia upon which the cell rested were stationary, or rather, non-motile. From an 8 day old rat. Unstained preparation; x II00.

Figure 2. Photomicrograph of tissue culture of the posterior lobe of an 8 day old rat. These are epithelial cells growing from the pars intermedia. There is a very close resemblance to the epithelial cells proliferating from the pars anterior as may be seen by comparing them with the cells in Plate II, Figure 3. There is the same polymorphism of cytoplsmic outline and variation in cell size. Note the deeply staining nucleoli.
Adaptation of Hortega's Double Silver Impregnation; x260.

Figure 3. Photomicrograph of a number of macrophages grown from the posterior lobe of an 8 day old pituitary. Stained 4 days after growth in vitro. It was unusual to encounter these macrophages (or microglia) in cultures of the pars anterior.
Adaptation of Hortega's Double Silver Impregnation; x 260.

PLATE I5

Figure I

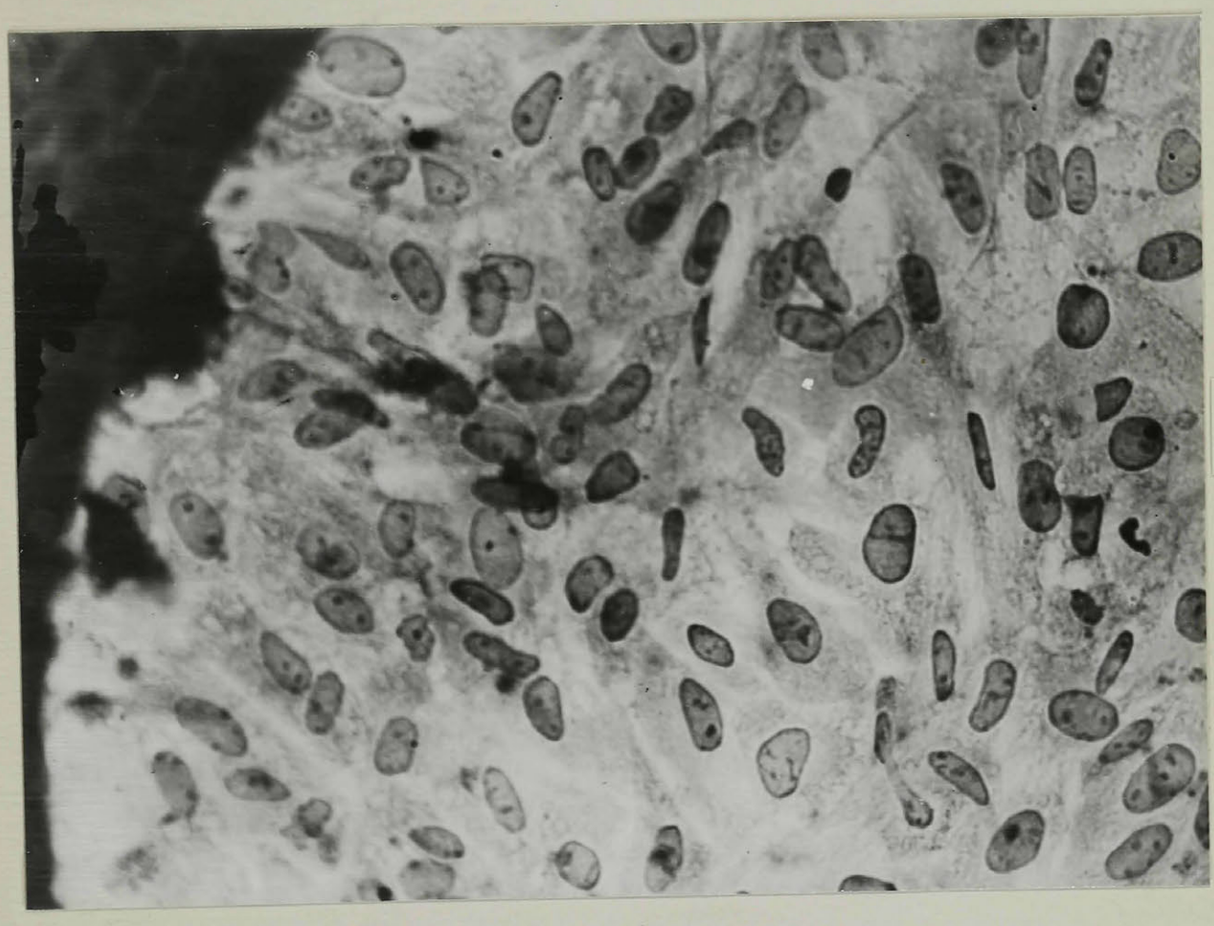


Figure 2

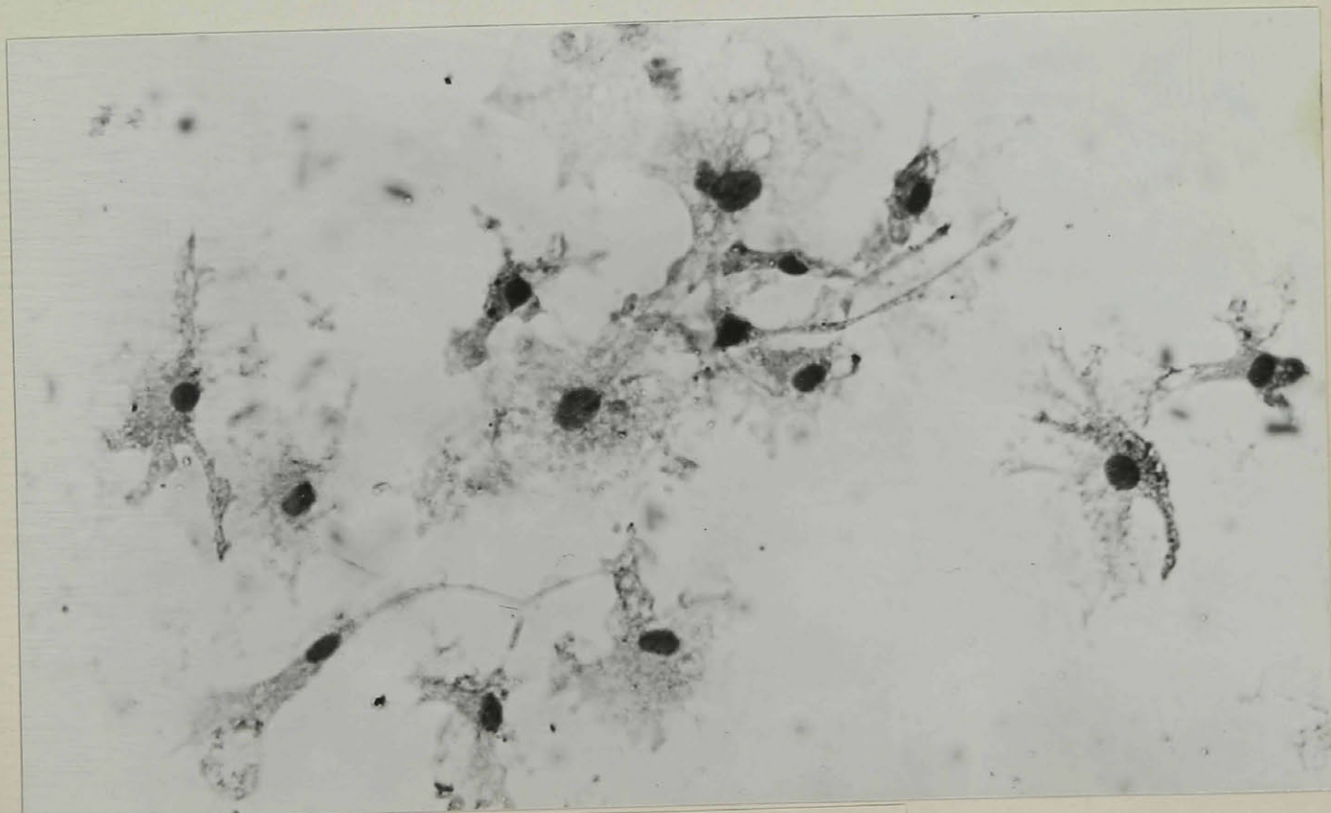


Figure 3

PLATE I6

Figure I. Photomicrograph of cells grown in tissue culture from the posterior lobe of an 8 day old rat. The larger cells (M) appear to be macrophages. For the sake of comparison the other cells are numbered as they are in Bucy's drawings in Figure 2. Cell II is a small multipolar cell with one longer extension possessing an end bud or foot. Cell IIa is a similar multipolar cell which has a few fine granules in its cytoplasm. Cell 8 is a larger bipolar cell containing granules in its cytoplasm. Adaptation of Hortega's Double Silver Impregnation x 260.

Figure 2. Bucy's drawings of pituicytes [(9) in text] taken from bovine posterior lobe. The cells grown in tissue culture are similar to the cells which he herein depicts. The cells grown in vitro are numbered as those in these drawings where a similarity exists.

Figure 3. Photomicrograph of cells grown in tissue culture from the posterior lobe of an 8 day old rat. Of smae stain and magnification as in Figure I. Cell A is a bipolar cell with fine processes showing terminal branching; it resembles an oligodendroglial cell. Cell B is another bipolar cell which has fine granules in its cytoplasmic processes; it suggests a bipolar spongioblast.

Figure 4. Photomicrograph of cells grown in tissue culture from the posterior lobe of an 8 day old rat. Cells In are intermediate lobe epithelial cells which seem to have taken on a syncytial structure. Evidence is strong that the outlying zone of cells in anterior and intermediate lobe cultures forms a syncytium is strong. Cell Ac resembles an astrocyte; cellAb is a lightly granular cell which has thicker processes. Cell 4 is a multipolar granular cell. Adaptation of Hortega's Double Silver Impregnation; x 260.

PLATE I6

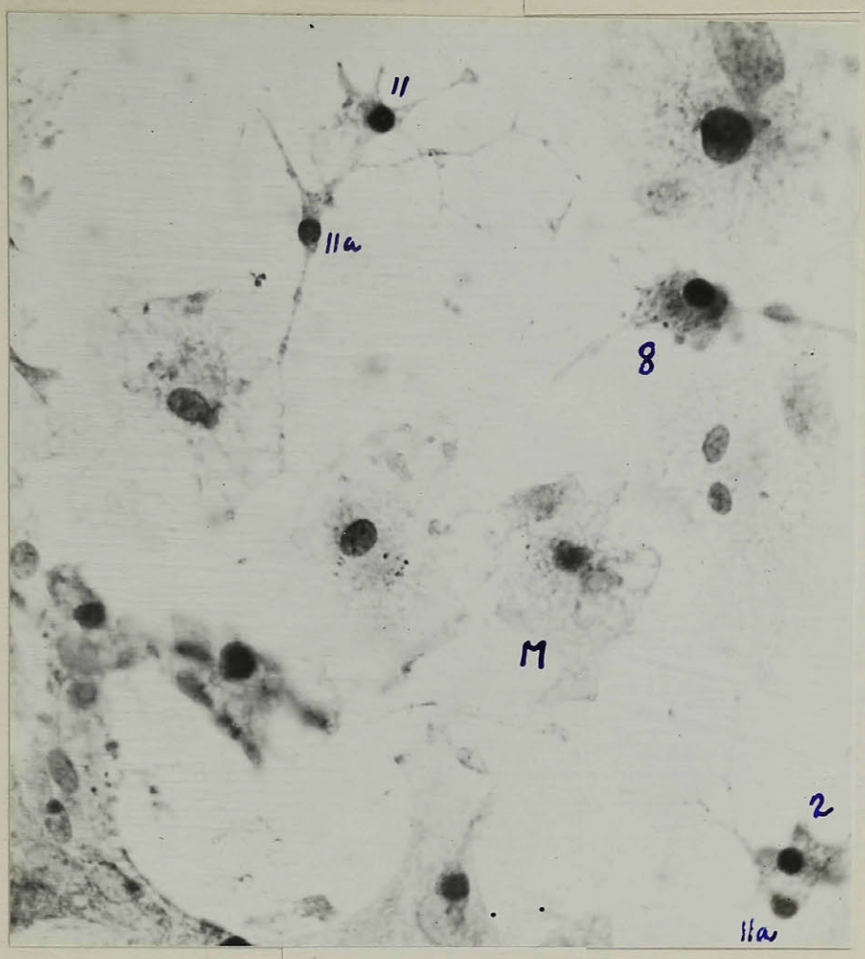


Figure I

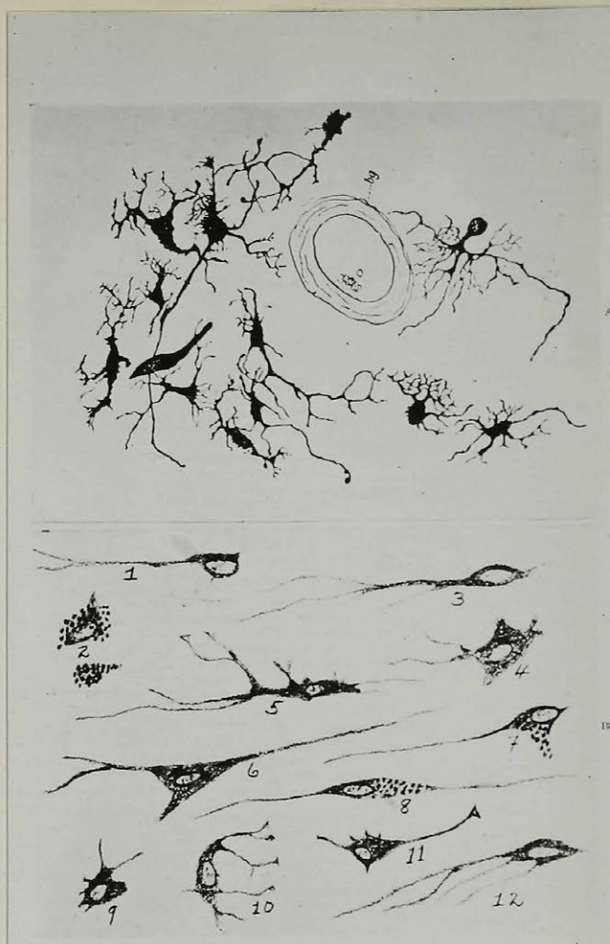


FIG. 2.—Pituicytes.

A. Showing similarity between the results of the Golgi method (after Retzius) and
B. The Penfield modification of Hortega's silver-carbonate method.

1. Similar to an astroblast with a large vascular foot and two small processes
2, 7, 8, pigmented cell (see Fig. 3 B); 3, 12, long processes with several branche
4, 9, 11, small multipolar cell; 5, 10, multipolar cell with several vascul
feet; 6, cell resembling a neurocyte; 8, bipolar cell.

[714]

Figure 2

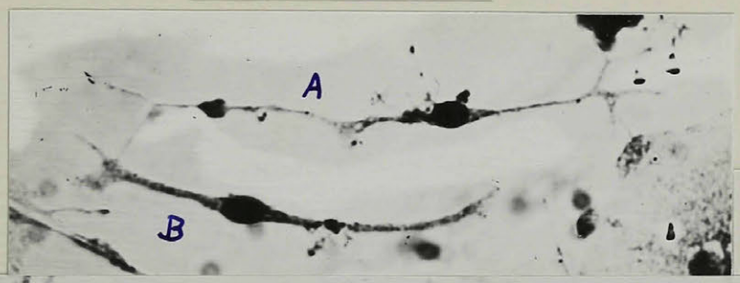
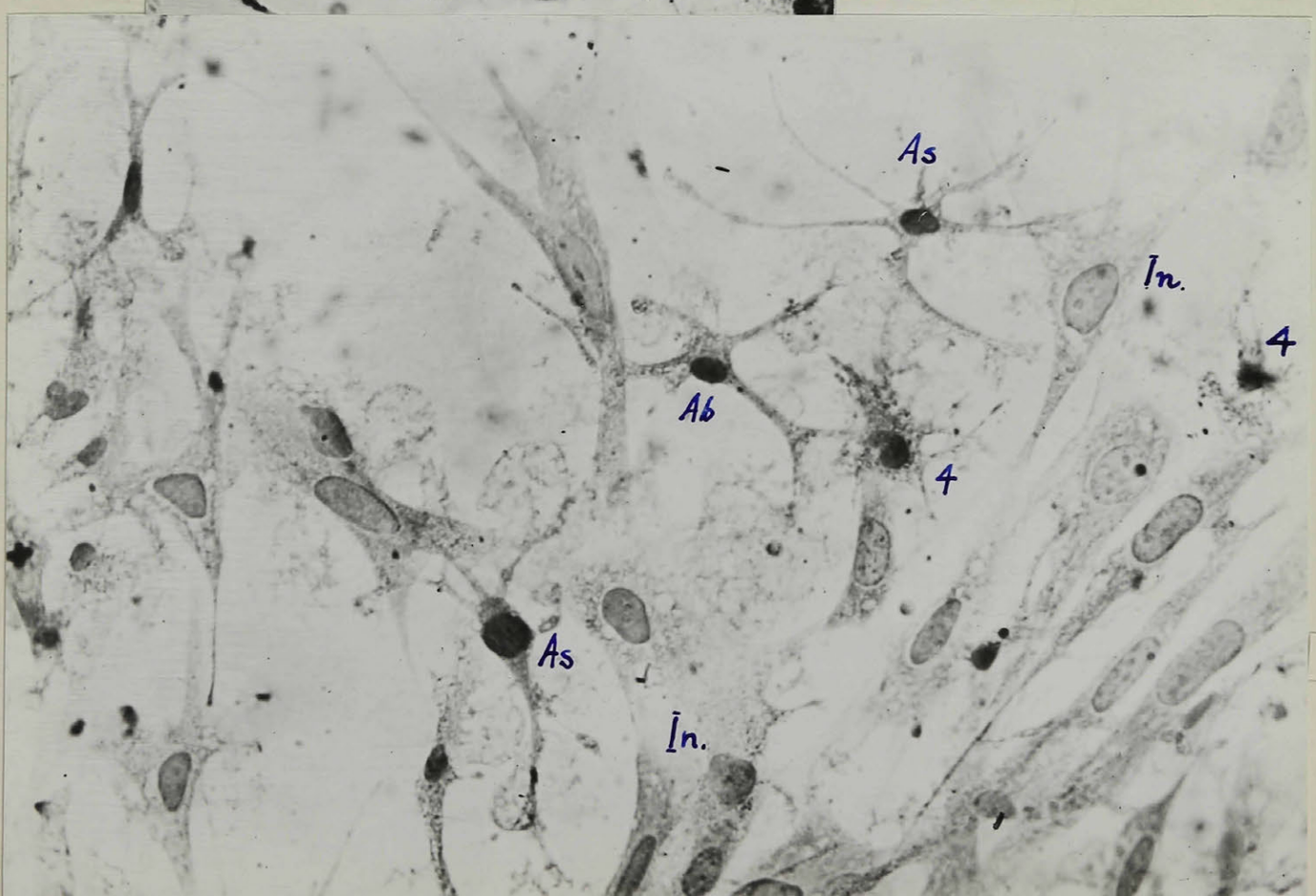


Figure 3



Figure

PLATE I7

Figure I. Photomicrograph of cells grown *in vitro* from the posterior lobe of an 8 day old rat. These, like the other cultures of cells of this type were grown for four days when staining was done. Cell M is a monopolar granular cell. Because of its granulation it would appear hazardous to liken it with cell types in the brain.

Adaptation of Hortega's Double Silver Impregnation; x 260.

Figures 2, 3 and 4. Photomicrographs of cells grown *in vitro* from the posterior lobe of an 8 day old rat. Cells In are intermediate lobe cells. Cell As strongly resembles an astrocyte; it possesses end processes which are not unlike vascular feet. Cells M are probably macrophages of differing stages. Cell IIa is a small multipolar type. Cell 2 is a small granular bipolar type of cell. The cell undergoing mitosis in Figure 4 is not identified (M).

Adaptation of Hortega's Double Impregnation; x 260.

PLATE I7

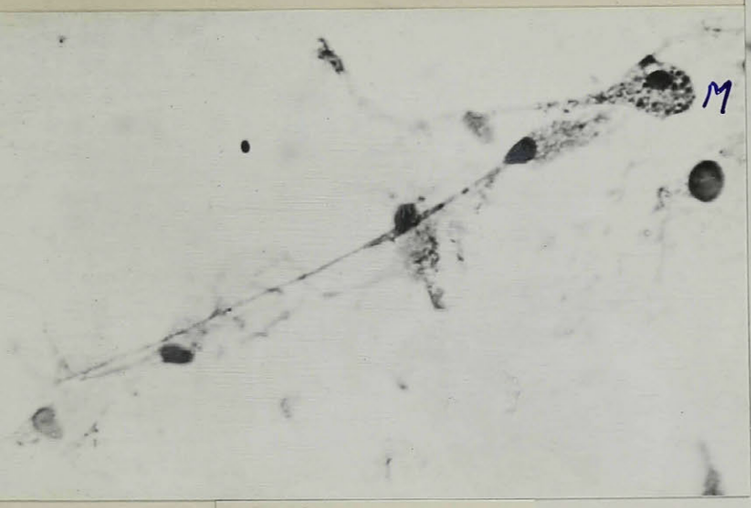


Figure I

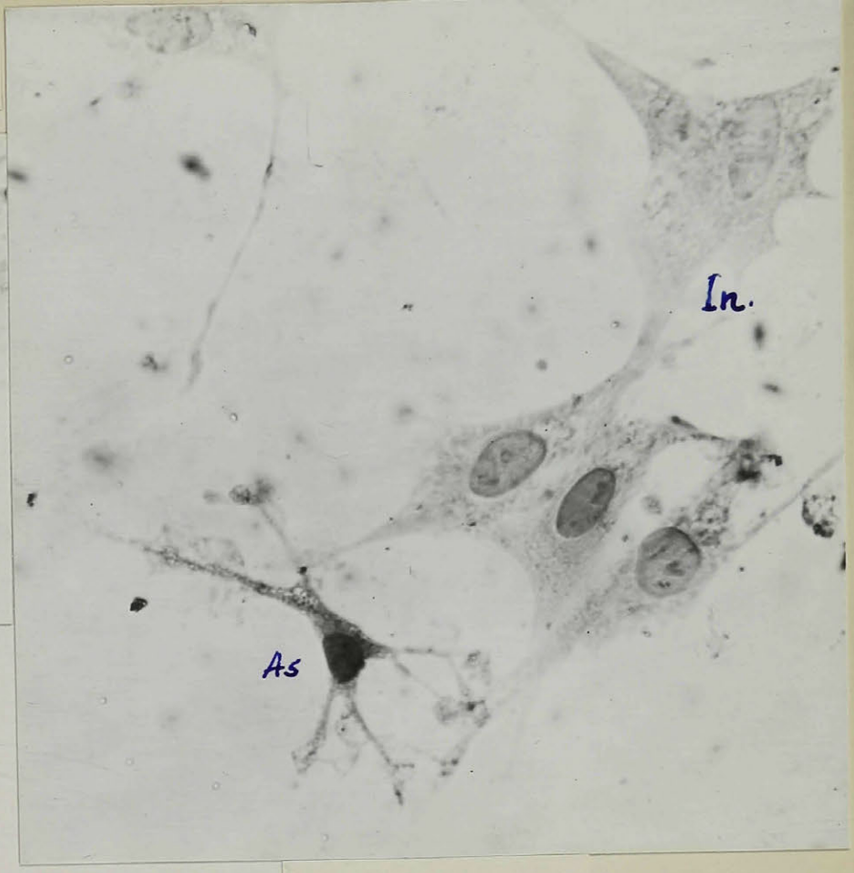


Figure 2

Figure 3

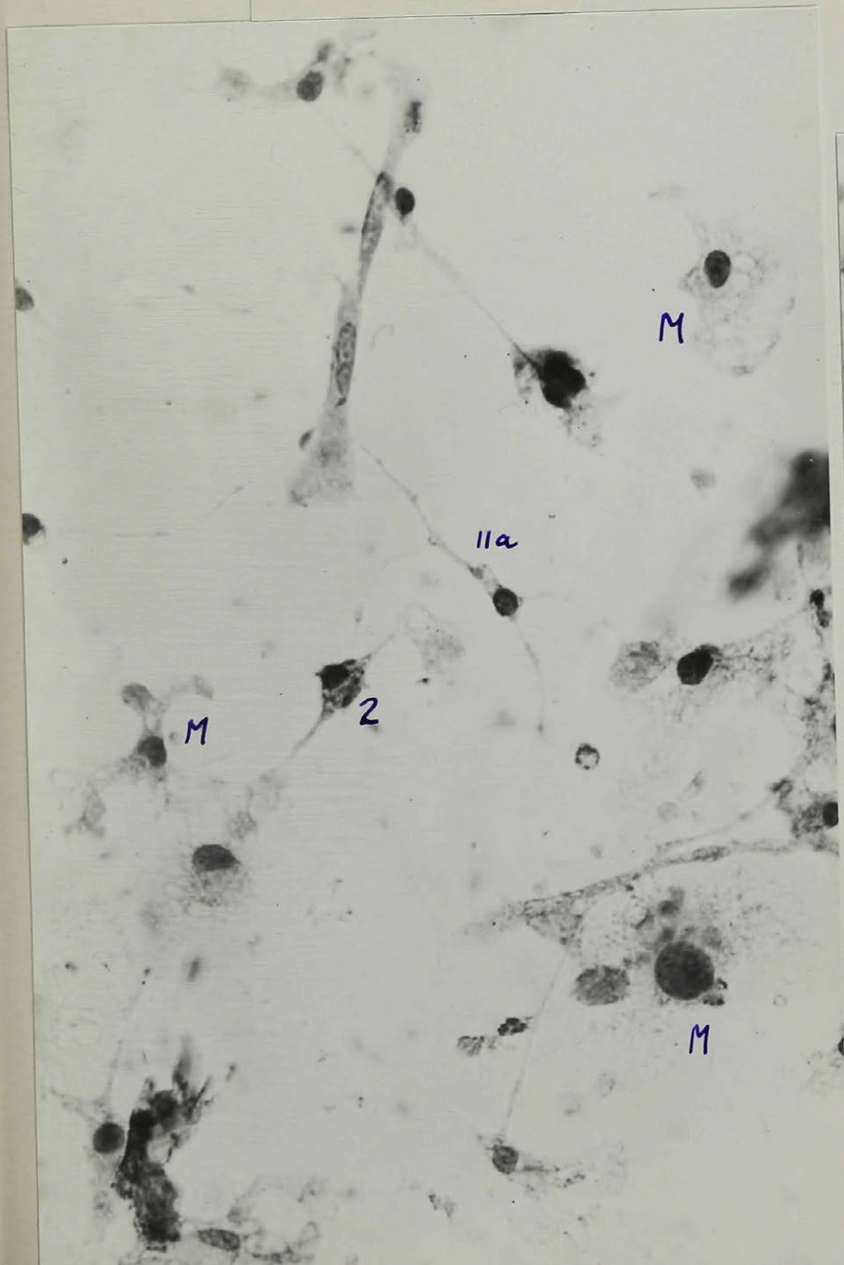


Figure 4

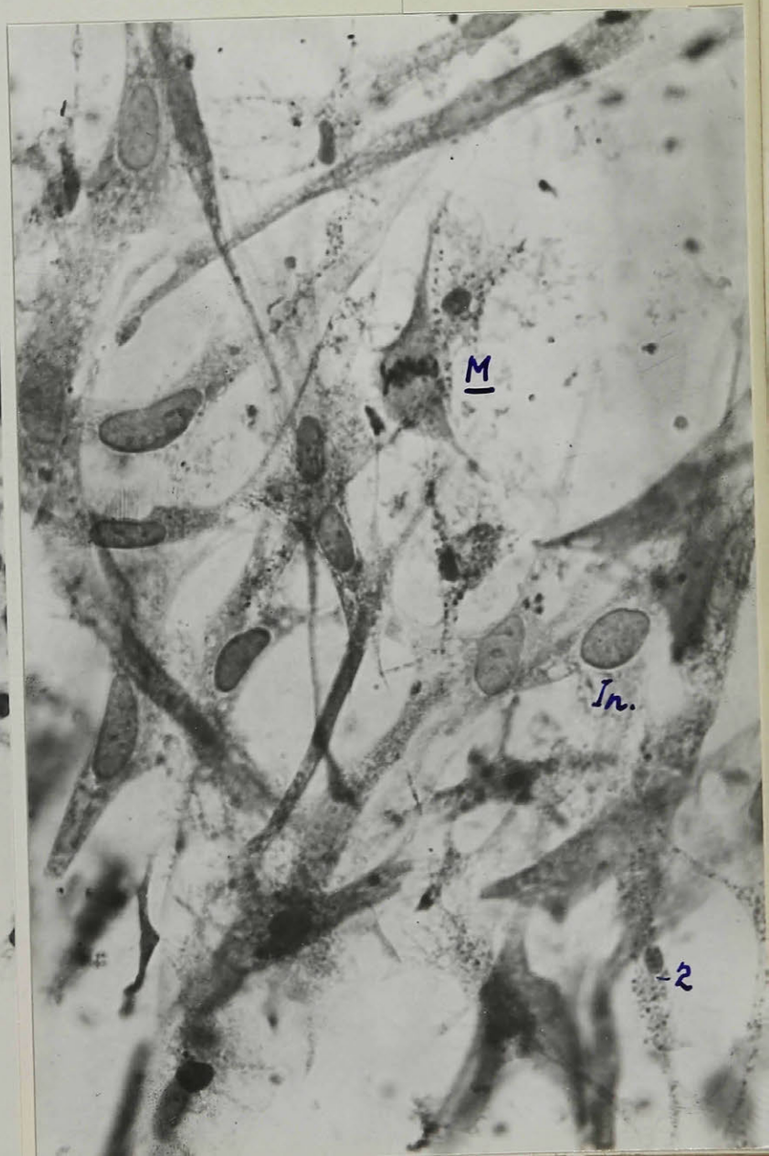


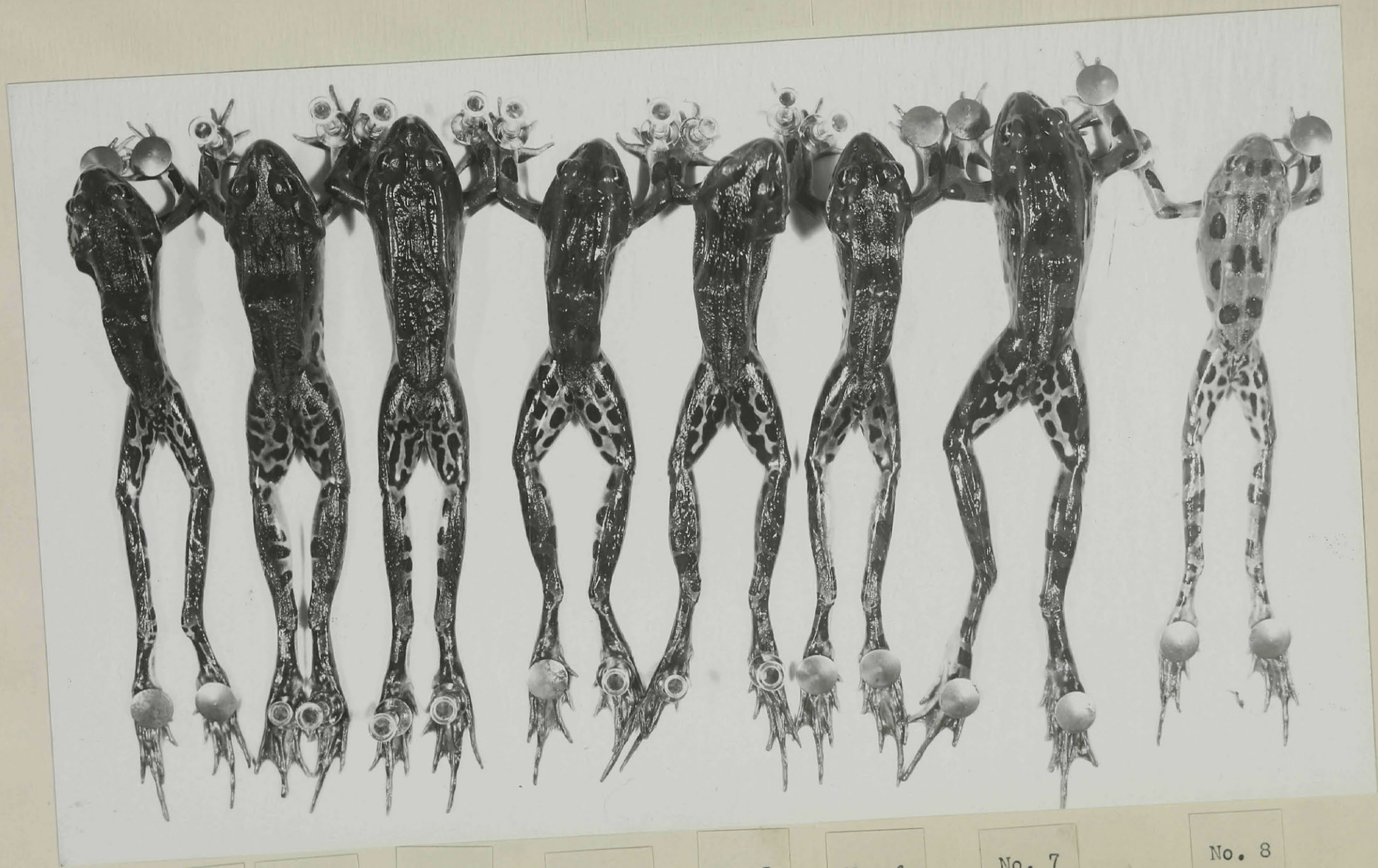
PLATE I8

Photograph of 8 frogs which have been used in testing the presence of melanophore-expanding principle in saline extracts of pars intermedia cells of the rat's pituitary. The frogs' skins were bleached before injections were begun. With injection the frogs have reacted by assuming various degrees of darkness. Frogs Nos. I, 2 and 3 were injected with rat posterior pituitary which had been grown in vitro and then extracted. The doses were equivalent to I/4, I/2 and 2 pituitaries. Frog No. 4 was injected with 0.05 units of pituitrin to serve as a basis of comparison. Frogs Nos. 5, 6 and 7 were injected with extracted rat posterior pituitary which was not cultivated in vitro. As in the case of Frogs Nos. I, 2 and 3 the dose of extracted material was I/4, I/2 and 2 posterior pituitaries. Frog No. 8 was uninjected.

By removing the photograph at some distance one can see that the skins of the frogs injected with pituitaries grown in vitro are darker than those of their respective controls. This indicates that hormones have been produced in vitro. This is shown more clearly in the photographs of frogs illustrated in Plate I9, in which series smaler doses were employed.

Photograph was taken 30 minutes after the injections.

PLATE I8



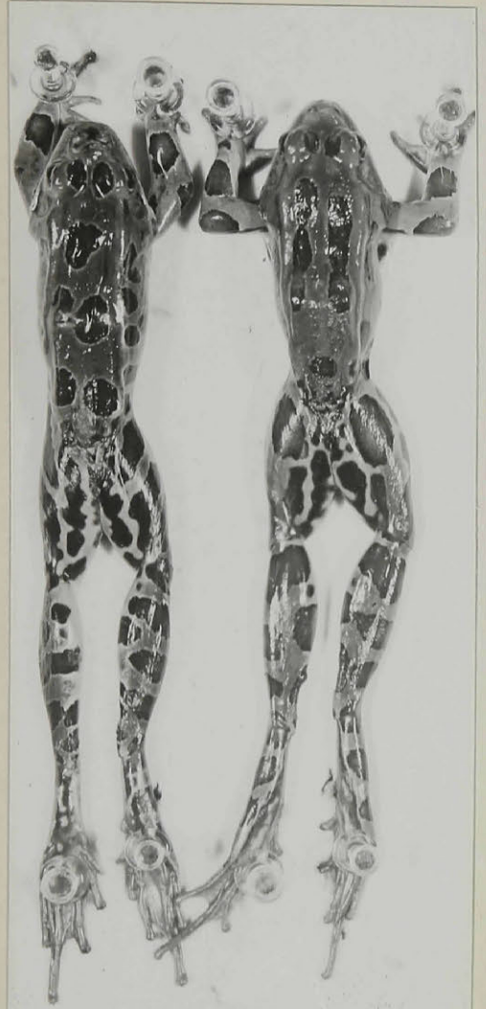
No. I | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | No. 7 | No. 8

PLATE I9

Photograph of ten frogs which have been used in testing the melanophore-expanding hormone (intermedin) content of rat posterior lobe without and with cultivation *in vitro*. It will be seen that those frogs injected with extract of posterior lobe which has not been grown *in vitro* are darker than those injected with extract of a similar number of pituitary fragments not so cultivated.

Frogs Nos. I, 3, 5 and 8 were injected with extract of I/80, I/40, I/20 and I/IO respectively of a single 8 day old rat posterior pituitary grown *in vitro* for six days. Frogs Nos. 2, 4, 6 and 9 were injected with I/80, I/40, I/20 and I/IO of a posterior not so cultivated. Frog No. 7 was untreated. Frog No. IO was injected with 0.05 unit of pituitrin.

PLATE I9



No. I	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. IO

TABLE 2 *

Frog No.	Weight in gms.	Material and amount injected	MELANOPHORE-EXPANDING EFFECT OF LOT 41 After 5 mins.	10 mins.	15 mins.	20 mins.	25 mins.	30 mins.	40 mins.
1	35	In vitro mat. ¼ post. pit.	slightly visible	visibly dark, +	visibly dark, ++	visibly dark, +++	visibly dark, +++	visibly dark, +++	visibly dark, ++
2	44	In vitro mat. ½ post. pit.	slightly visible	visibly dark, ++	visibly dark, +++	visibly dark, +++	visibly dark, +++	visibly dark, +++	visibly dark, +++
3	42	In vitro mat. 2 post. pits.	slightly visible	visibly dark, ++	visibly dark, +++	visibly dark, +++	visibly dark, ++++	visibly dark, +++	visibly dark, +++
4	37	.05 units Pituitrin	no change	visibly dark, ++	visibly dark, +++	visibly dark, +++	visibly dark, ++++	visibly dark, ++++	visibly dark, ++++
5	40	Non-cult.mat. ¼ post. pit.	no change	slightly visible	visibly dark, +	visibly dark, +	visibly dark, ++	visibly dark, +++	visibly dark, ++
6	36	Non-cult.mat. ½ post. pit.	no change	visibly dark, +	visibly dark, +	visibly dark, ++	visibly dark, ++	visibly dark, +++	visibly dark, ++
7	47	Non-cult.mat. 2 post. pits.	slightly visible	visibly dark, +	visibly dark, ++	visibly dark, +++	visibly dark, +++	visibly dark, +++	visibly dark, +++
8	20	None	no change	no change	no change	no change	no change	no change	no change

* Photograph of Frogs 30 minutes after injection may be seen on Plate 18.

TABLE 3 *

Frog No.	Weight in gms.	Material and amount injected	MELANOPHORE-EXPANDING EFFECT OF LOT 40 After 5 mins.	10 mins.	15 mins.	20 mins.	25 mins.
1	33	In vitro mat. 1/40 post. pit.	slightly visible	visibly dark, +	visibly dark, ++	visibly dark, +++	visibly dark, +++
2	35	Non-cult. mat. 1/40 post. pit.	no change	no change	no change	slightly visible	slightly visible
3	31	In vitro mat. 1/20 post. pit.	slightly visible	visibly dark, +	visibly dark, ++	visibly dark, +++	visibly dark, +++
4	27	Non-cult. mat. 1/20 post. pit.	no change	no change	no change	slightly visible	slightly visible
5	37	None	no change	no change	no change	no change	no change
6	41	In vitro mat. 1/10 post. pit.	visibly dark, +	visibly dark, ++	visibly dark, ++	visibly dark, +++	visibly dark, +++
7	34	Non-cult. mat. 1/10 post. pit.	visibly dark, +	visibly dark, +	visibly dark, ++	visibly dark, ++	visibly dark, +++
8	35	.05 unit Pituitrin	visibly dark, +	visibly dark, +	visibly dark, ++	visibly dark, +++	visibly dark, ++++
9	26	In vitro mat. 1/80 post. pit.	very slt. visible	slightly visible	visibly dark, +	visibly dark, +	visibly dark, ++
10	28	Non-cult. mat. 1/80 post. pit.	no change	no change	very slt. visible	slightly visible	slightly visible

* Photograph of Frogs 25 minutes after injection may be seen on Plate 19.

TABLE 4

Frog No.	Weight in gms.	Material and amount injected	MELANOPHORE-EXPANDING EFFECT OF LOT 40 After 5 mins.	10 mins.	15 mins.	20 mins.	25 mins.	30 mins.
1	34	In vitro mat. 1/320 post. pit.	no change	no change	no change	no change	very slt. visible	slightly visible
2	33	Non-cult. mat. 1/320 post. pit.	no change	no change	no change	no change	no change	no change
3	22	None	no change	no change	no change	no change	no change	no change
4	41	In vitro mat. 1/160 post. pit.	no change	no change	no change	very slt. visible	very slt. visible	very slt. visible
5	38	Non-cult. mat. 1/160 post. pit.	no change	no change	no change	no change	no change	no change
6	44	.05 unit Pituitrin	slightly visible	visibly dark, +	visibly dark, ++	visibly dark, +++	visibly dark, +++	visibly dark, ++++
7	38	In vitro mat. 1/80 post. pit.	no change	slightly visible	visibly dark,+(?)	visibly dark, +	visibly dark, +	slightly visible
8	35	Non-cult. mat. 1/80 post. pit.	no change	no change	no change	no change	very slt. visible	very slt. visible

TABLE 5

Frog No.	Weight in gms.	Material and amount injected	MELANOPHORE-EXPANDING EFFECT OF LOT 42 After 5 mins.	10 mins.	15 mins.	20 mins.	25 mins.
1	18	In vitro mat. 1/80 post. pit.	slightly visible	slightly visible	visibly dark, +	visibly dark, +	visibly dark, +
2	17	Non-cult. mat. 1/80 post. pit.	no change	no change	no change	no change	no change
3	41	In vitro mat. 1/40 post. pit.	slightly visible	slightly visible	visibly dark, +	visibly dark, +	visibly dark, +
4	37	Non-cult. mat. 1/40 post. pit.	no change	no change	no change	no change	no change
5	32	In vitro mat. 1/20 post. pit.	no change	no change	very slt. visible	slightly visible	slightly visible
6	22	None	no change	no change	no change	no change	no change
7	34	Non-cult. mat. 1/20 post. pit.	no change	no change	no change	very slt. visible	very slt. visible
8	36	In vitro mat. 1/10 post. pit.	slightly visible	visibly dark,+(?)	visibly dark, +	visibly dark, +	visibly dark, ++
9	34	Non-cult. mat. 1/10 post. pit.	very slt. visible	very slt. visible	slightly visible	visibly dark, +	visibly dark, +
10	49	.05 unit Pituitrin	slightly visible	visibly dark, +	visibly dark, ++	visibly dark, +++	visibly dark, +++