THE STRUCTURE AND DIFFERENTIATION OF THE SPECIFIC CELLULAR ELEMENTS OF THE PARS INTERMEDIA OF THE HYPOPHYSIS OF THE DOMESTIC PIG.*

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PLATE 5.

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Since the discovery by Oliver and Schäfer that the hypophysis contains a specific substance which causes, when injected into the blood serum, a rise of blood pressure, much interest has been displayed in the question as to the source of this material and the mode of its discharge from the gland.

The observations of Howell, which have been amply confirmed, definitely established the origin of the pressor material exclusively from the posterior lobe of the gland, but left unsettled the question whether the active portion of this lobe is the epithelial investment derived from the pouch of Rathke, or the nervous portion derived from the brain.

Many efforts have been made to recognize histologically the antecedents of the secretion in the several parts of the posterior lobe. Of particular interest in this connection are the observations of Herring (1908), since they have given rise to a conception of posterior lobe secretion which has been supported by later experimenters, and widely adopted by those interested in the problems of internal secretion.

Herring studied in particular the relations of the pars intermedia, or epithelial investment, to the nervous substance of the posterior lobe. In the former he saw frequently vesicles wholly or partly formed of epithelial cells and filled with a material which resembled, under the microscope, the colloid of the thyroid vesicles. In the pars nervosa also numerous small masses of a hyaline material sometimes homogeneous, sometimes finely granular, were found. These hyaline masses were found throughout the substance of the pars nervosa and gave the impression of materials flowing towards the recess of the infundibulum, in the cavity of which also similar deposits were found. Herring expressed himself with considerable

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reserve concerning the nature and origin of these hyaline bodies but considered two possibilities. He noted that they were found frequently between the ependymal cells near the ventricular cavity. He considered this as suggestive of their being the product of ependymal cells, but remarked that the hyaline was not always confined to this situation since it was frequently found among the cells of the epithelial investment, a fact pointing strongly to its origin from the epithelial cells. In some cases the hyaline bodies were apparently surrounded by a layer of cells resembling endothelium, as if they were contained in lymphatic vessels.

In a later study of the changes in the hypophysis produced by complete thyroidectomy in rabbits and dogs, Herring found proliferation of the cells of the pars intermedia with invasion of the pars nervosa, and an enormous quantity of granular, hyaline, or colloid bodies in the body of the pars nervosa and in the laminæ forming the floor of the third ventricle of the brain. Concerning these changes he says: "The significance of these changes is as yet undetermined. The colloid bodies appear to arise from the epithelial cells of the pars intermedia, and their extensive production to be an exaggeration of a normal process."

Crowe, Cushing, and Homans (1910) in their study of the results of partial and complete hypophysectomies in the dog reached similar conclusions. Referring to Herring's observations they say: "and we agree with him that the histological appearances point strongly toward the fact that this is an actual secretion and one which appears to be the product of activity of the cells of the epithelial investment (pars intermedia). The material seems to pass toward the infundibulum and in many cases may actually be seen passing into the cavity of the third ventricle." According to these authors neither pure intermedia substance nor the colloid of the pars intermedia vesicles has any pressor effect. They suggest, therefore, that the pars nervosa activates the hyaline bodies "at least if they are in any way related to the glandular colloid" during their passage toward the infundibular cavity.

Cushing and Goetsch (1910–11) meanwhile have confirmed the results of Herring's observations as to the increase of hyaline bodies, etc., after thyroidectomy, and found a similar increase after a number of other experimental procedures; for example, after total pancreatectomy, mechanical injuries, partial hypophysectomies, and after experimental obstruction of the stalk by means of a silver clip applied about it. These authors accept unreservedly Herring's conception of a secretory product passing through the pars nervosa and believe that it is discharged into the cerebrospinal fluid. They also state definitely that they believe the colloid accumulation in newly formed epithelial vesicles is merely a precursor of the hyaline bodies, though the latter apparently may originate by a direct transformation of individual wandering epithelial cells. These authors also obtained with human cerebrospinal fluid concentrated by evaporation, injected intravenously in dogs and rabbits, unmistakable evidences of the presence in the fluid of a pressor substance similar to that obtained from extracts of the posterior lobe of the hypophysis.

Carlson and Martin (1911–12), on the other hand, failed to get any pressor response whatever from the intravenous injection of dog cerebrospinal fluid into dogs. It must be noted, however, that these workers did not concentrate the cerebrospinal fluid prior to testing it, as did Cushing and Goetsch. Carlson and Martin suggest that the pressor response obtained by Cushing and Goetsch may have been due to their using foreign, pathological, and concentrated cerebrospinal fluid.

The net result of these various researches is an expansion of Herring's views. The pars intermedia elaborates a specific secretion which it discharges into the pars nervosa either in the form of colloid or by the degeneration of pars intermedia cells which have migrated into the pars nervosa. The secretion appears in the pars nervosa as hyaline bodies either free in the interstices of the glia or inside of lymphatic channels. It finally reaches the ventricle and is discharged into the cerebrospinal fluid. This product contains the pressor substance of Oliver and Schäfer. It is to be noted, however, that Herring recognizes the alternative possibility that the hyaline bodies may be a product of the activity of ependymal cells.

In order to establish this hypothesis it is necessary to prove that the active substance of the secretion is present in the epithelial structure from which it is supposed to take origin and also in the nervous tissue through which it passes en route to the ventricular cavity as well as in the cerebrospinal fluid itself. Unfortunately it is not easy to separate the colloid product of the pars intermedia from the epithelial cells which surround it or the pars nervosa from the pars intermedia. It is not surprising, therefore, that the results of these inquiries have not yielded wholly concordant results.

Crowe, Cushing, and Homans, as already mentioned, found the colloid obtained from cysts of the pars intermedia, and pure pars intermedia substance scraped off the pars nervosa, equally inactive, and adapted these findings to their hypothesis by assuming that the secretion was activated during its passage through the posterior lobe.

Lewis, Miller, and Matthews (1911) found the posterior part of the anterior lobe, pars intermedia, and pars nervosa, all active as regards the pressor substance, but found the colloid contained in the residual lumen of the gland only active when there was desquamation or autolysis of pars intermedia cells in the colloid. It must be pointed out, however, that the material contained in large cysts is hardly comparable to the colloid observed in small vesicles of the pars intermedia, since the former are lined in large measure by epithelial cells which have vestigial stomodeal characters, and it may be reasonably doubted whether any of the pars intermedia secretion of a specific nature is discharged into these cavities.

Biedl reports that in a number of cases he was able to separate an inactive pars nervosa from the surrounding pars intermedia, though he declares that this can rarely be accomplished on account of the anatomical difficulties involved. Biedl also found pars intermedia substance active in producing a pressor response, thus confirming the claim of Lewis, Miller, and Matthews.

On the basis of these investigations we may now consider that the presence of the pressor substance in the pars intermedia is established but that its relation to the colloid material of the pars intermedia or to the hyaline and granular masses of the pars nervosa remains a subject for further investigation.

The theory of the relation of colloid derived from pars intermedia to the hyaline and granular masses of the pars nervosa rests on the very unconvincing evidence afforded by the similarity in appearance in microscopic preparations, the frequent proximity of hyaline masses to epithelium, the fact that colloid in vesicles increases under experimental conditions which also increase the content of hyaline, and the fact that the vesicles containing colloid have not always a complete epithelial wall.

The theory that hyaline is derived from degenerating epithelial cells which have invaded the pars intermedia does not adequately explain the presence of hyaline in sites where epithelial invasion rarely occurs, as, for example, in the postoptic lamina and in the portions of the pars nervosa remote from the stalk. Furthermore, hyaline is abundant in the hypophysis of animals in which the pars intermedia is sharply delimited from the pars nervosa, and in which there is seldom, if ever, any epithelial invasion.

It is apparent from the foregoing review that Herring's theory of the mode of posterior lobe secretion as expanded by Cushing and his coworkers rests on a very precarious foundation. Its wide acceptance by investigators is doubtless due to the lack of evidence supporting the equally plausible hypothesis that the pars intermedia, like other endocrine organs, secretes its hormones directly into the circulating fluids. It is well to remember, however, that in constructing the Herring hypothesis as modified by subsequent observers certain assumptions are made which are themselves open to doubt. These are as follows: (1) Only epithelial cells are capable of secretory activity. (2) Amorphous masses occupying contiguous situations in histological preparations are chemically similar and are derived from a common source. (3) Masses forming a row in a microscopic preparation do so because they are moving in the same direction towards a common goal. (4) The increase in the amount of a given product under experimental conditions is an exaggeration of a normal process, a dis-

turbance of the equilibrium between secretion and export. In Cushing and Goetsch's silver clip experiment there is, in addition to obstruction of the recessus infundibuli, actual trauma and interference with the blood supply. The really cogent facts in support of the hypothesis are the presence of a specific pressor substance in the cells of the pars intermedia as demonstrated by Lewis, Miller, and Matthews, and confirmed by Biedl, and the presence of pressor substance in the cerebrospinal fluid. The latter is, however, contested by Carlson and Martin, and Wassing, working under Biedl's direction, was unable to demonstrate clearly the presence of vasoconstricting substances in the cerebrospinal fluid by the frog perfusion method.

The present paper is the result of a research undertaken to test the Herring-Cushing hypothesis, as compared with the alternative hypothesis of direct activity of the pars intermedia occupied with direct export of its product into the vascular channels. Before proceeding to a description of the technique employed and of the results obtained, in order that the point of view of the work may be understood, it may be well to point out that glandular cells of different types can only be distinguished from one another with certainty by the observation and study of the actual products of their activity. In the case of a mixed gland such a study must, of necessity, be made by microscopic methods applied to the living gland when possible and supplemented by observations on the precipitability of these products and on their staining reactions, or microchemical reactions if such be available in the fixed condition. The precarious foundation on which identification of gland cells by purely morphologic criteria rests is sufficiently illustrated by the long discussion not yet terminated of the nature of the demilunes of the salivary glands, and the error, most persistent in our text-books of histology and physiology, of describing the pyloric glands as serous glands. Accordingly the first question which presented itself was as follows: Can the pars intermedia cell be distinguished as a physiological unit by the nature of its chemical products from each and every type of cell in the anterior lobe of the hypophysis? This being answered in the affirmative and the characters of this product determined, the next phase of the problem is to determine whether there is any correlation in the embryo between the time of appearance of pressor activity in the hypophysis and that of

the specific product of the pars intermedia cells. Of necessity, the work must include a study of the other parts of the hypophysis as well as of the pars intermedia.

Technique.

Successful handling of hypophysis from the technical standpoint requires that the organ should be removed with the utmost speed as soon as the animal is dead and transferred with as little mechanical injury as possible to a suitable fixing fluid. We have found that postmortem changes proceed with great rapidity, particularly changes which involve the secretory antecedents in the cells of the pars intermedia. Also mechanical injury by pinching with forceps, compression, or traction cause obvious changes in cytological structure. In handling the adult material a certain amount of delay was inevitable on account of the abattoir processes, but usually it was possible to have the hypophysis in the fixing fluid within 5 minutes from the time the hog was hoisted on the wheel. As soon as the animal was bled, the top of the skull and brain were removed and the hypophysis was carefully dissected out of the sella turcica and dropped into a large quantity of fixing fluid. After a lapse of a few minutes, sufficient to stiffen the surface of the organ, the gland was removed with a spoon and carefully divided in the sagittal plane. The halves were allowed to remain in the fixing fluid for 24 hours in the dark, then dehydrated, cleared in bergamot, and imbedded in paraffin according to the usual practice.

For fixing fluids, formaldehyde 40 per cent, aqueous chrome sublimate (Zenker's fluid without acetic acid), and formalin-Zenker (Zenker's fluid without acetic acid nine parts, neutral formalin one part) were employed.

The following staining methods gave the best results.

1. The Acid Fuchsin-Acid Violet Method.—The stain consists of a mixture of 70 cc. of 1.7 per cent solution of Grübler's acid fuchsin in water, to which is added 30 cc. of a saturated aqueous solution of acid violet. After removal of the paraffin by benzene and transfer of the sections through alcohols to water, the sections are stained for 20 to 30 seconds in this mixture. They are then quickly blotted, dehydrated in anhydrous acetone, and cleared in anhydrous benzene. They are

next differentiated in a mixture of three parts of clove oil to one part of absolute alcohol, blotted, washed thoroughly in several changes of benzol, and mounted.

2. The Neutral Safranine-Acid Violet Method.—The dye is prepared as recommended by Bensley but is used in a different way. Sections 4 micra in thickness were cleared in benzene and passed through absolute alcohol to 70 per cent alcohol containing iodine, in which they remained until all mercury deposits were dissolved. The excess iodine was removed by further treatment with 95 per cent alcohol. Then 20 drops of the stock solution of the neutral dye were dropped on the alcohol-covered slide. 4 drops of distilled water were added and the whole was thoroughly mixed on the slide and allowed to remain for 40 to 50 minutes in the open room if the atmosphere was warm and moist or in a moist chamber if it was dry. The excess stain was poured off, the section blotted with a lintless filter paper, passed rapidly through 95 per cent alcohol to remove precipitated dye, dehydrated in absolute alcohol, and cleared in benzene. The sections were differentiated under the microscope with the oil of cloves-absolute alcohol mixture as described above. The beautiful differentiation of the cell elements of the anterior lobe after formalin-Zenker fixation obtained by this method is illustrated in Fig. 1.

3. The Neutral Gentian Method as Described by Bensley.—At this point it may be well to explain that the terms oxyphil, or eosinophil, and basophil as applied to the cells of the anterior lobe are not truly descriptive, since the cells in question stain with either acid or basic stains. It may even be questioned whether the cells described under these names in various animals or the same animal by different authors constitute a strictly comparable group. For this reason, in order to avoid confusion in our own identification of cell types, the following method was adopted. A section was first stained in safranine-acid violet. In this section a group of cells containing representatives of the different types of the anterior lobe was selected, carefully traced with the camera lucida, and each cell identified. The cover-glass was then removed, and the section restained with acid violet and acid fuchsin after extraction of the first stain. The cells were again identified and described, and the process repeated with neutral gentian and other stains.

To determine postmortem change, one half of the gland was placed in the fixing fluid and the other kept for 4 hours in blood serum at 18°C. Sections from the two halves were then compared after the same staining.

Cells of the Anterior Lobe.

In order to establish the fact that the pars intermedia is a functional unit in the hypophysis, it is necessary for comparison to describe and illustrate the cells of the anterior lobe. Five different types of cells are readily distinguished. Three of these are chromophil types and two chromophobe.

1. Chromophil cells.
   (*a*) Type 1. Eosinophil, oxyphil, or acidophil of other investigators.
   (*b*) Type 2. Basophil cells of other investigators.
   (*c*) Type 3. A hitherto undefined cell forming the main mass of the darker band of tissue extending down from the stalk on the anterior surface of the gland.
2. Chromophobe cells.
   (*a*) Chromophobe seen after formalin-Zenker and aqueous chrome sublimate fixation.
   (*b*) Chromophobe seen after formaldehyde fixation.

There is little to be gained by an attempt at description of the cell types since they do not possess cytologic differences either cytoplasmic or nuclear which would suffice to distinguish them with certainty in the absence of specific staining reactions of their organized secretion products. The reader is, therefore, referred to Fig. 1, which shows a section of anterior lobe, formalin-Zenker fixation, stained in safranine-acid violet, showing chromophobes and Types 1 and 2 of chromophil cells. In general, the granules in Type 1 (oxyphil cells) are more diffusely distributed throughout the cell substance than in Type 2 (basophils), and in the latter when the secretory content of the cell is small, it is usually collected into two or more oval or comma-shaped masses which are quite characteristic of this cell in the pig, and serve to identify it whatever the staining method. In both the oxyphils and basophils, so called, alongside of the nucleus may be recognized a mass which never contains secretion granules though in well filled cells it may be obscured by them. This is the sphere. It is always surrounded by a veil of cytoplasm containing many fine mitochondria and in preparations fixed in formalin-Zenker often is detached from the rest of the cytoplasm in part by clear spaces which are apparently similar to the canal-like structure described by Holmgren and others at one pole of the nucleus in epithelial cells. This is the macula described by Addison (1917) in the rat and by Rasmussen (1921) in the woodchuck in the basophil cells. It is not exclusively the property of the basophil cells but may be more conspicuous in these cells in some species. Such a structure is also present in the cells of the pars intermedia. The mitochondria in the oxyphil and basophil cells of the pig tend to be aggregated about the sphere and to some extent about the periphery of the nucleus, though they may

be more dispersed in cells containing a small amount of accumulated secretion, and there are always scattered mitochondria throughout the mass of secretion. These scattered mitochondria mark the location of the residual protoplasm of the cell. The mitochondria are in reality coextensive with the protoplasm and are condensed where the protoplasm is continuous, dispersed where the protoplasm is dispersed by the accumulation in it of secretion product. This may be taken as a criterion of what is essentially cytoplasmic in these cells, as distinguished from the casual and variable products of cell activity. The mitochondrium never intrudes itself into actual spaces of the cytoplasm occupied by a secretion droplet or a group of them. The characters just described, while interesting, are of little use for cell identification, which must depend solely on the properties of the specific secretion products or granules. These differences will be discussed later.

Type 3 of the chromophil cells (Fig. 2) is found in a strap-like layer that extends down the front of the anterior lobe a variable distance from the stalk. It can be clearly recognized in the fresh gland by its darker color and greater transparency than the rest of the anterior lobe. In sections this mass is sharply delimited from the rest of the anterior lobe even though a few anterior lobe elements of the other types may intrude. This division of the anterior lobe contains more connective tissue than the rest, and it is composed of the cells of Type 3, intermingled among which are some other chromophil cells. These cells are especially conspicuous in sections of material fixed in formalin-Zenker and stained with either the neutral safranine compound or acid violet-acid fuchsin. In both cases they stain intensely blue. The cells tend to a polygonal outline in section rather than to the spherical. The nucleus, not markedly different in type from that of the other two types, is placed at one end of the cell, and the cytoplasm presents an irregularly vacuolated appearance. In addition, the cytoplasm is filled with blue-stained granules. These granules are not colored by the stains here used after fixation with formaldehyde, while those of Types 1 and 2 are fairly well preserved.

Among the chromophobe cells in general two types are seen, though it is not certain that they are morphologically distinct. The first includes all of those seen after formalin-Zenker and aqueous chrome

sublimate fixation and is characterized by the fact that the cytoplasm contains no secretion antecedent granules and retains less color than that of the chromophil cells (Fig. 1). The shape is irregular, being determined by external pressures. The second type seen in formalin-fixed preparations is large and tends to be spherical; and the cytoplasm takes a light diffuse stain, but a more intense stain than the smaller chromophobes, and it may contain a few large granules which stain with acid violet. The real nature and relationship of these cells remain undetermined and they are only mentioned here to establish the dissimilarity of the chromophobes and the cells of the pars intermedia.

Cells of the Pars Intermedia.

The cells of the pars intermedia (Fig. 3) (leaving out of consideration the more or less indifferent cells of the residual lumen) are of two sorts, one of which, the secretory cell of the pars intermedia in the strict sense, is different from every other cellular element in the hypophysis and by its presence serves to delimit the pars intermedia. The other type is the colloid-producing cell which is common to the pars intermedia and that part of the gland surrounding the upper portion of the stalk. The colloid-producing cell (Fig. 3, *k*) being of least importance may be disposed of first. It is a small cubical or columnar cell found usually forming the epithelial lining of follicles containing transparent colloid material. It has a basal nucleus and mottled, poorly staining protoplasm, relatively poor in mitochondria. The cells usually show little evidence of secretory activity, but occasionally (Fig. 3) one sees in them small droplets of colloid similar to that in the follicles, showing that the follicular colloid is actually a product of these cells.

The other type of cell, the granular cell of the pars intermedia, constitutes the main bulk of this portion of the gland (Fig. 3). It is of a variable prismatic shape conditioned by mutual pressure, and the nucleus is located near one pole of the cell. Alongside of the nucleus or at some distance from it is a mass of deeply staining cytoplasm of about the same size as the nucleus which we presume contains the sphere and centrioles, though we have not actually demonstrated them. This mass is inconspicuous in preparations in which the secretion has

been stained but very obvious in cells from which the secretion antecedents have been removed, or in preparations in which both the secretion and the mitochondria are stained, for as in other cells of this gland the mitochondrium is coextensive as regards distribution with the real cytoplasm of the cell, and its concentrations in the cell indicate the locations of cytoplasmic condensations as distinguished from those parts of the cell where the cytoplasm is dispersed by the masses of secretion. Around this condensation the mitochondria are abundant in the form of short delicate rods. They are also abundant but less so in the perinuclear cytoplasm and they extend radially from the cytoplasmic mass to the periphery of the cell in lines which coincide with the strands of cytoplasm separating secretion-holding spaces. These same strands of cytoplasm are clearly seen in the cells in question from which the secretion has been dissolved as a result of postmortem change or the solvent action of the fixing fluid. In such cells the cytoplasm presents a coarse meshed network which has recently been well illustrated by Rasmussen (1921) in the cells of similar sort from the pars intermedia of the woodchuck.

The secretion in these cells is a highly labile material which appears in suitably fixed preparations in the form of small granules which are very difficult to stain. The amount in the individual cell is highly variable, and accordingly in preparations the pars intermedia presents a mottled appearance. These granules are not found in the juxtanuclear cytoplasm mass described above. The granules are smaller and stain blue in acid violet but less intensely than those of any of the cells of the anterior lobe. As will be seen later they disappear more rapidly from the cell, as a result of postmortem change, and are less resistant to the solvent action of fixing agents than any of the violet-staining granules of the anterior lobe. In preparations fixed by immersion, frequently only those pars intermedia cells which are near the surface of the piece have their secretion granules preserved, those more centrally placed having undergone in various degrees those changes which generally occur in the pars intermedia of glands kept several hours before fixation. Even here, however, the pars intermedia cell is a distinct secretory type because the granules in the cells of the anterior lobe are little affected.

Postmortem Changes in the Pars Intermedia Cell.

As described under Technique, glands were divided into two equal portions and one was fixed immediately while the other was kept in blood serum for 4 hours, then fixed, and the two lots were compared. In the sections of the piece which had been allowed to undergo this change, no granules were to be found in the cells of the pars intermedia (Fig. 4); instead the cell presented an irregularly vacuolated or rather reticulated appearance due to the withdrawal of the secretion antecedents. This fact possibly explains the variable experimental results obtained by different workers as regards the pressor action of extracts from the pars intermedia and neighboring parts of the hypophysis, for it is probable that so highly soluble a material may diffuse into neighboring sections of the organ to a variable extent, depending on the freshness of the gland and the promptness with which the several parts are separated after removal from the freshly killed animal.

These facts may be summarized as follows: The pars intermedia cells are characterized by their content of secretory granules. These granules are smaller than in any other of the granule-containing cells of the hypophysis. They stain a light shade of blue in the neutral combination of safranine and acid violet, or in the mixture of acid violet and acid fuchsin, or in Mallory's connective tissue stain, after fixation in formalin-Zenker. They are distinguished from all of the chromophil cells of the anterior lobe by the lability of the granules during postmortem change; from both types of blue-staining cells in the anterior lobe by the small size of the granules, and by the fact that the granules are stained lighter and are more diffusely distributed through the protoplasm than the granules of the so called basophils. The pars intermedia cell is distinguished from Type 3 chromophil cell by its lack of vacuoles and its much smaller, lighter stained granules. The cells of the pars intermedia are distinguished from all chromophobes of the anterior lobe by their positive granule content.

The two types of chromophobes are best distinguished from one another by their behavior in sections fixed in strong formaldehyde and formalin-Zenker; in the former large spherical chromophobes are usually abundant.

Study of the Embryonic Hypophysis.

The study of the embryonic hypophysis was undertaken to discover whether there was any relation in time between the appearance of the characteristic pressor effects of the posterior lobe extract and that of the granular secretion antecedents in the cells of the pars intermedia or of hyaline bodies or other structures which have been hypothetically related to the production of the pressor substance.

The technique followed was similar to that employed for the anterior lobe and pars intermedia of the adult.

The cells of the pars intermedia of pig embryos measuring 7.5 cm. in length have large nuclei and very little cytoplasm. The cell boundaries cannot be seen distinctly, and mitoses are abundant. In the 12.5 cm. stage the appearance of the cells is about the same, but vascularization of the mass is indicated by the ingrowth of mesenchyme and blood vessels from the investment of the pars nervosa. The cells are still difficult to delimit. Vascularization is still further advanced in the 17.5 cm. stage, and some of the cells of the deeper layers are increased in size, the increase being due to the expansion of the cytoplasmic body which now begins to show some fine granules (Fig. 5). In some cells these granules are abundant, the cells being fairly packed with them. The granules are minute and have the same staining reactions as the granules of the pars intermedia of the adult. In the later stages a progressively increasing number of cells of the pars intermedia shows this differentiation, and at full term the majority of cells is so differentiated, and the pars intermedia gives the impression of being greater in mass ratio to the other parts of the gland than at any other period of development or growth.

None of the fetal hypophyses contain, so far as we have been able to discover, hyaline bodies.

It is necessary to study serial sections to discover the colloid-containing vesicles, for frequently folds of the pars intermedia, the cavity of which communicates with the residual lumen, present in single sections the appearance of follicles. When actually present the follicles are found to be lined by cylindrical cells with clear non-granular protoplasm and basal nuclei.

These results may now be compared with the physiological action of extracts of the fetal gland as described by Lewis. Lewis found that extracts of the hypophysis of the 7.5 cm. pig were wholly inactive as regards pressor effect even though a very large number of hypophyses was employed in making the extracts. Similarly no definite effects were noted after the injection of extracts of the hypophyses of 12.5 cm. pig fetuses. On the contrary, a marked pressor effect followed injection of extracts made from the hypophyses of 17.5 cm. pigs. The conclusion is obvious—the pressor substance of the posterior lobe of the hypophysis is coincident in time of appearance with the granules or secretory antecedent of the pars intermedia. As an additional reason for assuming a relation between these granules of the pars intermedia cell, it may be pointed out that McCord and Fenger, using the oxytocic method, have found in the new-born calf and lamb that the extracts of the gland were more active per unit weight than in the adult.

CONCLUSIONS.

The bearing of these results on the Herring-Cushing theory of pituitary secretion is apparent. For the first time a true secretion antecedent has been demonstrated in the cells of the pars intermedia, an antecedent which appears in the cells at the same period of development at which active pressor effects may be obtained from the gland extracts. The route of export of this material from the gland to its point of utilization, however, is unknown; it may go by way of the blood or, as required by the Herring theory, by the transneural route to the third ventricle. The objections to the latter conclusion have been amply expanded in the introduction to this paper, but here may be emphasized the fact that the fetal pig hypophysis contains no hyaline bodies. Indeed they are rare in the adult, though there may be seen in the cells of the pars nervosa in the processes of its intrinsic cells, granular deposits which we believe to be the antecedents of the hyaline bodies but which in the pig rarely are discharged and aggregated into discrete masses as in other mammals. The fact that some observers have obtained positive pressor effects from the nervous part of the posterior lobe, exclusive of the pars intermedia, need not weigh very heavily in attempting to trace the course of the secretion,

when we reflect that the difficulties of making such a mechanical separation are almost insuperable, and that the chemical product of the pars intermedia is so soluble and vanishes from the cells so rapidly that it may well be diffusible through the thin membranes which intervene and penetrate post mortem into adjacent parts. We are inclined, therefore, to the view that the secretion leaves the gland by the vascular route rather than by way of the interfibrillar spaces of the pars nervosa.

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EXPLANATION OF PLATE 5.

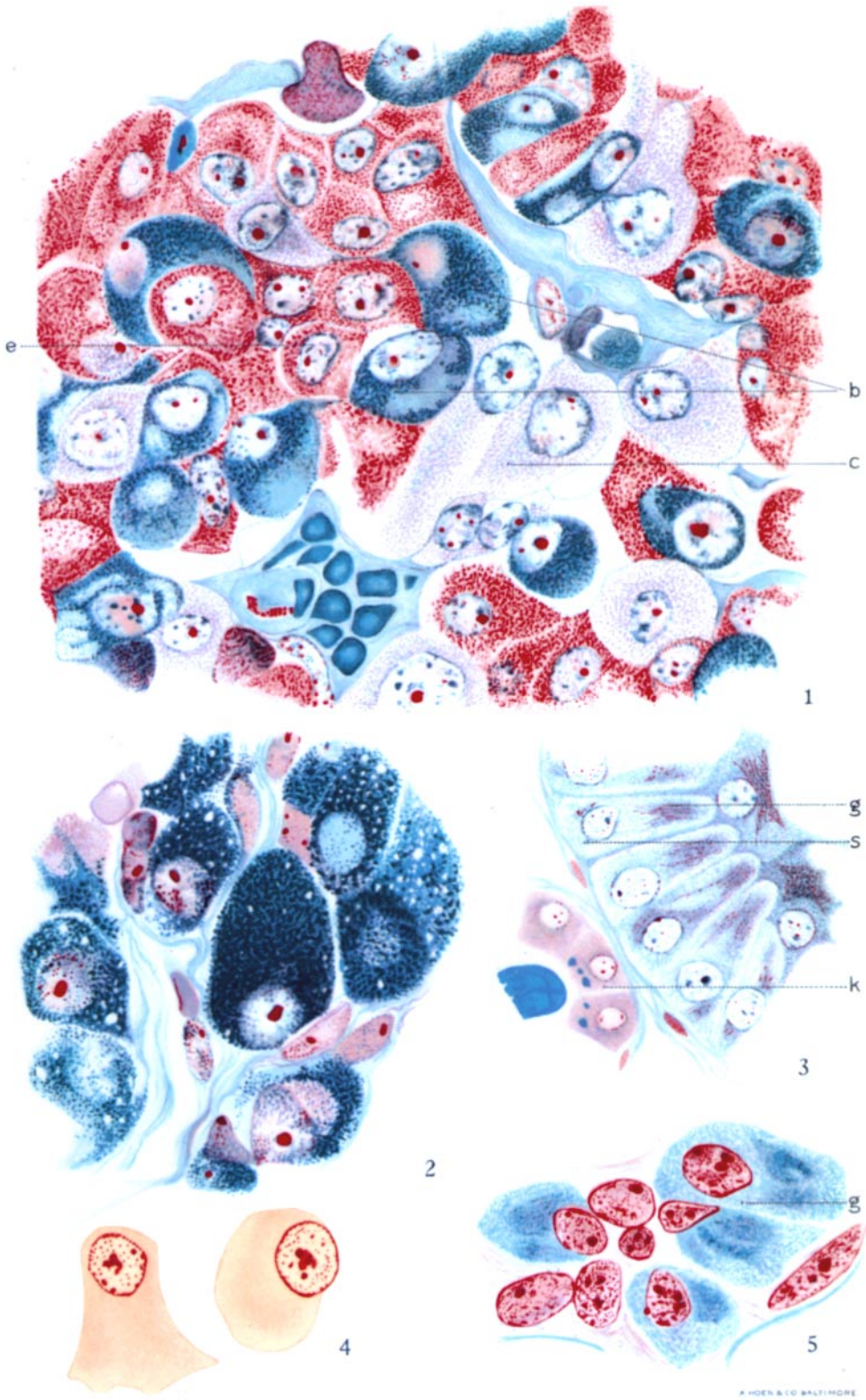
FIG. 1. Anterior lobe of pig hypophysis fixed in formalin-Zenker and stained with safranine-acid violet. (*e*) chromophil cell, Type 1 (eosinophil of authors); (*b*) chromophil cells, Type 2 (basophil of authors); (*c*) chromophobe cell.

FIG. 2. Chromophil cells, Type 3, from the anterior border of the hypophysis of the pig stained with safranine-acid violet.

FIG. 3. Pars intermedia of adult pig hypophysis. Formalin-Zenker; aniline-acid fuchsin and phosphomolybdic water blue. (*k*) part of the wall of a follicle containing colloid, showing colloid cells with colloid droplets at their free borders; (*g*) granular cell of the pars intermedia showing the blue-stained secretion and cytoplasmic mass containing mitochondria; (*s*) granular secretion.

FIG. 4. Two of the chromophobes of the anterior lobe as seen after formalin fixation; acid fuchsin-acid violet stain.

FIG. 5. Group of cells from the pars intermedia of the hypophysis of a fetal pig of 17.5 cm. Four of the cells already have the differentiated characters of the adult pars intermedia. Others are undifferentiated. Formalin-Zenker; acid fuchsin-phosphomolybdic water blue. (*g*) granular secretion antecedent.



(Maurer and Lewis: Pars intermedia of hypophysis.)