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Histological and Histochemical Studies on the Rat Pituitary Pars Intermedia.

ラット下垂体中間部の組織学的, 組織化学的研究.

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In spite of the recent progress in the endocrinology of the anterior pituitary, a specific part of the adenohypophysis which is in a close contact with the neurohypophysis, i.e., the pars intermedia, has been left in a state of incomplete understanding both in morphology and physiology. For example, early reports by ITANO (1936) and BAILLIF (1938) dealing with the rat pars intermedia, indicated morphologically the secretory activity of this tissue, but no physiological correlation with its fine structure could be established. A fact that the probable secretion from the pars intermedia might stimulate the melanophores of the frog skin was well known, but it is still doubted whether the control on the pigment metabolism by the intermedia hormone might occur in mammalia also. On the other hand, an extraordinary intimate contact between the neural lobe and the pars intermedia may suggest a close relationship between the neurosecretory activity of the hypothalamo-neurohypophyseal system and the probable functions of the pars intermedia.

We have reported several findings with the use of electron microscope on the ultrastructure of the rat intermedia cells and their morphological interrelation to the neural lobe (KUROSUMI et al. 1962). Simultaneous observations with the light microscope have revealed certain interesting facts concerning the histology and histochemistry of this tissue which have not been reported hitherto. Combining the light and electron microscopical findings, the result of the present observations may help to some extent to clarify the functional activities of the mammalian pars intermedia.

I. Material and Methods.

The hypophyses of the albino rats of Wistar strain were removed as soon as possible after the death by decapitation. The whole organ was fixed in various fixatives for about 48 hours, then washed, dehydrated and embedded in paraffin as the usual histological method. These were cut into serial sections about 4μ thick in the frontal direction for most preparations, but some in the sagittal direction. Some specimens were embedded in gelatine without dehydration, and cut at 10 to 15μ with a freezing microtome.

The following fixatives were used: LEVI's, CIACCIO's, ZENKER-formol,

BOUIN's, STIEVE's, CARNOY's, 10 % formaline, CAJAL's uranium-formol, and DA FANO's cobalt-formol. Most specimens fixed with CIACCIO's fluid were postchromicated with 3 % potassium dichromate for 1 week (abbreviated as CIACCIO-Cr in later chapters in this paper), and such treated sections were the best for general observations and lipid examinations.

The used stainings were as follows: HEIDENHAIN's azan and iron-hematoxylin (FeH), and hematoxylin (HANSEN's) and eosin for general purpose; for the detection of polysaccharide and for the differential demonstration of dark and light cell types, periodic acid-SCHIFF reaction (PAS) followed by counterstain of either HANSEN's hematoxylin, HEIDENHAIN's iron-hematoxylin or mercuric bromphenol blue (Hg-BPB) of MAZIA et al. (1953); Sudan black B with or without counterstain with carmalum, Sudan III with or without HANSEN's hematoxylin, and BAKER's acid hematein for the detection of lipids, these were used upon the preparations either CIACCIO-Cr paraffin sections or 10 % formol-fixed frozen sections; GOMORI's chrome-alum-hematoxin phloxin (CHP) and aldehyde fuchsin (AF) were used for the neurosecretory substance; for the examination of protein, MILLON reaction of SERRA (1946), ninhydrin-SCHIFF reaction of YASUMA and ICHIKAWA (1953), and Hg-BPB staining of MAZIA et al. (1953); to detect protein-bound sulfhydryl group CHÉVREMENT and FRÉDÉRIC's method (1943), and for disulfide group PEARSE's (1953) performic acid-SCHIFF reaction were used.

II. Observations.

a) *General Structure.*

The pars intermedia of the rat hypophysis is a belt-like tissue situated between the pars nervosa and pars distalis. It closely abuts the pars nervosa dorsally, but on the ventral aspect a residual lumen of RATHKE's pouch (briefly called lumen of RATHKE) separates it from the pars distalis (Fig. 1). Sometimes partial or total obliteration of RATHKE's lumen may appear.

Two distinct parts of the parenchymatous tissue are remarkable in the rat pars intermedia (Fig. 2). One of these is the *marginal zone* which is a relatively thin layer of a simple flattened epithelium lining the lumen of RATHKE. Another is a thick stratified epithelium consisting of polygonal glandular cells and frequently divided into lobules by thin partitions of connective tissue, which stain deep blue with azan and deep purple with PAS method, and contain a small number of slender blood vessels. This thick layer of stratified and lobulated epithelium may be called the *pars intermedia proper*. Connective tissue septa are inserted as a rule between the marginal zone and the pars intermedia proper. The marginal zone, therefore, may be considered as a flattened specific lobule of the pars intermedia, and is not yet differentiated at the birth. In other rodents such as mice, golden hamsters and guinea pigs, the marginal zone is not distinct, though the superficial cells facing the RATHKE's lumen are more or less flattened.

CIACCIO-Cr preparations stained with HEIDENHAIN's azan are the best for identifying the marginal zone, which shows a specific affinity for orange G in contrast to the underlying pars intermedia proper stained blue with aniline blue (Fig. 3). Pre-

Abbreviations in Figures.

C Cyst, *D* Pars distalis, *I* Pars intermedia, *Ip* Pars intermedia proper, *m* Marginal zone, *N* Pars nervosa, *R* RATHKE's lumen.

Fig. 1. Survey photomicrograph of a frontal section of the rat hypophysis. CIACCIO-Cr, CHP staining. $\times 110$.

Fig. 2. Pars intermedia of the rat hypophysis. RATHKE's lumen is filled with CH-stainable colloid, in which liberated cells and cell debris are floating. CIACCIO-Cr, CHP staining. $\times 600$.

parations with other fixations such as STEEVE's or LEVI's fluid do not show such a differential stainability, though in some cases a faint difference of color of the

Fig. 3. High power view of the pars intermedia. At the top, a half of a colloid-containing cyst is seen. At the point marked*, cells of marginal zone are disordered in arrangement and approach in nature to the pars intermedia proper. CIACCIO-Cr, azan staining. $\times 1,560$.

marginal cell cytoplasm from the pars intermedia proper can be recognized. PAS preparations may demonstrate most cells of the marginal zone to be negative or faintly positive to PAS reaction, in this connexion they resemble the light cells of the pars intermedia proper. When the mercuric bromphenol blue (Hg-BPB) is applied to PAS preparations instead of the counterstain with hematoxylin, PAS negative cells may intensely stain with Hg-BPB and therefore the blue tinted marginal zone may be distinguished clearly from the purple stained pars intermedia proper.

An alteration of a small number of marginal cells becoming basophilic with rounded contour which are very akin in appearance to the cells of the pars intermedia proper. In loci of such a change, the arrangement of the marginal cells becomes disordered; a rounded cell may bulge up into the pars intermedia proper and multi-layering of the marginal zone occurs (Fig. 3). In some parts of the marginal zone, tall columnar cells may appear. Such cells are usually pale after azan, PAS or CHP stainings. The connective tissue septa dividing the marginal zone from the underlying layer are not always continuous, but in some parts they may disappear. No blood capillary is found in each lobule of the pars intermedia besides small interlobular arterioles or venules.

In rats' hypophyses, partial defects of the pars intermedia proper are rather frequently observed (Figs. 4 and 5). At these defects, only the marginal zone is intact and therefore the neural lobe tissue directly abuts the marginal epithelium, into which CH-stainable substance (neurosecretory material) may invade occasionally (Fig. 5). Entrance of the CH-positive substance into the pars intermedia proper is frequently found (Fig. 6). Our previous observation with the electron microscope indi-

Fig. 4. Low power photomicrograph showing partial defects of the pars intermedia proper, indicated by arrows. CIACCIO-Cr, CHP staining. $\times 110$.

Fig. 5. High power view of the marginal zone at the defect of pars intermedia proper. Arrows indicate invasions of CH-stainable substance into the marginal zone. CIACCIO-Cr, CHP staining. $\times 1,560$.

cated that the axon with neurosecretory granules might invade into the cells of the pars intermedia (KUROSUMI et al. 1962). Therefore, the CH-positive substance found in the pars intermedia may indicate the nerve fibers laden with the neurosecretory substance, and not represent a fluid neurosecretory substance which may be liberated from the secretory nerve terminals in the pars nervosa and streaming through the in-

Fig. 6. Part of the pars intermedia proper near the pars nervosa. Arrows indicate invaded CH-stainable substance. CIACCIO-Cr, CHP staining. $\times 1,560$

Fig. 7. High power view of the marginal zone, some cells of which are ciliated (marked*). In the RATHKE's lumen, azan-blue colloid is observed. CIACCIO-Cr azan staining. $\times 1,560$.

tercellular spaces. Some ciliated cells are rarely observed in the rat hypophysis (Figs. 7 and 8). These were found among the marginal cells, at the superficial layer of the pars distalis just in the opposite side of the marginal zone and among the epithelial cells composing the wall of a cyst. In the last case, ciliated cells are intermingled

Fig. 8. A cyst occurring in the pars distalis. The wall consists of ciliated cells and goblet cells (PAS-positive and dark in appearance). CIACCIO-Cr, PAS-hematoxylin (HANSEN). $\times 1,500$.

with goblet cells whose supranuclear parts are intensely stained with PAS method (Fig. 8). Ciliated cells may occur separately or grouped, they are always columnar in shape and their cytoplasm appears very clear after azan or PAS stainings. Our previous observations with the electron microscope disclosed that the cilia in the rat hypophysis are the same in ultrastructure as the kinocilia of respiratory or female genital tracts (KUROSUMI et al. 1962). It is most probable that the ciliated cells are of rudimentary and ectopic occurrence, and are derived from the nasopharyngeal elements migrated during the early stages of development. The facts that the ciliated cells at the wall of the RATHKE's lumen or those of cysts are always tall columnar cells, and that the flattened marginal epithelium is not developed at the birth and appears later during the postnatal development suggest that the so-called marginal zone is a secondarily differentiated tissue and taller or rounded epithelial cells at this position are more primitive in nature.

b) *Dark and Light Cells.*

TRAUTMANN described two cell types of the animal pars intermedia as early as in 1911. He designated these 'dark and light cells (*dunkle und helle Zellen*)'. In our preparations, these two cell types are most easily distinguished on the PAS- or azan-stained sections after CIACCIO fixation (Figs. 9 and 10). In the former preparations, cells of the pars intermedia proper show various degrees of PAS reactivity. Cells with dark stained cytoplasm with fuchsin after PAS technique are coincident in all respects with the dark cells in azan preparations which appear deep blue or bluish purple as a result of strong affinity to aniline blue. In observations on PAS preparations, no marked line can be drawn between the light and dark cell types, because a gradual transition is apparent between these two types.

Fig. 9. Pars intermedia photographed at intermediate magnification. Various steps of darkening (PAS reactivity) of intermedia cell cytoplasm are apparent. RATHKE's lumen is obliterated. CIACCIO-Cr, PAS-hematoxylin (HANSEN). $\times 600$.

Fig. 10. Similar photomicrograph as Fig. 9. Dark cells are intensely stained with aniline blue. CIACCIO-Cr, azan staining. $\times 600$.

Some sections were digested with saliva prior to the staining with PAS method, but no significant difference was obtained as compared with the control section. The dark cells are generally smaller and more irregular in outline than the light cells. The nuclei as well as the cytoplasm are deeply stained with various dyes (Fig. 11). Hematoxylin and cosin stains dark cells, but the result was a little bad. HEIDEN-

Fig. 11. High power view of the pars intermedia. Dark cells are apparently shown. A cell at the lower left corner is strongly degenerated and tinted in an extreme intensity. CIACCIO-Cr, azan staining. $\times 1,560$.

HAIRN's iron-hematoxylin can weakly demonstrate the dark cells.

Preparations with various fixations followed by PAS reaction were examined. After fixations with CIACCIO's, LEVI's, CAJAL's, DA FANO's and BOUIN's fluid, the dark cells were well distinguished (Fig. 9), but after STIEVE's (Fig. 15) and ZENKER formol fixation, it was obscured and 10% formol and CARNOY's fixation missed to demonstrate the dark cells.

CIACCIO Cr paraffin sections were stained with various fat staining dyes. Both after Sudan black B and Sudan III stainings, the dark cells are weakly positive (Fig. 12). Counterstain with carmalum after Sudan black B intensely stains the cytoplasm and the nuclei of the dark cells, the result is rather superior to that of azan staining. The nuclei are tinted red with carmine and the cytoplasm shows greenish purple being stained with the both dyes.

CHP staining according to GOMORI demonstrated the dark cells though the degree of darkening was not so high (Fig. 13), but aldehyde fuchsin of GOMORI did not differentiate the two cell types (Fig. 16). After Hg-BPB staining for demonstration of protein, the dark cell cytoplasm was stained deeply as compared with that of the light cell.

c) *Cysts and Colloid.*

In the rat pars intermedia, colloid-containing cysts are often observed (Fig. 13). These are most frequently situated within the pars intermedia proper, but in some cases the cyst may bulge up into the pars nervosa. In only one case, a cyst was found in the anterior lobe. The wall of the cyst consists of a simple epithelium, the cells of which in most cases are flattened or cuboidal but rarely tall columnar. In a single cyst, a part of the wall consists of flattened cells but another part may be provided

Fig. 12. Pars intermedia and its neighbourhood at a high magnification. The cytoplasm of dark cells is weakly sudanophilic. Two cells at the right margin are pituicytes with dark stained lipid granules. CIACCIO-Cr, Sudan black B staining. $\times 1,560$.

Fig. 13. A cyst containing colloid stained with CH is shown. The stainability of colloid is slightly lower than that of the neurosecretory substance shown in the upper part of the figure. RATHKE's lumen is obliterated. CIACCIO-Cr, CHP staining. $\times 600$.

with columnar cells. In rare cases, ciliated cells and goblet cells may appear in the cyst-wall epithelium as described above (Fig. 8). A basement membrane or connective

tissue sheat may envelope the cyst-wall and separate it from the surrounding tissue such as the pars intermedia proper, neural or anterior lobes. The shape of cysts is mostly round or polygonal, but in some cases branching of a cyst can be observed. Direct continuity of the epithelium of the cyst-wall to the marginal zone was occasionally observed, but the free communication of the lumen of the cyst to the RATHKE's lumen was not found.

Colloid substance is filled in the cysts in almost all the cases, but the presence of the colloid in the lumen of RATHKE is rather rarely encountered. The staining behavior of the colloid with various dyes is not different between the both contained in the cysts and the residual lumen of RATHKE. Many vacuoles are usually seen in the colloid near the periphery of the lumen, looking like the peripheral vacuoles in the colloid of the thyroïdal follicles. In the colloid of the RATHKE's lumen, exfoliated cells and cell debris are frequently seen (Figs. 2 and 14).

Fig. 14. In RATHKE's lumen, PAS-positive colloid and exfoliated cells are contained. The pars intermedia proper is partially defective. CIACCIO-Cr, PAS-hematoxylin (HANSEN). $\times 600$.

After azan staining, the colloid is tinted usually blue, but red coloring with azocarmine occasionally occurs. In some cysts, a central part of the cyst was tinted red, while the other part was blue. Iron-hematoxylin stains the colloid very faintly. PAS reaction is always positive, but the degree of the reaction is higher in STEVE's-fixed preparations (Fig. 15) than the CIACCIO's (Fig. 14). GOMORI's CHP staining shows the affinity of the colloid to CH (Figs. 2 and 13), and aldehyde fuchsin also stains the colloid (Fig. 16). However, the staining intensity with CH or AF is weaker than that of the neurosecretory material of the neural lobe. Sometimes a part of the colloid may stain red with phloxin but others blue with CH after CHP staining. Furthermore, iron hematoxylin stains a part of the colloid (usually the center of the cyst), while the other part of the same colloid reacts with PAS in preparations of

Fig. 15. Two cysts are seen at the boundary between the neural and intermediate lobes. The colloid in the cysts is intensely positive to PAS reaction. Cells of the pars intermedia proper are diffusely stained with PAS technique, but differentiation between the dark and light cells is obscure. STEVE's-fixed, PAS-hematoxylin (HANSEN). $\times 600$.

Fig. 16. The neurosecretory substance in the pars nervosa (right) and the colloid in RATHKE's lumen (left) are positive to aldehyde fuchsin, but the former reacts more intensely. No differentiation between the dark and light cells is established. CIACCIO-Cr, AF-hematoxylin (HANSEN). $\times 600$.

Fig. 17. Cross arrows indicate a cyst containing colloid whose center stains extremely dark with FeH, while the peripheral part is gray in the photograph, being positive to PAS reaction. CIACCIO-Cr, PAS-iron hematoxylin (HEIDENHAIN). $\times 600$.

PAS-FeH (Fig. 17). These results may suggest a sort of segregation or condensation of a part of the colloid which occurs especially at the center of the cyst.

Reactions of the colloid are positive to the fat staining dyes on the CIACCIO Cr sections (Fig. 18): Sudan black B stains it moderately but the result of Sudan III

Fig. 18. Colloid in the lumen of RATHKE stains with Sudan black B. Lipid granules in pituitary cells are also sudanophilic (the upper right part). CIACCIO-Cr, Sudan black B staining. $\times 600$.

staining is weak. The intensity of sudanophile reaction, however, is less than that of the pure lipid (phospholipid) in the pituicytes of the pars nervosa. BAKER's acid hematein test is negative.

Histochemical tests to identify the protein were applied to the colloid. Results of ninhydrin-SCHIFF reaction of YASUMA and ICHIKAWA (1953) and of mercuric bromphenol blue staining (Hg-BPB) introduced by MAZIA et al. (1953) are both positive (Fig. 19), but MILLON reaction was negative. A CIACCIO-Cr preparation stained with Sudan III was counterstained with Hg-BPB. Such a preparation is useful

Fig. 19. Colloid in the RATHKE's lumen as well as dark cells of the pars intermedia proper are stainable with bromphenol blue (protein reaction). CIACCIO-Cr, Hg-BPB staining (MAZIA et al.). $\times 600$.

to demonstrate the distribution of lipids and proteins in a given section. The colloid stains deeply blue with Hg-BPB and is not affected by Sudan III, in a striking contrast to the neurosecretory substance of the pars nervosa which is well known to be glycolipoprotein (SCHIEBLER 1952) and stains brownish purple as a positive result to both dyes. In this preparation, phospholipid granules of the pituicyte stains orange red as the effect of Sudan III only. The result of this combined staining suggests that the probable lipid-content of the colloid in the lumen of RATHKE or the cysts in the pars intermedia may be lower than that of the neurosecretory substance.

Ferric ferricyanide method of CHÈVREMONT and FRÉDÉRIC (1943) for detection of SH group was examined on CIACCIO-Cr paraffin sections, and among parts of the entire hypophysis only colloid in the RATHKE's lumen showed positive reaction, that is, the formation of Prussian blue. Performic acid-SCHIFF reaction of PEARSE (1953) to detect SS group was positive in both the intermedia colloid and neurosecretory substance of pars nervosa.

III. Discussion.

In a morphological standpoint, colloid in the cysts and in the lumen of RATHKE is one of the most prominent features of the pars intermedia. A morphological similarity of colloid of the hypophysis to that of the thyroid gland made some endocrinologists to conceive an opinion that the pituitary colloid might bear the same physiological significance as does the colloid of the thyroid. Then many assumptions and discussions were raised, and possibilities that the colloid might be the secretion produced by the intermedia cells or other glandular elements of the hypophysis and might contain the hormone were actively disputed (see ROMEIS 1940). Because of a difficulty to collect the colloid in satisfactory volume for the chemical assay, the problem has been left in a vague state until the present days. Histochemical stainings are the only possible approach to the solution of this problem at the present level of technique, but only few findings have been reported in this field.

Through the observations described in this paper, similarities in various histochemical reactions may be noticed among the colloid of the pars intermedia, dark cell cytoplasm of the same tissue and the neurosecretory substance (HERRING bodies) in the pars nervosa. As shown in a summarized result given in Table 1., all of the three intensely react to PAS reaction, more or less contain lipids and proteins.

Table 1.

Stainings	Colloid in cysts & RATHKE's lumen	Dark cell cytoplasm	Neurosecretory substance
Periodic acid-SHIFF	+++	+++	+++
Chrome-alum hematoxylin	+	+ ~ ±	+++
Aldehyde fuchsin	+	—	+++
Detection of SS group	+	—	+
Detection of SH group	+	—	—
Mercuric bromphenol blue	+++	++	+++
Sudan black B	++	++	++
Sudan III	+	+	++

From these results at least two possibilities may be considered in connection to the origin of the colloid in the cysts or RATHKE's lumen: 1, The colloid may be produced by intermedia cells probably changing from light cells into dark cells and finally converting to the colloid. 2, The colloid of the pars intermedia may be derived from the neurosecretory substance, which may be released from the secretory nerve terminals in the pars nervosa and also in the pars intermedia, and transported through the intercellular spaces into the cysts or the lumen of RATHKE.

Some early investigators argued that the colloid in the RATHKE's lumen might originate from cells of the anterior lobe (ROMEIS 1940), but in the case of the rat, this possibility may not be tenable because occurrence of colloid in the pars distalis is very rare, and the cysts are found preferably at the side towards the pars nervosa.

The first possibility enumerated above was indicated by BAILLIF (1937) who described that in the hypophyses of rats exposed to cold, a portion of the intermedia

lobe was destroyed and the colloid was discharged into the residual lumen. ROMEIS (1940) classified the intermedia cells of animal hypophyses into three groups, viz., *undifferenzierte, typische und hyperchromatische Zellen*. The typical cell may correspond to the light cells and the hyperchromatic cells are the dark cells. He argued that the hyperchromatic cells are nothing but a regressive transformation (*Rückbildung*) from the typical cells. In our present observations, intensity of PAS stainability of the intermedia cells showed a marked variation, and various steps of transition from the quite clear (PAS-negative) light cells to the deeply stained (strong positive in PAS reaction) dark cells. Therefore, it seems possible that the light cell may convert into the dark cell as a result of accumulation of some material (glycolipoprotein?) within the cytoplasm. A fact that the colloid in the lumen of RATHKE frequently contains freely floating exfoliated cells and cell debris may suggest holocrine secretory mechanism as one of the means of production of the colloid, but this may not be the only way of colloid secretion.

Goblet cells may appear in the wall of the cyst (see also RASMUSSEN 1929), but this was observed in only one case, in which the cyst was found in the anterior lobe and the goblet cells were mixed with ciliated cells. As the latter are considered to be of rudimentary nature, the goblet cells accompanying them may also belong to the rudimentary apparatus which may occur as a result of an ectopic migration of the fetal nasopharyngeal mucosa. Goblet cells were neither observed in the marginal zone epithelium nor in the wall of the cysts occurring within the pars intermedia. Therefore, the mucous secretion from goblet cells may serve the formation of colloid in only exceptional cases.

Electron microscopical observations revealed that the dark cell contained a multitude of small particles measuring about 200 Å in diameter in its cytoplasm (KUROSUMI et al. 1962). These particles may be responsible for the dark staining behavior of this cell with various dyes. Irrespectively of these small particles, one can find round dense granules in the GOLGI area of the intermedia cells. The latter was assumed to be the secretory granules of this cell because of an apparent relationship with the GOLGI apparatus which was reminiscent of a similar phenomenon in anterior pituitary cells (FARQUHAR and WELLINGS 1957). No direct relation was observed under the electron microscope between the probable secretory granules and small particles of the dark cell cytoplasm. Even if it were the case that the colloid might originate from cells of the pars intermedia especially the dark cells, but this does not mean that the colloid might contain the hormone or some physiologically active products of the pars intermedia. There exists a possibility that the active substance, which may be directly concerned with the secretory granules found in electron micrographs, may be absorbed into the blood stream soon after its production and the colloid may represent an inert degeneration product of the intermedia cells.

In our unpublished observations on mice hypophyses with light and electron microscopes, cells of the strikingly well developed intermediate lobe of this animal were shown to possess many secretory granules, but there were very few if any typical dark cells and colloid containing cysts. The final solution of the problem whether the colloid may or may not be the normal, physiologically significant secretion of the pars intermedia must await the further investigations in future.

The second possibility concerns with the relationship between the neurosecretory substance (HERRING bodies or 'colloid in the posterior lobe' in old terminology) and the colloid in the pars intermedia. According to SCHIEBLER (1951, 1952), hypothalamic neurosecretory substance occurring in both the hypothalamus and posterior pituitary is PAS-positive, sudanophilic and protein-containing substance, being presumed as glycolipoprotein. Our additional findings on the neurosecretory substance of the rat neurohypophysis are essentially the same as those by SCHIEBLER. On the other hand, from our present observations, the colloid substance in the lumen of RATHKE and in the cysts always stains with chrome alum hematoxylin and also with aldehyde fuchsin of GOMORI both of which are well known to stain selectively the neurosecretory substance of the hypothalamo hypophyseal system. Furthermore, the colloid of the pars intermedia is PAS-positive, sudanophilic, protein-containing and performic acid-SCHIFF-positive (containing SS group) as is the neurosecretory substance of the posterior lobe. Such similarities in histochemical reactions may be enough to suspect a possibility that at least a part of the intermedia colloid may originate from the neurosecretory substance released from the nerve terminals and flowed into the pars intermedia.

However, the staining reaction of the colloid is not the same as that of the HERRING body, namely the intensity of CH- or AF-stainability of the colloid is less than that of the neurosecretory substance, and the result of examination of SH group is positive in only the colloid. Therefore, a possibility of admixture of neurosecretory substance into the intermedia colloid may be seeming, but this may not be the only mean of production of the colloid. Electron microscopy is powerless to solve this question, because the neurosecretory substance liberated from the nerve terminal is no longer electron opaque and therefore cannot be identified (KUROSUMI et al. 1962). Results of stalk section with careful histochemical examinations are expected to settle the problem that whether the colloid of the pars intermedia may be related with the neurosecretion or not.

IV. Summary.

The pars intermedia of the rat hypophysis was observed with the light microscope after staining with various histochemical methods. The results were compared with those of electron microscopy which have been published separately.

The pars intermedia may be divided into two parts, viz., the marginal zone facing the residual lumen of RATHKE's pouch and the pars intermedia proper situating dorsally to the former. When CIACCIO-fixed postchromicated paraffin sections are stained with HEIDENHAIN's azan method, the marginal zone preferably stains with orange G, while the pars intermedia proper with aniline blue. PAS staining followed by counterstain with mercuric bromphenol blue also differentiates the two parts, displaying the former blue and the latter purple.

Partial defect of the pars intermedia proper occurs frequently. In such a case the pars nervosa directly abuts the marginal zone. GOMORI-stainable substance probably corresponding to nerve fibers laden with neurosecretory substance frequently invades into the pars intermedia. In rare cases, ciliated cells occur among the epithelial cells lining the RATHKE's lumen or at the wall of cysts. In the latter case,

goblet cells may be intermingled with the ciliated cells.

Glandular cells of the pars intermedia proper may be classified into two types, the dark and light cells. The cytoplasm of the dark cell is PAS positive and sudanophilic, and deeply stains with mercuric bromphenol blue. Various steps of transition between the dark and light cells are apparent especially in PAS preparations.

Colloid substance occurring in the lumen of RATHKE and in the cyst is also PAS-positive, sudanophilic and protein containing. It reacts positively to tests for SS and SH groups. Probable origin of the colloid was discussed.

内 容 自 抄

各種の組織化学的染色を施したラット下垂体の中間部を光学顕微鏡により観察し、別に発表した電子顕微鏡像と比較した。

中間部は Rathke 嚢遺残腔に面する辺縁層（ほぼ1層の扁平上皮層）と、その背側に位置する固有中間部とに分けられる。Ciaccio液固定後クロム化したパラフィン切片に Heidenhain の azan 染色を行なうと、辺縁層は orange G に好染し、固有中間部は aniline blue により青染する。過ヨード酸 Schiff 反応を施した切片を水銀ブROMフェノール青にて後染すると、辺縁層は後者により青染し、比較的強く過ヨード酸 Schiff に反応する赤紫色の中間部から、容易に区別される。

固有中間部の一部欠損がかなり高頻度に認められ、その場合には神経葉が辺縁層に密接する。神経分泌物を含む神経線維に相当すると思われる Gomori 可染物質（CH 陽性物質）の中間部への侵入が屢々見られる。稀に Rathke 腔に面する上皮または嚢胞壁に繊毛細胞が出現し、後者の部位では杯細胞を混ずることもある。

固有中間部の腺細胞は明暗2種の細胞に分類される。暗細胞の細胞質は過ヨード酸 Schiff 陽性、スダン好性で、水銀ブROMフェノール青に濃染する。過ヨード酸 Schiff 標本では特に明瞭に明暗兩種細胞間の各種移行段階が観察される。

Rathke 腔内或は嚢胞内に現われるコロイドも亦過ヨード酸 Schiff 陽性、スダン好性で蛋白を含む。さらに SS および SH 基検出反応に陽性である。コロイドの起源について考察を加えた。

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