

THE DEVELOPMENT OF THE HYPOPHYSIS OF THE OX

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TWELVE TEXT FIGURES AND ONE PLATE (EIGHT FIGURES)

INTRODUCTION

The study ¹ of the ox hypophysis was undertaken primarily to trace the development of Wulzen's lobe which forms so constant and peculiar a feature of this gland. Wulzen ('14), it may be recalled, encountered in an examination of the hypophysis of the ox a peculiar protuberance of the pars intermedia extending into the residual lumen toward the pars distalis (pars glandularis). Histologically it was different from the rest of the pars intermedia. This enlargement is in the mid-sagittal plane about "one third of the distance from the dorsal to the ventral end of the residual lumen"; however, some variations in location were observed. Although generally cone-shaped, neither size nor form is constant. Occasionally this structure indents so deeply the substance of the pars distalis as almost or completely to obliterate the residual lumen. In but thirty-eight of 760 cases was the cone absent. One of its most interesting features, however, is the presence of the same cell types as are found in the pars distalis. The differentiation of these cell types in the pars intermedia would seem an even more significant occurrence than the projection itself which presents a purely morphogenetic problem; hence it is of more than passing interest to note that this phenomenon

¹The subject of this investigation was suggested by Dr. B. F. Kingsbury. The writer wishes to acknowledge his assistance throughout the investigation and in the preparation of the manuscript.

has been observed by others. Trautmann ('24) reported the presence of acidophiles in the pars intermedia not only in the ox but also in the sheep, dog, and pig. Zimmermann ('31) likewise found them in the ox, as did Berblinger ('39 a, b); but they make no mention of Wulzen's work. De Beer ('26) referred to the presence in the ox of Wulzen's lobe or cone — the term now generally accepted for this structure — and he further observed in the hypophysis of the pig a comparable structure containing acidophilic cells. The presence of a lobe of Wulzen might be expected in other ruminants, for example, the sheep, and indeed Ganfini ('22) illustrated a projection from the pars intermedia of the fetal sheep but made no comment on it in his text. Lubberhuizen, however, who later ('31) studied the development of the hypophysis in this animal, described the presence of an "eminencia cylindrica" which he recognized as the same type of structure first pointed out by Wulzen in the ox. Frey ('34), working with the dog, observed acidophiles in the pars intermedia as had Trautmann and De Beer. Thus was demonstrated the occurrence of this type of differentiation in totally unrelated mammals.

The presence of cells typical of the pars distalis in an otherwise well defined pars intermedia naturally indicates that differentiation between these two regions is not as sharp as was previously believed. On the other hand, in certain animals — the porpoise (Wislocki, '29), the whale (Wislocki and Geiling, '36), the armadillo (Wislocki, '38; Oldham, McCleery and Geiling, '38), the common fowl and other birds (Rahn, '39; Rahn and Painter, '41) — the pars intermedia is found to be lacking and there is a rather uniform distribution of the acidophile cells throughout the entire gland exclusive of the pars neuralis and the pars tuberalis. Thus, the ox might be looked upon as an intermediate form between those animals in which the pars intermedia is sharply defined and those in which this portion is lacking as such. It also may be noted that in the latter group the neural and epithelial lobes are separated by varying amounts of connective tissue and that the residual lumen is absent; in the "typical" mammal the

pars intermedia is in intimate contact with the pars neuralis, is but slightly vascular and is separated from the pars distalis by a residual lumen. It may therefore be inferred that underlying the condition in the ox and in those animals which lack a pars intermedia a variant in hypophyseal development will be found. Indeed, van Dyke ('39, p. 10) comments: "The embryonic development of the organ of the porpoise and the whale is therefore apparently different from that in other mammals."

Since a description of the embryology of the hypophysis of the ox could not be found in the literature, and particularly since the study of the first appearance of Wulzen's lobe indicated its dependency upon factors involved in the formation of other parts of the gland, a detailed account of the development of the hypophysis as a whole became necessary.² In order to demonstrate more effectively the changes taking place in the developing hypophysis and to visualize more clearly their relations to the adjoining structures which play an important role in the morphogenesis, a series of twelve median plane reconstructions were made from series cut in the sagittal plane of the head. The embryos utilized ranged in size from 7 mm. to 80 mm. in length. Since it is also important to appreciate the great growth which takes place and the transformations effected by it, all of the reconstructions were drawn at the same magnification. In the text the figures will often be referred to as "stages," taking each one as fairly representative of the conditions exhibited by all embryos of approximately the same size. To facilitate a ready appreciation of the relative "shiftings" accompanying the great growth, the reconstructions are reproduced (figs. 1-12) at

² For this study were used fifty-six series of fetal calves from the collection of the laboratory of Histology and Embryology, Cornell University. The range in size was from 2.5 mm. to 235 mm. The embryos were sectioned in sagittal, frontal or transverse planes. In addition to the fetal stages, a study was made of several adult glands which had been sectioned in various planes and stained with hematoxylin and eosin, Mallory's connective tissue stain or by the azocarmine method of Dawson. The adult material was received from the Wilson Laboratories through the kindness of Dr. David Klein.

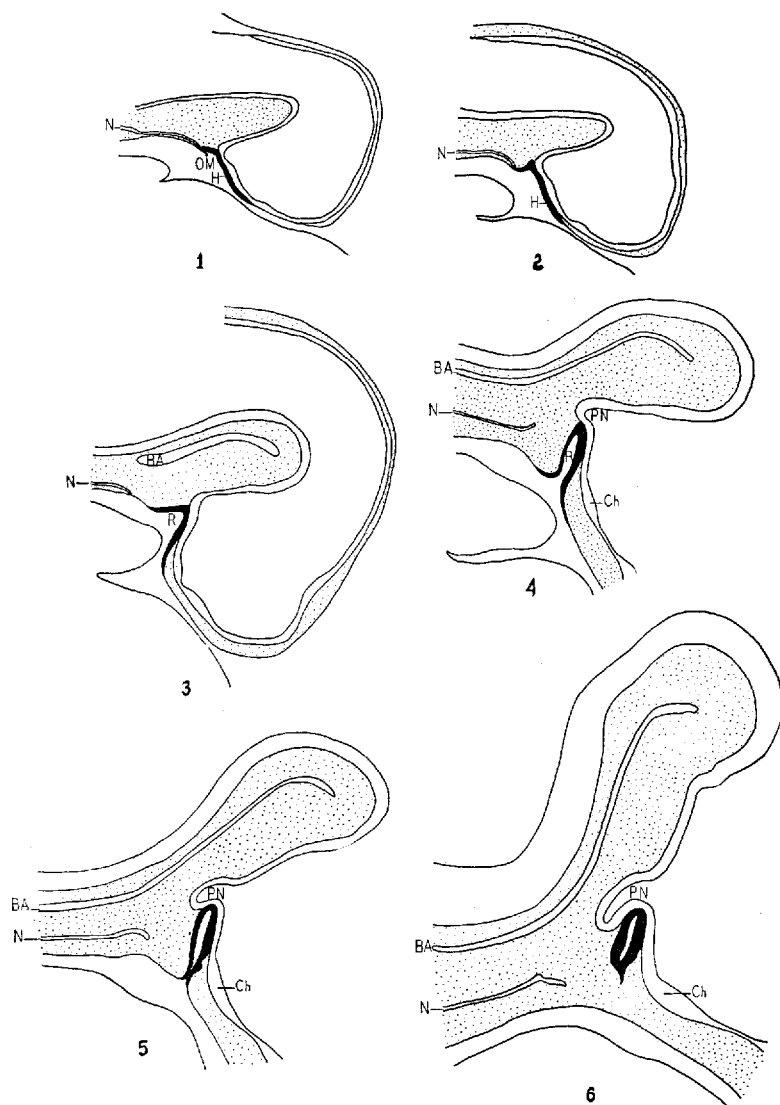
the same magnification ($\times 20$) and are all arranged with the long axis of the embryo³ horizontal, the dorsal aspect above, and the rostral end to the right.

OBSERVATIONS

Early development. The earliest available stages in the calf are strikingly similar to comparable stages in other mammals. The 7-mm. embryo was the youngest cut in the sagittal plane. In stage 1 (fig. 1) the pharyngeal membrane (oral plate) has recently broken. No definitive Rathke's pouch yet exists. The hypophyseal area, however, where the surface ectoderm is in intimate contact with the primary forebrain, is clearly shown. Here, as thickened ectoderm, it extends a short distance rostrally from the stump of the oro-pharyngeal membrane to the floor of the brain; thence, adherent to the latter, it passes ventro-rostrally to the point of earlier closure of the anterior neuropore at which level the thickening of the ectoderm ends. The anterior end of the notochord is still fused with the pharyngeal epithelium where the stub of the oro-pharyngeal membrane is seen to persist. It may be noted that no mesoderm has as yet appeared in the median plane in the region of the forebrain. In figure 2 the changes which lead to the formation of Rathke's pouch appear. At first these seem to consist mainly in the sharpening of the mesencephalic bend due to the continuation of the process of over-growth of the neural tube.

In stage 3 (fig. 3) the relative shifting of the "floor" of the forebrain, including the postoptic and optic (chiasmatic) regions, has begun. If we take a point at the tip of the infundibular recess in figure 2 as the apex of an angle formed by the

³ As was recognized by Lubberhuizen ('31), the application of directional terms for orientation is particularly difficult in the hypophyseal region. The extreme flexion in the forebrain and the early rotation accompanying it bring primarily dorsal material ventral and the early anterior end of the embryo ventral and relatively caudal. It has therefore seemed best at each stage to employ the terms dorsal and ventral without reference to the primary (morphological) significance; and to use as a line of reference the long axis of the embryo (at any stage) as the rostro-caudal axis. It is according to this axis that the reconstruction figures are oriented, the anterior (rostral) aspect being toward the right.



Figs. 1 to 6 Median plane reconstructions of the hypophyseal region, from series of calf embryos 7-, 9-, 7-, 9.5-, 14-, and 13-mm., crown-rump length respectively. $\times 20$. Abbreviations: BA — basilar artery; Ch — chiasmatic ridge; H — hypophyseal area; N — notochord; OM — oral membrane; PN — pars neuralis; R — Rathke's pouch; S — hypophyseal stalk.

premamillary area and the region of contact (the optic and postoptic regions) it will be seen that by stage 3 the angle has increased in size, due mainly to the relative dorsal movement of the diencephalic floor particularly in the optic region. Thus, since the ectoderm remains adherent to the brain floor it is carried with it. In this manner is produced a shallow pouch whose apex is directed dorso-rostrad. As is seen in figure 3, it is more capacious than in the preceding stage and is still widely open to the outside. The mesoderm has markedly increased and has appeared rostrally between hypophyseal area and optic (chiasmatic) ridge.

During these stages, another process of importance for the formation of Rathke's pouch occurs. If parasagittal sections of the 7-mm. embryo shown in figure 1 are examined, mesoderm is seen to be spreading from the territory of the ventral encephalic plica around the hypophyseal area. This tends to increase the distance between the brain floor and the superficial ectoderm. Mesoderm has in this stage already passed lateral to the hypophyseal area, but, as already noted, it has not yet reached the median plane. By stage 2 (fig. 2) the process of encirclement has been completed. By stage 3 the mesoderm has penetrated between the chiasmatic ridge and the rostral portion of the hypophyseal area (now rostral wall of Rathke's pouch). By comparing figures 4 and 5 the continued increase in the mesoderm rostral and caudal to the hypophyseal ectoderm is obvious. This is clearly a significant factor in the change in form of Rathke's pouch.

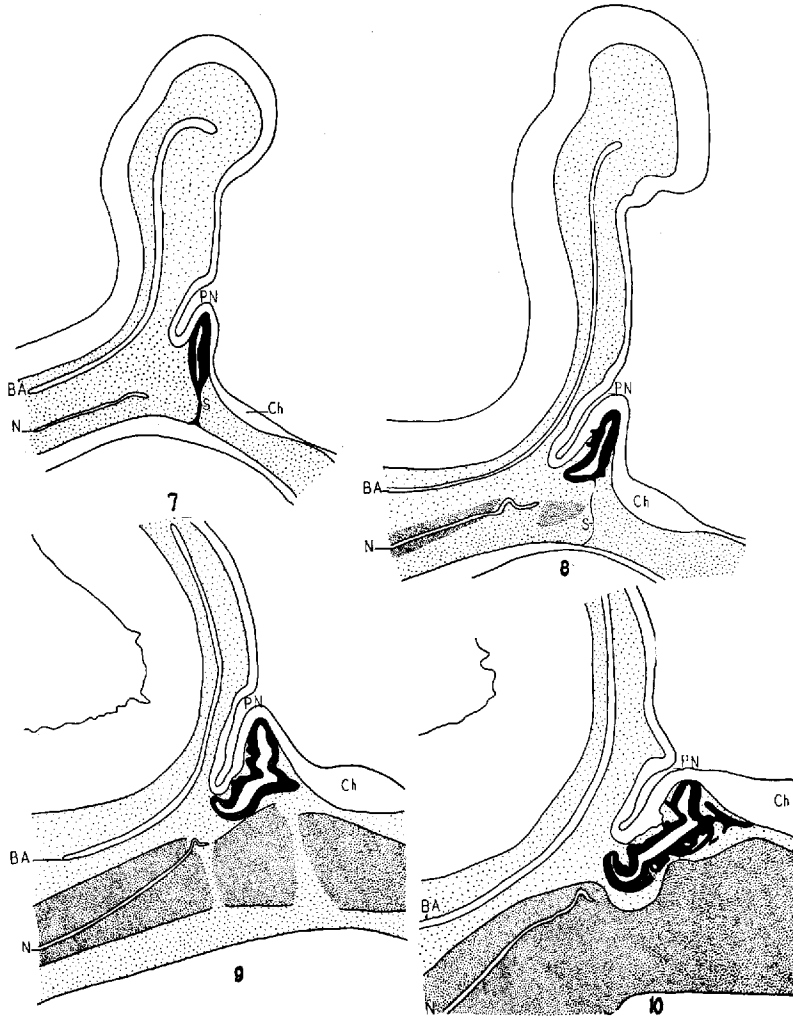
Two other changes in the general region may be noted at this point. In stage 3 (fig. 3) the distance between the end of the infundibular process and the tip of the notochord has slightly increased. Apparently, there has been some lengthening of the brain floor from the cervical to the mesencephalic flexures. This feature is of particular interest, for it may contribute to the elongation of the caudal wall of Rathke's pouch which in stages 1 and 2 was a very narrow strip extending from the site of rupture of the pharyngeal membrane to the infundibular recess. In stage 4 (fig. 4) the pontine bend,

which first appeared in stage 3, has become more marked. Also there has occurred a marked constriction of the lower end of Rathke's pouch. Again, if we compare figure 4 with figure 3, taking for reference the point of transition between the thicker hypophyseal and the thinner epithelium of the pharyngeal roof, it will be seen that an angle has been formed between the caudal wall of Rathke's pouch and the roof of the pharynx. That is, the caudal wall has been shifted through approximately 90 degrees.

In figure 5 Rathke's pouch is seen to be more elongated with a great constriction of the stalk. This is, again, a function of the increase in amount of mesenchyme as well as of the growth of the brain particularly in the optic (chiasmatic) region. These expansive processes, affecting the diencephalic floor adherent to the relatively passive epithelial pouch, tend to shift the region of contact dorsally.

As early as stage 3 but more noticeably in stages 4 and 5, certain areas of mesenchyme begin to condense, particularly in those regions central and dorso-caudal to the pouch. Examination of this material reveals it to be much less vascular than the looser mesenchyme immediately related to the pouch proper. The explanation has been offered by several recent authors that this condensation of mesenchyme, as a growing mass of precartilage, actively constricts the connection between the pouch and the roof of the stomodeum and is thus responsible for the final break which occurs. My observations lead me to suggest at least as an additional factor that this portion of the pouch ceases to grow. As the pouch is drawn out under the stress of the processes previously mentioned, the cells for a time continue to proliferate throughout the whole extent of the pouch wall. However, as the mesenchyme surrounding the lower part of the pouch becomes denser and less vascular, the cells exhibit fewer mitoses; cell proliferation fails to keep pace with the elongation and a break in the stalk occurs. There seems to be some variation in the time at which the break occurs, as may be seen by comparing figures 6, 7 and 8. It always takes place at the pharyngeal end,

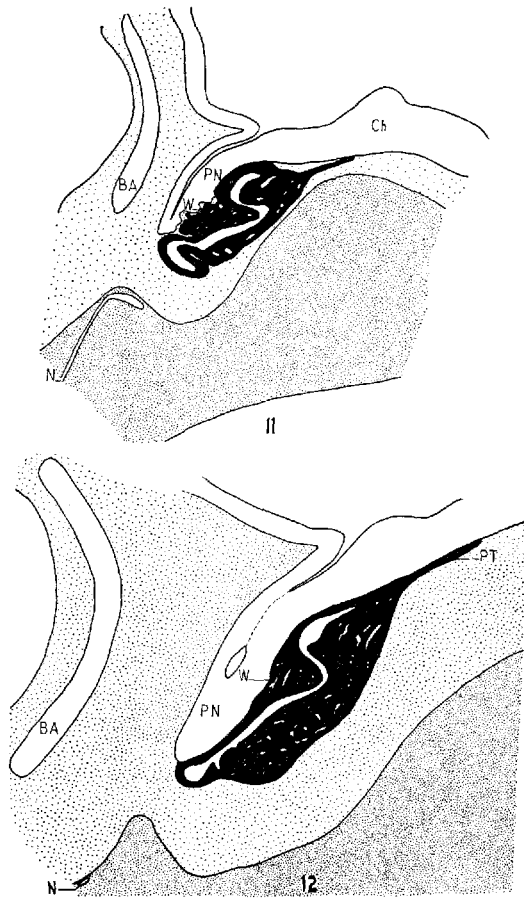
a fact already observed for other animals, leaving for a time a visible remnant attached to the dorsal portion of the pouch. In no case, it may be noted, was either a pharyngeal hypophysis (as in man and dog) or a para-hypophysis (as reported



Figs. 7 to 10 Median plane reconstructions of the hypophyseal region, from series of calf embryos respectively 14.5-, 17-, 25-, and 36-mm. crown-rump length. $\times 20$. Abbreviations: BA — basilar artery; Ch — chiasmatic ridge; N — notochord; PN — pars neuralis; S — hypophyseal stalk.

for the dog) observed in the late fetal or early postpartum stages.

Pars distalis. In stage 8 (figs. 8, 13) the condensation of mesenchyme in the region ventral to the pouch has now produced a dense mass of precartilag. In this stage, with the final severing of the stalk, the walls of the pouch begin an active proliferation. This tends to produce expansion. Along



Figs. 11 and 12 Median plane reconstructions of the hypophyseal region, from calf fetuses 48 and 80 mm. in length, respectively. $\times 20$. Abbreviations: BA — basilar artery; Ch — chiasmatic ridge; N — notochord; PN — pars neuralis; PT — pars tuberalis; W — Wulzen's lobe.

its long (dorso-ventral) axis this is blocked dorsally by the neural lobe (fig. 8) as it bends orally and caudally from the diencephalon. Elongation ventrally appears checked by contact with this dense material, the result being that the lower end of the pouch becomes somewhat flattened (figs. 8, 13). Extension of the pouch dorsally around the sides of the neural lobe is facilitated by the presence here of loose and vascular tissue. Expansion laterally, producing the so-called lateral lobes (fig. 18), also has set in. Rostrally, only a thin strip of mesenchyme separates the pouch from the brain (figs. 8, 13), the latter serving as a rather formidable barrier to growth in that direction. Thus it may be seen that from a very small portion of Rathke's pouch—a portion which at the stage represented in figure 6 lies just caudal to the attachment of the stalk to the pouch proper—gives rise to nearly two thirds of the pars distalis (figs. 8 to 12). The rostral third arises from that part of the pouch wall which, from stage 4 through stage 7 was in contact with the postoptic brain wall. Only after the relative rostral shifting of the optic (chiasmatic) region can this part of the pouch increase appreciable in extent (figs. 9 to 12).

When figures 11 and 12 are compared to some of the earlier ones, it will be seen that the long axis of Rathke's pouch has shifted from dorso-ventral toward rostro-caudal. Three suggestions for this change are offered: (1) the condensation of the mesenchyme of the basis cranii leaving in the region dorsal to it an area of looser material; (2) expansion of the region between forebrain and pons bend; (3) to a lesser degree, the relative shifting of the optic (chiasmatic) region dorsally and rostrally providing a space for ventral proliferation. It seems to be characteristic of the walls of the Rathke's pouch to proliferate cords or groups of cells when in contact with a bed of vascular tissue. As these cords become numerous, branching and anastomosis take place freely. Thus the ventro-rostral wall of the pouch becomes greatly thickened by a meshwork of cords. The mesenchyme enclosed within the

meshwork contains blood vessels which persist as the capillaries (sinusoids?) of the adult structure.

Pars intermedia. At the time of the primary neuro-epithelial contact (figs. 1 to 3) no definite neural lobe is present, but it is indicated as a small saccular bulge in the wall of the diencephalon — the infundibular recess (figs. 1 to 3). As the definitive pars neuralis begins to develop (fig. 4) its apex is directed caudally. With the advent of the pontine bend (fig. 5) and the occurrence of thickened strands of mesenchyme just dorsal to the apex of the neural lobe, the distal end of the lobe comes to point somewhat ventrally into a pocket of loose mesenchyme (figs. 5 to 7). In this way, the ventral wall of the neural lobe, so to speak, begins to "slip down" the caudal wall of Rathke's pouch. Between stages 3 and 4 the mesenchyme along that surface has become vascular. Thus, as the walls of the neural and epithelial portions of the hypophysis come into closer association, a variable amount of vascular mesenchyme lies between them (fig. 14). As the other walls proliferate cords into mesenchyme, so, similarly, from this portion buds extend into the rather small space between the lobes. Many of these buds at first contain extensions of the pouch cavity (fig. 17). Soon, however, these cavities disappear.

Almost as soon as the pars neuralis changes its direction, processes of the epithelial lobe tend to envelope it (figs. 16, 20). This tendency to surround the neural lobe may be interpreted as an expression of the shift in the position of the neural lobe and the expansion of the epithelial lobe. The process is probably begun as the pars neuralis in its extension "indents" the dorso-caudal surface of the pouch, the lateral edges of the pouch tending to push up along its sides. In the calf, however, envelopment is never complete, as it is for example in the dog and cat. The dorsal surface of the neural lobe remains throughout free of epithelial investment. Stendell ('14) made the same observation for the adult ox. This lack of inclosure may possibly be due to the rather dense connective tissue which early forms in contact with the dorsal

surface of the neural lobe. In the adult this material seems to be cartilage.

Mention has already been made of the flattening of the ventral end of Rathke's pouch (figs. 8, 13). This portion of the pouch also bends or curves, in this way producing a pocket in which loose mesenchyme is included between the epithelial pouch and the neural lobe (figs. 8, 9, 13). Rostrad of the bend or pocket association between neural lobe and pars intermedia becomes more intimate (figs. 9, 10, 11) while caudally the "mesenchymal bay" so produced remains for some time in open communication with the surrounding connective tissue. As soon as the "mesenchymal bay" has appeared, those portions of the intermediate lobe bordering it begin to proliferate into it (figs. 18, 20) until the area is a maze of branching and anastomosing cords (fig. 19). In the spaces between the cords is found vascular mesenchyme which for a time is continuous caudally with the mesenchyme surrounding the gland (figs. 9, 10, 11 and 16, 18, 20). It is apparent from the examination of later stages that in the bay thus occupied the vascular tissue comes to lie near the epithelium bordering the residual lumen. This structure is the primordium of Wulzen's lobe. Later growth in length caudally gives it a relatively more rostral position (fig. 12).

Up to and including the 163 mm. stage it is possible to trace blood vessels into the connective tissue lying between the lobes. These vessels give off branches to both neural and intermediate lobes. In the region of the Wulzen's cone, however, branches to the pars intermedia are more numerous.

Since Stendell ('14) and Frey ('34) have suggested that the shape of the pars intermedia is determined by the neural lobe, it might be thought that the enlargement of the pars intermedia as Wulzen's lobe is a function of a corresponding enlargement of the neural lobe. Figures 8 to 11 show, however, that changes taking place in the pars intermedia precede the appearance of the enlargement of the pars neuralis occasionally encountered in this same region (figs. 10, 11). They may, of course, be indirectly correlated.

Although sometimes in the adult a marked cone or lobe of Wulzen may not be visible, there is usually some thickening of the pars intermedia in the locality where the cone would be located and at least the outermost cells in this thickened area will exhibit a differentiation characteristic of the pars distalis (fig. 15). Insignificant though the amount of such differentiation may be, the connective tissue is more abundant and the blood supply is richer in that region.

Pars tuberalis. The pars tuberalis is present in the ox as a rather extensive lamina of cells which extends well out beneath the tuber cinereum toward the optic chiasma (fig. 12) and forms a complete investment for the infundibular stalk. In some animals the pars tuberalis has been traced back to the lateral lobes — two separate and distinct buds or hollow pockets (cat) arising from the ventral surface of the epithelial pouch on a ridge of tissue common to the lateral lobes and the epithelial stalk. The pars tuberalis in the calf is more comparable in its mode of development to that of the sheep as described by Lubberhuizen ('31). In the calf embryo lateral lobes are developed (fig. 18) and from the ventral aspect of these, cords pass in a rostral direction. At about the same time, however, a single median process arises from that portion of the ventro-rostral wall of the pouch which is between the lateral lobes. From this "anterior process" cords also are formed, but by the stage shown in figure 16 the cords from the three sources have become so united that the precise extent of the contribution by either the lateral lobes or the anterior process cannot be determined. The anterior process appears relatively late (fig. 9) from the rostral wall of Rathke's pouch well toward the ventral end and therefore near the place where in younger embryos the stalk was earlier attached (fig. 8). The characteristics of this region of the pouch were previously considered. Cords from the anterior process might be expected to attain their definitive position earlier; the more lateral cords (presumably from the lateral lobes), however, soon reach the brain even though the space between the more lateral wall of the pouch

and the brain is greater. The association between epithelial cords and mesenchyme becomes so intimate that it is often difficult in the material available to differentiate the two tissues. It is also difficult to determine to what extent a relative shifting of the material (mesenchyme and epithelioid cords) or a proliferation of the cords is responsible for the assumption of the final morphology of this unique component of the epithelial hypophysis. The complete investment of the infundibular stalk in the calf does not take place until after birth.

Pars neuralis. Gilbert ('34) furnished strong evidence that in the cat the appearance of the neural lobe is a passive process, the direct result of very rapid growth in the premammillary, the postoptic and optic regions as well as in the lateral wall of the diencephalon acting upon the relatively inactive infundibular recess. In 1935 the same author compared early development in several mammals, the calf among them. She reported that the primary development of the *pars neuralis* in the calf is fundamentally the same as in the cat.

The early development of the neural lobe was considered under the *pars intermedia* and its shift in direction noted (figs. 5 to 9). The shift is probably the result of several factors: first, as the material of the infundibular wall is "evaginated" under the influence of the adjoining more actively proliferating brain wall, the end of the diverticulum soon encounters rather dense strands of mesenchyme which seem to represent a portion of the primitive dura (fig. 14). This may possibly tend to deflect the neural lobe into a pocket of looser mesenchyme lying just dorsal to Rathke's pouch (fig. 14). Secondly, as the pontine bend approaches a right angle, the premammillary and postoptic regions expand (figs. 6 to 9) carrying the rostral end of the neural outpocketing slightly dorsad; at the same time, it should be noted, the apex of the neural lobe maintains a quite constant distance from a relatively fixed point—the rostral end of the notochord (figs. 4 to 11). The net result of this would incline the wall of the *pars neuralis* ventro-caudad.

Through the stage illustrated in figure 6 the elongation of the neural lobe is still apparently a passive one with the walls of the lobe remaining relatively the same thickness with mitoses few. However, in stage 8 there is evidence of much mitotic activity in the ependymal cells lining the cavity. Nuclei migrate from this region producing both an increase in thickness of the ventro-rostral wall of the lobe and an increase in the length of the lobe as a whole.

The ventro-rostral wall of the neural lobe is markedly thickened by the time the 30-mm. stage is reached. This is mainly a result of the "migration" from the actively growing ependymal layer. The lumen also has become narrower at its lower end. Between this stage and the one illustrated in figure 11 the character of the growth changes. Instead of the predominantly "cellular" nature of the wall as seen in the previous stages, nerve fibers begin to appear in the cephalic half. A large number apparently come in from the hypothalamus and course down the infundibular stalk. So extensive is this ingrowth that the connection between the third ventricle and the cavity within the lobe is almost obliterated. The caudal portion of the lobe, however, presents a picture of large nuclear masses external to the lining ependyma as increase in length proceeds by the addition of more cells. A small increment in thickness of the dorso-caudal wall also takes place with the appearance of fibrous material. Whether this represents small fiber tracts from the hypothalamus or the accumulation of neuroglial fibers could not be determined definitely.

As the fetus grows older, the fibrous character of the lobe becomes more obvious. In the 80-mm. stage shown in figure 12 the connection between the cavity in the lobe and the third ventricle is completely closed off, a small cleft remaining isolated in the middle of the lobe. This cavity is also obliterated at the 130-mm. stage.

In some of the series of older fetuses between 80 and 163 mm. long blood vessels were traced into the inferior (caudal) pole of the lobe. Beyond the latter stage the fetal

material at hand did not permit the determination of the persistence of the vessels. However, in the only adult in which the connective tissue relations remained undisturbed, evidence of an apical blood supply could be found.

DISCUSSION

The nature of the primitive neuro-epithelial contact which is involved in the development of the hypophysis is not easily determined. That fusion does not occur in the calf embryo is clear, since a line of demarcation between neural and surface ectoderm is evident. The fact, however, that the two tissues remain extensively adherent throughout the changes in the brain and the ingrowth of the mesenchyme into the region surrounding the hypophyseal area seems to indicate that the contact has some degree of firmness. Comparing the stages 1 to 9 (figs.) it seems that the primary contact in the calf fetus persists only in the proximal portion of the neural lobe and the adjacent postoptic region. The calf is thus different in this respect from the dog and the cat.

The later configuration of the gland has been shown to be influenced by two other conditions which arise, namely: first, a shift of the optic and postoptic regions from an approximate horizontal (rostro-caudal) position through a dorso-ventral orientation and again to a roughly horizontal direction (figs. 1 to 12). Examination of these figures also reveals the shift of the plica encephali ventralis from a horizontal (caudo-rostral) (figs. 1 to 4) to a dorso-ventral direction (figs. 5 to 9). In the later stages (figs. 10 to 12) the pons region approaches the hypophysis less closely as the intervening mesoderm increases in amount. These two growth changes appear linked with the change in the long axis of the epithelial hypophysis from a vertical to a roughly horizontal direction.

In early stages the walls of Rathke's pouch elongate, possibly due in part to the tension placed upon them by the shifting of the area of contact, and to the rather frequent mitoses which occur throughout. In the early stages, however — contrasting with the great growth of the head as a whole —

there is relatively slight growth of Rathke's pouch, as readily may be seen by comparing figures 4 to 8. It is not until the surrounding mesenchyme has become highly vascular that the extensive proliferative activity which characterizes the later stages really begins.

The second "influence" referred to above concerns the different relations of the mesenchyme — vascularization, condensation, and chondrification. The condensation and chondrification of the mesenchyme produces the basis cranii ventrally. Caudally a mesenchymal condensation bounds the hypophyseal area on that side; it is shown only in figures 13 and 14. The occurrence of the pre-cartilage of the prospective basis cranii has been previously mentioned in connection with the ventral flattening of the epithelial pouch. It was also earlier suggested that the mesodermal condensation caudal to the hypophysis limits the expansion of the epithelial lobe on that side. The effect of this condensation on the early morphology of the epithelial hypophyseal sack is indicated for certain other mammals; early in the cat (Brahms, '32, figs. 5, 6) and later in the dog (Kingsbury & Roemer, '40, figs. 6 to 9). In these two instances the pars intermedia is not involved. In the calf, at a relatively later stage (figs. 8, 9, 10) with mesoderm intruding between neural lobe and epithelial lobe, the caudal edge of the epithelial pouch is affected. In this way a bay of mesenchyme is formed behind the dorsal wall of the pouch. As far as known, in no other animal, with the possible exception of the sheep has there been produced a "buckling" in the incipient pars intermedia.

The vascularization of the "mesenchymal bay" and the penetration into it of epitheloid cords from the adjoining pars intermedia, establishing the cone of Wulzen, has been earlier described. The differentiation of cells typical of the pars distalis in a derivative of the pars intermedia which in perhaps most mammals contains only indifferent, faintly basophilic cells, required further comment. To account for the presence of acidophilic cells in the pars intermedia, we must accept one of two hypotheses: (1) the cells migrate into this central

region of the pars intermedia from the pars distalis through those areas of the gland where the two lobes adjoin at the periphery of the residual lumen; or — in cases where the pars intermedia is absent — the cells from the dorsal wall of the pouch are replaced by proliferation from the ventral wall as the residual lumen is obliterated or (2), all the cells of the pouch have the same potentialities for differentiation, failure to differentiate in a typical manner being the result of change in environmental conditions.

To support a contention for migration of cells, it would seem that in any of the portions of the pars intermedia located near the pars distalis occasional acidophilic cells might often be expected. In the calf, as far as could be determined, there is no evidence for migration. In the work of Oldham ('41) on the armadillo and Rahn ('39) on the fowl, the only embryological studies made on forms lacking a pars intermedia, no mention is made of any failure to grow and differentiate on the part of the tissue which earlier constituted the dorsal wall of the epithelial pouch. Furthermore, there were no observations of cell migration into this area from the constantly enlarging ventral wall.

Although no absolute proof for the differential potencies of the intermediate lobe has been established in higher forms through extirpation and transplantation of hypophyseal primordia, certain evidence has been furnished through the experiments of Atwell ('35, '37) on amphibia. His work points to the fact that cells typical of the pars distalis will differentiate when no definite pars intermedia is found. From this, then, it might be inferred that the reason for the relatively small volume of the intermediate lobe and its composition of more or less indifferent cells may be found in some variations in the environment.

There appear to be two such environmental differences which may have some bearing on the potentialities of the pars intermedia. The first is contact with the tissue of the neural lobe which might have some inhibiting effect upon cellular proliferation and differentiation; the second concerns the lack

of vascularity in this region. The possibility of the former cannot be discounted since there is no evidence at hand from which conclusions may be drawn. There is, however, ample indication that the latter plays a significant part.

Most recent authors have commented upon the relatively avascular condition of the pars intermedia. Atwell and Woodworth ('26) took particular cognizance of this fact in their paper on the relative volumes of the epithelial parts of the hypophysis. Such a condition is to be expected when one takes into account the close approximation of the pars intermedia and pars neuralis, at the same time appreciating the separation by the residual lumen of the pars intermedia from the highly vascular pars distalis. This is particularly true of those animals in which there is a complete investment of the pars neuralis as in the cat and dog. In these cases the only possibility of vascularization comes either from the infundibular stalk or at the apex of the pars neuralis through which the inferior hypophyseal arteries pass.

In Wulzen's cone, compared with the "typical" pars intermedia, conditions are different. Here the cells are in small groups or "nests" in the midst of fine vascular connective tissue, thus bringing individual cells into relation with the blood supply. The capillaries so enmeshed persist in the adult, retaining connection with the inferior hypophyseal arteries and in the cone of Wulzen cellular differentiation occurs; in all other localities adjoining the cone, epithelium typical of the pars intermedia is found.

The condition of greater vascularity in the cone was also noted by Lubberhuizen ('31) in the sheep. It may be mentioned that in the sheep the cone (*his eminentia cylindrica*) generally seems to separate from the pars intermedia and become so deeply imbedded in the substance of the pars distalis as to be indistinguishable from the latter. Although none of the calf material which was at my disposal gave evidence for such separation, one calf series showed almost complete obliteration of the residual lumen directly ventral to the cone, and another possessed a greatly drawn out cone attached to the

pars intermedia by a comparatively thin stalk. It is conceivable that, in subsequent development of these two glands, Wulzen's lobe might easily have become entirely lost to the pars distalis. This is mentioned as a possible explanation of the occasional absence of the cone.

In the case of the hypophysis, it can readily be appreciated that variations in any of the developmental conditions — in the size of the epithelial pouch, extent or time of formation of the denser mesoderm, or the changes in the caudal wall of the forebrain, etc. — could occur and markedly affect the early morphology of the gland. Apparently conditions remain fairly constant in animals of the same species, with development "normal." It may be assumed that should any of the developmental factors be markedly varied, or several of them varied to a lesser degree, the resulting hypophysis would exhibit characteristics not usually considered typical; as for example, the lack of a pars tuberalis (two- and three-toed sloths and anteater), or the lack of a pars intermedia (armadillo, birds in general, whale, porpoise, the manatee).

The condition of complete absence of a pars intermedia, as well as the presence of a lobe of Wulzen, it seems to me, is fundamentally related to the question of vascularization. The earliest stages illustrated by Oldham ('41) in her paper on the armadillo (figs. 15, 16, p. 311) show a well-defined Rathke's pouch with only a slight indication of a neural lobe in the region of the infundibular recess. In these stages (7 mm., 8.5 mm.) comparable to stage 4 (9.5 mm.) of the calf, a neuroectodermal contact is obvious. Earlier stages than those shown are undoubtedly similar to those typical for mammals in general. At 12.5 mm. (her fig. 18) intrusion of mesoderm between the neural and epithelial constituents has begun. Beyond this point Miss Oldham observed certain differences which appear to be of extreme importance for the definitive form of the armadillo hypophysis. Among these is the fact that the mesenchyme lying directly dorsal to the pouch is heavier and becomes more vascular than is typically the case. Thus, when the primordium of the pars neuralis begins to

expand, it is separated from the pars intermedia by a relatively denser, vascular mesenchyme. Whether the presence of this tissue is the sole factor in keeping these lobes apart; or whether there may be differences in the mode of growth determining the pars neuralis, is still uncertain. Whatever the cause(s), the part of the pouch which would typically become pars intermedia is from a rather early stage exposed to vascular mesenchyme which soon invades the wall of the pouch, breaking up the epithelium into small nests of cells. The proliferations in this area and the differentiation of cells typical of the pars distalis soon follows.

It would seem that the functions of the pars intermedia, which consist in the main of the production of a "melanophore-expanding" substance, in the mammals which lack a pars intermedia have been taken over by the cells of the pars distalis (Oldham, McCleery and Geiling, '38; Oldham, '41). This, from a physiological point of view, might further argue for a lack of sharp distinction between the pars intermedia and the pars distalis.

No attempt has been made to follow farther in the calf the cells which penetrate the pars nervosa from the pars intermedia, nor to determine their fate. The late development of the pars nervosa has not been followed and hence cannot be considered.

The pars tuberalis of mammals remains a most difficult portion of the hypophysis to interpret developmentally. Its place(s) of origin and its late establishment as a distinct part of the epithelial component suggest that growth alone does not determine its morphology. Accompanying shiftings are quite obscure. No attempt to formulate an adequate interpretation has been made by the writer. A comparative approach seems to be indicated, particularly as between forms in which it is present and forms in which it is absent. For the physiologist it is equally difficult.

Were the development of the hypophysis of the ox to be compared in detail with that determined for other mammals, two general conclusions might be established. First, the

hypophysis of the ox possesses its own distinctive morphogenetic features; second, there is a common developmental pattern. Here, as in mammals generally, the primary source of the epithelial constituent of the complex is the superficial ectoderm in contact with the floor of the forebrain. In the mammal this hypophyseal area of contact extends from (or near) the rostral end of the chiasmatic ridge (torus opticus) to the oro-pharyngeal membrane. By a complex of growth changes involving the ventral bending and expansion of the forebrain and a characteristic spread of the associated mesoderm, the ectoderm of the hypophyseal area becomes folded into a Rathke's pouch. The frequently expressed interpretation that the epithelial hypophysis arises as an (active) evagination of the stomodeum which fuses (secondarily) with an evagination of the brain is thus not strictly accurate. It still appears in textbooks. It is, however, confessedly difficult to state concisely the developmental transformations involved in the morphogenesis of this complex structure.

SUMMARY

1. The primary source of the epithelial component of the hypophysis is described.
2. The formation of Rathke's pouch is described and the growth factors influencing or determining it are considered.
3. The epithelial (buccal) stalk early disappears without trace. There is no pharyngeal hypophysis in the calf.
4. The long axis of Rathke's pouch during development shifts from dorso-ventral to rostro-caudal. The underlying growth factors are considered.
5. At the caudal end of the expanding epithelial pouch, at an early stage, an area of mesenchyme is included between the pars neuralis and the dorsal wall of the pouch (pars intermedia). Through vascularization of this mesenchyme and the growth into it of epitheloid cords from the pars intermedia, Wulzen's lobe is formed.
6. The conditions determining the differentiation of acidophile cells in the pars intermedia (Wulzen's lobe) are briefly discussed.

7. The early development in the calf of the pars distalis, pars tuberalis and the pars neuralis are briefly presented.

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PLATE 1

EXPLANATION OF FIGURES

A., acidophile cell	PH., pharynx	R.L., residual lumen
A.P., anterior process	P.N., pars neuralis	R.P., Rathke's pouch
L.L., lateral lobe	P.T., pars tuberalis	S., hypophyscal stalk
P.I., pars intermedia		

13 Calf series 23. Sagittal 21 mm. Approximately median section, caudal aspect to the left. The ventral end of the pouch is shown flattened against the precartilage of the basis cranii. Buds from the pars intermedia may also be seen. $\times 63$.

14 Calf series 16. Sagittal 14 mm. Approximately median section, showing the much constricted stalk at the lower end of Rathke's pouch. Relationship of the denser (meningeal) tissue to the dorsal wall of the neural lobe may be seen. Same series as figure 8. Caudal aspect to the left. $\times 63$.

15 Adult ox hypophysis. An enlarged portion of a very poorly developed lobe of Wulzen. The large acidophile cells are shown in black. $\times 507$.

16 Calf series 29. Frontal 27 mm. The intermingling of the cords from the anterior process and from the lateral lobes is shown. $\times 63$.

17 Calf series 20. Sagittal 19 mm. An enlarged portion near the tip of the neural lobe. A large amount of mesenchyme is seen between the neural and epithelial lobes. Extensions of the pouch cavity into the buds from the pars intermedia are also illustrated. $\times 253$.

18 Calf series 51. Frontal 18-19 mm. The early appearance of the anterior process is shown. On each side out-growths from the lateral lobes have appeared at this stage. $\times 63$.

19 Calf series 56. Frontal 27 mm. An enlarged portion of the "mesenchymal bay," showing cords from the pars intermedia. Considerable space in this area is occupied by them. $\times 200$.

20 Calf series 22 a. Frontal 20 mm. The section illustrated in this figure shows proliferations from the anterior process as well as from the lateral lobes. Cords from the pars intermedia which pass toward the neural lobe are also seen. $\times 63$.

