EARLY ONTOGENY OF THE PITUITARY COMPLEX IN *GALLUS DOMESTICUS*

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THESIS

EARLY ONTOGENY OF THE PITUITARY COMPLEX IN *GALLUS DOMESTICUS*

Submitted By

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY GLEN F. BIDDULPH ENTITLED *EARLY ONTOGENY OF THE PITUITARY COMPLEX IN GALLUS DOMESTICUS* **BE ACCEPTED AS FULFILLING PARTIAL REQUIREMENTS FOR THE SENIOR THESIS IN ZOOLOGY.**

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The means by which Rathke's pouch is formed is very complicated. Rearrangements of tissues in the region greatly affect the ectoderm of the the future pouch. The body forms the subcephalic pocket, folding epithelial ectoderm that was anterior and lateral back under the head. Anchoring of the epithelial tissue to neural tissue permits the movements of other head parts to move pouch ectoderm. The buccal ectoderm that eventually forms Rathke's pouch becomes thickened at this stage and begins to invaginate as early as stage 13 as mesenchyme commences to fold the epithelial sheet. These mesenchymal movements, along with forces created by cranial flexure and brain elongation, are the creative forces that direct the development of of Rathke's pouch.

In the past, the development of the pituitary gland in vertebrates has been described as two ectodermal plates developing separately but growing together to form the neuralepithelial complex of the adult pituitary. In concert with studies available on reptiles and mammals, the model of development in birds and amphibians described above may suggest a common pattern of pituitary development across all vertebrates.

INTRODUCTION

The chick embryo has long been a model organism for researchers in the field of developmental biology. The chick is ideal material for developmental embryology because embryos are available year round and the stage of differentiation can be closely timed and determined. The chick embryo is not influenced by maternal endocrine factors during development, except for the initial endocrine "charge" (Doskocil, 1979). Chick embryos can be removed easily from the egg unaccompanied by ill effects to the mother and development within the egg allows easier manipulation of and less damage to delicate tissues.

In an attempt to add to information available on the cat, sea turtle and the snake and to suggest a common pattern of pituitary development across vertebrates, I studied the early stages of development in the chick (Gallus domesticus) and quail (Coturnix coturnix) and two species of frogs (Xenopus laevis and Eleutherodactylus coqui).

In an attempt to correlate developmental events between different birds, quail eggs were obtained and incubated simultaneously with the chick eggs.

Although quail have a slightly shorter incubation time than do chicks, the early stages of development were closely parallel between the two species, and early stages of development were easily correlated.

Current literature concerning the developmental processes forming the chick pituitary contain a surprising number of individual findings that are explained in widely different ways. Contradictions between investigators concerning the differences in interpreting of ontogeny exist. It is accepted by most researchers that the early pituitary gland is derived from ectoderm and is made of two structurally different parts, one derived from pharyngeal epithelium and one from the ectodermal neural tube. Current textbooks of embryology (e.g., Schoenwolf and Watterson, 1989) as well as endocrinology (e.g., Saland and Rioch, 1986) describe the ontogeny as follows: At early stages of embryonic development, the adenohypophysis begins as an epithelial-lined pocket which extends out from the oral ectoderm. The anterior wall of the pouch develops into the pars distalis, the major part of the adult pituitary gland. The neurohypophysis arises as a down-growth (the saccus infundibuli) from the region of the diencephalon which forms the hypothalamus in later stages of development. Two lateral outgrowths of the upper end of Rathke's pouch grow forward and towards the midline, forming the pars tuberalis. According to Wingstrand (1951), Rathke's pouch and the saccus infundibuli are 'outgrowths' and the two structures develop separately but grow together to form the neural-epithelial complex of the full developed adult pituitary.

Martinez (1960) noted that this description is apparently not correct in all vertebrates. His work shows that during early development in cats (Felis catus), a hypophysial area could be found where the plates of ectoderm were in

close contact with each other. This contradicted early studies performed by Brahms (1932) and Dawson et al. (1942). Further studies (Pearson and Wurst, 1977; Pearson et al. 1980) have presented evidence corroborating Martinez's findings in studies with the snake (Thamnophis brachystoma) and the sea turtle (Caretta caretta). Correlated studies concentrating on the morphogenesis of Rathke's pouch by Jacobsen et al. (1979) have shown similar developmental processes. Although the model suggested above was not mentioned specifically, a number of photomicrographs allow the interpretation that the chick pituitary gland developed following similar pathways. In studies attempting to determine the details of pituitary development, researchers experimentally placed a barrier (e.g., a glass rod or a piece of paper) in the region where the pituitary would develop (Hammond, 1974; Tixier-Vidal and Follett, 1973). The disruption of tissues that resulted seemed to confirm the traditionally accepted view that Rathke's pouch and the saccus infundibuli were developing separately and growing together later in development. Studies on naturally developing embryos by Johnson and Listgarten (1972) looked at later stages, and the early stages of development were overlooked. Johnson and Listgarten inferred the developmental process by looking at tissue foldings in later stages of development and determined that the two tissue layers were developing separately and that the diverticulum formed by Rathke's pouch grew dorsad to make contact with the basal hypothalamus.

My results suggest a variation from the commonly accepted view and confirm that early pituitary development in birds and amphibians parallels closely the pattern described earlier for the cat, Thamnophis and Caretta.

MATERIALS AND METHODS

A breeding pair of Xenopus laevis was obtained from Carolina Biological Supply at the beginning of fall quarter 1988. They were bred in late September and tadpoles were fixed beginning with the 64 cell stage (stage 6). Additional specimens were removed at subsequently later stages until stage 32. Approximately 5 tadpoles of each stage were processed. The youngest embryos were at stage 6 and particular attention was paid to stages 12-18 when differentiation of the pituitary occurred. Fixation was in 10% formalin; specimens were stored in 70% ethanol until processing was completed.

Fertilized chicken and quail eggs were incubated during winter quarter in a Roll-X model incubator (Marsh Manufacturing, Garden Grove, CA) at temperatures varying from 37.5°C - 39.0°C. Approximately 40 specimens were removed and separated from the surrounding zona pellucida before being placed in Bouin's fixative. Age of the embryos was determined according to the 28 developmental stages outlined for the chick by Hamburger and Saunders. I examined embryos as early as stage 11 and as late as stage 21, emphasizing those specimens between stages 13-17 when initial development of the hypophysial region was observed.

All of the specimens were dehydrated in an alcohol series, cleared in xylene, and embedded in paraplast. They were sectioned with a rotary microtome at thicknesses varying from 7 to 10 um. Embryos were sectioned in sagittal, transverse and horizontal planes. Early Xenopus laevis embryos were stained with hematoxylin-eosin for preliminary observation; Brookes' triple stain was used in staining specimens in the later stages of development. The Xenopus laevis tadpoles were processed in December 1988; the chick and quail embryos

were processed in February and March 1989. In March of 1989, embryos and hatchlings of Eleutherodactylus coqui were received from Dr. Daniel S. Townsend (University of Scranton, PA). These specimens were subsequently processed following the procedures used for Xenopus.

RESULTS

The large number of slides produced and the limited time available forced me to concentrate my efforts on evaluation of the chick and quail tissues. In both the chick and quail, developmental stages were nearly parallel and similar pathways were noted during the early stages of development. In stage 6 chick embryos, the neural tissue of the forebrain and the epithelial cells of the stomodeum are already in close apposition. The two tissue layers are separated only by basal laminas. As early as stage 13, a thickening of the stomodeal epithelium was noted closely parallel to the curving floor of the diencephalon; this thickening was due to an alteration in the cell shapes within the stomodeal epithelium. The cells varied from very small cuboidal cells in the anterior region to very long columnar cells in the region of the bulge. This thickened region demarcates the posterior end of what will later develop as Rathke's pouch.

In early stage 14 embryos, the bulge of the buccal epithelium is extended further as the formation of Rathke's pouch begins (Fig. 1). Movements of the rapidly dividing mesenchyme cells in the region posterior to the oropharyngeal membrane cause a fold to begin directly posterior to the region of the bulging stomodeum.

Fig 1

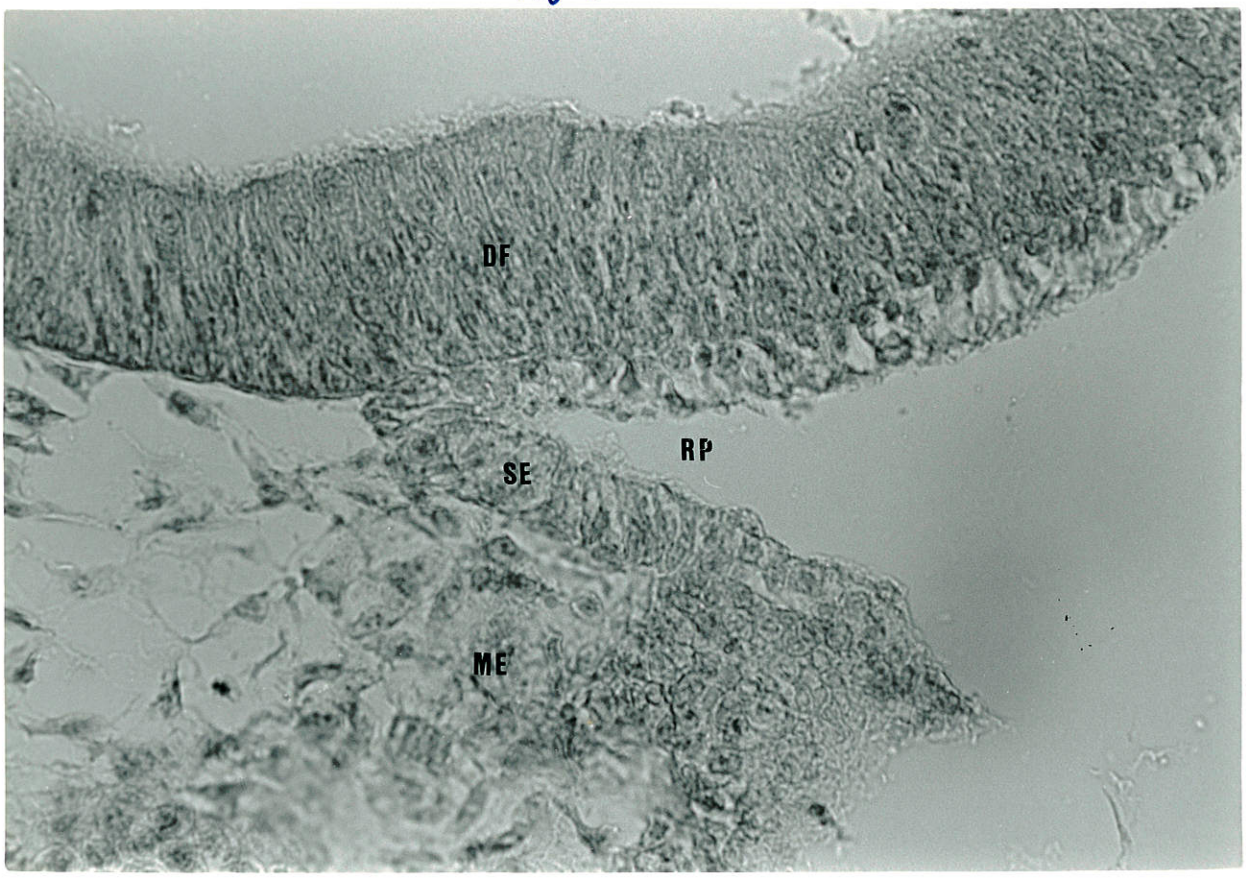

Fig. 1 (400x) Late stage 14 embryo showing the early formation of Rathke's pouch as dividing mesenchyme cells posterior to the oropharyngeal membrane create a fold in the bulging stomodium RP early formation of Rathke's pouch, ME mesenchyme cells, SE stomodeal epithelium, DF Diencephalic floor.

In stages 15 through 16, the continuing invasion of mesenchyme posterior to the diverticulum causes the formation of Rathke's pouch as it has been described traditionally. The ensuing diverticulum becomes elongated and continuous in shape with the curving diencephalon (Fig. 2). Frontal sections of these stages show that Rathke's pouch is narrowed laterally and the posterior region is slightly larger than the anterior. In stage 16 of the quail, Rathke's pouch was identifiable, but development was not as complete as in similar stages of the chick. Throughout this period of development, the stomodeum epithelium consisted of a single row of cuboidal epithelial cells in close apposition to

the diencephalon while the posterior end of Rathke's pouch consisted of the elongated columnar cells (Fig. 3). The thickness of the diencephalic floor varied and thickening occurred during late stage 16.

Fig. 2 (400x) Chick - Stage 15 As the posterior regions of the diverticulum are shaped by mesenchyme advancement, the traditional form of Rathke's pouch is identifiable.

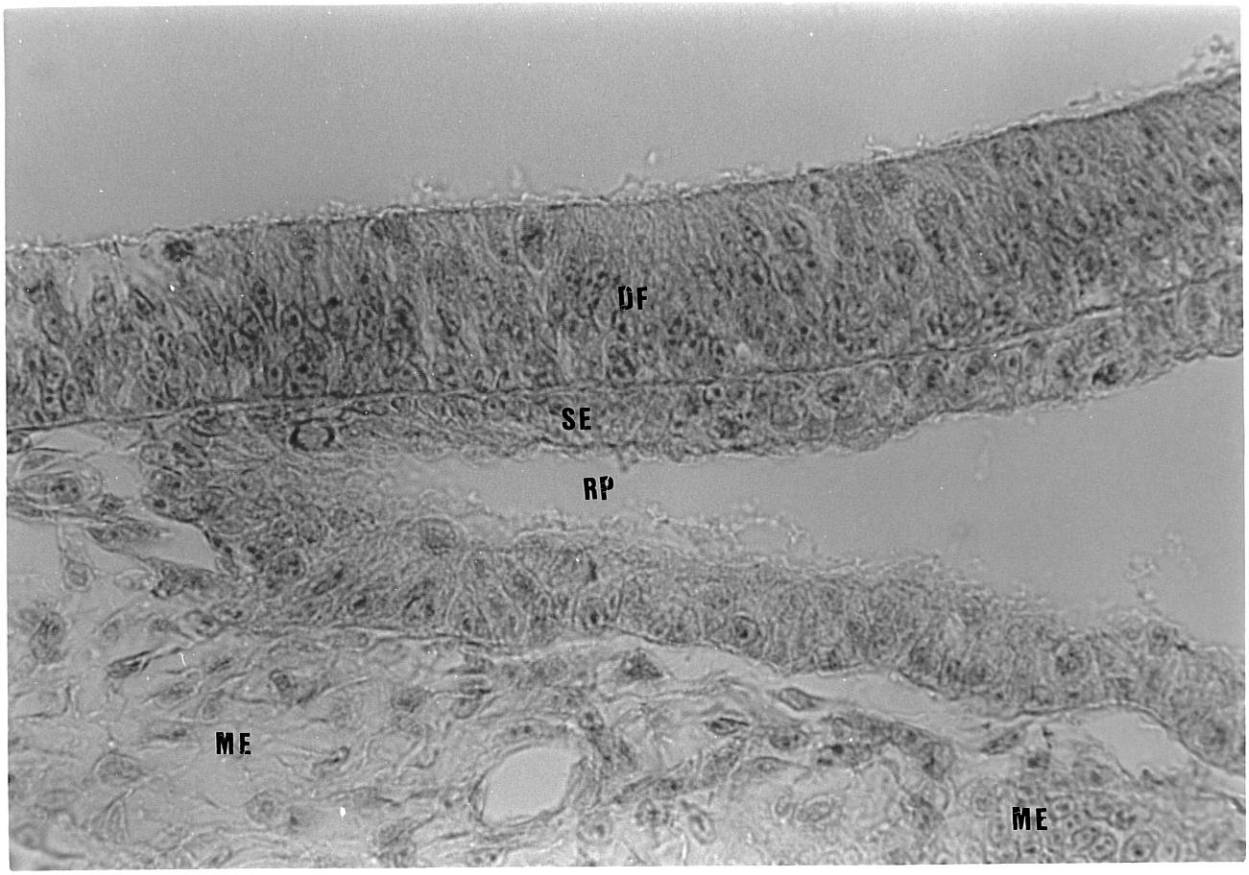

Fig 3 (400x) Chick - Stage 16 The posterior end of Rathke's pouch is shown anchored to the diencephalic floor. The cells at the end of the pouch are columnar epithelial, while cuboidal cells form the body of Rathke's pouch.

In stages 17-18, the folding of epithelial tissues delineating Rathke's pouch continued in both the chick and the quail. Mesenchyme advancement left a long, slender pouch that is easily identified in both bird species. In the anterior region of the hypophyseal anlage, mesenchyme cells appear in the region between the two cell layers, approaching the area of Rathke's pouch. The increased number of these cells separates the anterior region of epithelium from the diencephalon (Fig. 4). Posteriorly, the region which was earlier described as a bulge begins to separate from the diencephalic floor.

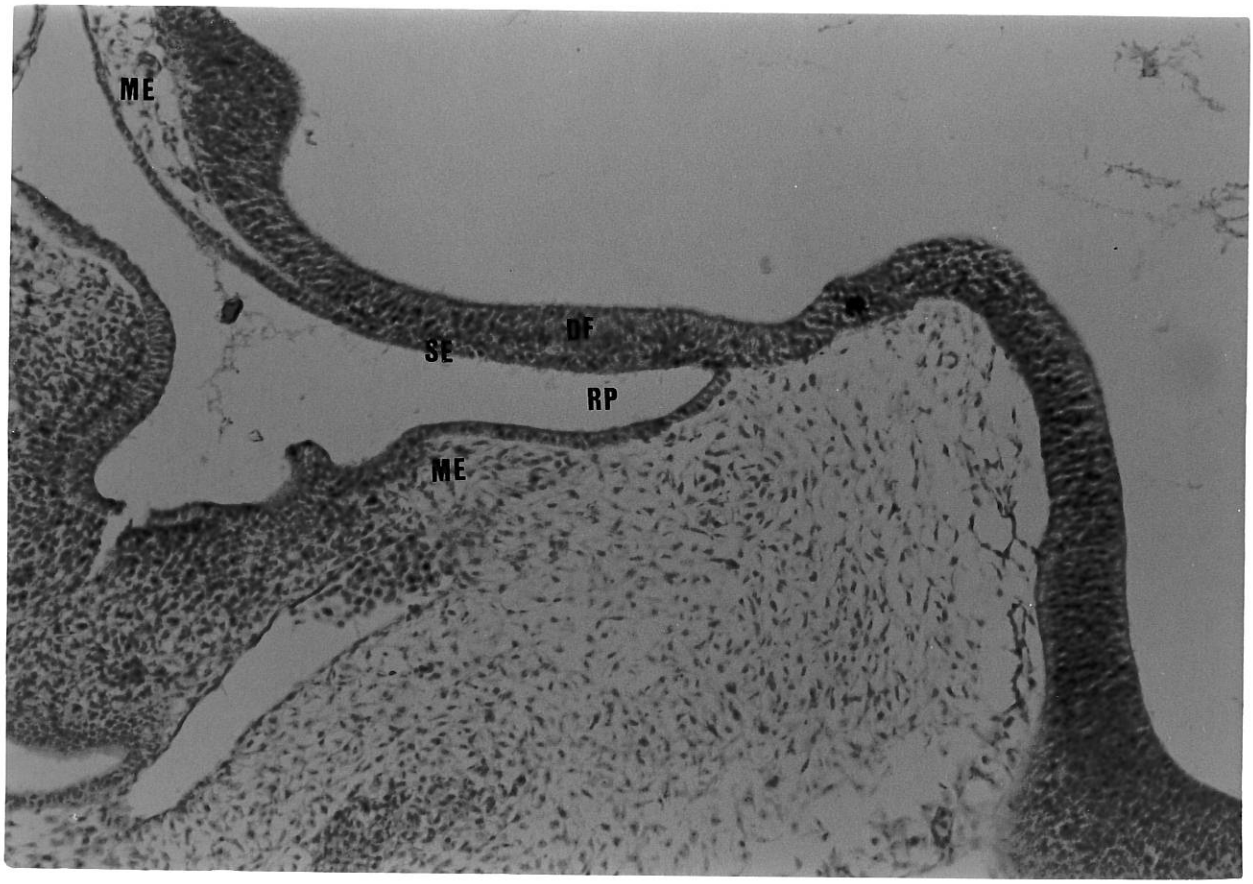

Fig 4 (100x) Chick - Stage 18 Mesenchymal proliferation in the anterior region of the hypophysial anlage begins to separate buccal epithelium from the diencephalon. Further advancement of mesenchyme modifies the posterior of Rathke's pouch into a long slender pouch.

Development of both avian species followed the same course to this point. In subsequent stages of chick (until stage 21), the formation of the pituitary gland continued as described by Doskocil (1970). In the quail, however, a striking difference was noticed. Late in stage 16, a slight thickening was observed on the bottom of Rathke's pouch; this continued to develop forming a diverticulum (Fig. 5). The invagination increased in size until stage 17 when Seessel's pouch was identified. In later stages, these two diverticula eventually met and formed what was presumed to be the fully formed Seessel's pouch. The last quail embryo sampled was stage 18, and continuing development could not be followed. Subsequent studies will investigate further what may be occurring in the developing quail that has not been identified in vertebrates previously examined.

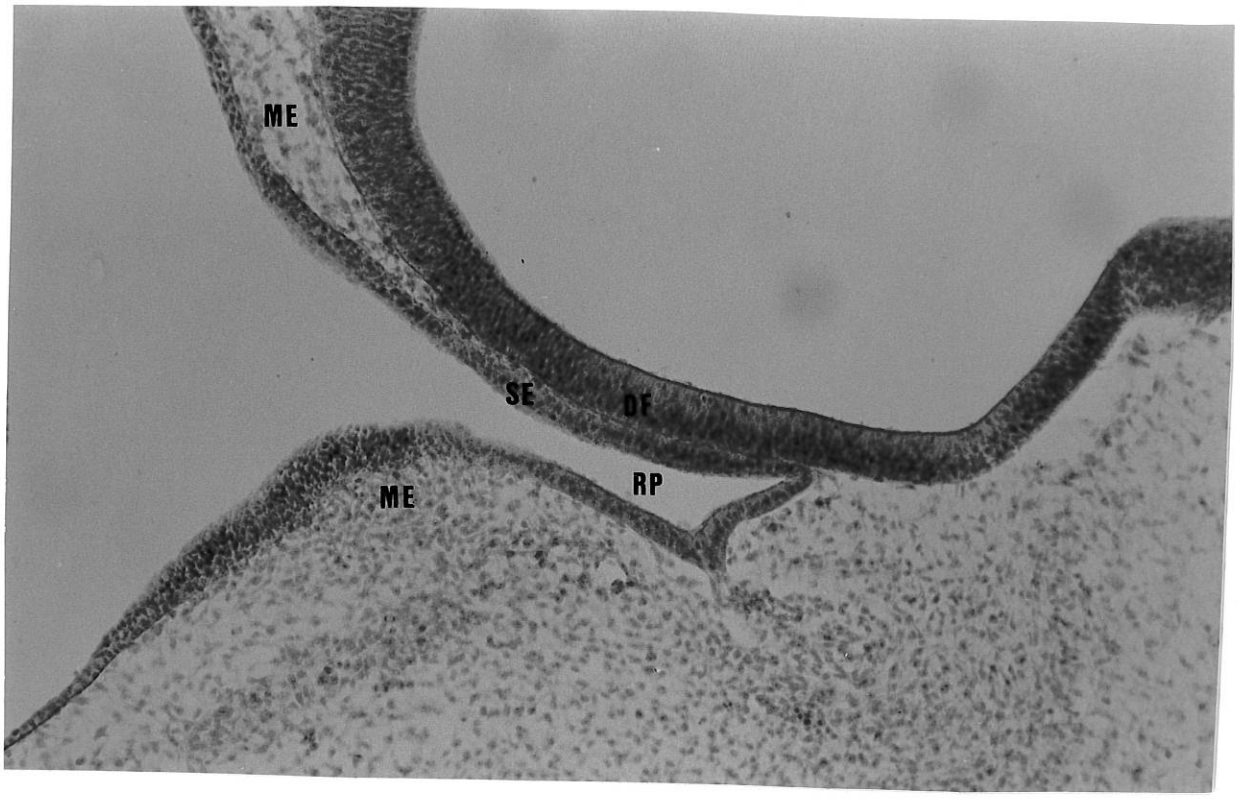

Fig. 5 (100x) Quail - Stage 16 (compare Fig. 4) Quail pituitary development proceeds similar to that of the chick until stage 16 when a small diverticulum forms on the bottom of Rathke's pouch. Seessel's pouch was seen to extend and presumed to develop along with Rathke's, although later specimens were unavailable for investigation.

DISCUSSION

It is generally accepted by developmental biologists that the diverticulum originally described by Rathke forms posterior to the diencephalon and is constructed of stomodeal epithelium. Few investigators have realized, however, that from the time of its inception, part of Rathke's pouch is intimately associated with the brain floor. There has been some question by others as to whether the pouch arises entirely from stomodeal ectoderm as originally proposed by Rathke. Seessel (1877) described the pocket that presently bears his name and suggested that it contributed to the anterior pituitary. This was contradicted by the observations of many subsequent investigators and conclusive

evidence that the pouch arises from ectoderm was provided from the chick-quail experiments of Ferrand and Hraoui (1973) and Takor Takor and Pearse (1975). Studies by Pearson et al. (1983) once again brought up the suggestion that endoderm may contribute cells to the lateral lobes of the developing Caretta pituitary subsequent to rupture of the oropharyngeal membrane.

I found that early sagittal sections of stage 12 embryos revealed that a deep subcephalic pocket had formed and the future ectoderm of Rathke's pouch lay in the region beneath the developing brain and anterior to the oropharynx. The somatic Rathke's pouch is marked by the point of attachment of the prechordial plate at the juncture of foregut and brain (Jacobsen et al. 1979). The pouch tip is the point around which other parts of the embryo fold to create the pouch. Doskocil (1966, 1970) questioned whether Rathke's pouch actually invaginated and grew in, or whether the pouch was the result of bending and rearrangements of surrounding tissue. This latter arrangement was consistent with embryos I studied and may suggest an explanation as to the requirement of anchoring the tissue of the pouch as seen in later stage embryos.

In stage 13 embryos, the epithelial tissue begins to indent, with the tip of Rathke's pouch closely adherent to the floor of the diencephalon. The roof, tip and floor of the pouch form a diverticulum that extends along the base of the diencephalon. The driving force that forms the folding is the movement of mesenchyme around the stomodeum and foregut. The initial folding of epithelial tissues that eventually form the pouch coincide with cranial flexure that takes place during the same period. In stages 11-12, the head of the embryo extends unbent and a slight longitudinal furrow lying between the two aortae on the side of the head mark the location of the

future floor of Rathke's pouch. Most of the bending called cranial flexure is accomplished in the period of development separating stages 12-14 (Goodrum, 1977). This is the period that Rathke's pouch is transformed from a flat layer of tissue into an elongated pouch lying beneath the floor of the brain. Cranial flexure seemed to be an important factor in the development of the pouch in this study.

Experimental examination of this hypothesis by Doskocil (1967, 1970) and Denis'evskii and Bozhok (1983) led to conflicting conclusions. Doskocil (1967, 1970) attempted to prevent cranial flexure by inserting a tungsten rod in the cerebral cavities at stage 10-13 embryos. The floor of the brain was flattened as a consequence of such treatment, but a small, somewhat undeveloped pouch did form. Doskocil concluded that cranial flexure is not an important factor in the formation of Rathke's pouch. He suggested that the pouch developed as a consequence of proliferative ingrowth of the pouch ectoderm. Denis'evskii and Bozhok performed an experiment that separated the pouch rudiment and adjacent brain floor from the rest of the brain by placing a narrow tin foil plate through the anterior end of the brain and into the foregut of stage 11-13 embryos. It was determined in these experiments that neither cranial flexure nor Rathke's pouch formed. The results were interpreted to show that cranial flexure is in fact an important factor in the development of Rathke's pouch.

In recent modifications of Denis'evskii and Bozhok's experiment, cranial flexure was further suggested as a force in the formation of Rathke's pouch. These researchers (Denis'evskii and Bozhok 1974) used glass rods instead of tin foil and they were inserted into a small slit in the floor of the prosencephalon so that the rod passed back in the foregut, then out the anterior intestinal

portal in stages 9-12 embryos. The easy passage of the rod was the criterion that the rod was within the foregut and had not penetrated into surrounding tissues. The embryos were allowed to develop to stage 16-17, then fixation and the removal of the rod was performed. Minimal disruption of the tissue occurred. Cranial flexure was inhibited by the front end of the rod left outside the anterior end of the head. In control experiments, the end of the rod was pushed into the cerebral cavity, and the brain folded around the end of the rod and cranial flexure was almost normal. In these experiments, the ventral ectoderm was also folded to form an almost normal looking Rathke's pouch. The fold that resulted when the rod went through the top of the brain also resulted in a nearly normal looking Rathke's pouch, however, the depth of the pouches that were formed was much greater than in the embryos that were not manipulated. Results of this experiment showed that the rod itself does not stop pouch formation. Rather, the flexure of the brain over the rod supported evidence that cranial flexure is an important factor in the development of Rathke's pouch. Observation of my sectioned embryos seemed to corroborate the results of Denis'evskii and Bozhok (1967, 1970) and Jacobsen (1979).

In stage 13-14 embryos, the extension of Rathke's pouch continues along the base of the diencephalon. Gilbert (1934, 1935) suggested that the lateral pouch walls are formed as a consequence of an accumulation of mesenchyme that results in the ventral bulging of the ectoderm. Further investigation in snake pituitary development by Pearson and Wurst (1977) suggest that the folding of the buccal epithelium is directly correlated with movements of mesenchyme in the region directly posterior to the formed diverticulum. Close adhesion between the pouch roof and the brain floor continue through this stage of development. Tracing studies have shown that most of the mesenchyme originates

from cranial neural crest cells that have migrated ventrally from the neural folds into the region around the pouch (Johnson and Listgarten, 1972). The concept of folding epithelial tissues by mesenchymal movements has been shown to be consistent with other embryological processes in developing embryos (Schoenwolf and Watterson, 1989).

Doskocil (1970) proposed that proliferating pouch epithelium has an active role in the formation of Rathke's pouch. His study in embryos incubated from two to five days showed that the pouch ectoderm had higher mitotic activity than nearby epithelium of Seessel's pocket. After three days of incubation, there was a higher mitotic activity in the pouch roof than in the floor, although differences after two days were small. Unfortunately, Doskocil did not stage his embryos, nor did he have data for the third day of incubation that corresponded with the stage of development that was investigated in this study. Because of his sampling times, between two, four, and five days, Doskocil does not give data on mitosis near the time of pouch formation which occurs at stages 13-17 and falls between the incubation days of 2 and 3.

According to Goodrum (1977), a better measurement would be total pouch tissue volume through time. Once the development of the pouch has begun, measurements can be made from sagittal sections by projecting and drawing every section of the embryo that includes pouch tissue, then cutting out and weighing the images of the pouch tissue. By calculating volume from section thickness, tissue volume can be determined. In Goodrum's investigation of chick embryos from stage 12-19, the volume of pouch ectoderm increased by 1.5 fold. By comparison, over the same period, the total tissue in prosencephalon and mesencephalon increased two-fold. The increase in tissue volume may be described as a result to the pouch roof's being stretched as a response of diencephalic elongation. Goodrum's experiment illustrates that mitotic proliferation is

most likely not a factor in the development of Rathke's pouch early in development. My observations appear to support the conclusions of Goodrum, although tissue volume was not measured.

In stage 16-17 embryos, an invagination of the diencephalon begins. Studies by Patten (1958) have shown that this is not the true infundibulum associated with Rathke's pouch that contributes to posterior pituitary development, but that this invagination disappears in later stages. The true infundibulum appears by stage 26 and forms directly above the tip of Rathke's pouch. Observation by Jacobsen et. al. (1979) show that in some sections a remnant of the false infundibulum may also be seen as the true infundibulum begins to form. Rathke's pouch begins to separate anteriorly from the diencephalon as mesenchyme continues to extend down and enter the region that separates the two ectodermal layers.

Sections from older embryos that I studied matched the observations of previous researchers. Development proceeded as described by Schoenwolf and Watterson (1989). The last stage that I studied was approximately stage 22, and development continued as has been described traditionally.

Development of the quail paralleled that of the chick until stage 15. At this stage of development a striking difference was noted in the floor of Rathke's pouch. As the mesenchyme folds, the layers of epithelial tissue at the posterior end of Rathke's pouch, a small bulge forms in the region directly anterior to the tip. The invagination continues to lengthen, extending posteriorly along the floor of Rathke's pouch in subsequent stages. At stage 17, Seessel's pouch begins to develop posteriorly and grows anteriorly and dorsally until, in late stage 18, the epithelial and neural cell layers meet and grow together. Unfortunately, the last stage of quail embryos

processed was stage 18, so further development could not be followed. Formation of this second pouch represents a process not described in other vertebrates and investigations are continuing into this apparently unique aspect of quail pituitary development.

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