Neuroectodermal origin of avian hypothalamo-hypophyseal complex: the role of the ventral neural ridge

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SUMMARY

Embryonic discs from White Leghorn chick embryos (presomite to 12-somite stages) were examined in serial transverse and longitudinal sections. Later stages, up to 21 days, were also examined. The following observations were made:

1. Lateral folding rather than head folding is the mechanism by which the avian embryo is delimited. The process takes place at two terminal points, one cephalic the other caudal.

2. Thickening of the ventral ectoderm around the tip of the presumptive anterior neuropore, beginning at the 4-somite stage, subsequently extends the dorsal neural ridge to form a ventral neural ridge.

3. The cephalic portion of the ventral neural ridge, extending from anterior neuropore to optic chiasma, is mainly incorporated into the alar plates of the diencephalon.

4. The caudal portion, extending from optic chiasma to the stomodeum, gives rise to Rathke's pouch and thus to the adenohypophysis.

We conclude that the latter is to be regarded as of neuroectodermal rather than ectodermal (stomodeal) origin, and that some or all of the neuroendocrine nuclei of the hypothalamus are similarly derived from the neuroectoderm of the neural ridge.

The hypothalamo-hypophyseal complex is thus to be regarded as a single rather than a composite entity.

INTRODUCTION

At the present time there is virtually complete acceptance of the view that the adenohypophysis and its six endocrine cell types are of ectodermal origin. This state of affairs has existed almost since 1838 when Rathke first described the pouch, which now bears his name, as ectodermal (stomodeal). Doubts have sometimes been expressed as to the precise contribution made by the stomodeum, and De Beer (1924) – noting that, in amphibia and actinopterygians, the pituitary anlage arises before the stomodeum appears – supported the idea of a non-stomodeal origin for the pituitary in the ancestors of the gnathostomes. Wingstrand (1966) believed that many questions concerning the ancestral form of the adenohypophysis, including the matter of its origins, remained un-
answered but, in spite of these and other doubts, there has been no essential alteration to the original stomodeal hypothesis.

Because of their cytochemical characteristics the corticotrophs and melanotrophs of the adenohypophysis were classified with the so-called APUD (Amine Precursor Uptake and Decarboxylation) series of endocrine polypeptide cells (Pearse, 1966, 1968), from the earliest days of the concept. The members of this series are either of proven or presumptive neuroectodermal origin. In the absence of experimental proof it was considered that corticotrophs and melanotrophs could be derived either from neuroectoderm (neural crest) or from presumptive ectoderm (Rathke's pouch). With the demonstration by Takor Takor & Pearse (1973), however, that human and murine somatotrophs possessed APUD characteristics, it was possible to doubt the contribution of the neural crest to the APUD cells of the adenohypophysis and these doubts were not entirely dispelled by the results of studies carried out by Ferrand, Pearse, Polak & Le Douarin (1974). Using chorioallantoic grafts of chick Rathke's pouch at different ages, these authors showed that immunofluorescent ACTH cells appeared in the developing pituitary primordium only when the graft contained cephalic mesenchyme (mesectoderm, ectomesoderm). It was not possible to decide whether the contribution of the latter was by specific induction of potential ACTH cells in the graft or through inoculation of neuroectodermal endocrine cells capable of differentiation into ACTH cells.

Development of the hypothalamus and other components of the diencephalon has been an altogether less controversial matter. The hypothalamus is considered to be derived from the central alar plates of the primitive diencephalon and, according to Wingstrand (1966) 'the neurohypophyseal region of all vertebrates is differentiated ... in the floor of the post optic hypothalamus'.

The present study was undertaken, together with parallel studies in amphibian and mammalian embryos which are not reported in this paper, in order to establish the precise origin of Rathke's pouch and, if possible, to resolve the extent of neuroectodermal participation in the development of hypophysis and hypothalamus. It was evident from the outset that we should need to concentrate particularly on the earliest stages in the development of both structures, since it is these which have in the past been most neglected.

**MATERIAL AND METHODS**

Eggs of White Leghorn hens were incubated artificially at 37.6 °C and 87 % humidity for 18 h up to 21 days. The embryonic discs from the early stages were dissected from the surrounding yolk in a trough of water. They were then rinsed in normal saline and fixed for 12 h in 10 % formal-saline. After dehydration through graded alcohols and clearing for 48 h in cedar-wood oil they were embedded in paraffin wax. Serial sections were cut at 6 μm in either the longitudinal or transverse planes, and mounted on glass slides. They were stained...
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with haematoxylin and eosin, examined microscopically, photographed and, where necessary, drawn, to provide material for reconstructive diagrams.

Staging of embryos by the hours of incubation was supplemented by a somite count and the two were expressed in the Hamburger–Hamilton (1951) formula.

RESULTS

These are given below under separate headings for individual stages. They are illustrated in two dimensional diagrams in Figs 2, 5, 12 and 15, which have been drawn directly from the microscopic image. Some of these stages are presented also as three dimensional reconstructions (Fig. 13).

20 h; presomite: Hamburger–Hamilton stage 5

This is a late gastrula stage in which the three germ layers are demonstrable. Longitudinal sections show at one extremity of the neural plate a small inden-
tation or groove. At this point, the cephalic pole, we observed a columnar cap of mesoderm cells lying on the unicellular layer of endoderm (Fig. 1). This cap of cells was destined to grow as Seessel’s (1877) pouch. Transverse sections (Fig. 2, stage 4 and Fig. 13.1) demonstrated the neural plate, flat at this stage but with slight folds at its sides.

24 h; 2 somites: Hamburger–Hamilton stage 6

The late gastrula is transformed into a neurula by the process of lateral folding (Figs. 13.2, 13.3). This commences at a point, designated the cephalic terminal point (CTP) in Figures 1, 3 and 13.3, at the head end of the neural plate and the endoderm. By the progress caudad of lateral folding the embryonic disc is delimited from the surrounding membranes. At a later stage (14-somites) lateral folding begins at the caudal terminal point. The lateral fold is usually more prominent on one side (Fig. 13.2) so that the point of ventral union of the two folds occurs on one side of the midline (Figs. 4, 13.3). Transverse sections showed that the presumptive prosencephalon had now a navicular outline (Fig. 2, stage 6). The process, as seen in longitudinal sections, is usually described in embryological texts as Head Folding. We found no evidence of head folding as such, but only a progressive lateral folding which extended from the anterior neuropore to the mid-region of the embryonic disc.

27 h; 4 somites: Hamburger–Hamilton stage 8

At this stage the progress caudad of lateral folding is seen to convert the mesodermal cap of cells, lying on the single layer of endoderm, into the definitive Seessel’s pouch (Fig. 5, stage 8), thus delimiting the upper foregut and the stomodeum. The ventral surface of the embryo is lined by ectoderm from the anterior neuropore to the region of the stomodeum (Fig. 13.4). Dorsally, the edges of the prosencephalic neural plate evolve into the neural folds (Fig. 5,
stages 7 and 8) and its rapid growth, combined with the beginning of the cephalic flexure, can be seen to push Seessel's pouch and the foregut in a caudal direction.

![Diagram](image1.png)

Fig. 1. Longitudinal section at prosencephalic region of an 18 h chick embryo showing lateral folding in the three primary layers. × 320.

Fig. 3. Longitudinal section through the cephalic region of 24 h chick embryo showing Seessel's pouch and the ventral neural ridge anlage. × 230.

**ABBREVIATIONS TO FIGURES**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>a.n.</td>
<td>Anterior neuropore</td>
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<td>c.t.p.</td>
<td>Cephalic terminal plate</td>
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<td>d.n.r.</td>
<td>Dorsal neural ridge</td>
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<tr>
<td>ect.</td>
<td>Ectoderm</td>
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<td>end.</td>
<td>Endoderm</td>
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<td>for.</td>
<td>Foregut</td>
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<td>l.f.</td>
<td>Lateral folds</td>
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<td>mes.</td>
<td>Mesoderm</td>
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<td>n.c.</td>
<td>Neural crest</td>
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<td>n.f.</td>
<td>Neural fold</td>
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<td>n.g.</td>
<td>Neural groove</td>
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<td>not.</td>
<td>Notochord</td>
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<td>n.p.</td>
<td>Neural plate</td>
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<td>n.s.</td>
<td>Neural sulcus</td>
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<td>or.</td>
<td>Oral membrane</td>
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<td>prosen.</td>
<td>Proencephalon</td>
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<td>proso.</td>
<td>Prosocoele</td>
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<td>R.p.</td>
<td>Rathke's pouch</td>
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<td>S.sc.</td>
<td>Seessel's cell cap</td>
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<tr>
<td>S.p.</td>
<td>Seessel's pouch</td>
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<tr>
<td>v.n.r.</td>
<td>Ventral neural ridge</td>
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<tr>
<td>v.n.r. (ce.)</td>
<td>Ventral neural ridge (cephalic part)</td>
</tr>
<tr>
<td>v.n.r. (po.)</td>
<td>Ventral neural ridge (postchiasmatic or Rathke's)</td>
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<tr>
<td>z.c.l.</td>
<td>Zone of cell lysis</td>
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30 h; 6 somites; Hamburger–Hamilton stage 9

Thickening of the ventral ectoderm of the head occurs in the midline (Fig. 13.5) and this thickening progresses in a caudal direction from the anterior neuropore until, at the 7-somite stage, it reaches the stomodeum (Fig. 6). This specialized ectoderm is to be regarded as an extension of the dorsal neural ridge.
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(Horstadius, 1950) which, in its dorsal expression, contains the neural crest prior to its dispersal. At the 6/7-somite stage the edges of the neural ridge begin to fold in a ventral direction changing its flat profile (Fig. 5, stage 9, and Fig. 7)

![Diagram of chick embryo development](image)

**Fig. 2.** Drawings of transverse sections through the prosencephalic regions of 17–24 h chick embryos demonstrating the progress of lateral folding.

... into an inverted U-shape (Figs. 5, stage 9, 8 and 13.6). The groove, which thus develops, can be called the *neural sulcus* (Figs. 5, stage 9, and Fig. 9). This feature extends from the anterior neuropore to the stomodeum, where it ends.
The formation of the neural sulcus, the ventral equivalent of the neural groove, and the arching effect due to dorsal neural folding in the region of the prosencephalon causes the floor of the latter to become apposed to the roof of the neural ridge (Figs. 9, 13.6). This juxtaposition leads to union of the two neuroectodermal elements (Fig. 10 and Fig. 5, stage 9), followed by cell death and lysis. The dehiscence which then occurs opens the prosocoele ventrally into the neural sulcus (Figs. 11, 12, stage 9, and Fig. 13.7). The neural ridge and the prosencephalon are thus split ventrally and each half of the ridge becomes aligned and attached to the corresponding half of the prosencephalon (Fig. 12, stage 10, and Fig. 13.8). This process of alignment and attachment moves caudad from the anterior neuropore to extend by the 10-somite stage as far as the optic chiasma (Fig. 13.9). Those parts of the cephalic neural ridge which are not incorporated into the floor of the forebrain are dispersed into the surrounding mesectoderm. An important feature of this stage is observed in the extension of the neural crest over the margins of the anterior neuropore and into the ventral surface of the embryo. There is thus complete continuity between the dorsal and ventral ridges.

Caudad to the optic chiasma the neural ridge continues to proliferate and to thicken, forming a neural sulcus, but there is no attachment to the ventral surface of the diencephalon (Figs. 13.8, 13.9). This caudal (post-chiasmatic) portion of the ventral neural ridge is transformed by the rapid growth of the diencephalon, before the 13-somite stage, into the classical Rathke's pouch (Figs. 13.10–13.13). The pouch, in its conversion from the neural sulcus, changes its position from being ventral to the diencephalon to become posterior to that region (Fig. 13.13).

36 h; 10 somites; Hamburger–Hamilton stage 10

By this stage the process of neural folding is completed in prosencephalon, mesencephalon and upper rhombencephalon, where the edges of the neural folds meet in the dorsal midline (Fig. 12, stage 10, and Fig. 13.9). There are large masses of neural crest on either side of the neural tube (Fig. 12, stage 10, and Fig. 14) and the anterior neuropore is clearly defined. Ventrally the communication of the prosocoele with the neural sulcus has reached the optic chiasma and the caudal neural ridge is growing in a direction posterior to the diencephalon (Fig. 13.9).

38 h; 12 somites: Hamburger–Hamilton stage 10

Rapid growth of the prosencephalon takes place and the neural ridges become better aligned. Dorsally, the neural crest is dispersed to intermingle with the somatic mesoderm and the neural tube is closed from the anterior neuropore to the otic vesicles. Ventrally the edges of the neural ridges meet in the midline, unite and separate the prosocoele from the neural sulcus (Figs. 13.10, 13.11, and 15, stages 10 and 11). The prosencephalon and cranial diencephalon are thus
reconstituted with the incorporation of the neural ridge to form the floor in each region (Fig. 16).

Some of the cells of the cephalic neural ridge are not incorporated into the prosencephalon and diencephalon but are dispersed into the neighbouring mesoderm. The caudal portion of the ventral neural ridge thus proliferates and bulges into the space between the diencephalon and the roof of Seessel's pouch. In this way it takes on the classical form of Rathke's pouch, ultimately extending as far as the oral plate.

49 h to 21 days

The prosencephalic and diencephalic floor, which has incorporated the neural ridges, proliferates in a cranial direction. In the adult this region constitutes the lamina terminalis, hypothalamus and optic chiasma. There is no connexion between the neural ridge and the olfactory apparatus.

DISCUSSION

We conclude, from the results presented here, that:

(1) Lateral folding, rather than head folding, is the operative mechanism for delimitation of the avian embryo.

(2) Part of the floor and ventral alar plates of the diencephalon, rostral to the optic chiasma, is contributed by those portions of the cephalic ventral neural ridges which are incorporated into the neural tube at the 6-somite stage.

(3) Rathke's pouch is not to be considered as a derivative of stomodeal ectoderm but rather of the neuroectoderm of the caudal ventral neural ridge.
Fig. 5. Drawings of transverse sections through the prosencephalic region in chick embryos 24-30 h showing the formation of the ventral neural ridge.
Fig. 6. Longitudinal section at cephalic region of 30 h chick. The ventral neural ridge stretches from the anterior neuropore to the stomodaeum. × 200.

Fig. 7. Transverse section at the prosencephalic region of a stage-9 30 h 6-somite chick embryo. The ectoderm is thickened to form the ventral neural ridge. × 200.

Fig. 8. Transverse section through the prosencephalic region of a stage-9 33 h 6-somite chick embryo demonstrating the apposition of the ventral neural ridge to the neural plate and the formation of the neural sulcus. × 200.

Fig. 9. Transverse section at prosencephalic region of a stage-9 33 h 6-somite chick embryo showing the union of the ventral neural ridge and the neural plate. × 200.
The pouch described by Seessel (1877), and considered by him to contribute to the formation of the hypophysis, is constituted by a thick cap of mesectodermal cells lying on the unicellular layer of endoderm which represents the rostral extension of the foregut.

It is impossible to assess the total contribution made by the ventral neural

![Image](image.png)

Fig. 10. Transverse section at prosencephalic level in a stage-9 33 h 6-somite chick embryo illustrating cell death at zone of union of ventral neural ridge and neural plate. × 200.

Fig. 11. Transverse section through prosencephalic region of a stage-9 33 h 6-somite chick. The ventral neural ridges are aligned with the neural plates and the prosocoel drains to the exterior through the neural sulcus. × 215.

ridges to structures in the floor of the IIIrd ventricle. This contribution may include some, or even all, of the neurosecretory neurons of the hypothalamus.

The formation of Rathke’s pouch is seen to be a relatively late stage in the development of the adenohypophysis. In this respect our observations agree with those of De Beer (1924) and it is clear that the earliest stages of pituitary development in Amphibia, Angatha and Actinopterygii should be re-examined. The derivation of Rathke’s pouch from specialized neuroectoderm, and its development in close association with the diencephalon, provide a satisfactory explanation for the common possession of APUD characteristics by corticotrophs, melanotrophs, somatotrophs and, most probably, lactotrophs. It becomes necessary, instead, to explain why the gonadotrophs (in mammals) and thyrotrophs apparently do not possess these characteristics.
Ferrand et al. (1974) showed that Rathke's pouch chorio-allantoic homografts develop ACTH cells only if accompanied by their normal complement of mesectoderm. They put forward three hypotheses to explain their experimental results:

(a) The differentiation of ACTH/MSH cells might require a late inductive influence from the hypothalamic primordium.
Fig. 13. Three-dimensional reconstructions of the cephalic region of chick embryos (18–40 h) illustrating lateral folding and the formation and fate of the ventral neural ridge.
(b) The cephalic mesenchyme might be responsible for the differentiation of ACTH/MSH cells by a specific inductive action on the primordium.

(c) The cephalic mesenchyme might contain cells derived from the neural crest which could play a direct role in the development of the pituitary primordium by giving rise to specific endocrine cells.

Originally, the third hypothesis was regarded as the most attractive of the three but, in the light of our present observations, it is no longer necessary to postulate their neural crest origin in order to explain the APUD qualities of the ACTH/MSH cells. The first hypothesis therefore appears most likely to be true. Comparative pituitary ontogenetic studies support the view that ACTH/MSH cells do indeed require the direct influence of the diencephalon for their full expression. A cellular contribution from the cephalic crest to the adenohypophysis is not, of course, precluded by the evidence presented here and there is ample evidence that cells from the peripheral stream of the neural crest do reach the area of the developing hypophysis and hypothalamus. There the majority presumably contribute to the general mesoderm (mesectoderm) of the region.

We found no evidence to support any contribution by Seessel’s pouch to the adenohypophysis. On the contrary, in line with the currently accepted view, and in complete agreement with the conclusive results of experimental work with
Fig. 15. Drawings of transverse sections through the prosencephalic region of 40 h chick embryos illustrating the incorporation of the ventral neural ridge into the prosencephalic floor.

chick-quail chimaeras carried out by Ferrand and Hraoui (1973), we observed its gradual regression. It is therefore necessary to postulate a neuroectodermal derivation for all the endocrine cells of the adenohypophysis and to regard the whole hypothalamo-hypophyseal complex as a neuroendocrine derivative of the ventral neural ridge. Acceptance of this view has important implications not only for embryologists but equally for endocrine physiologists and pathologists.

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REFERENCES


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