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The avian prechordal head region: a morphological study

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ABSTRACT

The axial mesoderm of the anterior head region was investigated in young chick and quail embryos by light and electron microscopy. Semithin sections showed that the axial head mesoderm consists of the head process and prechordal mesoderm. At the anterior end of the prechordal mesoderm, a group of columnar epithelial cells formed a pit-like structure. The bases of these columnar cells extended to the neural plate, thus limiting the prechordal mesoderm anteriorly. The cells lining the pit-like structure at its anterior end joined a cell accumulation made up of cells of mesenchymal character. Electron microscopy revealed that the columnar cells forming the pit-like structure were covered by a basal lamina which was discontinuous on its anterior aspect. No basal lamina was recognisable between the columnar epithelial cells and mesenchymal cells joining them anteriorly. The columnar epithelial cells bordering the prechordal mesoderm anteriorly were therefore assumed to be part of the endodermal germ layer. In agreement with the findings of other authors, it is proposed to term these axially located columnar cells of the endoderm the prechordal plate and to distinguish them from the prechordal mesoderm arising during gastrulation. For the mesenchymal cell accumulation anterior to the prechordal plate, participation in the formation of the prosencephalic mesenchyme is assumed. This implies that the definitive endodermal germ layer, like the ectodermal one represented by the neural crest, may also be able to contribute to mesenchyme formation in the head.

INTRODUCTION

In the avian embryo, the mesoderm arises during gastrulation by invagination through the primitive streak. In the literature the term mesoderm for this germ layer has been used synonymously with the term mesoblast which stresses its proliferative activity. After gastrulation, a paraxially as well as an axially located mesoderm can be distinguished within the prospective head region of the young avian embryo, as well as in the trunk region. The paraxial head mesoderm has been the subject of several investigations (Noden, 1988; Couly et al. 1992). A metameric pattern comparable to that of the paraxial mesoderm of the trunk is still under discussion (Meier, 1979; Jacobson, 1988).

Mesodermal cells are capable of differentiating in order to form the mesenchyme which consists of cells detached from the organised germ layer, showing irregularity of shape and bearing cytoplasmic processes (Patten, 1971). From stage 9 onwards, according to the classification of Hamburger & Hamilton (HH) (1951), the ectodermally derived neural crest cells are also able to differentiate into mesenchyme and to contribute to the formation of the paraxial head mesoderm in the chick embryo (Noden, 1975; Le Douarin, 1982). Therefore, from this time of development onwards, the head tissue between ectoderm and endoderm, excluding the axial part of the mesoderm, is termed head mesenchyme.

The axially located part of the head mesoderm arises from HH stage 4 onwards by invagination through Hensen's node, the most anterior part of the primitive streak. Most of the axial head mesoderm will contribute to the formation of the notochord. We propose to use the term head process for that part of the axial head mesoderm contributing to the notochord up to its demarcation from the surrounding tissues by the notochordal sheath. The notochordal sheath is seen as a dense mat of extracellular fibrils

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around the entire notochord in the chick embryo initially at HH stage 11 (Jurand, 1962; Bancroft & Bellairs, 1976).

Adelmann (1922, 1926) distinguished a region located axially but anterior to the head process in the chick embryo. He described this region as a site of mesodermal proliferation immediately anterior to the notochord and called the tissue the 'prechordal plate', a term which was introduced by Oppel (1890). Hillman & Hillman (1965) and Meier (1979) described this region as prechordal plate mesoderm and stressed the mesenchymal character of the tissue. Rosenquist (1983) assumed that all the axial mesoderm anterior to Hensen's node contributed to the notochord. Hammond (1974), in young chick embryos, described an axially located prechordal mesoderm anterior to the notochord whose anterior part appeared to be incorporated into the endoderm of the dorsal wall of the foregut. Hamilton (1965) described the prechordal plate as a thickened mass of cells immediately anterior to the notochord and dorsal to the oral plate in the 4-somite chick embryo, where the endoderm of the gut and the mesoderm are indistinguishable. He therefore favoured an endodermal origin for the prechordal plate. Using both light and electron microscopy, Jacob et al. (1984) described the region anterior to the prospective notochord in the chick embryo as a continuation of the mesodermal head process. Spratt (1955, 1957), Nicolet (1970) and Rosenquist (1983) considered that this mesoderm arises by invagination during gastrulation through Hensen's node. Jacob et al. (1984) therefore proposed the term 'prechordal mesoderm' for this particular part of the axial head mesoderm. It does not contribute to the formation of the notochord, but in later stages the premandibular head cavities are formed within the lateral masses of the prechordal mesoderm (Jacob et al. 1984, 1986).

The term prechordal plate is also used in other vertebrate embryos to describe a median thickening of the endodermal epithelium anterior to the notochord. A derivation from the endodermal germ layer is assumed (see O'Rahilly & Müller, 1987, for the human embryo, Aasar, 1931, for the rabbit embryo, and Parker, 1917, for the marsupial embryo).

Since the term 'prechordal plate' needs further specification, the axially located region anterior to the notochord was investigated in chick and quail embryos using light and electron microscopy. Based on these findings the different parts of the region are described as derivatives of the endodermal or mesodermal germ layers.

MATERIALS AND METHODS

Eggs of White Leghorn hens (*Gallus gallus domesticus*) and Japanese quail (*Coturnix coturnix japonica*) were incubated at 38.7 °C and 60 % humidity for up to 3 d. A total of 19 chick and 22 quail embryos from the head fold stage to 22-somite embryos were investigated using light and electron microscopy.

Semithin sections and electron microscopy

Specimens were fixed in 4–6 % glutaraldehyde in 0.12 M cacodylate buffer (pH 7.4) for at least 12 h. In some cases 0.5 % tannic acid was added to give better contrast in the extracellular material. After fixation, the specimens were washed in Hanks' solution, postfixed in 1 % osmium tetroxide (Dalton, 1955) and, for semithin and thin sections, dehydrated in graded acetone solutions. This was followed by en bloc staining in 1 % uranyl acetate and embedding in Durcupan ACM (Fluka). Semithin sections were stained with 1 % methylene blue. Thin sections for transmission electron microscopy (TEM) were mounted on Formvar-supported grids, stained with uranyl acetate and lead citrate (Venable & Coggeshall, 1965) and examined using a Philips EM 420 electron microscope.

Specimens intended for scanning electron microscopy (SEM) were dehydrated in graded propanol solutions and critical point dried with carbon dioxide (Reimer & Pfefferkorn, 1977). Dried specimens were coated with gold and observed and photographed using a JEOL JSM 35 scanning electron microscope. In some cases the ectodermal or endodermal layers were removed from the already dried specimen with tungsten needles and very small pieces of adhesive tape.

RESULTS

For the staging of the chick embryos the criteria of Hamburger & Hamilton (HH) (1951) were used. Following these criteria, Zacchei (1961) also suggested a normal table for quail embryos, but since her staging was started some time after laying, it differs from that of Hamburger & Hamilton inasmuch as her stage 1 corresponds to HH stage 3. We therefore prefer not to use numbered stages but to orient ourselves by the morphological characteristics of each stage in order to describe and compare the embryos of both species, so that the head process stage = HH stage 5 = Zacchei stage 3, and the head fold stage = HH stage 6 = Zacchei stage 4.

The following stages are based primarily on the numbers of clearly visible pairs of somites. A new stage is assigned for every 3 pairs of somites; embryos with intervening numbers of somites are designated by adding a plus sign (plus 1 pair of somites) or a minus sign (minus 1 pair of somites) in relation to the respective stages. Thus 1-somite stage = HH stage 7 = Zacchei stage 5, and 4-somite stage = HH stage 8 = Zacchei stage 6 and so on up to the 22-somite stage.

The prechordal mesoderm

The head region anterior to the notochord was first studied using scanning electron microscopy. To examine the head mesoderm, the dorsal ectoderm and neuroectoderm had to be removed. They could most easily be detached from the underlying mesoderm after fixation, the basal side of the ectoderm showing a relatively smooth basal lamina, the extracellular material remaining largely attached to the mesoderm. When looking at the mesoderm from the dorsal aspect, in the 2-somite quail embryo, paraxial and axial mesoderm could be distinguished (Fig. 1*a*). In the most anterior part of the embryo, i.e. in the prospective head, the axial mesoderm appeared to be widened. This part corresponds to the one described by Jacob et al. (1984) in the chick embryo and termed the prechordal mesoderm. In line with their observations, the prechordal mesoderm borders at its anterior end on cells covered by a large amount of extracellular material (Fig. 1*b*). Jacob et al. (1984) assumed that these cells were part of the endoderm reaching into this area as far as the neural plate. Laterally, the prechordal mesoderm seemed to turn into the paraxial head mesoderm. In the transitional region some cells which were oriented in a mediolateral direction were observed (Fig. 1*c*). Caudally, the prechordal mesoderm joined the head process which later contributed to the notochord. Its demarcation from the paraxial mesoderm was not yet obvious in the 2-somite quail embryo (Fig. 1*d*).

Light microscope appearance of the axial head mesoderm

For further studies of the axial head region, sagittal and transverse semithin sections of quail and chick embryos were examined. In the 1-somite chick embryo, the prechordal mesoderm consisted of irregularly oriented cells lying on a flat endodermal epithelium (Fig. 2). The prechordal mesodermal cells seemed to accumulate anterior to a point where the endodermal epithelium, the prospective roof of the foregut, changed into a columnar epithelium adjacent to the neural plate. In line with Hamilton (1965), the term neural plate is used for the floor of the anterior neuropore until further development of the prosencephalon.

In the 5-somite chick embryo the head process was continuous with the prechordal mesoderm which, after the formation of the foregut, came to lie on its dorsal wall. At its anterior end the flat endoderm of the foregut could not be traced anteriorly, but some columnar cells were observed at this site. They seemed to continue anteriorly as a cell mass bulging into the lumen of the foregut (Fig. 3). Within the cell mass the cells could not be distinguished as to their mesodermal or endodermal origin. The cell mass extended to the clearly visible high columnar endodermal epithelium of the anterior extremity of the foregut. From transverse sections anterior to the prechordal mesoderm, it became obvious that the cell mass was limited to the median part of the dorsal wall of the foregut, continuing laterally into the adjacent head mesenchyme (Fig. 4). The columnar cells directly adjacent and anterior to the prechordal mesoderm appeared to form a pit-like structure made up of high columnar cells (Fig. 5). In the quail embryo the situation was similar, but in the 5-somite quail embryo the head process already showed notochordal features and appeared to be demarcated from the prechordal mesoderm (Fig. 6). The prechordal mesoderm bordered at its anterior end on a pit-like structure lined by columnar cells possibly derived from the endodermal roof of the foregut and contacting the neural plate. Spreading anteriorly from the pit-like structure, cells can be seen filling up the space between the anterior end of the foregut and the neural plate. They appeared to intermingle with the prosencephalic head mesenchyme. A demarcation of this mesenchyme towards the endoderm of the roof of the gut is not distinguishable up to the high columnar epithelium of the tip of the foregut. In a transverse section through the anterior extremity of the foregut wall, between dorsal neuroectoderm and ventral ectoderm a cell mass could be recognised which did not allow any distinction between head mesenchyme and endoderm of the foregut (Fig. 7). Moreover, both tissues seemed to be continuous with the surrounding head mesenchyme. At a slightly more caudal level, in transverse section, a cell accumulation situated in the median part of the dorsal wall of the foregut was visible. Its cells could not be assigned to the endodermal or mesodermal germ layer (Fig. 8). A more caudal

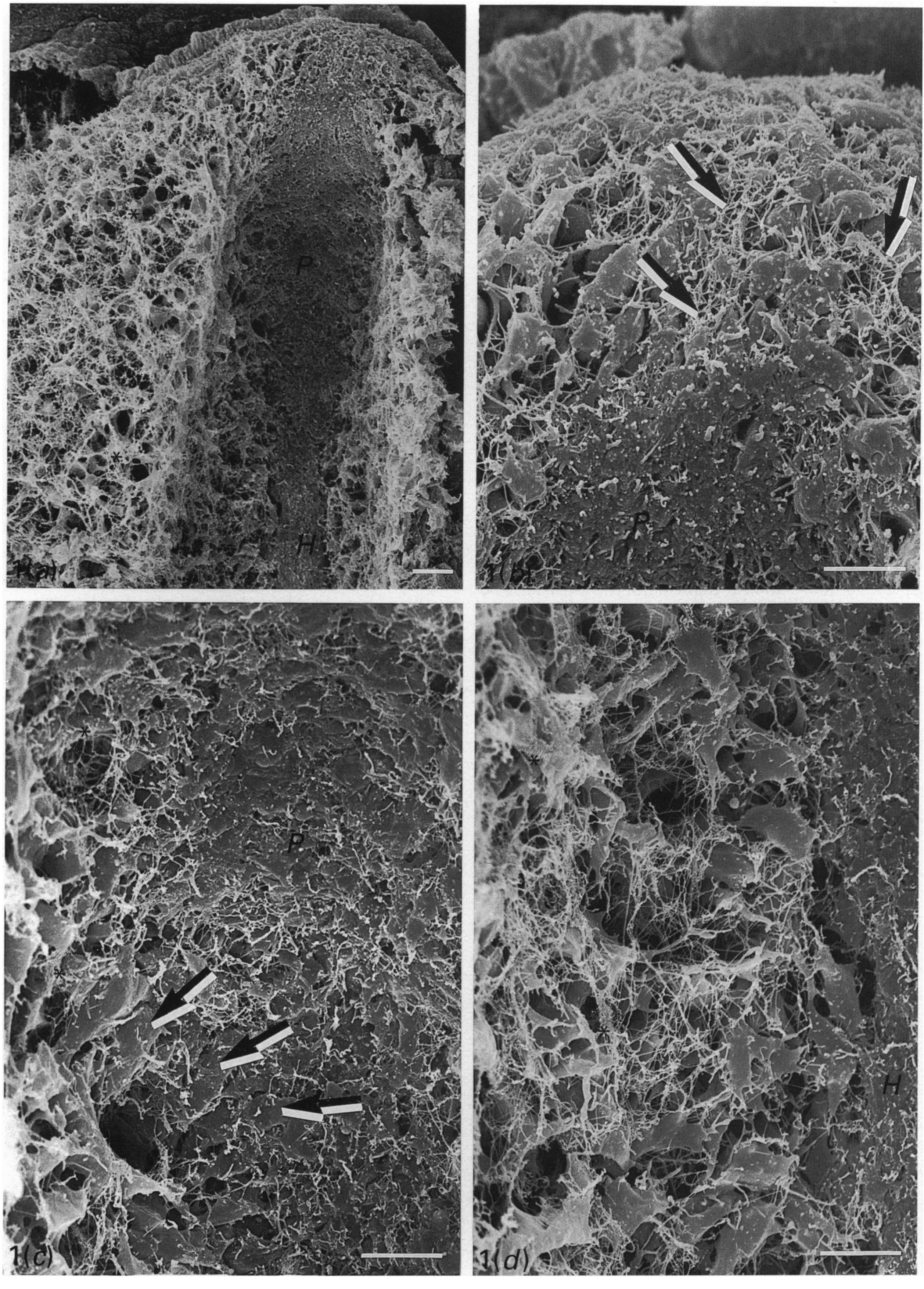
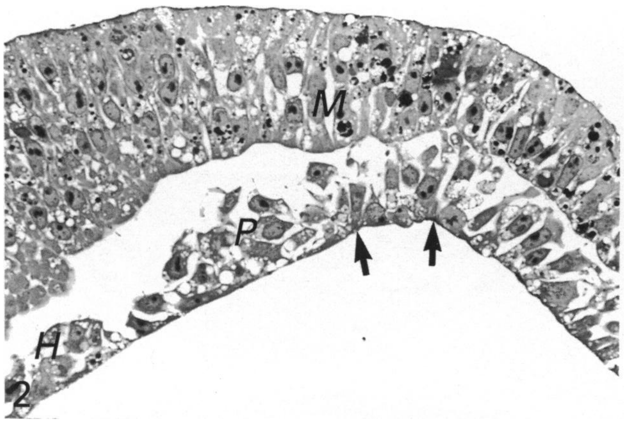
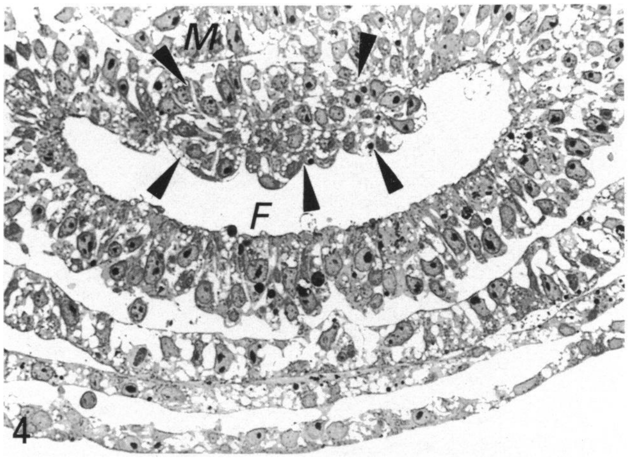
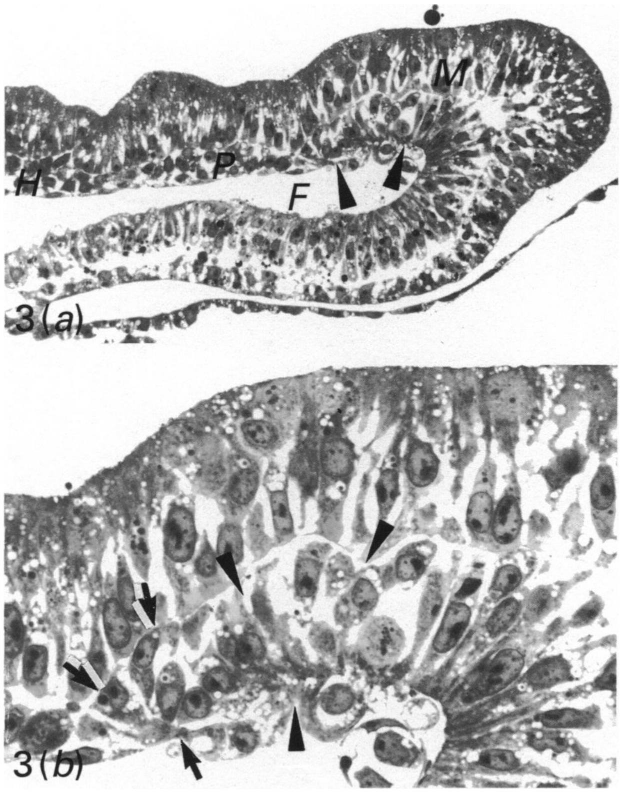


Fig. 1. (*a*) SEM photograph of the head region of a 2-somite quail embryo from a dorsal view after removal of the dorsal ectoderm and neuroectoderm. Axially located head process (*H*) and prechordal mesoderm (*P*) can be distinguished from paraxial head mesoderm (asterisks). Bar, 20 μm. (*b*) The prechordal mesoderm (*P*), recognisable because of its cell density and the small amount of extracellular







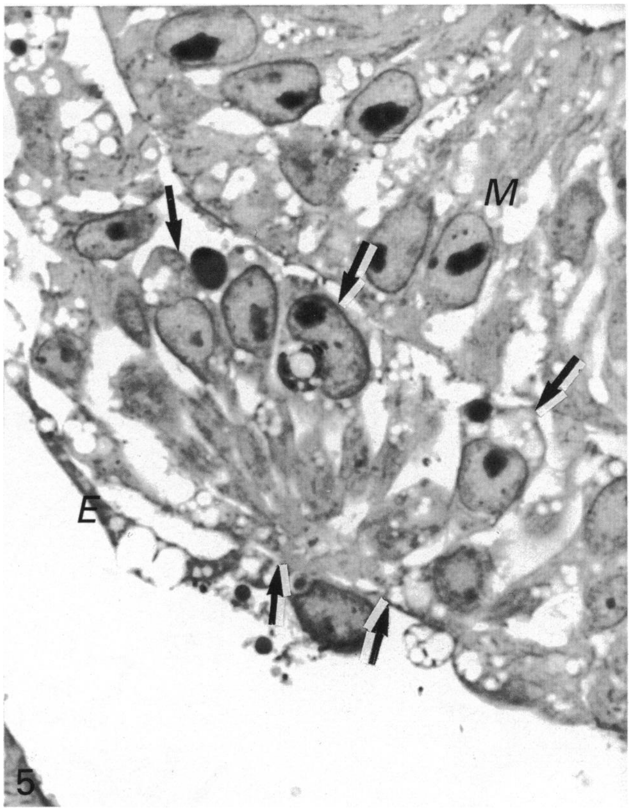


Fig. 2. Median sagittal semithin section through a 1-somite chick embryo. The prechordal mesoderm (*P*) consists of loosely packed mesenchymal cells and is continuous with the head process (*H*). It accumulates anteriorly at a point where the flat endodermal epithelium of the prospective roof of the foregut changes into a columnar epithelium (arrows) contacting the neural plate (*M*). × 300.

Fig. 3. (*a*) Median sagittal semithin section of a 3-somite chick embryo. The prechordal mesoderm (*P*) is continuous with the head process (*H*) and lies between neural plate (*M*) and flat endoderm of the roof of the foregut (*F*). At its anterior end it borders on a cell mass (arrowheads) reaching to the anterior extremity of the foregut. × 225. (*b*) In more detail, the cell mass appears to consist of columnar cells (arrows) followed by a cell mass (arrowheads) in which endoderm and mesoderm cannot be distinguished. × 600.

Fig. 4. Transverse semithin section of a 4-somite chick embryo anterior to the prechordal mesoderm. A cell mass (arrowheads) can be seen in the median part of the dorsal wall of the foregut (*F*), bulging into the gut lumen and reaching to the neural plate (*M*). × 320.

Fig. 5. Transverse semithin section of a 4-somite chick embryo at a slightly more caudal level than Figure 4. The cell mass is joined caudally by some columnar epithelial cells (arrows) lying in the median part of the roof of the foregut, intermingling with its flat endodermal epithelium (*E*) and extending to the neural plate (*M*). × 1200.

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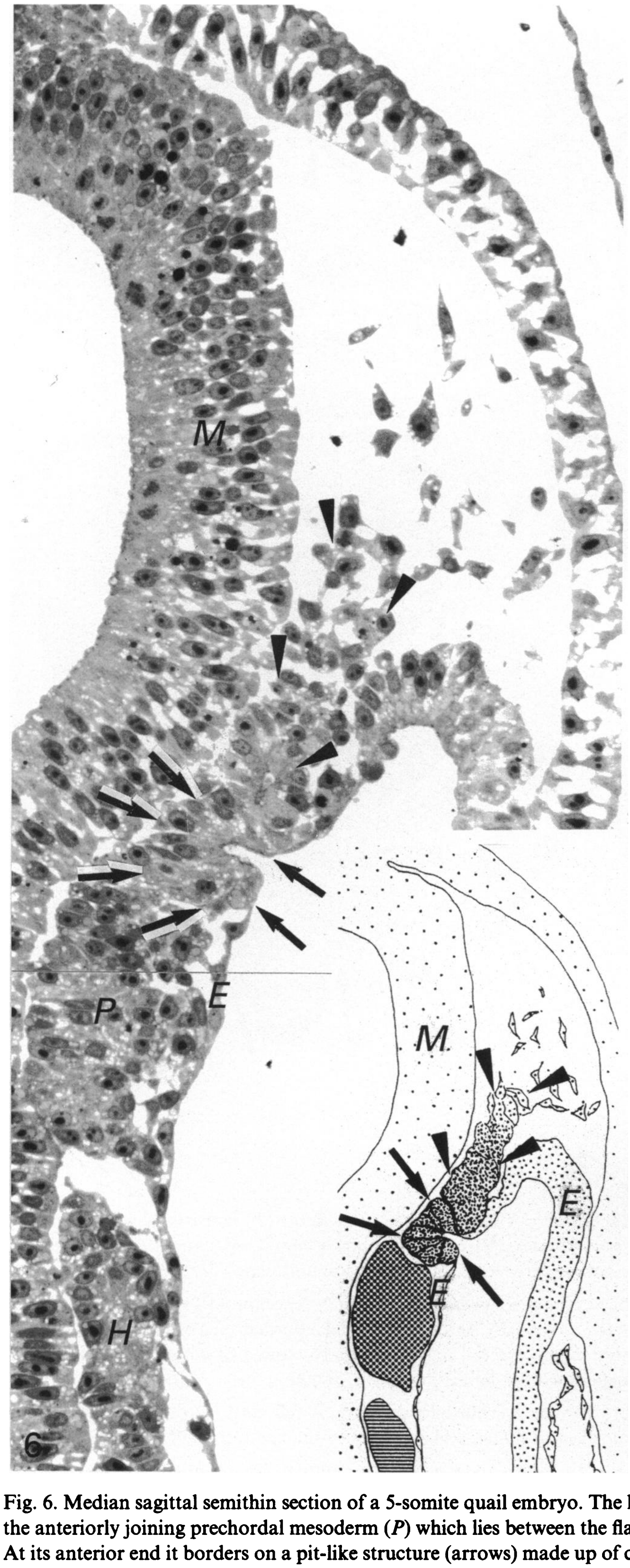
material, borders anteriorly on cells covered by a larger amount of extracellular material (arrows). Bar, 10 µm. (*c*) Laterally, the prechordal mesoderm (*P*) appears to be continuous with the paraxial mesoderm (asterisks). There some mediolaterally oriented cells can be seen (arrows). Bar, 10 µm. (*d*) Caudally, the prechordal mesoderm appears to be continuous with the head process (*H*) which is not yet well demarcated from the paraxial mesoderm (asterisks) in the 2-somite quail embryo. Bar, 10 µm.



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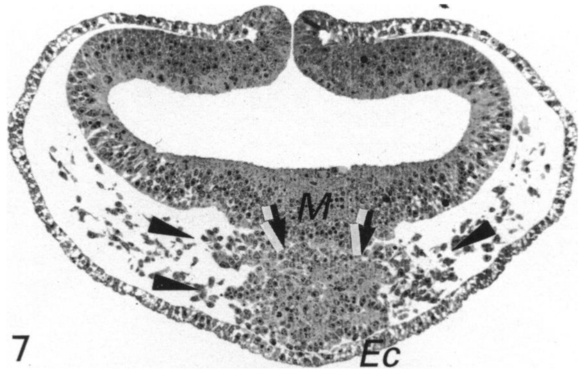

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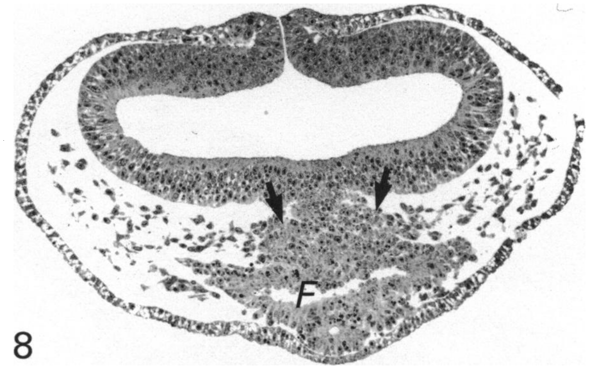

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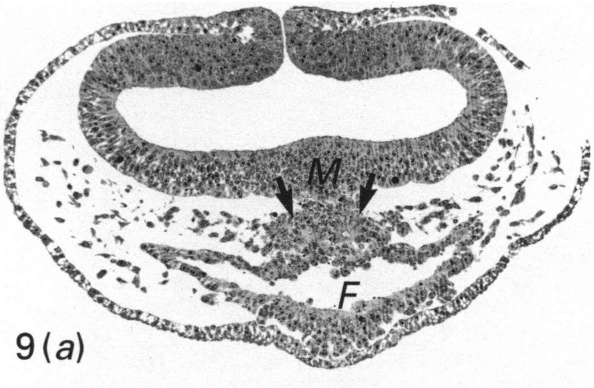

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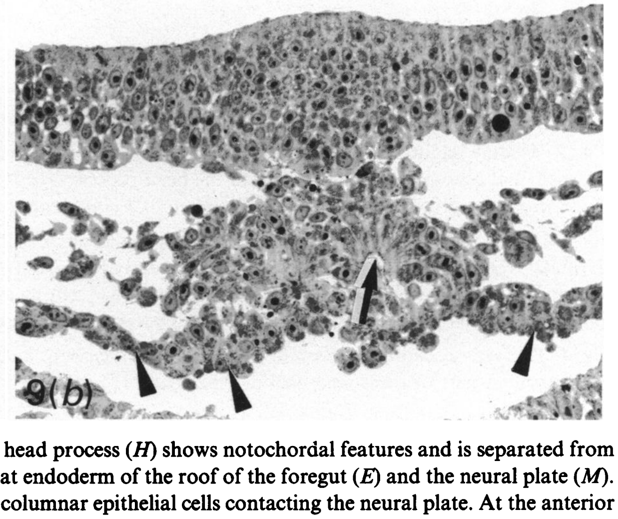

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Fig. 6. Median sagittal semithin section of a 5-somite quail embryo. The head process (*H*) shows notochordal features and is separated from the anteriorly joining prechordal mesoderm (*P*) which lies between the flat endoderm of the roof of the foregut (*E*) and the neural plate (*M*). At its anterior end it borders on a pit-like structure (arrows) made up of columnar epithelial cells contacting the neural plate. At the anterior end of the pit-like structure the cells lining it spread anteriorly into the prosencephalic mesenchyme (arrowheads). × 410. Inset. Schematic drawing of the section. *M*, neural plate; *E*, foregut endoderm. The hatched part of the axial head mesoderm represents the head process, the dotted part the prechordal mesoderm. The pit-like structure (arrows) is marked with irregular stippling and corresponds to the prechordal plate of Figure 18. Arrowheads, prosencephalic mesenchyme.

transverse section, directly anterior to the prechordal mesoderm, showed the closed foregut and, on the median part of its dorsal wall, a cell mass continuous with the surrounding head mesenchyme (Fig. 9). The demarcation between this cell mass and the underlying roof of the foregut could not be well defined. Within the cell mass some columnar epithelial cells lining a lumen were visible. It most likely represents a part of the pit-like structure seen in the sagittal sections.

Up to the 7-somite chick and quail embryos, the appearances of the axial head mesoderm essentially do not change.

Electron microscope appearance of the axial head mesoderm

To study the roof of the foregut as seen from the ventral aspect with SEM, it was necessary to remove the head fold, at least partly. In the 3-somite quail embryo the flat endodermal epithelium of the roof of the foregut could be seen. In its most anterior part, a cell mass appeared which bulged with spherical protrusions into the foregut lumen (Fig. 10). At the posterior end of this mass its entrance into a fissure can be assumed. After further removal of the head fold, including the anterior extremity of the foregut, a zone was reached where the endoderm could not be removed easily from the overlying prosencephalic floor. Looking at this zone from a ventral and anterior direction, in the midline of the dorsal wall of the foregut a single layer of high columnar cells was observed, with their apical spherical parts apparently bulging into the gut lumen (Fig. 11). In a 4-somite quail embryo after removal of the head fold and the lateral parts of the foregut, it was possible to look at the median anterior part of the dorsal wall of the foregut from a lateral direction (Fig. 12). Head mesenchyme could be distinguished between the neural plate and the roof of the foregut. Looking more closely, it became obvious that the medially and anteriorly located cells within the roof of the foregut seemed to be in close contact with the neighbouring cells of the head mesenchyme.

In a nearly median sagittal thin section of a 5-somite chick embryo, the prechordal mesoderm lay on the flat epithelium of the roof of the foregut, as described previously by Jacob et al. (1984, 1986). Anteriorly it bordered on a pit-like structure made up of columnar epithelial cells reaching the overlying prosencephalic floor (Fig. 13). A basal lamina was observed covering the base of the high columnar epithelial cells, partly fusing with that of the neural plate (Fig. 14*a*, *b*). At the anterior aspect of the pit-like structure, where the endoderm seemed to flatten again, a basal lamina was not observed, but it reappeared at the anterior extremity of the foregut, covering the high columnar endoderm. In the area where a basal lamina seemed to be lacking, the cells forming the pit-like structure made contact on their basal surfaces with cells of the anterior axial head region. The cells of the anterior axial head region showed typical mesenchymal features, exhibiting irregular shapes and cytoplasmic processes. No basal lamina was found at the boundaries between the epithelial cells forming the pit-like structure and the cells of mesenchymal character (Fig. 14*c*). The studies performed on quail embryos yielded similar results.

The microscopic observations of the axial head region are summarised in a schematic drawing reconstructed from serial transverse sections of the 5-somite quail embryo (Fig. 18). The drawing shows, anterior to the notochord and the head process, the prechordal mesoderm bordering anteriorly on a part of the endodermal roof of the foregut which at that point extends to the neural plate. This is the site of the above-mentioned pit-like structure which is probably composed of endodermal cells from the roof of the foregut. This structure is referred to as the 'prechordal plate' and is assumed to spread anteriorly into the prosencephalic mesenchyme.

Further development of the axial head region

To observe the further development of the axial head mesoderm, embryos of both species up to the 22-somite stage were investigated by light microscopy. Semithin sections showed that from the 10-somite

Fig. 7. In a transverse section of a 5-somite quail embryo through the anterior extremity of the foregut wall, a cell mass (arrows) can be seen lying between neural plate *M* and ventral ectoderm *Ec*. Within the cell mass, foregut endoderm and axially located mesoderm cannot be distinguished. Both tissues seem to be continuous with the surrounding head mesenchyme (arrowheads). × 120.

Fig. 8. Slightly more caudal to Figure 7, a transverse section from the same embryo shows the cell mass (arrows) lying in the median part of the dorsal foregut wall (*F*) and bulging into its lumen. × 120.

Fig. 9. (*a*) Transverse section of the embryo of Figure 7 directly anterior to the prechordal mesoderm. Between the neural plate (*M*) and the foregut (*F*) a cell mass is visible (arrows), which is continuous with the adjacent head mesenchyme. × 120. (*b*) In more detail the cells of the cell mass are seen intermingling with the endodermal cells of the roof of the foregut (arrowheads). Within the cell mass a lumen made of columnar epithelial cells can be seen (arrow). × 300.

stage onwards the appearance of the axial head mesoderm changed. The head process of 10-somite quail and chick embryos had already differentiated into the most anterior part of the notochord (Fig. 15). The prechordal mesoderm persisted as a median strand of cells of mesenchymal character extending to the anterior extremity of the foregut. The epithelial cells of the roof of the foregut changed shape from flat to columnar at the point where the head process now formed the tip of the notochord. A pit-like structure extending to the neural plate could no longer be observed. The cell accumulation with apical protrusions bulging into the gut lumen had disappeared. At the anterior extremity of the foregut the endodermal cells did not appear to be well demarcated from the cells of the prosencephalic mesenchyme. The oral membrane, consisting of clearly distinguishable ectoderm and endoderm, appeared at the ventral side of the embryonic head.

The 16-somite chick and quail embryos showed a similar situation (Fig. 16). A pit-like structure made up of columnar cells of the roof of the foregut was lacking, but in the chick embryo a new structure appeared. This appeared to be a dorsal evagination of the flat part of the endodermal roof of the foregut, extending to the tip of the notochord (Fig. 16*a*). A comparable structure has not yet been found in the quail embryo.

In the 22-somite chick and quail embryos the forebrain had grown forward, the mesencephalic flexure having developed up to an angle of 90° between the forebrain on one side and the hindbrain on the other (Fig. 17). The notochord did not reach the site of the infundibulum, the anlage of the neurohypophysis, completely but ended at the site of the angle created by the mesencephalic flexure. The median strand of prechordal mesodermal cells was demarcated from the tip of the notochord and extended to an invagination of the ectoderm anterior to the oral membrane at the ventral side of the embryo, the anlage of Rathke's pouch (Rathke, 1838).

DISCUSSION

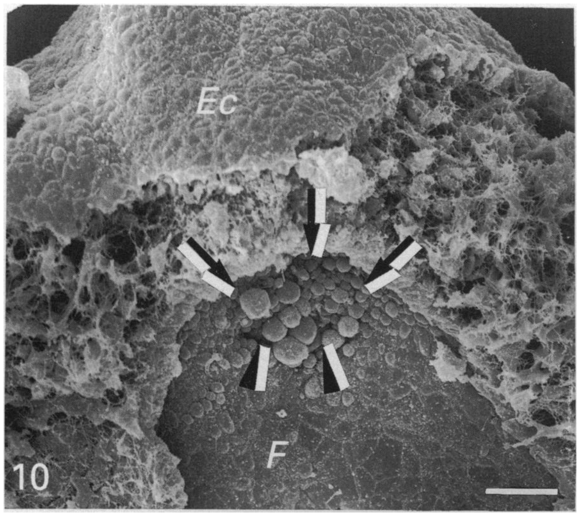
The prechordal mesoderm

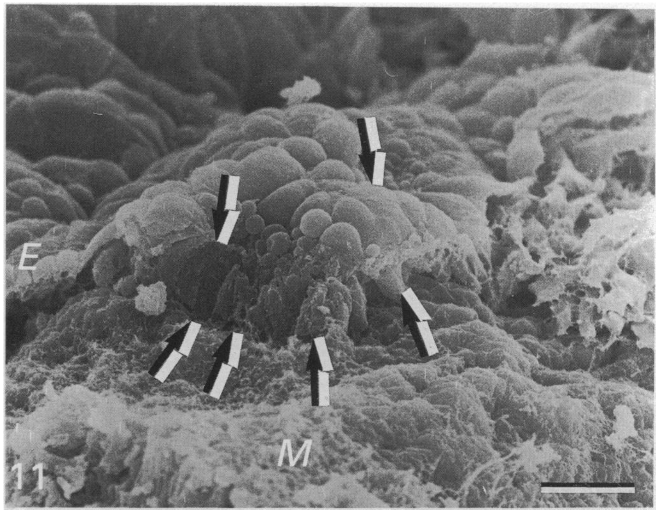
The existence of a prechordal mesoderm in the chick embryo, i.e. consisting of the most anterior part of the axial head mesoderm and not contributing to the notochord, as described by Jacob et al. (1984, 1986), has now generally been accepted (Noden, 1988; Couly et al. 1992). It is assumed to arise during gastrulation as part of the mesodermal germ layer, as experimental and labelling studies using tritiated thymidine or carbon particles (Spratt, 1955, 1957; Nicolet, 1970; Rosenquist, 1983) have indicated. Our scanning electron microscopic studies demonstrate that the prechordal mesoderm of the 2-somite quail embryo greatly resembles that of a chick embryo in the headfold stage as shown by Jacob et al. (1984, 1986). Laterally, it turns into the paraxial mesoderm. Here some mediolaterally oriented cells can be observed. This is in line with the findings of Jacob et al. in the 4-somite chick embryo (1984). They considered that the mediolaterally oriented cells colonise the head mesenchyme and give rise to the extrinsic eye muscles. This was established by the experimental studies of Wachtler et al. (1984), Wachtler & Jacob (1986) and Couly et al. (1992).

Light microscope appearances of the axial head mesoderm

Our studies of semithin sections reveal a similar development of the axial head mesoderm in quail and chick embryos. The prechordal mesoderm as described by Jacob et al. (1984) in the chick embryo can also be identified in the quail embryo. A pit-like structure at the anterior end of the prechordal mesoderm is prominent in both species. Since the pit-like structure extends to the neural plate, the cells of the prechordal mesoderm seem to accumulate here. In semithin sections the pit-like structure is seen to be formed by columnar endodermal cells in the roof of the foregut. More anteriorly, the columnar epithelial cells seem to be continuous with a mesenchymal cell mass in which endodermal and mesodermal cell layers cannot be distinguished. The epithelial cells of the pit-like structure therefore appear to be continuous, laterally and anteriorly, with the prosencephalic mesenchyme spreading into a space between the anterior part of the foregut and the neural plate. The space appears to be more distinct in the quail embryo than in the chick embryo of the relevant stage. This might be due to the fact that the development of the head region of quail embryos precedes that of chick embryos at the stages used here, as was shown recently (Seifert et al. 1992).

A pit-like structure as was observed in the sagittal and transverse sections of the avian embryo has previously been described in the duck embryo (Rex, 1897) and was interpreted as an evagination of the dorsal wall of the endodermal foregut. Poelmann (1981) also observed an epithelial structure in the





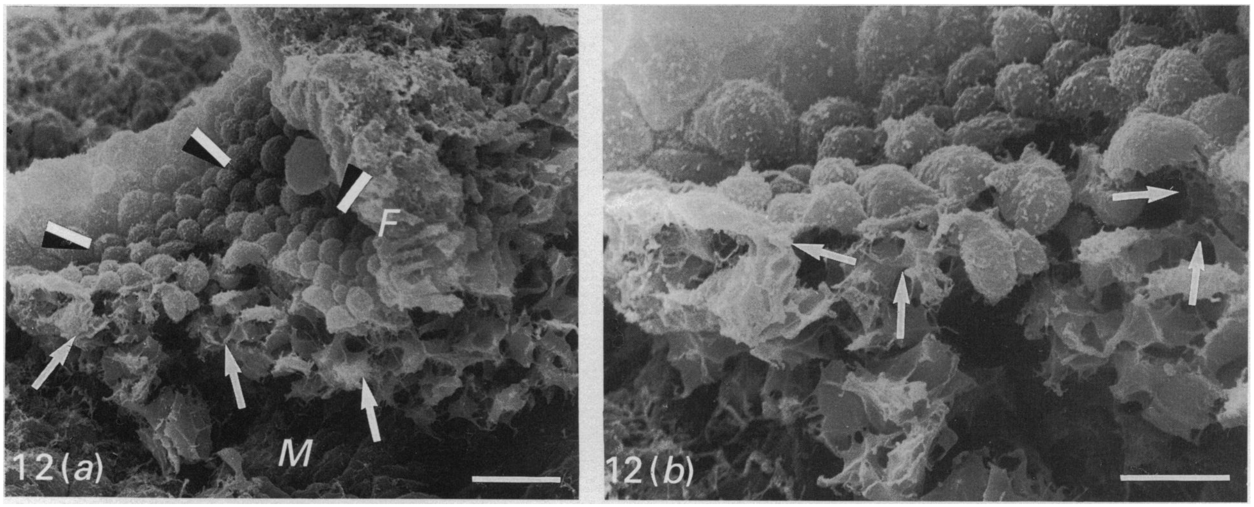


Fig. 10. SEM photograph of a 3-somite quail embryo from a ventral view after removal of the head fold and the ventral wall of the foregut. The ventral ectoderm (*Ec*) and the head mesenchyme are distinguishable. The dorsal wall of the foregut (*F*) is made up of flat epithelial cells. At its most anterior and dorsal part, cells are visible bulging with apical spherical protrusions into the gut lumen (arrows), and the entrance into a fissure might be assumed (arrowheads). Bar, 20 µm.

Fig. 11. The additional removal of the anterior extremity of the foregut allows an anterior view onto the cells bulging with apical protrusions into the gut lumen. These cells form a single layer of columnar epithelial cells (arrows) in the median part of the roof of the foregut and contact the neural plate (*M*) with their basal aspects. Laterally, the flat endoderm of the foregut (*E*) can be seen. Bar, 20 µm.

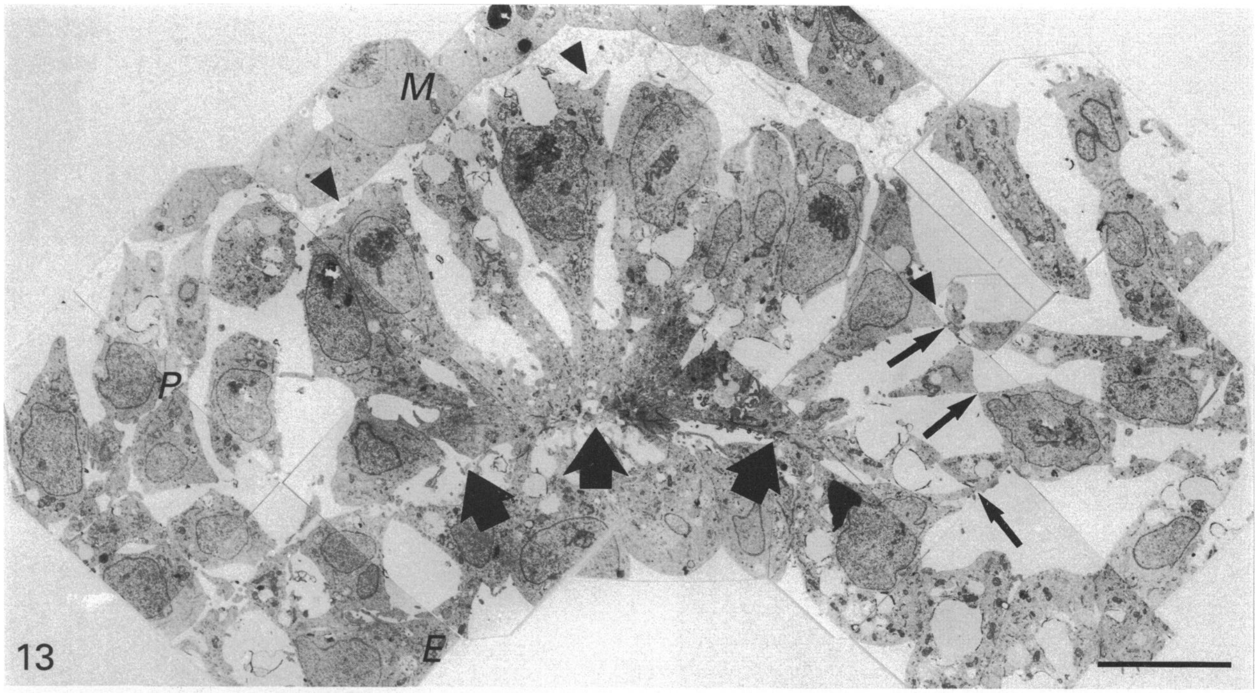
Fig. 12. (*a*) Viewing a 4-somite quail embryo from the lateral aspect after further removal of the lateral wall of the foregut, the cells in the anterior part of the roof of the foregut (arrowheads) are studied. Here the endodermal cells appear to be in contact with the adjacent cells of the head mesenchyme (arrows) which are spread between the anterior extremity of the foregut (*F*) and the neural plate (*M*). Bar, 20 µm. This is more clearly visible in more detail in (*b*). Bar, 10 µm.

mouse embryo similar to that seen in our transverse sections of the chick embryo. He assumed that this structure is a derivation of the mesodermal head process contacting the primary endoderm of the yolk sac. He therefore suggested that the mesodermal head process might contribute to the formation of the endoderm of the gut in the mouse embryo.

From our morphological studies we assume that the epithelial cells forming the pit-like structure are part of the foregut endoderm, since the definite endoderm of the foregut in the avian embryo, in contrast to that of the mouse embryo, has already been established. Whether the epithelial cells lining the pit-like structure as well as the cell mass anterior to it derive from the mesodermal or endodermal germ layer may be revealed by our electron microscopic studies.

Electron microscope appearances of the axial head mesoderm

The SEM observations support the implications from the light microscopic observations. The pit-like struc-



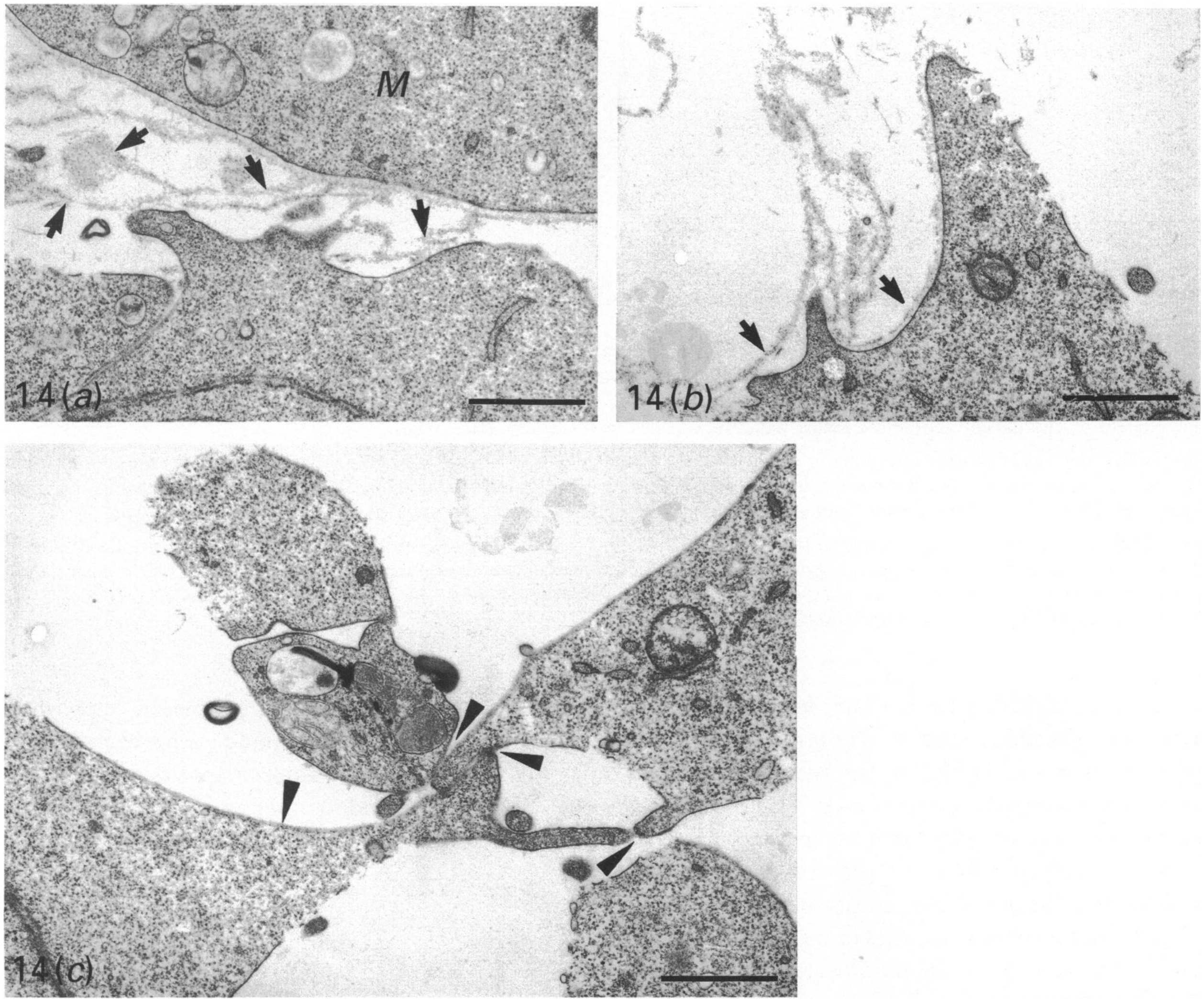


Fig. 13. TEM photograph of a nearly median sagittal section of a 5-somite chick embryo. It shows the pit-like structure (large arrows) made up of columnar epithelial cells extending to the neural plate (*M*). On the left side, part of the prechordal mesoderm (*P*) lying on the flat endodermal epithelium (*E*) of the roof of the foregut can be seen. At the anterior side (arrows) the cells of the pit-like structure are in contact

ture appears to be a median thickening of the endodermal roof of the foregut anterior to the prechordal mesoderm. These cells directly contact the neural plate on their basal surfaces. At their apical surfaces they show spherical protrusions bulging into the gut lumen. The endodermal cells of the most anterior part of the roof of the foregut appear to be in contact with the adjacent head mesenchyme. In the TEM studies the high columnar cells anterior to the prechordal mesoderm are seen to be covered by a basal lamina which seems to be continuous with that of the flat endoderm underlying the prechordal mesoderm, as was described by Jacob et al. (1984, 1986) in more detail. Our electron microscopic studies therefore provide evidence that the columnar epithelial cells lining the pit-like structure in the roof of the foregut anterior to the prechordal mesoderm probably have to be assigned to the endodermal germ layer. In contrast to that finding, the origin of the mesenchymal cells within the cell mass joining anteriorly remains uncertain. The morphological criteria do not permit a distinction between endoderm and mesoderm-derived cells.

Our findings match those of Adelmann (1922, 1926) and Hamilton (1965) in the chick embryo, those of Hill & Florian (1931) and O'Rahilly & Müller (1981) in the human embryo and those of Aasar (1931) in the rabbit embryo. These authors also described a median endodermal thickening anterior to the notochord and termed this structure the prechordal or prochordal plate. The prechordal plate as it was described by Adelmann (1922, 1926) seems to be the same as the prechordal plate mesoderm described by Hammond (1974). In both descriptions the axial head region of the chick embryo appears to contain various tissues: on the one hand the prechordal mesoderm which has been described by Jacob et al. (1984, 1986) as originating during gastrulation as part of the mesodermal germ layer, on the other hand the endodermal thickening described in this paper. Thus we propose using the term 'prechordal plate' only for the median thickening of the endodermal roof of the foregut and to distinguish it from the prechordal mesoderm (Fig. 18).

Our observations also give some indication that the endoderm of the prechordal plate probably contributes to the prosencephalic mesenchyme, at least in the 3 to 7-somite avian embryo. It is known that the ventral part of the head mesenchyme arises from the mesectodermal cells of the neural crest (Le Lièvre & Le Douarin, 1975; Noden, 1975; Le Douarin, 1982). According to Couly & Le Douarin (1985, 1987), the anterior part of the prosencephalic neural ridge does not give rise to mesenchyme itself but the prosencephalic mesenchyme of the facial area is assumed to derive partly from the posterior prosencephalic and mainly from the mesencephalic neural crest. The sources of the mesenchymal cells of the dorsomedian prosencephalic region are therefore not yet quite clear. It is possible that the prechordal plate may contribute to this part of the prosencephalic mesenchyme. This would mean that the prosencephalic mesenchyme consists of cells deriving from different germ layers, the mesoderm, the ectoderm and the endoderm, all filling up the space around the rapidly growing prosencephalon. Moreover, it implies the possibility of the secondary formation of mesenchyme also out of the definitive endodermal germ layer in analogy to the ectoderm-derived mesenchyme of the neural crest. In that our findings match those of other authors (Veini & Bellairs, 1991; Griffith et al. 1992), whose observations also indicate that the potential of the germ layers to differentiate into specific tissues is not finally determined at the time of gastrulation.

Further development of the axial head region

From the 10-somite quail and chick embryos onwards a pit-like structure could no longer be observed. The prechordal mesoderm persists as a median strand of cells with mesenchymal character during the formation of the head cavities in its lateral parts, as was reported earlier by Adelmann (1926), Wachtler & Jacob (1986) and Jacob et al. (1986). At the point where prechordal mesodermal cells, head mesenchyme and the endoderm of the anterior extremity of the foregut meet each other, the demarcations of these tissues remain unclear. According to Jacobson et al. (1979) this area might be working as a 'pivot' for the increasing mesencephalic flexure due to the extensive growth and rearrangement of the prosencephalon.

It is remarkable that in the 16-somite chick embryo

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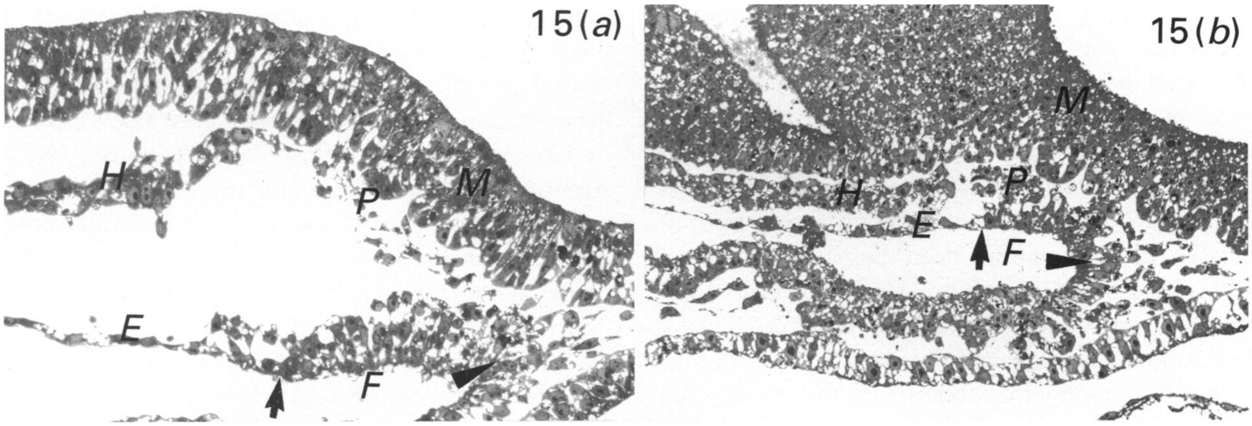
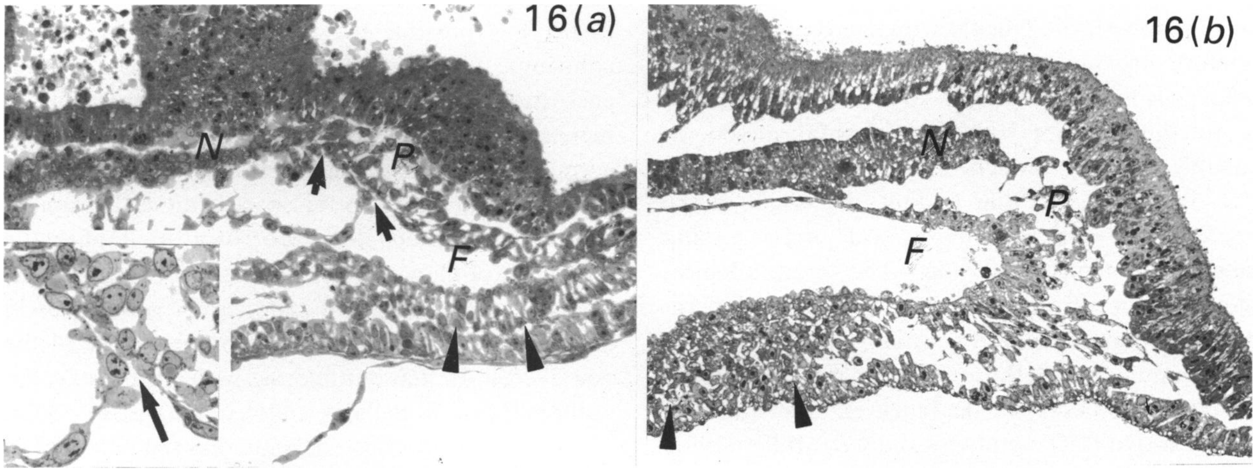
at their basal sides with cells of the prosencephalic mesenchyme. Apical parts of cells which underlie the columnar cells of the pit-like structure indicate that the section is slightly turned at the sagittal level. Arrowheads, details shown in Figure 14. Bar, 10 µm.

Fig. 14. (*a* and *b*) In more detail (arrowheads in Figure 13), it can be seen that the columnar cells of the pit-like structure are covered by a basal lamina on their basal surfaces (arrows). At the top of the pit-like structure the basal lamina fuses with that of the neural plate (*M*). (*c*) At the points where the columnar cells on the anterior aspect of the pit-like structure (arrowhead in Figure 13) contact cells of mesenchymal character, no basal lamina is present (arrowheads). Bars, 1 µm.





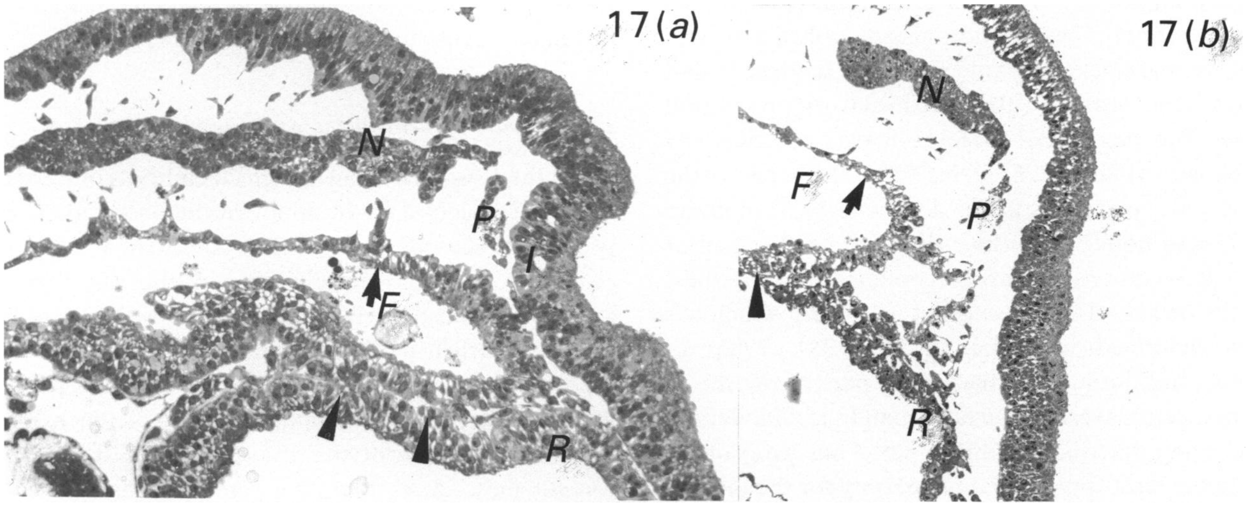


Fig. 15. Median sagittal semithin section of (*a*) a 10-somite chick embryo and (*b*) a 10-somite quail embryo. The head process (*H*) has differentiated into the notochord with its tip ending at the prosencephalon (*M*) which has just started to bend. The epithelium (*E*) of the dorsal wall of the foregut (*F*) changes at some distance from its anterior end from a flat into a columnar epithelium (arrows) without forming a pit-like structure. Anterior to the notochord the prechordal mesoderm (*P*) is visible, forming a median strand of cells of mesenchymal character extending to the anterior end of the foregut (arrowheads). There the borders to the adjacent tissues are not clearly visible. *a* and *b*, ×250.

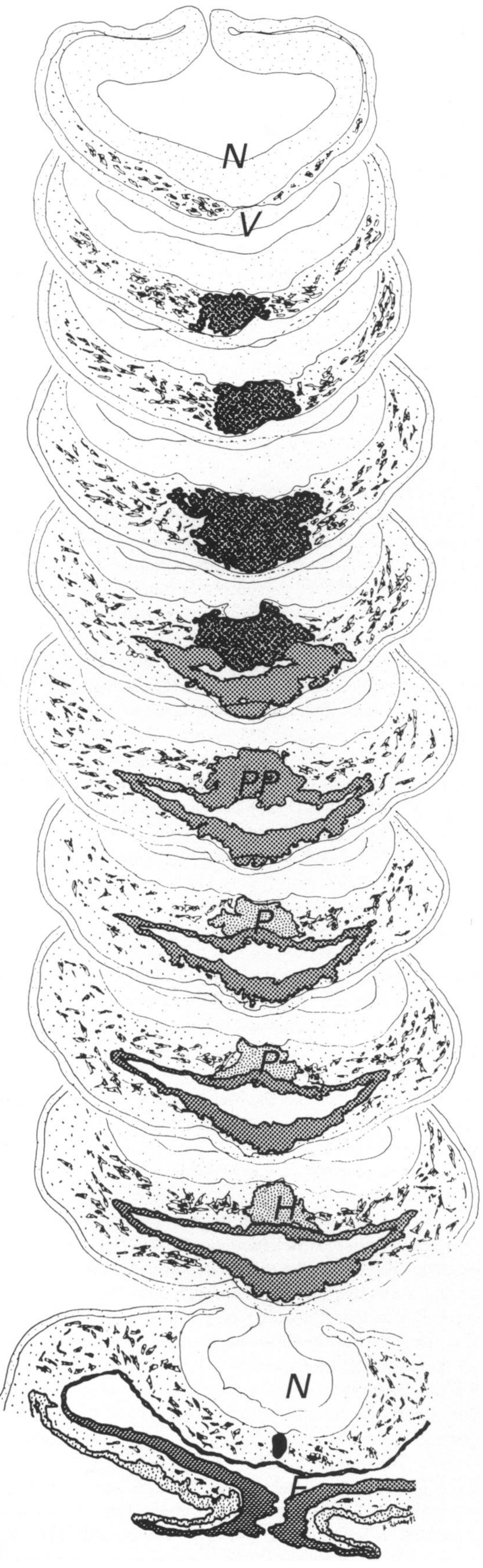
Fig. 16. Median sagittal semithin section of (*a*) a 16-somite chick embryo and (*b*) a 16-somite quail embryo. The tip of the notochord (*N*) now extends more anteriorly than in the 10-somite embryos, and in the quail embryo even up to the anterior end of the foregut (*F*). On the ventral side of the head, the oral membrane can be seen (arrowheads). No pit-like structure appears, but in the chick embryo another structure is evident: it appears to be an evagination of the dorsal wall of the flat epithelium of the foregut in the direction of the tip of the notochord (arrows). This is more clearly visible in the inset. *a*, ×220; inset, ×430; *b*, ×190.

Fig. 17. Median sagittal semithin section of (*a*) a 22-somite chick embryo and (*b*) a 22-somite quail embryo. The flexure of the forebrain has progressed. In the chick embryo the infundibulum (*I*), the anlage of the neurohypophysis, can be made out, the notochord (*N*) almost reaching it. The prechordal mesoderm (*P*) persists as a median strand of cells of mesenchymal character anterior to the notochord. The endoderm of the roof of the foregut (*F*) changes from a flat into a columnar epithelium (arrows) at some distance from the anterior end of

a new structure arises. This structure seems to be an evagination within the flat part of the endoderm of the dorsal wall of the foregut extending to the anterior tip of the notochord. In accordance with Aasar's studies in the rabbit embryo (Aasar, 1931) it might be interpreted as Seessel's pouch, although Seessel (1877) himself described the pouch as appearing only in slightly older chick embryos. So far, our studies indicate that a similar structure is lacking in quail embryos.

Conclusions

In summary, the following conclusions arise out of our morphological investigations of the axial head mesoderm. (1) The prechordal mesoderm has to be regarded as being of mesodermal origin. In young avian embryos it is continuous with the head process which later contributes to the notochord. The prechordal mesoderm itself does not contribute to the notochord. It persists as a median mesenchymal strand extending to the anterior extremity of the foregut. (2) Anteriorly, it borders on the prechordal plate made up most probably of a localised thickening of the endoderm of the dorsal wall of the foregut. In young avian embryos it is established only transiently and was observed in 3-somite to 8-somite chick and quail embryos. (3) The prechordal plate has to be regarded as being of endodermal origin. Since it has contacts with cells of the adjacent head mesenchyme, a contribution of the definitive endodermal germ layer



18

Fig. 18. Schematic drawing of serial transverse sections of the head region of a 5-somite quail embryo. Sections 4–6 from the top of the page are derived from Figures 7–9. The most caudal section is at the bottom, the most anterior at the top. The most caudal section is situated about 500 μm caudally to the second, whereas the sections following anteriorly are about 15 μm apart. In the most caudal section the notochord is seen lying in the midline between the prosencephalic neural tube (*N*) and the dorsal wall of the foregut (*F*). More anteriorly, the notochord continues into the head process (*H*) and it, in turn, further anteriorly into the prechordal mesoderm (*P*). Both tissues, lightly dotted, are of mesodermal origin. The prechordal mesoderm ends at the prechordal plate (*PP*) which is assumed to be part of the endodermal roof of the foregut. The tissues of endodermal origin are marked with larger dots. The prechordal plate can be described as a pit-like structure made up of columnar endodermal cells. The prechordal plate spreads more anteriorly into the axial prosencephalic mesenchyme (heavily dotted) ending where the prosencephalic floor of the neural tube (*N*) and the ventral ectoderm (*V*) meet each other in the midline, as shown in the most anterior section.

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the foregut without forming a pit-like structure. In the ventral ectoderm anterior to the oral membrane (arrowheads), the anlage of Rathke's pouch (*R*) is visible. Where prechordal mesodermal and endodermal foregut cells meet each other the demarcation of these tissues appears to be indistinct. *a*, × 180; *b*, × 150.

to the formation of the head mesenchyme of the fast growing prospective prosencephalon during this developmental period might be assumed.

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