Studies on the Development of the Foregut in the Chick Blastoderm

1. The Presumptive Foregut Area

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With one plate

Introduction

Little attention has hitherto been paid to the early stages in the development of the foregut in the chick. This paper is the first of a series concerned with an investigation into how it develops during the period between the primitive streak stage and the stage of an embryo with about ten pairs of somites. The term 'foregut' refers throughout to the blind diverticulum extending forward into the developing head from the anterior intestinal portal. The present communication opens with a brief consideration of the gross morphological changes which take place; the rest of the paper is concerned with the location in primitive streak and head process stage blastoderms of the presumptive area from which the foregut of the ten somite embryo will develop.

Morphology

The following account has been compiled from the study of serial sections of twenty embryos and from the publications of Duval (1889), Adelmann (1922), and Wetzel (1929).

Between the time of laying and the stage when the head process begins to form, the endoderm of the area pellucida lies as a thin sheet of cubical cells below the epiblast. At the borders of the area pellucida it is continuous with the yolky endoderm of the area opaca (Text-fig. 1A). The whole endodermal layer takes part in the general expansion of the blastoderm over the yolk.

The earliest visible changes concerned with foregut formation can be seen at the beginning of the head-fold stage. These changes are as follows:

1. The formation of the most anterior part of the foregut. This develops at the anterior end of the head process as a wide endodermal diverticulum surrounded by mesoderm; it extends forwards into the developing head and opens posteriorly into the yolk sac (Text-fig. 1B). Its most anterior end and all its floor are thick.

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and consist of columnar epithelium, but its roof gradually becomes thinner behind the anterior thickened zone.

2. **Thickening of the endoderm on either side of the head process.** The thick floor of the anterior diverticulum is continued posteriorly as a thickened region on either side of the head process. Later this thickening gradually spreads backwards in the endoderm lying beneath the splanchnic mesoderm. The endoderm here becomes about twice as thick as that which lies beneath the somites and notochord (Text-figs. 1c and 2). The cells become large and arranged in a columnar epithelium, the nuclei tending to lie on the mesodermal side. This thick epithelium becomes even thicker as development proceeds.

3. **The assumption of a dorsally concave, ‘trough-like’ form by the endoderm lying medially between the two thickened regions** (Text-figs. 1c and 2). The cells here, which were formerly cubical, now become squamous with flattened and widely separated nuclei.

As the formation of the embryonic axis proceeds the thickened parts of the
endoderm swing ventro-medially and meet in the midline where they fuse to form the backward continuation of the floor of the foregut (Text-figs. 1c, 1d, and 3). At this junction of tissues contact of the foregut floor with the adjacent

extra-embryonic endoderm is lost and the latter forms the endodermal roof to the yolk sac. This formation of the foregut floor begins anteriorly and gradually spreads backwards. The floor is thick whilst the roof remains thin. The foregut as a whole is dorso-ventrally flattened, but the floor is concave dorsally like the already ‘trough-shaped’ roof. It retains this characteristic appearance until the stage of about 15–20 pairs of somites, when the first steps occur in the regional differentiation of the gut.

The movements by which the future floor of the foregut is brought medio-

TEXT-FIG. 2. Diagram of the endoderm in the early embryo. The anterior half of the area pellucida is represented as if seen from above after removal of most of the ectoderm and mesoderm. The areas outlined by broken lines indicate the extent of the thickened endoderm.

TEXT-FIG. 3. Diagram representing a T.S. across the developing foregut region. On the left side the thickened region is swinging ventro-medially to form the foregut floor. On the right side the corresponding part of the endoderm, already thicker, is represented as having almost reached the midline. The interrupted lines represent stages in the displacement of the future foregut floor, the arrows the direction of the movement.
ventrally also serve to bring the associated splanchnic mesoderm into a corresponding medial and ventral position. This close association of the two layers has been emphasized in a recent paper by Rudnick (1952).

**THE PRESumptive FOREGUT AREA**

*Method*

Maps showing the location of the presumptive areas of the epiblast have been presented by Wetzel (1929), Gräper (1929), Pasteels (1937), and Spratt (1952). They were prepared either entirely, or partially as in the case of Spratt’s, from the results of vital marking experiments carried out upon the blastoderm lying in situ above the yolk. The inaccessibility of the endoderm in its normal situation precludes the possibility of using the same technique to determine the position of the presumptive foregut. In all cases therefore, in the present investigation, vital marking was carried out upon blastoderms which had been removed from the yolk and explanted on a plasma clot (technique of Waddington, 1932). Specimens were placed with the epiblast surface against the clot and the endoderm uppermost. Vital dyes were not used because of the difficulty, notorious in the chick, of retaining the colour throughout the preparation of serial sections. Instead finely powdered carbon (animal charcoal) was put on a selected region of the endoderm to act as a mark.

In the present work I found it advantageous to remove as much saline as possible from the exposed tissue before applying the mark, otherwise the carbon tended to float off. As reported by Spratt (1946) for the epiblast layer, the dry carbon adheres to the surface of the cells most tenaciously. Jacobson (1938) placed drops of Indian ink on the endodermal surface of blastoderms and reported phagocytosis of the carbon particles. In the present experiments also, small carbon particles were sometimes found within the cytoplasm of the endoderm cells, though the position of the ingested material usually corresponded well with that of the main body of the mark itself (Plate, fig. B). Only small patches of carbon were used as marks (about 5\(\mu\) to 50\(\mu\) in diameter). Larger masses tended to fall through the endoderm and become adherent to the mesoderm (Jacobson, 1938).

Waddington (1950) has objected to the use of carbon as a marker because it is ‘by no means certain that the solid particles always remain attached to the cell’. Because of this criticism I have performed large numbers of experiments and these have given fairly consistent results. Had the carbon been moved independently of the cells somewhat erratic answers might have been expected. It is improbable that the marking technique had any toxic influence on the embryo, for no difference could be detected between the development of experimental cultures and of unmarked control blastoderms.

After each specimen had been marked it was drawn to scale and the position of the carbon patch plotted. After further incubation for 24 to 36 hours the embryo was redrawn and, where it was visible, the position of the carbon was
again noted (Plate, fig. A). The embryos were subsequently fixed in Bouin's fluid, sectioned at 10\(\mu\), stained with Weigert's haematoxylin, and examined for traces of carbon in the foregut (Plate, fig. C).

![Text-Fig. 4. Marking experiments.](image)

**A and C.** The arbitrary regions which have been distinguished in the endoderm at the long streak and head process stages respectively.

**B and D.** The results obtained at the long streak and head process stages respectively. The number of specimens in which inclusion in the foregut of a marked group of cells was (+) and was not (−) obtained is indicated by the numerals in each region.

Marking experiments have been carried out upon blastoderms at two stages, the long or definitive primitive streak stage and the head process stage, as defined by Waddington (1932) and Abercrombie (1950).
To make it possible to describe and compare the marks in different blastoderms accurately, a number of arbitrary regions were distinguished in each specimen (Text-fig. 4, A and C). The variation in size between individuals is considerable at these stages, so direct measurements have been avoided for fixing the extent of each region. Morphological landmarks were employed where possible; otherwise the regions were assessed as fractions of the total width or length of the area pellucida. Thus the long streak stage blastoderm was considered as consisting of five longitudinal strips: strip 3 enclosed the primitive streak; strips 2 and 4 were the regions on either side, and they extended half of the way to the area opaca; strips 1 and 5 were the lateral parts of the area pellucida (Text-fig. 4A).

These strips were in their turn considered as being subdivided into six regions across the primitive streak of equal antero-posterior length (C, D, E, F, G, and H) and two equal ones (A and B) anterior to the primitive node. In this way the area pellucida of the long streak stage was treated as forty different parts.

Similarly the head process stage (Text-fig. 4C) was considered as possessing five longitudinal strips assessed in a comparable manner, which were subdivided transversely into seven regions. Level a lay anterior to the head process; levels b and c each contained half of the head process; level d included the primitive node; levels e, f, and g each incorporated a third of the primitive streak.

**Results**

The results of the marking experiments are summarized in Text-fig. 4, B and D. Taking region C4 in Text-fig. 4B as an example, the convention used is as follows: 4 + means that in four specimens the carbon mark, when placed in this position, became included in the foregut; and 2 – means that in two specimens the mark did not become included in the foregut.

In the long streak blastoderms it will be noticed that the positive results are distributed around the anterior end of the primitive streak. In no case were laterally situated marks in strips 1 and 5 at any level enclosed in the foregut. In the regions close to, and on either side of, the anterior end of the primitive streak (i.e. regions 2 and 4 of B, C, D, and E) 33 from a total of 60 marks became enclosed in the gut. Most of the negative results were obtained from specimens in which marks were placed at the lateral edges of these regions and in these the marked cells were usually found eventually in the yolk-sac endoderm. Carbon placed more medially in regions 2 and 4 usually became included in the foregut. The occurrence in a given region of positive results from some specimens and negative ones from others may have been due in part to experimental error in fixing the boundaries of the regions, but it is also likely that the lateral extent of the presumptive area varies according to the specimen.

A similar variation from individual to individual may also be a feature of the posterior end of the presumptive area, for here too the positive and negative results overlap. To some extent, however, the fate of a marked group of cells depended on the amount of development which had occurred in that embryo before fixation. It is likely, for example, that in the two negative specimens shown
in D3 the marked cells would have become part of the foregut had development proceeded further. In regions D and E, however, other types of individual differences also affected the results.

Firstly, there was sometimes a backward or sideward movement of cells. Fourteen specimens were marked in region E3, but the carbon was subsequently found in the foregut of five embryos only. In 8 of the other 9 cases the mark was discovered at the hind end of the primitive streak, i.e. the marked cells had migrated posteriorly. In regions E2 and 4, of the 11 marks which did not become

![Text-Fig. 5. The presumptive foregut area indicated by carbon marking experiments. A. The long streak stage. B. The head process stage.](image)

included in the foregut, 4 were found to have migrated laterally and 2 to have moved posteriorly. In three other specimens a second circumstance connected with the non-inclusion of midline marks in regions D and E may be noted; marks placed in position E2 or E3 were subsequently found on the yolk-sac endoderm in the midline ventral to the heart. The carbon extended forward for 20 μ from the level of the anterior intestinal portal.

Text-fig. 5A shows the region in which positive results were usually obtained at the long streak stage, i.e. the presumptive foregut area. Although for convenience precise limits are shown, the borders of the presumptive foregut in any given blastoderm may not coincide exactly with these boundaries.

In head process stage blastoderms the results obtained from marking are summarized in Text-fig. 4D. The distribution of the positive results is similar to that obtained at the long streak stage. The lateral extent is comparable: in regions 2 and 4 of levels a, b, c, d, and e, 34 out of a total of 59 marks became enclosed in the foregut. Although the positive results appear to extend farther anteriorly in the head process than in the long streak stage, the region of positive results is morphologically comparable at the two stages in that it reaches to about the most anterior limit of the invaginated mesoderm (Adelmann, 1922; Wetzel,
1929). Posterior migration of the medially situated marks occurred as in the long streak stage, e.g. of 12 specimens marked at e3, the marks became enclosed in 3, moved posteriorly in 6, laterally in 2, and remained apparently in the same place in the remaining embryo. Of the 8 blastoderms marked in regions f2 and f4, 5 showed a migration in a postero-lateral direction.

Text-fig. 5b shows the area in which positive results were obtained, i.e. the presumptive foregut region at the head process stage.

DISCUSSION

Rudnick & Rawles (1937) have investigated the ability of isolated fragments of blastoderms to develop gut when cultured upon the chorio-allantois. After 8 to 10 days many of their specimens had grown into tissues which could be recognized histologically as different levels of the gut, e.g. small or large intestine. The time during which blastoderms may be maintained in tissue culture by the method I have used is much shorter and differentiation does not proceed so far. There is no evidence in my results to show which part of the simple foregut tube will give rise to a particular region, such as the oesophagus, or even to indicate if a certain level of future adult gut was yet included in the foregut when the experiment was terminated at the time of fixation. It may well be that the small intestine lies farther posteriorly than the presumptive area shown in my maps. Despite these differences in technique, however, it is interesting to compare the endodermal potencies of fragments on the chorio-allantois with the results of the present marking experiments.

Using the long streak stage Rudnick & Rawles (1937) divided the blastoderm into four portions. From each of these gut was subsequently obtained. According to my results, each of their pieces contained a fragment of the presumptive foregut area.

With the head process stage, however, Rudnick & Rawles obtained very few cases of gut (about 6 per cent.) from isolates taken from the 'anterior embryonic and node fields', i.e. the region which is presumably equivalent to a, b, c, and d in Text-fig. 4c. In grafts from the streak field, however (i.e. my regions e, f, g, and h?), they obtained 'organized gut' in 20 per cent. of their isolates. In the posterior parts of this field gut formed in median isolates only, whereas in the level 0.3–0.7 mm. behind the pit, lateral pieces as well had this potency.

The failure of Rudnick & Rawles to obtain more than a few cases of gut from the 'anterior embryonic and node field', that is from the very heart of the presumptive area, is perhaps significant, for ordinarily the potency region for a given tissue may be expected to include its presumptive area. If, however, certain parts of the presumptive area fail to differentiate when isolated (i.e. are not included in the region of potency for that particular tissue), the lack of some condition necessary for their differentiation is indicated. Rudnick & Rawles report that the pharynx and oesophagus were never found as organs. It may be that these structures normally develop in the 'anterior embryonic and node field'
under the influence of neighbouring tissues. The results of Rudnick & Rawles are at least highly suggestive of some difference existing between the head process and the anterior end of the primitive streak in head process stage blastoderms which affects the ability of the foregut to differentiate under the conditions of chorio-allantoic grafting.

It has frequently been suggested that the development of the gut from undifferentiated endoderm is dependent on the presence of mesoderm (Rudnick & Rawles, 1937; Hunt, 1937; Rudnick, 1944; Waddington, 1952). Despite the valuable marking experiments of Graper (1929), Wetzel (1929), Pasteels (1937), and Spratt & Condon (1947), the distribution of mesoderm at these stages is not fully understood. It would not be out of place, however, to emphasize here the close correspondence which exists between the distribution of the presumptive foregut and the presumptive neural plate (Pasteels, 1937; Spratt, 1952). Neural plate is believed to be induced by the underlying mesoderm (Waddington, 1932, 1933); and it may well be that this mesoderm performs a double function by also influencing the development of subjacent endoderm. This concept of foregut induction will be discussed more fully in a subsequent paper.

**SUMMARY**

1. A brief morphological account is given of the changes which take place in the endoderm during the first stages of foregut formation. The period of development concerned is from the stage of the primitive streak to that of the embryo with about ten pairs of somites.

2. Experiments are described in which the endoderm of chick blastoderms grown *in vitro* was marked with carbon particles. By this means the presumptive foregut area was found to lie around the anterior end of the primitive streak in the long streak stage, and around the head process and anterior end of the primitive streak at a slightly later stage of development.

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**REFERENCES**


R. BELLAIRS—THE DEVELOPMENT OF THE FOREGUT


EXPLANATION OF PLATE

FIG. A. A specimen fixed 24 hours after marking with carbon particles. Two carbon marks, which did not become enclosed in the foregut, can be seen on the yolk-sac endoderm.

FIG. B. Carbon particles ingested into the endodermal cells; they are closely associated with the main part of the carbon mark.

FIG. C. Transverse section across the embryo shown in fig. B. The carbon is in the floor of the foregut. The neural plate is somewhat distorted, because the blastoderm was explanted with its dorsal surface against the clot.