Topography of the Presumptive Rudiments in the Endoderm of the Anuran Neurula

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WITH ONE PLATE

INTRODUCTION

In previous papers (Nakamura & Tahara, 1953, 1954), evidence was presented on the formation of the anuran stomach and intestines. The first paper demonstrated the origin of the stomach from the posterior part of the fore-gut of the neurula. The second disproved the archenteric origin of the intestine: contrary to Goette's (1875) long-accepted theory, the archenteron was shown to close up throughout the whole length of the mid-gut. The definitive cavity of the intestine is newly formed by the splitting of the mass of yolk cells.

These and more recent findings make it possible to determine the position occupied in the neurula by the materials which form the various endodermal organs. In the present paper, the data are fully reported and a map of the presumptive materials is produced.

MATERIAL AND METHODS

Embryos of a common Japanese frog, Rana nigromaculata nigromaculata, were used. This species was preferred to others because of its quick healing and its undisturbed development after the operation.

To stain the endoderm already invaginated an incision was made in the neurula at the place of the presumptive sucker. Through it, small pieces of agar containing neutral red or Nile blue were inserted into the archenteron and put directly upon the part to be marked. The endoderm was stained in a few minutes. When it was necessary to prevent the transmission of the dye to the other walls of the archenteron, the agar was covered with a piece of cellophane. Slight modifications of this procedure were required for staining some special areas; these will be described at the appropriate place.

Most specimens were reared until the digestive organs had formed and were then dissected after fixation with 10 per cent. formalin. Some specimens were sectioned, after fixation in Zenker's solution without acetic acid and dehydration with dioxan, to study microscopically the exact position of the stain.

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Anatomical and histological observations

Anatomical and histological features of the development of the digestive organs in the present species tally on the whole with the classical description by Goette (1875) of the embryo of *Bombinator igneus*, but there are a few points of considerable difference. It is desirable, therefore, to present a brief sketch of the phenomena in our species, with definitions of the terms to be applied, before describing the results of vital staining.

We begin with the endoderm of the neurula in stage 14 (after Tahara's (1959) table for *R. japonica*). The archenteron may be divided, as was done by Goette, into three parts, i.e. fore-gut, mid-gut, and hind-gut (Text-fig. 1A, B). The fore-gut lies under the brain region of the neural plate and in front of the yolk mass. It expands considerably both laterally and ventrally. It has also an antero-ventral outpocketing just below the transverse neural fold and a postero-ventral outgrowth beneath the anterior end of the yolk mass, the former usually termed the ‘oral evagination’ and the latter the ‘liver diverticulum’. Adjacent to the posterior end of the latter is a remnant of the blastocoel. The partition separating it from the liver diverticulum is formed of a single layer of yolk cells.

The wall of the fore-gut is one cell thick throughout, except at its posterior side. For convenience of description we adopt a sixfold division of the wall—anterior, posterior, dorsal, ventral, right, and left.

The anterior wall ascends postero-dorsally from the level of the transverse neural fold to that of the middle of the brain region. Posteriorly to it, the dorsal wall (the roof) lies horizontally beneath the hindbrain. On the other hand, the ventral wall (the floor) descends from the antero-dorsal edge of the oral evagination to the posterior end of the liver diverticulum. The posterior wall is identical with the dorsal wall of the latter. The lateral (right and left) walls are thin layers of the endoderm limiting the lateral expansions of the fore-gut.

The hind-gut is the hindmost portion of the archenteron descending to the posterior side of the yolk mass. The lining of the dorsal lip of the blastopore forms its dorsal wall, while that of the ventral lip makes its posterior wall.

The mid-gut is a narrow canal dorsal to the yolk mass, which connects the fore-gut with the hind-gut.

When the tail is somewhat elongated and the external gills begin to appear (stage 19), there are considerable changes in the three portions of the archenteron, which has become remarkably extended in the antero-posterior direction (Text-fig. 1c).

In the fore-gut region the pharynx is distinguishable from the rest, forming the visceral pouches on its lateral walls. The liver diverticulum and the remnant of the blastocoel become united to form a very deep pocket of the endoderm. The mid-gut is so narrowed that it is found only as a vertical slit in cross-section. The dorsal wall of the hind-gut extends posteriorly into the tail, forming the ‘post-anal gut’ under the notochord. The remnant of the blastopore, the ‘neurenteric canal’, is already obliterated and the anus is newly open.
TEXT-FIG. 1. (See opposite.)
At the stage of completion of the external gills (stage 21), each part of the digestive tract becomes fairly distinct. Text-fig. 1D is a diagrammatic illustration of the tract at this stage. The mouth opening is formed at the anterior extremity of the fore-gut. From the hind end of the floor of the pharynx develops a small saccular evagination which will give rise to the lungs and trachea. The portion of the fore-gut posterior to this evagination descends postero-ventrally as a tube of the endoderm which is referred to as the "gastro-duodenal tube". It consists of the rudiment of the oesophagus, stomach, and duodenum, but the boundary of each component is not distinguishable by external appearance. Histologically, the rudiment of the stomach is distinguished from that of the oesophagus by a difference in its glands and from that of the duodenum by the position of the opening of the bile-duct. The liver develops as a large evagination projecting antero-ventrally from the hind end of the gastro-duodenal tube. A small vesicle posterior to it is the precursor of the gall-bladder.

On the left side of the liver there is found a small evagination, the 'ventral pancreas'. In subsequent stages it is incorporated to form the pancreas with the 'dorsal pancreas', which arises from the part of the archenteric roof covering the boundary between the fore-gut and the mid-gut.

The cavity of the mid-gut is quite vestigial (Plate, fig. A), its anterior end descending ventrally to approach the remnant of the blastocoel. In the hind-gut region, there is no noticeable change other than the obliteration of the post-anal gut.

As the operculum develops and the external gills are reduced, the intestine begins to form the double coil characteristic of the frog tadpole (stage 25). Each component of the digestive system becomes quite distinct (Text-fig. 1E). In the fore-gut region the oesophagus, stomach, and the anterior part of the duodenum are distinguishable by their external appearance. The dorsal rudiment of the pancreas unites with the ventral. The bile-duct is found leading from the gall-bladder into the anterior end of the mid-gut.

The most remarkable changes take place in the mid-gut region. The archenteric cavity in this region is entirely obliterated (Plate, fig. B). The definitive cavity of the intestine is newly formed by a split in the solid mass of yolk cells.
which begins at both ends of the mid-gut and effects a junction in the middle portion (Plate, fig. c).

Histological observation of the newly opened intestine reveals that its wall is formed of one layer of columnar cells abounding in yolk granules. Held between the inner tips of these columnar cells there are also yolk cells, undifferentiated histologically and small in size. They may be identified as food cells, because similar cells are often observed degenerating in the intestinal cavity (Plate, figs. d, e). The hind-gut forms the rectal portion running antero-posteriorly across the dorsal side of the coiled intestine.

RESULTS OF VITAL STAINING

Ventral and posterior walls

Staining of the fore-gut

RF 541. The midline of the ventral wall of the fore-gut of a neurula at stage 14 was stained blue (Text-fig. 2A). Both ends of the wall were left unstained and the other walls were protected from the dye by cellophane. The specimen was dissected after the formation of the external gills (stage 20a) and the mark was found in the following areas (Text-fig. 2B): the ventral wall of the pharynx and oesophagus, the rudiments of the lung, the ventral wall of the gastro-duodenal tube, and the anterior wall of the growing liver. No sign of the dye was detected in the gall-bladder, in the ventral pancreas, or in the ventral portion of the liver.

RF 542. The original mark was placed as in RF 541, except that it reached to the posterior end of the ventral and lateral walls, i.e. into the liver diverticulum. In this case the gall-bladder, the ventral pancreas, and the ventral portion of the liver were also dyed.

RF 543. The anterior half of the ventral wall was stained. The anterior end of the stained area coincided with that of the ventral wall, while the posterior end lay near the opening of the liver diverticulum (Text-fig. 3A). A small mark was also made on the posterior wall by transmission of the dye. In the final stage of external gill formation (stage 21), there was found a long band of dye extending
from the mouth to the end of the gastro-duodenal tube (Text-fig. 3B). The liver was quite free from stain. The mark on the posterior wall was traced to the ventral wall of the intestine (perhaps duodenum).

A B

TEXT-FIG. 3. Another example of the staining of the ventral wall of the fore-gut (RF 543). A, area stained originally. B, location of the mark in stage-21 larva.

RFM 551. A piece of blue agar was applied, through an incision at the level of the remnant of the blastocoel, to the ventral surface of the endoderm (Text-fig. 4A). At the same time a red piece was inserted into the fore-gut through another incision in the oral region, to mark the greater part of the posterior wall and the anterior half of the ventral wall as well as the lateral walls connecting these two areas. At the beginning of spiral formation by the intestine (stage 23) a small portion of the blue mark lay in the gall-bladder and the posterior end of the liver, while the rest was found as a long band in the ventral wall of the duodenum and small intestine (Text-fig. 4 B, C, D).

From these results the following conclusions may be drawn:
1. The anterior half of the ventral wall of the fore-gut forms the ventral wall of the digestive tract from the mouth to the anterior half of the duodenum; details of the fates of its various parts are illustrated in Text-fig. 22.

2. The posterior half of the ventral wall of the fore-gut, i.e. the floor of the liver diverticulum, develops for the most part into the liver, its hindmost part giving rise to the gall-bladder and the ventral pancreas.

3. The posterior wall of the fore-gut incorporates the posterior end of the ventral wall to form the ventral wall of the posterior half of the duodenum.

**Dorsal wall**

A series of operations was performed on neurulae at stage 14 to investigate the dorsal and anterior walls of the fore-gut. In each specimen, an incision was made through the neural plate and its substratum. The wound was plugged with a small piece of dyed agar, which was taken out after a few minutes. By this method the staining of the endoderm could be restricted to a small area. The operated levels varied according to the specimen. The following three (RF 546-8; Text-fig. 5A) are well worth describing: the anterior end (I), the middle (II), and the posterior end (III) of the narrowed portion of the plate. The final position of the marks in stage 21 are summarized in the Table and illustrated in Text-fig. 5B, C, and D.

**Table**

Results of staining of the dorsal wall of fore-gut

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Text-figures</th>
<th>Levels marked originally</th>
<th>Final situation of the stain</th>
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<tr>
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<td>RF 547</td>
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<td>RF 548</td>
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Text-fig. 5. Staining of the dorsal wall of the fore-gut (RF 546-8). A, levels stained originally. Arrows indicate the portions in which the coloured agar was inserted. B, C, and D, situation of the mark in stage-21 larvae. A is left side view; C and D are dorsal views. The stomach is pulled out anteriorly in C.
fig. 5 B, C, D respectively. The stain was always found in the dorsal wall of the digestive tract and sometimes in the dorsal pancreas. The dorsal pancreas is derived from the endoderm just beneath the middle of the narrow portion of the plate, the gastro-duodenal tube originating from the area just anterior to it and the intestine from the region posterior to it (see Table).

Anterior and lateral walls

RF 544. A blue mark (mark 1 in Text-fig. 6A) covered originally the endoderm of the oral evagination. Another mark, red and very large (mark 2 in Text-fig. 6A), was placed posteriorly to it, covering the whole region of the dorsal and left walls of the fore-gut and the anterior part of the mid-gut as well. The specimen was dissected in the early stage of external gill-formation (stage 19). The blue stain was found restricted to the endoderm of the oral plate. The red mark not only spread over the dorsal and left walls of the pharynx, but also extended to the walls of the mid-gut (Text-fig. 6B).

RF 545. Two marks were arranged on the left wall of the fore-gut, the blue one anteriorly and the red one posteriorly (Text-fig. 7A). Both marks reached partly to the dorsal wall, so that the boundary between their dorsal portions
underlay the midbrain region of the neural plate. The specimen was reared until its external gills appeared (stage 21). The blue mark was traced to the left wall of the pharynx and the red one to the left wall of the gastro-duodenal tube (Text-fig. 7B). A portion of the latter was also detected in the dorsal pancreas. It was thus clearly shown that the boundary between the two marks coincides with the posterior end of the oesophagus.

**TEXT-FIG. 8.** Staining of the lateral wall of the fore-gut (RF 552). A, area stained originally. B, location of the mark viewed dorsally in stage-21 larva.

**RF 552.** As shown in Text-fig. 8A, the ectoderm and mesoderm on the lateral side of a neurula were incised in a L-shaped and the flap thus formed was lifted to expose the endoderm, against which dyed agar was pressed. By this method, the posterior end of the lateral wall of the fore-gut was stained from the outside. Afterwards, the stain was traced to the left side of the dorsal pancreas as well as to the lateral wall of the stomach and intestine (Text-fig. 8B).

It is seen from this result that the presumptive region of the dorsal pancreas is not restricted to the dorsal wall but extends to the lateral walls. Comparative study of the results described above leads us to the conclusion that the presumptive rudiments in the fore-gut region are located as illustrated in Text-fig. 22.

**Staining of the mid-gut**

It is well known that the greater part of the mid-gut is involved in the formation of the small intestine. The most important problem is the fate of the walls of the mid-gut at the time of the new formation of the intestinal cavity following the closure of the archenteron. The following experiments were therefore performed.

**RM 541** (Text-fig. 9). A mark was placed in the midline of the anterior part of the floor of the mid-gut. Transmission of the dye to the roof was avoided by the cellophane-cover method. After the development of the intestine (stage 24), the stain was found on the dorsal wall of the small intestine, and not on the ventral one. A part of the dye was also detected on the dorsal wall of the duodenum.

RM 542 (Text-fig. 10). A mark originally restricted to the roof of the mid-gut was traced on to the dorsal wall of the intestine and to the dorsal pancreas. In other examples, in which similar marking was done in the middle and caudal regions of the mid-gut, results were obtained in accord with those described above. All of the marks placed on the inside of the mid-gut were traced to the dorsal wall of the intestine.

TEXT-FIG. 10. Staining of the roof of the mid-gut (RM 542). A, area stained originally. B, location of the mark viewed ventrally in stage-23 larva. C, cross-section of the intestine on the same level with the broken line in B. ar, remnant of the archenteron of the mid-gut exceptionally present on the dorsal side of the true intestinal cavity.

These facts demonstrate clearly that, in consequence of the closure of the mid-gut, its floor and lateral walls join with its roof to form the dorsal wall of the definite intestinal cavity, which is newly formed beneath the floor of the mid-gut, penetrating through the mass of yolk cells. Where then does the material for the lateral and ventral walls of the intestine originate? The answer will be found in the following specimens.

RM 551 (Text-fig. 11). The ventral ectoderm of a neurula was incised together with the underlying mesoderm. Through the wound, a piece of dyed agar was inserted to stain the antero-ventral surface of the yolk mass. After the formation of the intestine (stage 24), the stain was found stretched in a line on the surface of the most anterior portion of the ventral wall of the small intestine.

RM 552 (Text-fig. 12). Through a wound made in the ecto- and mesoderm on the right side of a neurula, a piece of coloured agar was applied to the right surface of the yolk mass. When the intestine was formed (stage 24), there was found a rather long and somewhat twisted stain on the right wall of the gut, extending from the duodenum to the anterior part of the small intestine.


RM 553 (Text-fig. 13). A mark somewhat larger in size was placed on the endoderm of the side opposite to that of the preceding case. At the time of dissection it was found on the left wall of the digestive tract as a long band running from the stomach to the small intestine. It is clearly shown by these examples that the lateral and ventral portions of the yolk mass contribute to the formation of the intestinal walls of the lateral and ventral sides respectively.

On the other hand, it has been mentioned (p. 144) that the material of the ventral wall of the duodenum derives also from the posterior wall of the foregut, i.e. the roof of the liver diverticulum. The epithelium of the duodenum therefore comes from two sources. The relation of these materials in the duodenal wall is illustrated in Text-fig. 4B. The stain originally placed on the roof of the liver diverticulum occupies almost the whole thickness of the wall but shows little extension, while the other, originally placed on the ventral surface of the yolk mass, stretches as a long line on the outer surface of the wall.
Text-fig. 13. Staining of the left surface of the yolk mass (RM 553). A, area stained originally. B, location of the mark viewed ventrally in stage-23 larva.

**Opening of the intestinal cavity into the fore-gut**

As mentioned above, the archenteric cavity in the mid-gut is gradually closed up, a definitive cavity of the intestine being newly formed through the mass of yolk cells. Where is the opening of the latter into the fore-gut made? The following two examples suggest an answer to this question.

Text-fig. 14. Staining of the walls of the liver diverticulum (RMF 554). A, areas stained originally. B, location of the marks in stage-24 larva. C, cross-section of the duodenum at the level of the broken line in B. 1 and 3, red marks; 2 and 4, blue marks; mes, mesentery.

**RMF 554.** A red piece of agar was inserted into the liver diverticulum by breaking through the septum between the latter and the rudimentary blastocoel. Another piece, blue in colour, was put anteriorly in close contact with it. Consequently, both dorsal and ventral walls of the inner part of the liver diverticulum were stained with red dye (marks 1 and 3 in Text-fig. 14A) while those near the entrance of the diverticulum were dyed blue (marks 2 and 4 in Text-fig. 14A).

The specimen was examined after the formation of the definitive intestinal cavity (stage 24). The red stain was detected in the liver, pancreas, gall-bladder, and also on the ventral wall of the small intestine; on the other hand, the blue dye was found only in the pharyngeal endoderm, and not in any portion of the
stomach or intestine (Text-fig. 14 B, C). From the data already presented it may be inferred that the blue stain originated from mark 4 in Text-fig. 14A, which was placed on the ventral wall of the fore-gut. Where then is mark 2, originally placed on the posterior wall? It was so dark that it can hardly be supposed to have faded out. It must have been obliterated as the result of the degeneration of the cells containing it at the time of perforation of the surface of the yolk mass. The area occupied by mark 2 may therefore be the site where the formation of the intestinal cavity begins. This conclusion is in quite good harmony with the fact that the mark on the anterior edge of the yolk mass was found on the dorsal wall of the anteriormost part of the small intestine (see Text-fig. 9B), whereas mark 1 in Text-fig. 14A was displaced to the ventral wall of the same part of the tract.

**TEXT-FIG. 15.** Staining of the walls of the liver diverticulum (RMF 555). A, semi-diagrammatic representation of the posterior half of the fore-gut of the neurula viewed anteriorly, showing the areas stained originally, B, part of the duodenum and the most anterior part of the small intestine cut off from stage-24 larva. C, cross-section of the duodenum at the level of the broken line in B. 1 and 3, blue marks; 2, red mark; *ect*, ectoderm; *fg.l*, lateral wall of fore-gut; *fg.p*, posterior wall of fore-gut; *me*, mesoderm; *mes*, mesentery.

**RMF 555.** As illustrated in Text-fig. 15A, three marks—blue, red, and blue—were arranged laterally across the posterior wall of the fore-gut. Afterwards, all of them were traced on to the ventral wall of the entrance of the intestine, keeping their original arrangement (Text-fig. 15 B, C). Such a fact demonstrates that the aperture of the intestine is formed in the lateral direction across the dorsal half of the posterior wall of the fore-gut.

**Staining of the hind-gut**

The closure of the archenteric cavity does not take place in the hind-gut region. The prospective fate of each part of the walls of the hind-gut was studied as follows.

**RH 551.** A piece of dyed agar was inserted into the cavity of the hind-gut, so that the anterior, ventral, and posterior walls were stained in their median portion. The endoderm surrounding the blastopore was also stained
(Text-fig. 16A). At the appearance of the external gills (stage 19) the dye was found not only in the roof and floor of the hindmost part of the archenteron but also in the whole region of the post-anal gut (Text-fig. 16B).

**Text-fig. 16.** Staining of the walls of the hind-gut (RH 551). A, area stained originally. B, longitudinal section of the posterior trunk and tail regions in stage-19 larva, showing the situation of the mark. ch, notochord; nt, neural tube.

*RH 552.* The stain was applied as in the preceding example. The specimen was reared until the spiral formation of the intestine had proceeded to a considerable degree (stage 24). The dye was detected mostly on the lateral walls on both sides extending from the posterior part of the small intestine to the anus. The dorsal and ventral walls were found stained only in the end portion of the rectal tube (Text-fig. 17 A–D).

**Text-fig. 17.** Another example of the same staining as shown in Text-fig. 16 (RH 552). A, situation of the mark viewed dorsally in stage-24 larva. B, right side view of the rectum cut off from the same specimen, showing the situation of the mark. C and D, cross-section of the rectum at the level of the broken lines in A, X-X' and Y-Y', respectively. mes, mesentery.

*RH 541* (Text-fig. 18). By the method of cellophane-cover, the stain was restricted to the anterior wall of the hind-gut. In the stage of external gill appearance (stage 23), the mark was located within the yolk cells in the posterior portion of the intestine, which still did not have its definitive cavity.
The following conclusions may be drawn from these results:

1. The dorsal wall of the hind-gut, i.e. the endoderm lining the dorsal lip of the blastopore, forms the dorsal wall of the tract extending from the hind end of the small intestine to the rectum.

2. The anterior wall is split along its median line (in the dorso-ventral direction) by the opening of a definitive intestinal cavity into the hind-gut, so that its main portion is divided into right and left halves and involved in the formation of the hindmost part of the intestine.

3. The ventral wall is incorporated into the formation of the ventral wall of the rectum.

4. The post-anal gut arises from the endoderm at the margin of the blastopore, for the most part from the endoderm lining the ventral lip.
Formation of the anal tube

There still remains a problem unaccounted for in the preceding description, namely, the formation of the anal tube. It is well known that in Anura the anus does not originate from the blastopore, but is newly formed by perforation of the posterior wall of the hind-gut. In the present study, the location of its rudiment in the neurula and the site where the perforation occurs were investigated by vital staining. In order to examine the final situation of the dye exactly, specimens were sectioned and observed microscopically.

**RP 552.** A rectangular mark was placed on the ventral lip of the blastopore of a neurula in the middle stage (Text-fig. 20A). Its length was twice that of the blastopore (in the dorso-ventral direction) and its width 1½ times that of the blastopore (in the lateral direction). Its ventral edge was at nearly the same level as the ventral wall of the hind-gut. Just after the closure of the neural folds it was found in the groove formed on the ventral side of the blastopore (Text-fig. 20B). On the lower end of the mark there appeared a small pit which soon became perforated to give rise to the aperture of the anus. For a brief space of time the blastopore and the anal pit were observed side by side, keeping a short distance from each other. Soon afterwards, the former was covered by the epidermis to form the neurenteric canal, and the latter alone was left open. When the tail was somewhat elongated (stage 19) the dye was found on the ectodermal wall of the anal tube and in the somites of the tail end as well as in the mesenchyme cells and epidermis of the ventral fin (Text-fig. 20C).

**RP 551.** Dorsal and lateral lips of the blastopore were stained in the middle stage of the neurula (Text-fig. 21A). When the tail had grown somewhat (stage 19) a microscopical examination was made, revealing the dye in the somites and neural tube of the tail region and also at the caudal end of the notochord. No trace of the dye was found in the anal tube (Text-fig. 21B).

These results show clearly that the posterior wall of the hind-gut is perforated...
through its ventral half to form the anus. The material for the proctodaeum is located on the ventral side of the blastopore and not on the dorsal or on the lateral side.

CONCLUSIONS

The results of the present study have been synthesized into a map of the prospective digestive organs in the neurula, shown as Text-fig. 22.

Pharyngeal endoderm. The greater part of the fore-gut is devoted to the formation of the pharynx. The oral evagination, lying just below the transverse fold, is actually the rudiment of the mouth, as its name signifies. The lateral walls on both sides give rise to the branchial pouches. It is probable that the lungs are
derived from the area of the floor ventral to the hindmost pair of the pouches. The oesophagus will arise from the boundary between both pharyngeal and gastric regions.

**Gastric endoderm.** The posterior end of the fore-gut is occupied by two presumptive rudiments, both having the shape of very large but extremely slender rings. The anterior one is that of the stomach, and the posterior one that of the anterior half of the duodenum. The uppermost part of the anterior ring which underlies the rhombencephalic region of the neural plate will form the dorsal wall of the stomach, while the lowest part which is located about the entrance of the liver diverticulum will form the ventral wall. In consequence, the intermediate parts on both sides of the fore-gut will become the lateral walls of the stomach.

**Duodenal endoderm.** The material for the duodenum is contained partly in the fore-gut and partly in the mid-gut region. The part in the fore-gut is the posterior ring mentioned above. It is at first involved in the formation of the gastro-duodenal loop and afterwards forms the anterior half of the duodenum which reaches from the end of the stomach to a level somewhat anterior to the aperture of the bile-duct. The other occupies the most anterior part of the mid-gut region, but does not coincide with the mid-gut itself as a portion of the archenteron. The mid-gut is closed up later, and the definitive cavity of the duodenum is newly perforated beneath the floor of it. Consequently, not only the roof of the mid-gut, but also the walls and even the floor are incorporated into the dorsal wall of the posterior half of the duodenum. The cells on the lateral surface of the yolk mass contribute to the formation of the lateral walls, and those of the roof of the liver diverticulum as well as those on the ventral side of the vestigial blastocoel are concerned with the formation of the ventral wall.

In the anuran tadpole the duodenum is not distinct either from the remainder of the intestine or from the pylorus of the stomach, and it is hardly possible to designate both ends of the duodenum with accuracy. In the present map the extent of the presumptive area of the duodenum is shown only approximately.

**Hepatic endoderm.** The presumptive region of the liver occupies the greater part of both lateral and ventral walls of the liver diverticulum, being bordered by the material for the duodenum. In the hindmost part of this region are situated, besides the material for the hepatic lobes, those for the ventral pancreas, gall-bladder, and bile-duct. The ventral pancreas originates from the left wall and the gall-bladder from the ventral wall. It is probable that the material for the bile-duct is closely connected with that for the gall-bladder, but the actual location of it has not yet been determined.

**Pancreatic endoderm.** The presumptive rudiment of the pancreas consists of two components, dorsal and ventral. The latter has been described above; the former is located on the dorsal and lateral walls of the archenteron in the region underlying the most anterior part of the spinal cord and forms the boundary between fore-gut and mid-gut.
Intestinal endoderm. The greater part of the endodermal cells in the mid-gut region, including those of the yolk mass, is concerned with the formation of the small intestine. As a consequence of the closure of the archenteron in this region and the new formation of the intestinal cavity, the floor of the mid-gut, incorporating the walls and roof, contributes to the formation of the dorsal wall of the intestine. The rest of the intestinal wall is derived from the other portions of the yolk mass; the lateral walls from the lateral portions and the ventral wall from the ventral portion, respectively.

The anterior opening of the intestinal cavity is formed as a horizontal slit in the dorsal half of the posterior wall of the fore-gut, just below the anterior edge of the yolk mass. On the other hand, the posterior aperture is perforated as a vertical slit along the median line of the anterior wall of the hind-gut. In the present map, the openings are connected by broken lines drawn across the yolk mass. These lines represent the prospective site of the definitive cavity of the intestine. However, neither the details of its course nor its width is known for certain. The only fact that is absolutely certain is that the intestinal cavity is newly formed through the yolk mass.

Rectal endoderm. The hind-gut is responsible for the formation of the rectum. Its roof develops into the dorsal wall of the latter, while its floor and subjacent yolk endoderm give rise to the ventral wall. The main portion of the anterior wall of the hind-gut, except for the median portion to be perforated, is involved, together with the endodermal walls on both sides, in the formation of the lateral wall of the rectum.

Post-anal endoderm. The endodermal rod in the tail, known as the ‘post-anal gut’, receives a contribution from the endoderm on the margin of the blastopore, for the most part from that lining the ventral lip. In the neurula stage the material for the post-anal gut is closely connected with that for the ventral wall of the rectum. These two materials are, however, separated later by the anus, which originates from the ectoderm of the ventral blastoporal lip.

DISCUSSION

Balinsky (1947) was the first investigator to apply the technique of local vital staining to the endoderm of the urodele neurula and to map the presumptive areas of the digestive organs. The most important of his findings is the closure of the mid-gut accompanied by the new formation of the intestinal cavity through the yolk mass. The present results show that the same is true in anuran embryos. Such a state of affairs seems to favour the old theory of Remak (1850) and to be out of harmony with the long-accepted idea of Goette (1875), who claimed the archenteric origin of the intestinal cavity. The discrepancy between these ancient authors may, however, be attributable to the different species adopted as their materials. In fact, we recorded in a previous paper (1954) an observation suggesting that the closure of the archenteron might be
much delayed in Bombinator igneus, the species used by Goette, with the result that the remainder of the mid-gut would incorporate the newly formed split in the yolk mass. In this way the new formation of the cavity is recognized as the general principle of the development of the amphibian intestine.

In spite of this agreement in principle, there are considerable differences between the present map for an anuran and Balinsky's for a urodele. In the latter, the liver diverticulum is designated as a rudiment of the digestive tract, including the stomach, the duodenum, and the foremost part of the small intestine. It goes on invaginating, according to Balinsky, deeper and deeper into the yolk mass till these primordia are shifted to their final situations. In the Anura, on the contrary, the same diverticulum is shown to be the material for the liver, as its name signifies. It was repeatedly substantiated in the present study that the greater part of the lateral and ventral walls of the diverticulum are devoted to the formation of the liver, the dorsal wall alone taking part in the formation of the duodenum. The presumptive rudiments of the stomach and of the anterior half of the duodenum were found in the fore-gut, occupying the hindmost part of the expanded wall, one-cell thick. These rudiments attain their destinations as a result of a drastic stretching and folding taking place in the anterior part of the archenteron in the course of subsequent development.

In order to throw more light on this apparent difference between Urodela and Anura, we have repeated the investigation on Japanese species of urodele. The results, to be published shortly, were very similar to those obtained in the present study on an anuran.

SUMMARY

1. The topography of the presumptive rudiments of the endodermal organs in the neurula of R. nigromaculata nigromaculata was studied by local vital staining. The results obtained are summarized in Text-fig. 22.

2. The main portion of the fore-gut develops into the pharynx, oesophagus, stomach, and the anterior half of the duodenum.

3. The lateral and ventral walls of the liver diverticulum give rise not only to the liver, but also to the gall-bladder, the bile-duct, and the ventral pancreas. The dorsal pancreas is derived from the dorsal and lateral archenteric walls at the boundary between fore-gut and mid-gut.

4. The yolk mass subjacent to the mid-gut is the material for the small intestine and the posterior half of the duodenum. The whole endoderm originally lining the mid-gut is incorporated into the dorsal wall in consequence of the closure of the archenteron and the perforation of the definitive cavity of the intestine through the yolk mass.

5. The hind-gut mostly forms the rectum; only a small portion at the margin of the blastopore becomes the post-anal gut.
RESUME

Topographie des ébauches présomptives de l'endoderme chez la neurula des Anoures

1. La topographie des ébauches présomptives des organes endodermiques a été étudiée par des colorations vitales localisées chez Rana nigromaculata nigromaculata. Les résultats obtenus sont résumés dans la fig. 22 du texte.

2. La plus grande partie de l'enteron antérieur se développe en pharynx, œsophage, estomac et la moitié antérieure du duodénum.

3. Les parois latérales et ventrale du diverticule hépatique ne forment pas seulement le foie mais aussi la vésicule et les voies biliaires, ainsi que le pancréas ventral. Le pancréas dorsal provient des parois dorsale et latérales de l'archenteron mais à la limite entre l'enteron antérieur et moyen.

4. La masse vitelline sous-jacente à l'enteron moyen représente le matériel pour l'intestin grêle et la moitié caudale du duodénum. La totalité de l'endoderme revêtant primitivement l'enteron moyen est incorporée dans la paroi dorsale de ces mêmes segments du tube digestif en conséquence de l'oblitération de la cavité archenterique et de la perforation de la lumière intestinale définitive au travers de la masse vitelline.

5. L'enteron postérieur forme le rectum; seule une petite portion adjacente au blastopore en est dévolue au canal anal.

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REFERENCES


EXPLANATION OF PLATE

Fig. A. Cross-section of the mid-gut region, showing the archenteron just before closure. ×160.

Fig. B. Cross-section of the mid-gut region of an embryo with its archenteron completely closed. ×120.

Fig. C. Frontal section of the intestinal region, showing the new cavity appearing as a split. ×130.

Fig. D. Cross-section of the small intestine just after the new formation of its cavity, showing the columnar epithelium of the intestine and the degenerating yolk cells. ×130.

Fig. E. Cross-section of the small intestine with its cavity well defined, showing the columnar epithelium of the intestine and the degenerating yolk cells. ×120.

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