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Some Aspects of the Shortening of the Small Intestine in *Rana catesbeiana* Larvae during Metamorphosis

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ABSTRACT The shortening of the small intestine in *Rana catesbeiana* larvae during spontaneous metamorphosis was studied from anatomical, biochemical and histological aspects. The part of the small intestine which undergoes an intensive shortening, the part with a high cathepsin D activity, and the part with abundant dense granular area (DGA) with acid phosphatase activity shifted from the anterior to the posterior part of the intestine with a critical boundary at stage XIX. There seems to be a causative relationship among these three phenomena. (*Zool. Mag. 89: 176-182, 1980*)

The shortening of the small intestine accompanying histolysis of its epithelium is one of the remarkable events during amphibian metamorphosis.

Bowers (1909) described the histogenesis and histolysis of small intestinal epithelium on the light microscopic level. Bonneville (1963), Brown and Millington (1968) and Hourdry and Dauca (1977) followed these phenomena with histochemical and electronmicrographic methods. Hourdry (1972, 1974) also reported that lysosomal enzymes reached maximum levels at the climax of metamorphosis.

In the present study, the correlations among the change in the intestinal length, the activity of lysosomal enzymes and the appearance of autolytic vacuoles in epithelia have been pursued.

Materials and Methods

The bullfrog, *Rana catesbeiana* tadpoles undergoing spontaneous metamorphosis were obtained from a local animal supply center (Tokyo). They were fed on Swimmy (Nippon Pet Food Co.) and were maintained in the laboratory at 22±2°C.

The number of tadpole used through this investigation was 467 in total and its body weight and total length were 9.35 g and 10.13 cm, on the average, respectively.

Tadpoles were staged according to Taylor and Kollrors (1946). After the animals were pithed, the double-coiled small intestine between the hepato-pancreatic duct and large intestine was taken out and stretched. The part of small intestine prior to the top of the coil was defined as A in the first half and as B in the latter half. The part posterior to the top of the coil was defined as C and length of each part was measured (Fig. 1). The intestinal contents were removed by gentle pressure with forceps in Holtfreter's solution (pH 7.8).

10% (w/v) homogenate of small intestine in 0.02 M Tris-HCl buffer (pH 7.2) containing 0.25 M sucrose was prepared as the enzyme sample.

Cathepsin D was assayed according to the method of Barrett (1967) using acid denatured bovine hemoglobin (Sigma, Type II) as a substrate. The incubation mixture consisted of 0.25 ml of 1.0 M formate buffer (pH 3.0), 0.5 ml of 3% (w/v) substrate solution and 0.25

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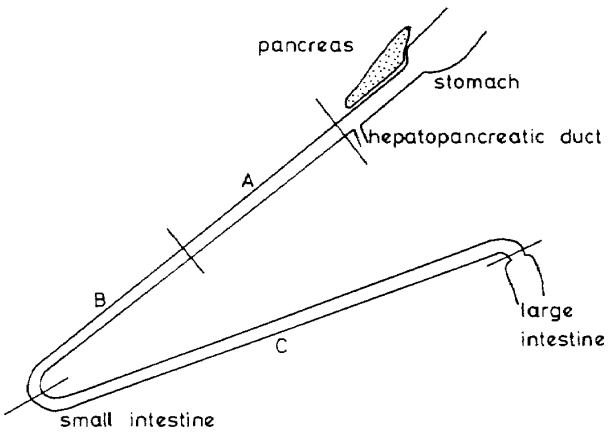

Fig. 1. Schematic diagram of the mode of dissection of small intestine. The part of intestine anterior to the top of the coil was dissected into two equal parts: A and B.

m*l* of enzyme sample (0.2-0.5 mg protein/m*l*). After the incubation for 30 min at 37°C, the reaction was terminated by the addition of 5 m*l* of 3% trichloroacetic acid (TCA) and then filtered. The filtrates were processed through the Folin-Lowry reaction and the products were measured colorimetrically by Hitachi model 124 spectrophotometer at 700 nm. Acid phosphatase was assayed by a modification of Lowry's method (1957). The reaction mixture consisted of 2 m*l* of 0.1 M citrate buffer (pH 5.0), 0.5 m*l* of DW, 0.5 m*l* of the enzyme sample and 1 m*l* of 0.003 M p-nitrophenylphosphate. After 30 min incubation at 37°C, the reaction was terminated by the addition of 1 m*l* of 20% TCA, then filtered. Saturated Na_2CO_3 was added to the filtrate. The developed colour was measured at 420 nm. Protein concentrations were estimated by the method of Lowry *et al.* (1951).

For routine histological observations, the intestinal tissues were fixed with Bouin's fluid, embedded in paraffin and cut 4 μm thick. The sections were stained with glychemalun and periodic acid-Schiff (PAS) reaction (Mowry, 1958). Histochemical detection of acid phosphatase was carried out according to the method of Gomori (1950) using β-glycerophosphate as a substrate.

Results

I. Shortening of small intestine during metamorphosis

The length of small intestine increased from stage V to XVIII. However, after stage XIX, it diminished in length. It came to one eighth of its maximum length after metamorphosis (stage XXV) (Fig. 2).

Accompanying the shortening, the position of the small intestine in the body shifted from left (to stage XX) to center (stage XXII) and then to the right side (stage XXIV) as shown in Table 1.

When the ratio of the length of the anterior (A+B) to the posterior (C) part of

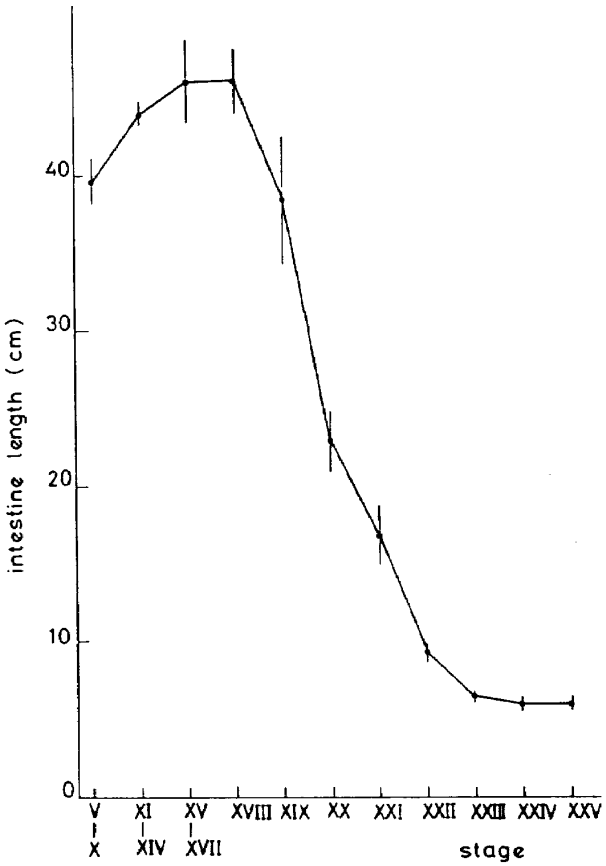

Fig. 2. Intestine length during growth and metamorphosis. The numbers of tadpoles used were 32 (stage V-X), 51(XI-XIV), 25(XV-XVII), 23(XVIII), 10(XIX), 19(XX), 6(XXI), 12(XXII), 11(XXIII), 13(XXIV), and 21(XXV), respectively.

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Table 1. Position of small intestine in the body

Stage	Position
-XX	left
XXI	left-center
XXII	center
XXIII	center-right
XXIV-XXV	right
adult	right

the small intestine (see Fig. 1) was plotted against the stage, the curve descended from stage XVIII to XIX and ascended from stage XIX to XXII (Fig. 3). This means that the shortening in the anterior part from stage XVIII to XIX is more prominent than that in the posterior part, and that after stage XIX, the shortening in the anterior part is not as prominent as that of the posterior part. In other words, the main locus of intestinal shortening shifted from the anterior to the posterior part with a boundary at stage XIX, i.e. directly prior to the onset of climax of metamorphosis.

II. Cathepsin D and acid phosphatase activities in three parts of small intestine

The specific activity of cathepsin D in the small intestine reached the maximum level at stage XVIII (2180 μg tyrosine released/hr/mg protein) and decreased thereafter.

When the small intestine was dissected into three parts as shown in Fig. 1, and the specific activity of cathepsin D in each part was measured as shown in Fig. 4, the cathepsin D activity of part A, which was the highest at stage V-XIV, decreased gradually until stage XXV. The activity of B decreased also until stage XXV except at stage XX. On the contrary, the activity of C, which was the highest at stage XX, decreased at stage XXII. It increased again until stage XXIV. This means that the major locus of cathepsin D activity in small intestine shifted from the anterior to the posterior part.

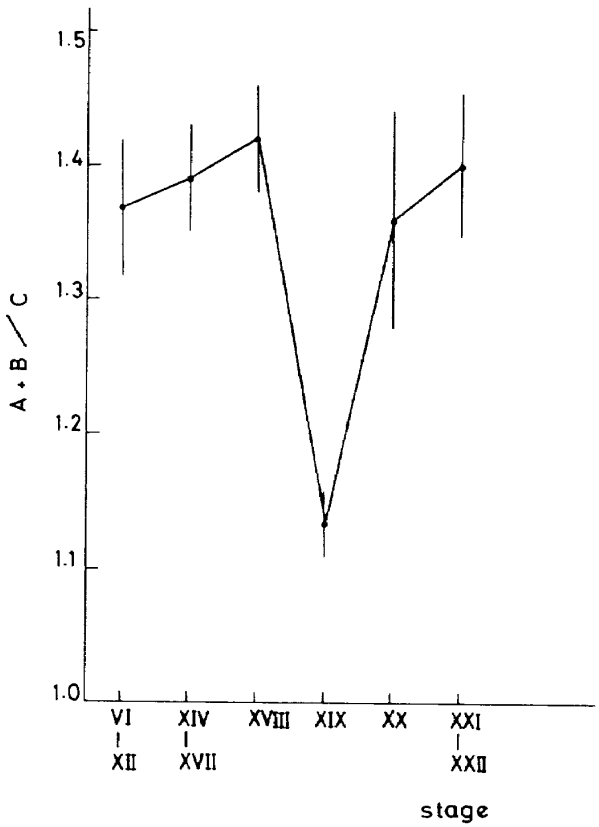

Fig. 3. Ratio of the length of anterior to posterior part of the small intestine during growth and metamorphosis. The numbers of tadpoles used were 29(stage VI-XII), 22(XIV-XVII), 6(XVIII), 4(XIX), 5(XX) and 5(XXI-XXII), respectively.

Acid phosphatase activity did not show as remarkable a change as cathepsin D did. However, the shift in the major site of activity from anterior to posterior part was clear with the progress of metamorphosis.

III. Histological examination of small intestine during metamorphosis

For histological examinations, central portions of each of small intestine pieces A, B and C were used.

With the shortening of small intestine, the mesenchymal layer became thick, while the epithelium underwent degenerative changes, shed off into the lumen and was substituted by the secondary epithelium.

Bonneville (1963) found the existence of

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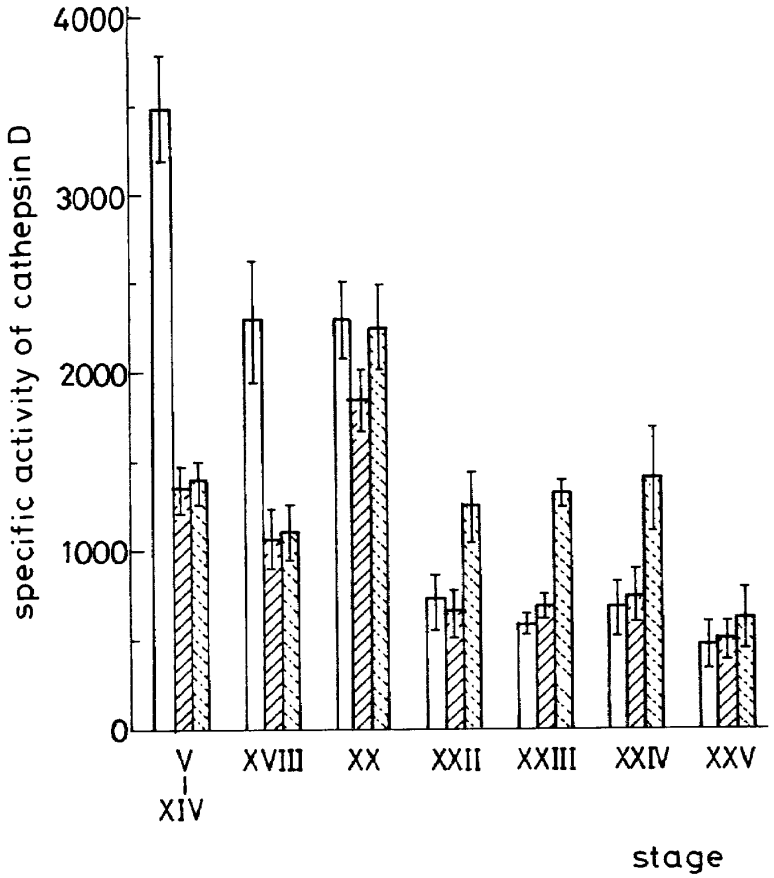

Fig. 4. Specific activity of cathepsin D of A, B and C in different stages. The numbers of tadpoles used were 15(stage V-XIV), 5(XVIII), 3(XX), 2(XXII), and 3(XXIII-XXV), respectively. The specific activity was shown in terms of μg tyrosine/hr/mg protein. ▭ A, ▨ B, ▩ C.

dense granular areas (DGA) in the intestinal epithelium of *Rana catesbeiana* larvae under the phase contrast microscope. This dense granular area can be identified as a chromophilic granular area when stained with PAS. Figure 5 represents the change in the number of DGA per cross section of each part of the small intestine during growth and metamorphosis. Prior to stage XIX, DGA is most abundant in part A (Fig. 6). However they disappeared at stage XXI (Fig. 7). After stage XIX, the most abundant part shifted to part C. At stage XX, in part C, the degeneration of epithelium containing abundant DGA was observed (Fig. 8). From stage XXI to XXII, DGA was present only in part C, but after stage XXIII, DGA could not be observed in the whole length of the small intestine. The above mentioned stages at which DGA is lost into lumen corresponded to the stages when the secondary epithelium develops.

To characterize the nature of DGA, histochemistry of acid phosphatase was tested on the sections of small intestine. DGA was positively stained by Gomori's method (Fig. 9). Controls prepared by incubating either in the presence of an inhibitor of acid phosphatase (0.01 M NaF) or in the absence of substrate were negatively stained.

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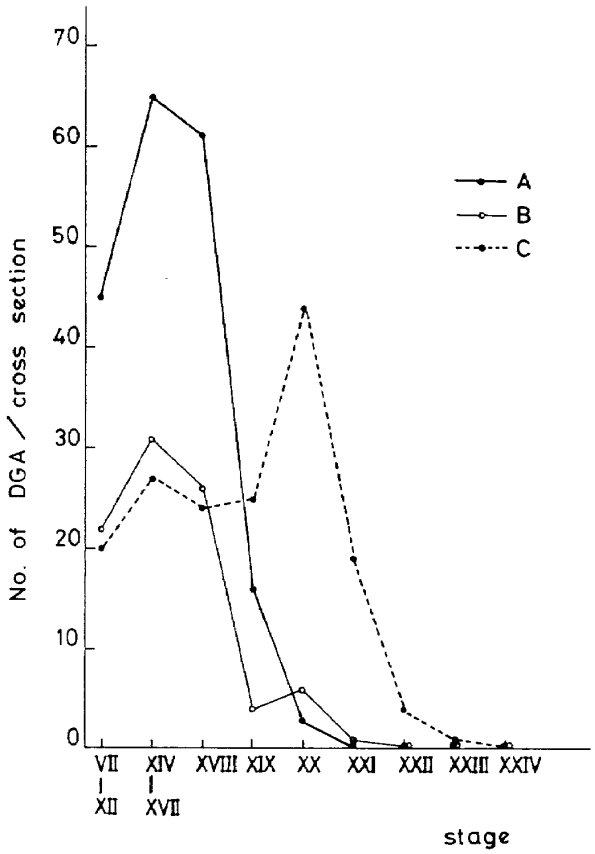

Fig. 5. Nunber of DGA per cross section in each part of the small intestine. The numbers of tadpoles used were 6(stage VII-XII), 5(XIV-XVII), 3(XVIII-XXI), 4(XXII), 3(XXIII), and 2(XXIV), respectively. The numbers of DGA were counted by the use of the hematocytometer (Bürker-Türk counting chamber).

Discussion

There was a parallelism among the three phenomena described above:

(1) localization of the shortening in intestine,
(2) catheptic activity in each part and
(3) distribution pattern of DGA in the epithelium.

The synchronous timing of these three events is probably significant. The parallelism suggests that epithelial cell degeneration is one of the causative factors in the shortening of small intestine, that cathepsin D plays some roles in this degenerative process, and that the DGA, which is lysosomal in nature, is the site of catheptic activity which plays a part in the autolysis of epithelia.

Stage XIX is a critical stage when the intestine begins to shorten (Fig. 2). At this stage, the locus of high cathepsin D activity and the locus rich in DGA shift from the anterior to the posterior part of the small intestine (Fig. 4 and 5). Simultaneously, tadpoles stop feeding and the intestinal contents begin to be eliminated.

Since acid phosphatase is a marker enzyme of lysosome (De Duve *et al.*, 1955), dense granular areas are thought to be lysosomal in nature. Cathepsin D and acid phosphatase, detected biochemically as mentioned above, both of which are lysosomal enzymes, are possibly located in these areas. These DGAs are thought to correspond to the lysosomal autolytic vacuoles in anuran intestine described by Hourdry and Dauca (1977).

Seshimo *et al.* (1977) reported that cathepsin D plays a major role in the hormone-induced regression of tadpole tail fin in culture. Sakai and Horiuchi (1979a, b) also suggested the major role of cathepsin D in the tail regression of *Rana catesbeiana* larvae. In the present study, a possible role of cathepsin D in epithelial cell death in small intestine was noted, but whether it plays a major role as in tail regression is further to be studied.

The most part of the cathepsin D activity measured in the present study is epithelial in origin. Cathepsin D activity in isolated epithelium was six times as high as that in isolated mesenchyme (Sumiya, unpublished data). This is in agreement with the distribution of DGAs which are exclusively located in the epithelium.

The trigger which initiates the shortening of intestine is thyroid hormone (Hourdry and Dauca, 1977); however, the factor which causes the regional difference in intestinal response to thyroid hormone is yet unknown.

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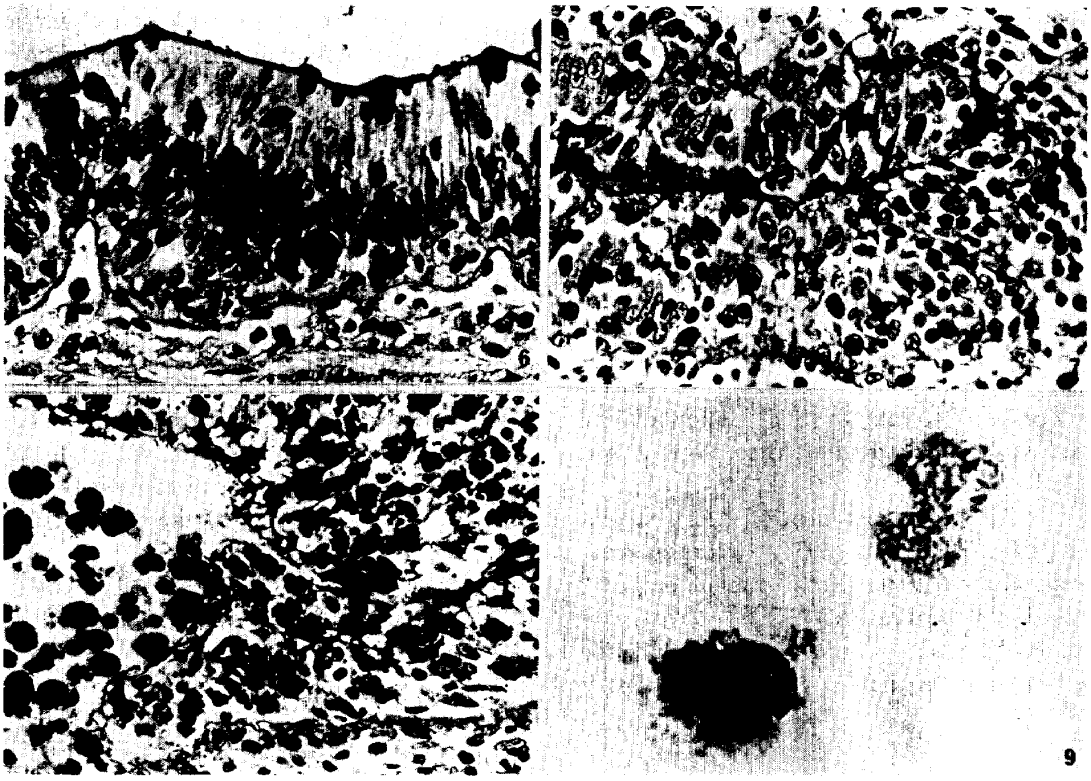

Fig. 6. Central portion of the part A of stage XVIII small intestine. Abundant chromophilic DGAs are observed. ×400.

Fig. 7. Central portion of the part A of stage XXI small intestine. Note the absence of DGA. ×400.

Fig. 8. Central portion of the part C of stage XX small intestine. Degenerating epithelium is being shed off into the lumen. ×400.

Fig. 9. Central portion of the part A of stage XVIII small intestine. DGA was visualized as an accumulation of acid phosphatase-containing granules. Stained with Gomori's method. ×1200.

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