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Light Microscopic Study of the Alligator (*Alligator mississippiensis*) Spleen With Special Reference to Vascular Architecture

YASUKAZU TANAKA[1]* AND RUTH M. ELSEY[2]
[1]*Pathological Anatomy Service, Department of Clinical Pathology, Ohme Municipal General Hospital, Tokyo, Japan*
[2]*Fur and Refuge Division, Department of Wildlife and Fisheries, Rockefeller Wildlife Refuge, Grand Chenier, Louisiana 70643*

ABSTRACT The spleen of the American alligator (*Alligator mississippiensis*) was studied histologically. The alligator spleen is a bean-shaped organ covered by a thick capsule. The concave side of the spleen faces the pancreas. Many venous vessels are present in the capsule. The stem segment of a large intestinal artery, the lieno-intestinal artery, enters the organ from its upper pole, runs within the organ at the axial center (axial artery) and leaves it from the lower pole. Many peripheral branches originate from the axial artery towards the capsule, but the artery has no associated collateral veins. The capsule/trabecula and white and red pulp may be distinguished. The capsular veins appear to be continuous with venous vessels that sheathe the axial artery and its peripheral branches. Surrounding the axial artery are trabeculae containing leiomyocytes and nerves. The white pulp consists of lymphoid tissue and occurs around terminal arterioles and sheathed capillaries. The materials examined do not show germinal centers. The large red pulp is composed of venous vessels and splenic cords rich in reticular fibers. Two venous routes, hilar and capsular, are present. The structural characteristics of the alligator spleen are similar to spleens of other reptiles; however, its vascular architecture is primitive, suggesting that the alligator spleen may be a portal spleen. J Morphol *233*:43–52, 1997.

The structure of the crocodilian spleen has been mentioned by only a few authors (Tauchi, '20; Dittmann, '69; Tanaka, in press). In this report, information supplementary to the earlier reports (Tauchi, '20; Dittmann, '69) and a summary prepared by one of the authors in a review article (Tanaka, in press) is provided. In addition, the possible significance of the alligator spleen in establishing an early extra-intestinal spleen in ancestral vertebrates is considered. The alligator spleen may be one type of portal spleen, a primitive form otherwise reported only in adult lungfishes (all three genera: Tanaka, '85a) and *Polypterus* (Tanaka, '85b).

MATERIALS AND METHODS

Spleens from wild and captive alligators (*Alligator mississippiensis*) were collected at Rockefeller Refuge in Grand Chenier, Louisiana. Four spleens were from adult males (2.0–3.2 m total length) and six were from juveniles (four females, two probable males; 0.8–0.9 m total length). The size, weight and sex of the animals (fresh samples) and splenic weights (after fixation) are listed in Table 1. After recording the size, weight and general appearance, the spleens were histologically examined. The spleens were removed from the body and were cut parallel to obtain better fixation, and fixed with 10% formalin.

The tissues were washed, dehydrated, cleared by xylene, and embedded in paraffin. Sections 3 μm thick were cut and stained with hematoxylin-eosin (HE), the periodic acid-Schiff (PAS) procedure, van Gieson (e-vG) procedure, elastica Masson's trichrome (e-Masson) procedure, the Perls' method for

*Correspondence to: Yasukazu Tanaka, M.D., Clinical Pathology Department, Ohme Municipal General Hospital, 4–16–5 Higashiohme, Ohme, Tokyo 198, Japan.

TABLE 1. S/B ratios of materials used

No.	Sex	B.L.	B.W.	S.W.	S/B ratio
1	M	208 cm	n.a.	20.6 g	—
2	M	187 cm	n.a.	17.7 g	—
3	M	215 cm	n.a.	20.1 g	—
4	M	312 cm	n.a.	85.6 g	—
5	F	83 cm	1.61 kg	1.3 g	1/1,240 (0.081%)
6	F	88 cm	2.04 kg	1.4 g	1/1,460 (0.069%)
7	M?	81 cm	1.47 kg	1.1 g	1/1,340 (0.075%)
8	F	88 cm	1.96 kg	1.2 g	1/1,630 (0.061%)
9	M?	83 cm	1.76 kg	1.1 g	1/1,600 (0.063%)
10	F	90 cm	2.07 kg	1.3 g	1/1,592 (0.063%)

No., specimen number, 1–4, wild adults; 5–10, juveniles; B.L., body length; B.W., body weight; S.W., spleen weight; n.a., data not available.

tissue iron, or the silver impregnation method for reticular fibers (Lillie, '65).

RESULTS

The weight ratio of the spleen and body

Weights of the spleen and body and the spleen/body weight ratio (S/B ratio) of the materials examined are listed in Table 1. The S/B ratio is available for only juveniles as adult alligators were not weighed. The S/B ratio of juveniles ranged from 1/1,240 (0.081%) to 1/1,630 (0.061%).

Macroscopic appearance of the alligator spleen

The exact topography of the alligator spleen is not discussed in this report because the spleens studied were removed from the body at a remote field station. Thus, further confirmation is required on its gross anatomy, particularly the vascular relationships to large intestinal vessels.

The spleens of adult *Alligator mississippiensis* are bean- or sausage-shaped, and have one pole larger than the other (Fig. 1). A fragment of pancreatic tissue is attached near the site of arterial entry (or exit). The smaller pole appears to be free from the surrounding tissue. In juveniles, both poles appear nearly equal in size. The spleen lies within the dorsal mesentery, being covered by the stomach and duodenum. Its caudal end lies close to the mesenteric root, its ventral side is slightly convex and its dorsal side is concave (Dittmann, '69). The lieno-intestinal artery (the post-gastric segment of the gastro-lieno-intestinal artery) enters the spleen from its upper pole and leaves it from the lower pole. Because the spleen is formed around a stem segment of the gastro-lieno-intestinal artery, the artery corresponding to the splenic artery of other vertebrates is absent in this species. This unique feature confirms an earlier description made on the splenic artery by Hafferl ('33). No collateral veins parallel the splenic artery; however, many larger veins lie on the splenic surface (Fig. 1). These correspond to veins histologically observed on the capsular surface (Fig. 4) and tend to gather from the convex margin to the concave side (Fig. 1). They are assumed to join to larger extrasplenic veins, which are distal segments of the hepatic portal vein. Dittmann ('69) reported a venous plexus made of v. lienalis and its branches on the convex ventral face. This venous plexus was not confirmed in the present study. No definitive splenic vein(s) was found around the spleen at the gross anatomical level.

Histology of the alligator spleen

As spleens from six juveniles were small (1.1–1.4 g, Table 1), histological analysis was made mainly on spleens from adults although juvenile samples were studied for comparison. Structurally, no essential differences were noted between spleens of juveniles and adults. The weight of the four adult spleens varied from 17.7 g to 85.6 g (Table 1). They were fixed relatively well except for the largest one (number 4), which showed advanced autolysis at the organ center. Thus the analysis was chiefly made on the remaining three samples (numbers 1–3). Quality of fixation was acceptable in all six spleens from juveniles (numbers 5–10).

Histologically, the spleen may be divided into the capsule/trabecula and the splenic pulp. The splenic pulp is further divided into lymphoid tissue (white pulp) and a vascular zone (red pulp). The density of reticular fibers differs in white and red pulp; thus, a lobular pattern is evident in splenic pulp in silver impregnated specimens (Figs. 2, 3, 7).

The capsule/trabecula

The capsule is approximately 0.5–0.7 mm or thicker in adults (Figs. 3, 6a) and 0.2–0.3 mm thick in juveniles (Figs. 5, 6b). The capsule is loosely attached to the serosa/subserosa tissue (Fig. 6a,b). Where no serosal covering is present, the capsule is continuous with either connective or fatty tissue of the mesentery. The capsule consists of two layers (Fig. 6a,b). The outer layer comprised of dense collagen fibers and a coarse net of elastic fibers is thin and indistinct in juvenile spleens (Figs. 5, 6b); however, it is a

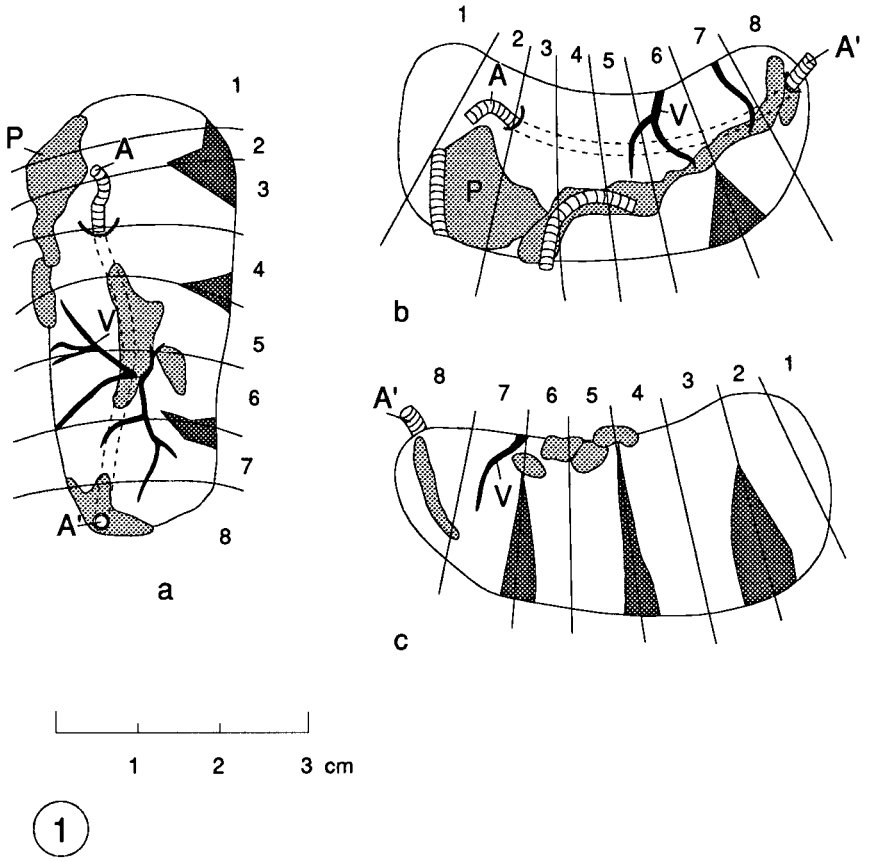


Fig. 1. *Alligator mississippiensis.* Schemata illustrating a macroscopic view of a spleen (number 2 specimen in Table 1) from three different sides. In this figure, the artery is drawn to enter the spleen from the larger pole (see text). Note presence of several veins (V) on the splenic surface. They lie on the splenic capsule (extrasplenic position) and join with capsular veins (not shown in this figure). **a**: View on the concave (dorsal) face. **b**: View from the right side. **c**: View from the left side. A, splenic artery at entry site; A′, splenic artery at exit site; P, pancreas. Numbers (1–8) indicate the number of each tissue slice used for histological examination (see Fig. 2).

distinctive layer in adult spleens (Figs. 3, 6a). The inner layer is composed of bundles of leiomyocytes, finer elastic fibers and modest amounts of collagen. Leiomyocytes are distributed rather evenly within the capsule except in areas around capsular veins.

The artery (the stem segment of the gastrointestinal artery) enters the spleen obliquely from the lateral face of the upper pole (Fig. 1). This feature is not clear in juvenile samples, wherein the artery seems to enter the spleen from the top of the upper pole. The artery reaches to the center of the spleen, runs along the splenic axis toward the opposite pole and leaves the spleen from there (Figs. 1, 2). Nerves are associated closely with the artery. Large capsular veins appear to envelop the artery at the entry site (Figs. 3, 5). They run along the axial artery in a sheath form and branch along arteries toward the periphery. The inner layer of the capsule lies peripheral to arteries and reinforces the vessels (Fig. 3). Bundles of leiomyocytes, which are unrelated to arteries and veins, occur in this reinforced area (Fig. 10). Thus, this reinforcement can be regarded as the trabecula. Unlike in mammalian spleens, trabeculae originating from the capsule independent from sites of vascular entry and coursing to the splenic center are not observed. In the trabecula, a dense net of elastic fibers encompasses the artery, separating it from other elements. Nerve fibers are also present in the trabecula (Fig. 10). As stated

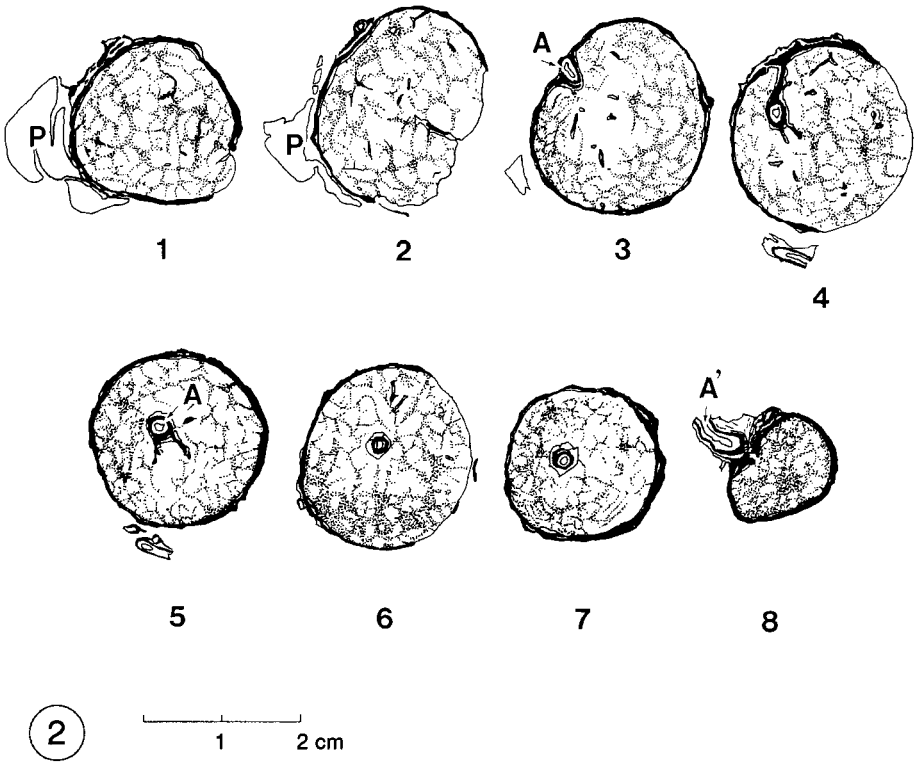


Fig. 2. *Alligator mississippiensis.* Schemata illustrating histology of tissue slices (1–8 in Fig. 1) impregnated by silver. A, splenic artery at entry site; A′, splenic artery at exit site; P, pancreas. Note that the artery (A) enters the spleen (3) and runs its axis as an axial artery and leaves the spleen from the end segment (8). A right lower corner of the spleen is not shown in 2, 4 and 6 due to prefixation cut given to ensure better fixation. A coarse network of a darker area is a venous zone of the red pulp rich in reticulin fibers (see Figs. 3, 7, 11).

earlier, the axial artery leaves the spleen from the lower pole (Fig. 1, 2). At this site, veins that anastomose with capsular veins ensheath the artery (Fig. 7). Around slit-like veins, a structure made of fine elastic fibers and leiomyocytes occurs. This may be the venous wall.

Independent from the axial artery, smaller arteries (Fig. 8) and a bundle of nerves (Fig. 9) enter the spleen through the capsule at arbitrary positions. This phenomenon is common particularly in areas where no axial artery exists, e.g., at the upper pole, or in a spleen that has a smaller axial artery.

Many arterial branches originate from the axial artery and run toward the capsule. These arterial branches have a definitive wall comprised of single-layered endothelial cells, an internal elastic lamina, leiomyocytic media and a well-developed external elastic lamina. Collagen tissue forms around the arteries and nerve fibers commonly present in this area. To the periphery, nerve fibers separate from arteries and their surroundings, and course into the red pulp being enveloped by a thin layer of collagen.

Branches of the peripheral arteries divide a few times, abruptly lose both leiomyocytic media and external elastic lamina and become terminal arteries (capillaries). Slit-like veins are observed around arteries of this level. Terminal arteries give off many sheathed capillaries (ellipsoids) (Fig. 11). From this level to the periphery, the veins course around nodular lymphoid tissue (white pulp) and the white pulp contains no veins.

The white pulp

The white pulp consists of lymphoid tissue that occurs around distal segments of terminal arteries and sheathed capillaries (Figs. 11, 12, 13). The white pulp is distributed

more densely at the subcapsular region than the organ center. Lymphoid tissue appears nodular around terminal arteries and is uniformly thick around ellipsoids (Fig. 13). Nodular lymphoid tissue formed by medium-sized lymphocytes lies around terminal arteries (Fig. 13). However, no germinal center occurs. Lymphocytes around ellipsoids are of the small type (Fig. 13).

Sheathed capillaries communicate with venous vessels of the red pulp in a closed manner (Fig. 12). Conversely, Dittmann ('69) reported that the arterial terminals end in the pulp cord in an open manner. Accumulation of cells with acidophilic granules (granulated cells) occurs around the distal segment of sheathed capillaries (Fig. 14).

The red pulp

The red pulp is wide and made of venous vessels of various sizes and a wide pulp cord (Figs. 3, 7, 11). The pulp cord is comprised of a coarse network of reticular fibers (Figs. 11, 12) and interstitial cells (Fig. 13). Extravasated erythrocytes, macrophages with iron-positive cytoplasmic inclusions (Fig. 15) and granulated cells commonly occur. As stated earlier, granulated cells are observed most frequently around terminals of sheathed capillaries. Juveniles commonly exhibit accumulation of granulated cells in both trabeculae and the capsule.

Two venous routes are distinguished in the red pulp. One is a route directed to the hilus. As stated earlier, the veins of this group ensheath the axial artery and its peripheral branches. Though they run parallel with arteries, they are not collateral veins. The other route is directed to the capsule. They are usually smaller vessels that run in the pulp cord independently from the artery (Fig. 11) and eventually join with capsular veins. These two routes probably join within the capsule, or at the hilus, although they may drain splenic blood independently to extrasplenic veins.

Plexiform veins are distinguished histologically outside of the capsule, particularly at an area adjacent to the pancreas near the site of arterial entry (Fig. 4). Capsular veins stated above end by anastomosing directly with these plexiform veins. It is likely that capsular veins of alligator spleens act as the drainage vessel because definitive splenic vein(s) are lacking.

DISCUSSION

The weight ratio of the spleen and body

The spleen/body weight ratio may be a good parameter to estimate a level of splenic differentiation (or evolution). The S/B ratio decreases with derived state in vertebrates. It is higher in cartilaginous fishes and lower in reptiles and birds (Tanaka, in press). The S/B weight ratios obtained in our alligator samples are apparently lower than those presented by Dittmann ('69) on two juveniles; 1/959 (0.10%) and 1/567 (0.18%) respectively. This difference is probably due to a difference in preparation of the materials. In the present study, tissue slices were prefixed to ensure better fixation. By this procedure, peripheral blood could be lost from the spleen resulting in a decrease in splenic weights after fixation. It has been stated that the S/B weight ratio decreases with increasing body size and reaches to 0.05% in a 99 kg sample (Coulson and Hernandez, '83).

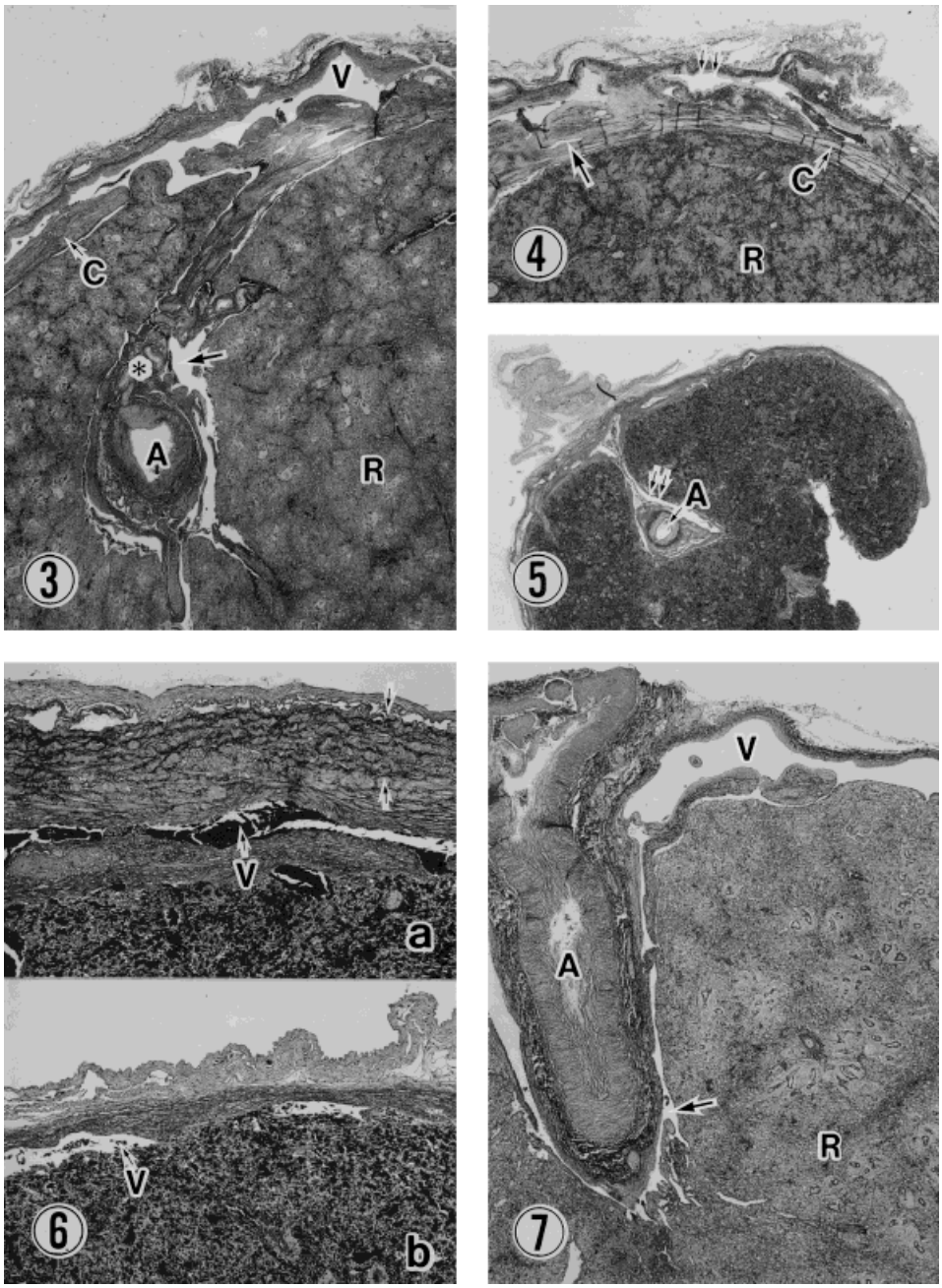
Structural characteristics

Earlier, Kanesada ('56) stated that he examined two samples of *Alligator sinensis;* however, no data were included in his report. A histological report on two juvenile spleens of *Alligator mississippiensis* (total length, 1.01 cm and 0.78 cm respectively) is that of Dittmann ('69). Structures of alligator spleens here described are essentially the same as those described by Dittman ('69); however, structures unique for alligator spleens are presented in more detail. Some features important for the alligator spleen are as follows.

First, the alligator spleen has a unique vascular architecture; the major splenic artery is the stem segment of a large intestinal artery. This feature is exceptional and not noted in spleens of other vertebrate species except the lungfish. In reptiles, the splenic artery originates as independent branches (rami lienales) from a peripheral intestinal artery in testudines (a. lieno-intestinalis; Schabadasch, '35), lizards (a. lieno-pancreatico-gastrica; Hochstetter, 1898) and snake (a. lieno-pancreatico-vesica fellea; Schabadasch, '35). In lungfishes, the spleen lies within the intestinal wall (intestinal spleen) and may be the most primitive form (Tanaka, '85a). The axial artery of the lungfish spleen is either the coeliac or mesenteric segment of the coeliaco-mesenteric artery located within the intestinal wall. The spleen is formed around these arterial segments. The

drainage vein of these intestinal spleens is actually a distal segment of the hepatic portal vein (Tanaka, '85a).

Second, the alligator spleen has no definitive splenic vein. Venous vessels occur along arterial branches. These veins drain blood toward the hilus but fail to form the splenic vein(s). As is common in other reptile spleens, two venous routes, hilar and capsular, occur in the alligator spleen. These two routes



Figures 3–7

apparently join the capsular vein, which, in turn, drains blood directly to extrasplenic veins, most probably distal segments of the hepatic portal vein. Dittmann ('69) observed a venous plexus made of v. lienalis (splenic vein) and its branches on the ventral splenic face. We are unable to confirm his observation. No definitive splenic vein was observed in our ten samples. Several venous vessels observed on the splenic surface varied in numbers in samples. These tended to gather from the convex to the concave margin (see an example in Fig. 1). Venous vessels from the spleen (mostly capsular veins) seemed to join a larger vein(s), possibly v. pancreaticolienalis, observed at the concave margin adjacent to the pancreas. The relationship of the spleen with the intestinal vasculature should be examined in a future study.

A unique architecture of splenic vessels in alligators indicates that the spleen is formed around the stem of a large intestinal artery. Absence of definitive splenic vein(s) suggests that splenic blood might be drained by capsular veins directly to the hepatic portal vein or its major branches. These features suggests that the alligator spleen may be a portal spleen (see discussion in the next section).

Phylogenetic significance of the alligator spleen

Ontogenic studies on the spleen in the lungfish, amphibians (*Megalobatrachus japonicus*) and birds (*Gallus gallus*) demonstrate three splenic forms: sinusoid, portal and separate spleens. These forms are distinguished by relation to the formation of the hepatic portal vein (Miki, '65; Saito, '84). The sinusoid spleen is a hemopoietic nest occurring around a major intestinal artery. It is made of sinusoidal veins and blood cells. Analogy of this form in adults (or larvae) is sought in the hemopoietic nest of ammocoetes typhlosole. In the next developmental stage, sinusoidal veins anastomose with each other and form the hepatic portal vein. The hemopoietic nest at this stage entirely surrounds the completed hepatic portal vein. The spleen of this stage is named the (hepatic) portal spleen. Analogy of this form in adults is sought in the intestinal spleen of the lungfish. The lungfish spleen is an intra-intestinal spleen. In contrast, most spleens of modern vertebrates are of an extra-intestinal type. An extra-intestinal type of portal spleen is rare in adults of most modern vertebrates although a close relationship between the splenic anlage and the hepatic portal vein has been described in ontogeny of fishes (*Acanthias vulgaris,* Laguesse, 1890; Maximow, '23; *Trutta fario,* Laguesse, 1890; Léon, '32; *Salmo iridens, S. solar,* Léon, '32; *Amia calva,* Brunie, '37) and amphibians (Gymnophiona, Weilacher, '33; *Megalobatrachus japonicus,* Miki, '63). An example of this stage in adults (or larvae) is *Polypterus* (Tanaka, '85b). The next stage is marked by establishment of both the splenic artery and vein(s). During this process, the hemopoietic nest (the spleen) becomes separated from the hepatic portal vein. A spleen of this type is termed a separate spleen. The separate spleen is most commonly observed in adult vertebrates. Early splenic forms (sinusoid

Fig. 3. *Alligator mississippiensis.* Splenic artery (A) (a stem segment of the intestinal artery) after entry in the splenic parenchyma. The artery is covered by connective tissue (*, trabecula) that includes nerve fibers. The trabecula is further ensheathed by dilated veins (arrow) that eventually communicate to a large vein (V) that locates outside of the capsule (C). Note presence of lobular pattern in the splenic pulp (R). e-Masson, ×7.5.

Fig. 4. *Alligator mississippiensis.* Large veins (extrasplenic, double arrows) on the capsule (C) noted near the site of arterial entry. Note presence of developed elastic fibers in the venous wall. Capsular veins (arrow) communicate with these outside veins. R, splenic pulp. e-vG, ×7.5.

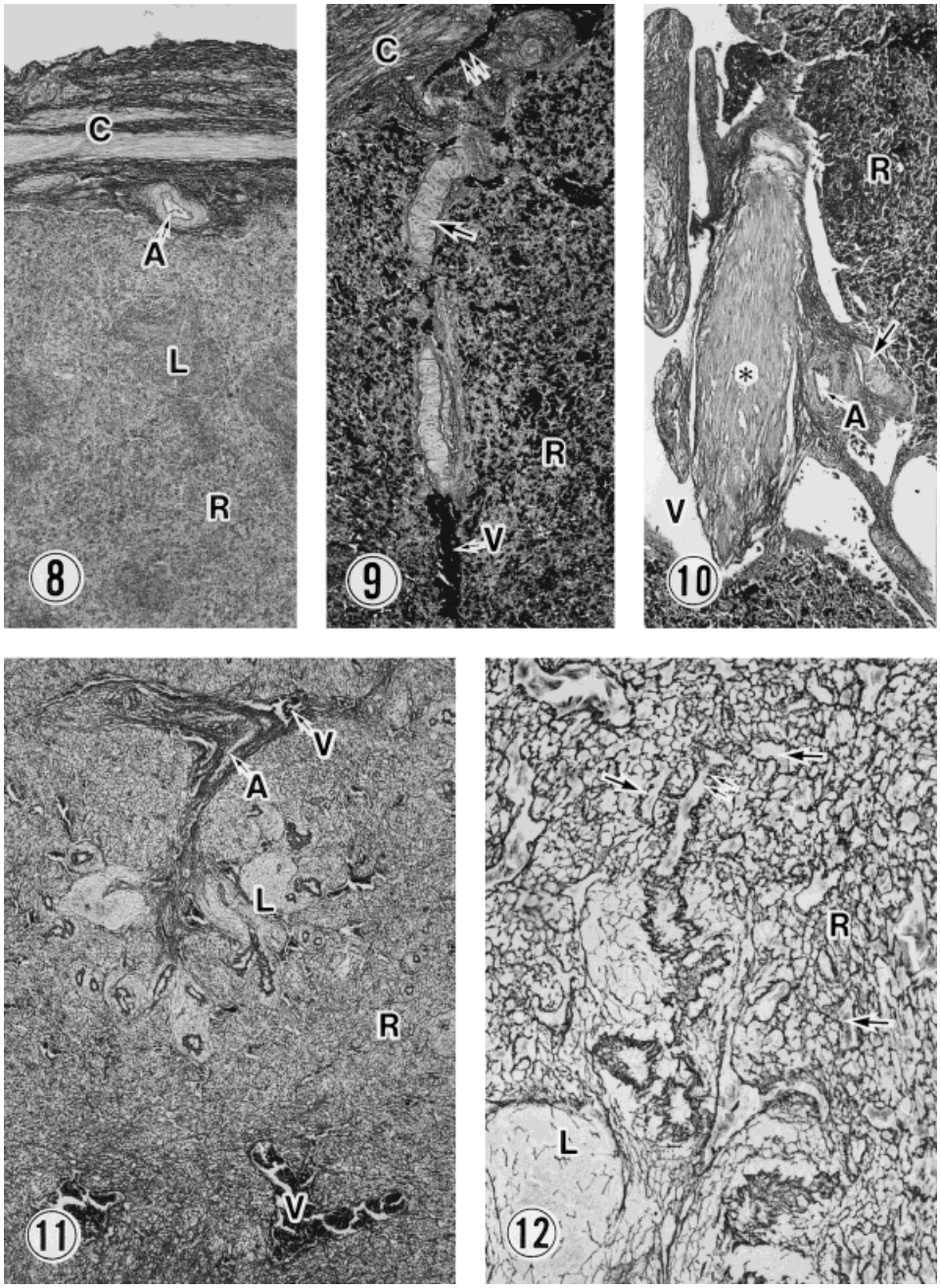
Fig. 5. *Alligator mississippiensis.* A juvenile spleen showing an axial artery (A) near the site of entry. The same magnification as Figures 3 and 4. A defective right corner is due to a cut made prior to fixation. Splenic structure is essentially the same as that of adult spleens. Note presence of a venous lumen (double arrows) around the trabecula. e-Masson, ×7.5.

Fig. 6. *Alligator mississippiensis.* **a**: Capsule of an adult spleen showing a double-layered architecture. Serosal surface on top. The outer layer (between two small arrows) is made of collagen and coarse elastic fibers and continues to the subserosal connective tissue. **b**: Capsule of a juvenile spleen. Serosal surface on top. A double-layered architecture of the capsule is not clear at this magnification. Note a loose connection between the capsule and the subserosal connective tissue. V, capsular veins. e-Masson, ×60.

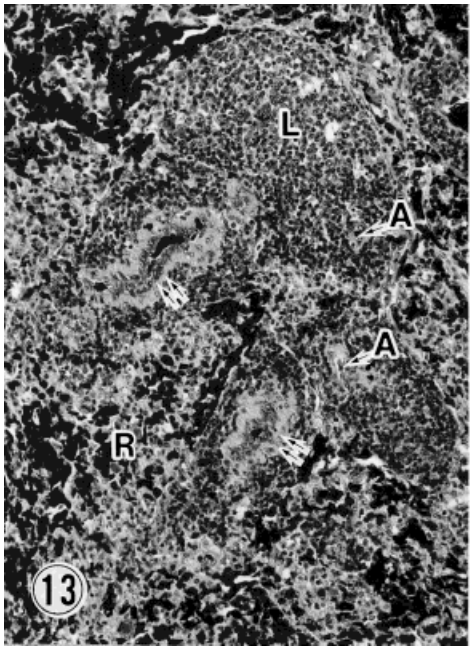
Fig. 7. *Alligator mississippiensis.* Artery (A) about to leave the spleen (corresponds to the A′ artery of Figure 1, 2). The trabecula around the artery is thin compared to that of the entry site (Fig. 3). Note presence of a slit-like vein (arrow) around the artery. This vein apparently communicates with the capsular vein (V). In the splenic pulp (R), a lobular pattern made of a lighter arterial zone and a darker venous zone is evident. This pattern is schematically shown in Figure 2. Silver impregnation, ×15.

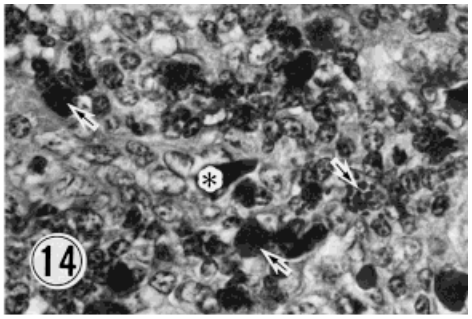
and portal spleens) occur only briefly in ontogeny of differentiated vertebrates and are nearly absent in mammalian ontogeny (Saito, '84).

The unique vascular architecture of the alligator spleen suggests that the form of the spleen of this species is primitive and might be a portal spleen. As stated earlier, the portal spleen is rare in adult vertebrates. So far, an intra-intestinal type of portal spleen has been described in three genera of living lungfishes (Tanaka, '85a) and an extra-



Figures 8–12





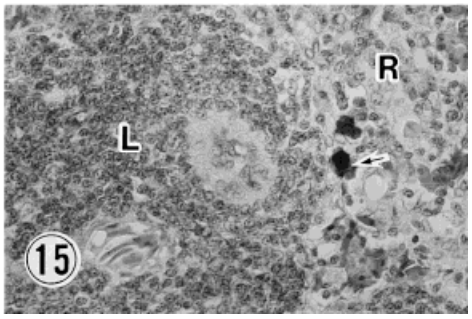


Fig. 13. *Alligator mississippiensis.* Lymphoid tissue around arterial terminals and surrounding red pulp (R). Nodular lymphoid (L) tissue is present around terminal arteries (A). Two sheathed capillaries (double arrows) are covered by a lymphocyte cuff. The surrounding red pulp is made of many venous vessels (dark areas) and interstitial cords rich in stromal cells. e-Masson, ×150.

Fig. 14. *Alligator mississippiensis.* High magnification of a distal segment of a sheathed capillary (*) showing accumulation of granulated cells (arrows). e-Masson, ×600.

Fig. 15. *Alligator mississippiensis.* Sheathed capillary with surrounding lymphoid tissue (L) and the red pulp (R). Iron-positive macrophages (arrow) are observed in the latter. Perl's stain, ×300.

intestinal type in *Polypterus* (Tanaka, '85b). The presence of this unique form in alligators contributes further understanding on early splenogenesis since the process how extra-intestinal spleens were established from the intra-intestinal form is not clearly understood at this time. Further study of this topic is warranted.

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Fig. 8. *Alligator mississippiensis.* Small artery (A, arrow) independent from the axial artery about to enter the spleen. Note absence of the slit-like vein around this small artery. C, capsule; L, lymphoid tissue; R, splenic pulp. e-vG, ×60.

Fig. 9. *Alligator mississippiensis.* Nerve fiber (arrow) independent from the axial artery entered into the splenic parenchyma. Veins (V) associate closely with this nerve. Double arrows, capsular vein; C, capsule; R, spleic pulp. e-Masson, ×60.

Fig. 10. *Alligator mississippiensis.* A bundle of leiomyocytes (*) in the trabecula. Small artery (A) and nerve bundles (arrow) are also noted in the trabecula. R, splenic pulp; V, slit-like vein. e-Masson, ×60.

Fig. 11. *Alligator mississippiensis.* Low power view of the spleen in silver impregnated specimens showing an arterial (top) and venous (bottom) zone. Presence of more reticular fibers contributes a darker appearance to the latter zone. A peripheral artery divides and gives off a terminal artery (A), which eventually extends sheathed capillaries. Slit-like veins (V) are also observed. Lymphoid tissue (L) with sparse reticular fibers occurs around both terminal arteries and sheathed capillaries. The wide red pulp (R) made of venous vessels and pulp cord rich in reticulin fibers is observed around the white pulp. In the venous area, two large drainage veins (V) are noted. Silver impregnation, ×40.

Fig. 12. *Alligator mississippiensis.* Several sheathed capillaries and red pulp (R). One of the sheathed capillaries communicates directly with a pulp vein (double arrows). Many venous vessels (arrow) are observed in the surrounding red pulp. L, nodular lymphoid tissue. Silver impregnation, ×150.

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