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COMPARATIVE STUDIES OF THE SPLEEN IN SUBMAMMALIAN VERTEBRATES*

II. MINUTE STRUCTURE OF THE SPLEEN, WITH SPECIAL REFERENCE TO THE PERIARTERIAL LYMPHOID SHEATH

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INTRODUCTION

The present work is an attepmt to give a bird's-eye view concerning the minute structure of the spleen in lower vertebrates, from Cyclostomata to Aves. In this research special attention was directed to the structural elements of the spleen which are phylogenetically of major significance, to the periarterial lymphoid tissue in particular, and to the role of spleen in hemocytopoiesis in submammalian vertebrates.

Comparative study of the spleens of lower vertebrates has been reported by many investigators, but is generally confined to some classes or orders of the vertebrate series. Much of the earlier literature has been reviewed by *Yoffey* (1928–29) who made an extensive survey of the spleen in fishes, and more recently by *Klemperer* in his article dealing with the spleens of the entire vertebrate series in *Downey*'s Handbook of Hematology (Vol. III, Sect, XXI, 1938).

As regards the role of spleen in hemocytopoiesis in lower vertebrates, a series of morphological and experimental investigations have been reported by *Jordan* and *Speidel* (1924a, 1924b, 1927–28, 1929, 1930a, and 1930b), who claim that in lower vertebrates the spleen is the chief site of production of erythrocytes and thrombocytes. The literature pertaining to this problem has been reviewed by *Jordan* in his article in *Downey*'s Handbook of Hematology (Vol. II, Sect. XII, 1938).

MATERIALS AND METHODS

The spleens of thirty species representing all the classes of the submammalian vertebrates were studied (Tables 1 and 2), including Cyclostomata.** In most in-

* A preliminary report covering certain phases of this series of studies was read by *Osogoe* at the autum meeting of the Japan Hematological Society in Tokyo, October 1953 (Symposium on Hematology, 7 : 1-35, 1954).

** As representative of Cyclostomata, *Entosphenus japonicus*, *Lampetra planeri* and *Ammocetes branchialis* (the larval stage of Lampetra) were examined.

stances they were taken from adult animals.*

The materials were generally fixed in 10% formol, *Zenker*-formol or in *Susa*, followed by the usual process of dehydration and embeddding in paraffin. Then they were sectioned serially at 5–10μ thick. The sections were divided into groups and stained by the following methods: *Mayer*'s acid hemalum and eosin or eosion azur II, *Giema*'s stain, alum-carmine, *Heidenhain*'s azan stain, *Van Gieson*'s connective tissue stain, *Weigert*'s elastic stain, *Bielschowsky-Maresch* silver method, and *Gömöri*'s method for silver impregnation of reticulum (*Gömöri*, 1937). For microchemical detection of iron the *Berlin* blue reaction was used.

The technique for vital staining of the reticulo-endothelial cells and other methods employed will be briefly described in the respective sections.

MAJOR STRUCTURAL ELEMENTS OF THE SPLEEN

Before describing the minute structure of the spleen, it was felt necessary to consider the structural elements of this organ which are phylogenetically of major significance, and to survey variations of these elements among different classes and orders or suborders.

In the present research special attention was focused upon the following elements: (1) *Periarterial lymphoid sheath (or lymphoid tissue)* which envelops the central artery. In some species of Pisces, Reptilia and Aves, lymphoid tissue also occurs surrounding the sheathed capillary (ellipsoid). Such lymphoid tissue was called "periellipsoidal lymphoid sheath" in the present research, since this was considered to correspond to the periarterial lymphoid sheath. (2) *Sheathed capillary or ellipsoid.* This is often as well developed in the spleens of lower vertebrates as in the mammalian spleens. (3) *Red pulp.* Of particular interest are the splenic cords (*Billroth*'s cords), reticular stroma, venous sinuses and blood cells, notably the immature forms. (4) *Capsule and trabeculae.* The spleen in lower vertebrates generally lacks trabeculae.

Among these, the first three were called "major structural elements" in this research and the variations in these elements among different groups of lower vertebrates will be briefly described below.

(a) *Cyclostomes.*

It is generally accepted that the mesenchymal tissue of the spiral fold of the intestine of larval Lampetra may be considered to be analogous to the spleen of higher vertebrates (*Jordan*, 1930; *Osogoe*, 1953 and 1954; *Kanesada*, 1956). As described previously, a longitudinal artery, corresponding to the central artery

* The majority of the animals were collected for study during the period from April to August. Every species obtained is indigenous to Japan.

of the spleen of higher vertebrates, runs through the entire length of the spiral fold. The artery is surrounded by a mass of densely aggregated lymphoid cells, which closely resembles the periarterial lymphoid tissue of mammalian spleen (Fig. 1). In the more peripheral portion of the spiral fold there are numerous capilliform venous sinusoids embedded in the reticular stroma. In the sections stained with hemalum and eosin, this portion takes reddish color like the red pulp of the spleen of higher vertebrates.

As outlined above, the spiral fold of larval Lampetra shows the essential elements characteristic of spleen, that is, the central artery, the periarterial lymphoid tissue and the red pulp; but these are very primitive in structure and arrangement. It should be noticed here that the blood forming tissue of the spiral fold of larval Lampetra is chiefly concerned with granulocytopoiesis, and that it disappears almost completely in the adult stage. It is also noteworthy that the sheathed capillary (ellipsoid) is not yet differentiated in the splenic tissue of the Lampetra.

Of particular interest in this connection is the spleen of a lung-fish, Calamoichty, described by *Yoffey* (1928–29). It consists of: a central artery which runs throughout the entire length of the organ, being accompanied by a vein, placed side by side; a mass of lymphoid tissue surrounding the central artery and vein; and the red pulp encircling the lymphoid tissue. It has no sheathed capillary. Thus, the spleen of Calamoichty is also very primitive in structure and arrangement, like that of larval Lampetra. Especially noteworthy is the fact that in such a spleen only a single central artery runs through the entire length of the organ and the lymphoid tissue surrounding the central artery constitutes a single, continuous mass.

b) *Elasmobranchs.*

The spleen of elasmobranchs shows much complexity in arterial ramifications. The artery divides into numerous small branches, each of which becomes surrounded by a mantle of lymphoid tissue (Figs. 2–3) and is termed the central artery. The central artery, after leaving the lymphoid tissue, enters into the red pulp and gives off many branches, which are called the penicillary arteries. A portion of the penicillary artery is surrounded by an ellipsoidal sheath which consists of reticular tissue (Figs. 4–8). For this reason it is termed the sheathed capillary or ellipsoid. The sheathed capillary then ends by opening into the pulp spaces (Fig. 6). Thus, the spleen in elasmobranchs has the three major elements —the periarterial lymphoid sheath, the ellipsoid, and the red pulp. Moreover, the periarterial lymphoid sheath and the ellipsoid are so well developed that the histological picture of the spleen closely resembles that of mammlian spleen. The red pulp contains no definite foci of immature blood cells of either the erythrocytic or the granulocytic series.

c) *Teleost fishes.*

The arterial ramifications in the spleen become more complex than in elasmobranchs. However, lymphoid tissue is generally less well developed than in elasmobranchs. In most instances, lymphoid cells are scattered diffusely in the splenic pulp and form no dense aggregations surrounding the central arteries. In contrast, ellipsoids are very well developed in the spleens of all species examined (cf. Table 1 and Fig. 9). The red pulp is more extensive than in elasmobranchs. In some marine fishes (e.g., grey mullet, *Mugil cephalus*), cell nests like erythrocytopoietic foci were found scattered in the red pulp, but there was no evidence of granulocytopoiesis in this region.

The occurrence of relatively large pigment nodules, which are round or oval in shape, brown in color and located near the central arteries, is another feature of the spleen in teleost fishes (Fig. 10). The histochemical test for iron showed that they have a high iron content. Such pigment nodules have already been described in detail by *Yoffey* (1928–29) in his extensive study of the spleen in fishes.

In some teleost fishes, e.g., *Carassius auratus*, pancreatic tissue fragments are constantly present, incorporated within the spleen (Fig. 11).

d) *Urodeles.*

Lymphoid tissue also tends to be rather diffuse in urodeles as in teleost fishes. Only in *Hynobius nigrescens* (Fig. 13) and *Hynobius tokyoensis* it tends to form definite nodules around the central arteries and ellipsoids, whereas in many others lymphoid tissue is almost indistinguishable. In contrast, ellipsoids are constantly found in the spleens of all species examined, though generally in lesser numbers than in fishes (cf. Table 1). In the giant salamander ellipsoid is especially well developed (Fig. 14). The splenic red pulp is very extensive, as in the spleen of teleost fishes. In the newt it contains difinite foci of erythrocytopoiesis (Fig. 15). It is of interest to note that the erythrocytopoietic activity in the splenic pulp seems to undergo a marked seasonal variation, because the erythrocytopoietic cells are very numerous in Spring but they become scanty in Summer (*Kanesada*, 1956). There was no evidence to indicate that the spleen of urodeles does participate in granulocytopoiesis.

e) *Anurans.*

In the spleen of anurans, lymphoid tissue shows more tendency toward formation of definite nodules, that is, periarterial lymphoid sheaths, than in the spleen of urodeles. This tendency is particularly evident in the spleen of the bullfrog (Fig. 17). No ellipsoid is discernible in the spleen in any species examined, however. The red pulp is very extensive, but it contains no definite foci of either erythrocytopoiesis or granulocytopoiesis.

f) *Reptiles.*

There are essential differences in structure between the spleens of tortoises and those of snakes.

In the spleen of tortoises, lymphoid tissue is very well developed and shows much more tendency to form definite nodules surrounding the central arteries, in comparison to the spleen of anurans. The lymphoid tissue is composed of densely aggregated small lymphoid cells around the central arteries (periarterial lymphoid sheaths) (Fig. 18). Similar lymphoid tissue also surrounds the ellipsoids, which occur very numerously and are relatively large in size (periellipsoidal lymphoid sheaths) (Figs. 19–20). This is also the case in the spleens of urodeles, teleost fishes and elasmobranchs, but to a much lesser degree. Conversely proportional to relative increase in the mass of lymphoid tissue, the red pulp become relatively narrower than in the spleens of anurans, urodeles and teleost fishes. No definite foci for production of either red cells or granulocytes are seen in this region. The spleen of lizards lacks ellipsoids.

The spleen of snakes consists of diffuse lymphoid tissue, divided into lobules by connective tissue; it has neither central artery nor red pulp (Fig. 21). Consequently, it can hardly be regarded as being homologous to mammalian spleen, and for this reason it was not included in the present research.

g) *Birds.*

In the spleen of birds, lymphoid tissue reaches its highest degree of development throughout the submammalian vertebrate series. However, the red pulp is less extensive than in the spleen of tortoises. Ellipsoids also occur very numerously and constitute an important constituent of the spleen (Figs. 22–23). As is the case in reptilian spleen, lymphoid tissue surrounds not only the central arteries but also the ellipsoids (periellipsoidal lymphoid sheaths) (Figs. 22–23). Of particular interest is the fact that lymphoid sheaths surrounding the ellipsoids occur much more numerously than those surrounding the central arteries. Similar tendency is also seen in reptilian spleen.

The most striking feature of the lymphoid tissue of avian spleen is the occurrence of secondary nodules (germinal centers) which are never seen in the spleens of reptiles and other lower vertebrates (Fig. 24).

In no case was there any trace of either erythrocytopoiesis or granulocytopoiesis in the red pulp of avian spleens.

The findings on the degree of development of the lymphoid tissue and the ellipsoid in individual species from various classes and orders or suborders of the submammalian vertebrate series are summarized in Table 1. It can be stated that both elements are essential constituents of the spleen in the majority of sub-

Table 1. Comparison of the chief structural elements of the spleen

Class	Order or suborder	Species	Lymphoid
			Periarterial lymphoid sheath
Pisces	Elasmobranchii	*Mustelus manazo*	++++
	〃	*Dasybatus akajei*	++++
	Teleostei	*Cyprinus carpio*	±
	〃	*Carassius auratus*	+
	〃	*Mugil cephalus*	+
	〃	*Sebastodes tokionis*	±
	〃	*Scomber japonicus*	+
	〃	*Lateolabrax japonicus*	±
	〃	*Sparus macrocephalus*	±
	〃	*Sillago sihama*	±
Amphibia	Urodela	*Megalobatrachus japonicus*	±
	〃	*Triturus pyrrhogaster*	±
	〃	*Hynobius lichenatus*	±
	〃	*Hynobius nigrescens*	+
	〃	*Hynobius tokyoensis*	+
	〃	*Hynobius dunni*	±
	Anura	*Bufo vulgaris japonicus*	+
	〃	*Rana nigromaculata*	±
	〃	*Rana catesbiana*	+～++
Reptilia	Chelonia	*Amyda japonica*	++
	〃	*Clemmys japonica*	++
	Lacertilia	*Eumeces latiscutatus*	++
	Ophidia	*Elaphe quadrivirgata*	−
Aves	Galliformes	*Gallus domesticus*	++++
	〃	*Coturnix coturnix japonica*	++++
	Anseriformes	*Anas platyrhynchos domestica*	++++
	Charadriiformes	*Columba livia domestica*	++
Mammalia	Rodentia	*Oryctolagus cuniculus var. domesticus*	++++

* Lymphoid tissue surrounding the sheathed capillary or ellipsoid.

in various vertebrate animals.

tissue		Sheathed capillary or ellipsoid	
Secondary nodule	Periellipsoidal lymphoid sheath*	Number per cm^2 of section	Average length and width in μ
−	+	2792	190 × 51
−	±	1609	573 × 96
−	−	++	
−	±	++	
−	±	4391	41 × 51
−	+	1742	68 × 25
−	+	++	
−	+	+++	
−	−	++	
−	±	11219	95 × 21
−	+	513	274 × 64
−	+	+	
−	+	+	
−	+	++	
−	+	+	
−	+	+	
−	−	−	
−	−	−	
−	−	−	
−	+++	2103	156 × 32
−	+++	1217	210 × 39
−	−	−	
−	−	−	
+	++	+++	
+～++	+++	6354	73 × 32
+	+++	1760	75 × 35
+	+++	3247	85 × 38
+++	−	−	

mammalian vertebrates. The degree of development of the red pulp is, roughly speeking, inversely proportional to that of the lymphoid tissue.

The red splenic pulp in lower vertebrates generally shows no evidence of either erythrocytopoiesis or granulocytopoiesis, except in the spleens of newts and of some marine teleost fishes which seem to participate in erythrocytopoiesis.

MINUTE STRUCTURE OF THE SPLEEN

1. Capsule and Trabeculae

In the submammalian vertebrates, the spleen has no trabeculae and its capsule

Table 2. Capsule and trabeculae of the spleen in various vertebrate animals.

Class	Order or Suborder	Species	Capsule			Trabeculae	Septa
			Thickness (μ)	Smooth muscle fibers	Elastic fibers		
Pisces	Elasmobranchii	*Mustelus manazo*	10-20	−	+	−	++
	//	*Dasybatus akajei*	33-66	−	−	−	−
	Teleostei	*Cyprinus carpio*	10-20	−	−	−	−
	//	*Carassius auratus*	40-47	+	−	−	−
	//	*Mugil cephalus*	10-13	+	−	−	−
	//	*Sebastodes tokionis*	3-10	−	−	−	−
	//	*Sparus macrocephalus*	7-13	+	+	−	−
Amphibia	Urodela	*Megalobatrachus japonicus*	17-40	−	−	−	−
	//	*Triturus pyrrhogaster*	7-12	−	−	−	−
	//	*Hynobius lichenatus*	10-20	+	−	−	−
	//	*Hynobius dunni*	5-10	+	−	−	−
	//	*Hynobius nigrescens*	7	+	−	−	−
	Anura	*Eufo vulgaris japonicus*	7-13	−	−	−	−
	//	*Rana nigromaculata*	10-20	−	+	−	−
	//	*Rana catesbiana*	13-23	−	+	−	−
Reptilia	Chelonica	*Amyda japonica*	10-23	++	+	−	−
	//	*Clemmys japonica*	10-47	++++	+	−	−
	Ophidia	*Elaphe quadrivirgata*	23-53	++	+	−	++
Aves	Anseriformes	*Anas platyrhynchos domestica*	23-40	++++	+	+	−
	Galliformes	*Gallus domesticus*	50-80	++++	+	−	−
	Charadriiformes	*Columba livia domestica*	23-40	−	++++	−	−
Mammalia	Rodentia	*Oryctolagus cuniculus* var. *domesticus*	33-67	−	−	++++	−

is generally thin. The connective tissue of the capsules is characterized by paucity of elastic fibers and of smooth mucle fibers. The capsule is usually covered by flat mesothelium but in some instance by cuboidal or columnar mesothelium. Of interest is the fact that the mesothelial cells of the tortoise spleen vigorously store trypan blue in their cytoplasm. A similar finding in *Phrynosoma solare*, a kind of lizard, is reported by *Jordan* and *Speidal* (1929). The findings on the capsule of the spleen in the individual species examined are summarized in Table 2.

2. *Reticular Stroma*

a) *Reticular (argyrophil) fibers.*

The framework of argyrophil fibers is generally well developed in the splenic pulp throughout the entire vertebrate series. In the spiral fold of the intestine of larval Lampetra, the argyrophil fibers radiate from the wall of the central artery and form a relatively fine network. In the spleen of elasmobranchs, these fibers themselves are slightly finer but the meshes of the network are of greater caliber than in the spiral fold of Lampetra. Of particular interest is the fact that the fibrillar framework is strikingly condensed in the ellipsoids (sheathed capillaries), especially at their peripheral boundary. Thus, in sections impregnated with silver for reticular fibers the ellipsoids are very clearly delimited (Fig. 8).

In Teleostei and Urodela, the network of argyrophil fibers in the splenic pulp shows much similarity to that in Elasmobranchii. It is notworthy, however, that the argyrophil fibers in the spleen of the giant salamander are very thick. This may be correlated with the fact that in the giant salamander the tissue and blood cells are generally much large in size than in other animals.

In Anura, Reptilia and Aves, the network of argyrophil fibers in the spleen tends to become finer, as compared with that in lower vertebrates (Fig. 16).

As already stated, the denser arrangement of argyrophil fibers within the ellipsoids is one of the most important features of these structures, not only in spleens in Elasmobranchii but also in those in higher vertebrates such as Teleostei, Urodela, Chelonia, and Aves.

b) *Reticulum cells.*

In the present research, special attention was paid to the dye-storing capacity of the fixed cells of the reticular stroma. As vital dyes, trypan blue and lithium carmine were employed.

It was first attempted to determine whether or not reticulum cells which vigorously store vital dyes are present in the splenic tissue of cyclostomes (in the spiral fold of the intestine of larval Lampetra). However, the animals did not survive the repeated injections of 1% trypan blue solution in a dose of 4 ml per

kilogram body weight for a sufficiently long period. Since they usually died within 24 hours, we failed to demonstrate dye-storing cells in the splenic and other tissues, except in the kidney in which the epithelial cells of renal convoluted tubules evidently showed dye-storing ability.

In animals higher than Pisces, we encountered no difficulties in performing repeated injections of trypan blue or lithium carmine for a sufficiently long period. Usually, 1% or 2% trypan blue aqueous solution, or 2.5% or 5% lithium carmine aqueous solution, was injected intraperitoneally or intravenously once a day in a dose of 3–4 ml per kilogram body weight. The injection was repeated 8–13 times (for 8–10 days). The animals thus treated were: crusian carps (Teleostei), newts (Urodela), frogs and bullfrogs (Anura), tortoises (Amphibia), and domestic fowls (Aves).

Histological examinations of the spleens of these animals revealed a striking fact that the fixed cells of the reticular stroma of the spleen in lower vetebrates generally show only a slight degree of dye-storing capacity. Namely, these cells did not vigorously take up the dye, except in the spleens of crusian carps and domestic fowls. Of particular interest in this connection is the finding that, in frogs, the reticulum cells scarcely take up trypan blue, whereas they show a slight degree of dye-storing ability against lithium carmine. It should be emphasized here that, in contrast with the weak storage capacity of the reticulum cells of the spleen, a striking degree of accumulation of the dyes was definitely seen in the stellate cells of *Kupffer* in the liver as well as in the epithelial cells of the renal convoluted tubules.

As an exception to this, an interesting finding should be added that the liver of the crusian carp lacks the cells corresponding to the "stellate cells of *Kupffer*", because cells capable of storing vital dye could scarcely be demonstrated in the liver, although in the splenic pulp there occurred numerous cells which vigorously take up vital dyes (Fig. 12). A detailed account of this interesting finding will be published elsewhere.

3. Arterial System

The arterial ramifications of the splenic artery in lower vertebrates may be divided into two main portions: the central artery and the penicillary artery. The penicillary artery may be further subdivided into arteriolar, sheathed, and terminal capillary portions. As mentioned already, the spleen of lower vertebrates generally lacks trabeculae. The artery corresponding to the trabecular artery enters directly into the substance of the spleen but runs therein only for a short distance and divides into branches which are called the "central arteries".

a) *Central artery.*

Because this artery is surrounded by a mantle of lymphatic tissue, it is termed

the central artery (Figs. 17–18). Already in elasmobranchs, both the central artery and the lymhatic tissue envelope surrounding it are well developed, as noted in the foregoing section. In teleost fishes, urodeles and anurans, however, lymphoid tissue shows little tendency toward formation of definite nodules surrounding the central arteries. In reptiles and birds, on the other hand, lymphoid tissue is again very well developed and forms definite nodules surrounding not only the central arteries but also the ellipsoids.

b) *Sheathed capillary (or ellipsoid).*

After emerging from the lymphoid tissue, the central artery divides into branches, called penicillary arteries, and radiates in all directions into the red pulp, constituting an arterial tree. The terminal portion of the penicillary artery become surrounded by an elliptical sheath of reticular tissue (Fig. 4). Here the vessel wall has lost its muscle layer and consists of endothel only. Since it was first described in detail by *Schweigger-Seidel* (1862, 1863), the terminal portion of the penicillary artery with an elliptical sheath is referred to in the literature as *Schweigger-Seidel*'s capillary sheath or ellipsoid (cf. *Solnitsky*, 1938 and *Snook*, 1950).

The above-menthioned arterial ramification of the splenic artery and the *Schweigger-Seidel*'s ellipsoid are well developed at the evolutional levels higher than Elasmobranchii. It is surprising that in the sting-rays ellipsoids are very well developed and their size is greatest both in length and width throughout the entire vertebrate series.*

Ellipsoids are constantly found in both cartilaginous and bony fishes, though highly variable in size and number. They also constantly occur in the spleens of Urodela among Amphibia, Chelonia among Reptilia, and Aves, whereas they are quite absent in the spleens of Anura among Amphibia, and Lacertilia and Ophidia among Reptilia. The average number of ellipsoids per cm^2 of sections and their average size in the spleens of different groups are summarized in Table 1.

In the spleens of Chelonia and Aves, both lymphoid tissue and ellipsoid are so well developed that the former tissue surrounds not only the central arteries but also the ellipsoids (Fig. 19–20, 22–23). In the present research, the lymphoid sheath which surrounds the ellipsoid is termed "periellipsoidal lymphoid sheath", in contrast to "periarterial lymphoid sheath" which surrounds the central artery.

The morphological features of the ellipsoids in the spleen of lower vertebrates are quite similar to those in mammalian spleens. The ellipsoids may be single or branched. Their general distribusion is shown in Figures 4, 5, 9, 22 and 23. They are characterized by the presence of relatively large cells with a round or

* *Snook* (1950) reported the largest ellipsoids in mammalian spleen are found in the mole spleen, which measure $218 \times 90\mu$ in length and width on the average; whereas the corresponding figures for the spleen of the sting-ray in the present study was $573 \times 90\mu$ (cf. Table 1).

oval nuclei and abundant cytoplasm* (Figs. 6, 7, 19, 22) and by an extremely close-meshed net work of reticular fibers. In sections impregnated for reticulum, therefore, the ellipsoids are very clearly delimited as seen in Figure 8.

After intravenous or intracardial injection of a larger amount of *India*-ink, there occurred a striking elective accumlation of carbon particles in the ellipsoids immediately after injection. This was particularly evident in the spleens of sting-rays (Fig. 7), crusian carps, giant salamanders, tortoises (Fig. 22) and domestic fowls (Fig. 23), in which ellipsoids are very well developed. A detailed description of these interesting findings will be published in another paper.

c) *Terminal capillary.*

In mammalian spleen, the capillary which, after having left the ellipsoid, courses for a short distance within the reticulum is termed the "terminal capillary". In the spleen of lower vertebrates, this capillary could not be demonstrated with certainty. In the spleen of sting-rays the sheathed capillary ends by opening directly into the pulp spaces (Fig. 6). In his extensive study of the spleen in fishes, *Yoffey* (1928-29) has demonstrated that the ellipsoids open directly into the spaces of the spongy pulp reticulum. In the spleen of Reptilia and Aves, the sheathed capillary also appears to terminate freely into the meshes of the reticulum (Fig. 19).

4. Venous System

The venous system commences as sinus-like splenic venules among the meshes of the reticulum. The splenic venules lead to larger collecting veins. In the spleen of elasmobranchs, the collecting vein enters into the periarterial lymphoid tissue soon after its formation, and runs throught it, being accompanied by the central artery. In the higher evolutional levels, beginning with Teleostei, the collecting veins do not enter into the lymphoid tissue but course exclusively through the red pulp, and finally converge into a single vein, the splenic vein, which accompanies the splenic artery.

The true "venous sinus" having characteristic anular fibers (cf. *Snook*, 1950) does not appear to occur in the spleen of submammalian vertebrates.

5. Lymph Vessel System

The lymph vessel system is not yet differentiated in elasmobranchs. According to *Glaser* (1933) and *Kihara* (1940), this system becomes differentiated for the first

* Definite cell boundaries are usually absent. These cells would appear, therefore, to be syncytical in nature.

time at the evolutional levels of bony fishes. However, the writer could not demonstrate the presence of lymph vessels in the spleens of bony fishes and higher vertebrates.

6. *White Pulp and Red Pulp*

In mammalian spleen, there is usualy a fairly sharp demarcation of white pulp from red pulp, the fromer being composed of lymphoid tissue and the latter of splenic cords (*Billroth*'s cords) and venous sinuses. In the spleens in some classes, orders or suborders of lower vertebrates, lymphoid tissue is very well developed and forms definite nodules surrounding the central arteries, so that the demarcation between white pulp and red pulp is also relatively sharp (e.g., in the spleens of Elasmobranchii, Chelonia and Aves); whereas in the spleens of others, lymphoid cells are scattered diffusely in the pulp without forming dense aggregations around the central arteries and, for this reason, white pulp is hardly distinguishable from red pulp (e.g., in the spleens of Teleostei, Urodela and Anura).

It should be noticed here that in the spleens of reptiles and birds, lymphoid tissue surrounds not only the central arteries but also the ellipsoids, and that lymphoid sheaths surrounding the ellipsoids predominate over those surrounding the central arteries. In the spleens of these animals, therefore, the "periellipsoidal" lymphoid tissue constitutes a greater portion of the white pulp than does the "periarterial" lymphoid tissue.

DISCUSSION

1. *Periarterial Lymphoid Sheath*

In the present study special attention was paid to the degree of development of the lymphoid tissue enveloping a central artery, the so-called "periarterial lymphoid sheath", in the spleens of different species of lower vertebrates, from Cyclostomata to Aves.

It has been demonstrated that the spleens of Elasmoblanchii has the three major structural elements—the periarterial lymphoid sheath, the ellipsoid, and the red pulp—which also constitute the essential elements of mammalian spleen. Especially notworthy is the fact that the periarterial lymphoid sheath and the ellipsoid are so well developed that the histological picture of the spleen of Elasmobranchii closely resembles that of mammalian spleen.

At the next higher evolutional levels represented by Teleostei, Urodela and Anura, the periarterial lymmphoid tissue in the spleen is greatly reduced in amount and shows less tendency of forming definite nodules; whereas the ellipsoid is fairly well developed except in the spleen of Anura.

In Reptilia and Aves, both the periarterial lymphoid sheath and the ellipsoid in the spleen are again very well developed, except in snakes in which the spleen shows a peculiar structure quite different from the spleen of other vertebrates.

It is therefore evident that both structures, together with the red pulp, constitute essential structural elements which are the homologues of the corresponding structures of mammalian spleen.

As outlined above, the lymphoid tissue surrounding a central artery, though variable in the degree of development, is of universal occurrence in the spleen throughout the vertebrate series higher than Elasmobranchii.*

At this point, reference should be made to the work of *Jordan* and *Speidel* (1924a), who believe that the spleen of elasmobranchs constitute essentially a mass of myeloid tissue. This is incorrect because in elasmobranchs granulocytopoiesis is almost completely shifted from the spleen to the stroma of gonads (*Kanesada*, 1956). As regard the spleen of Teleostei, the previous observations of *Jordan* and *Speidel* (1924a) and *Yoffey* (1928–29) agree with the present findings in that the lymphoid tissue sheaths around the central arteries, though they tend to be more diffuse in Teleostei than in Elasmobranchii, are the homologues of the periarterial lymphoid sheaths of mammalian spleen. In his extensive comparative survey of the hemopoietic loci in urodele amphibia, *Burret* (1939) reports that lymphoid tissue, though highly variable in amount and density, constantly occurs in the spleen of all the animals examined. This author is of opinion that the observed variation in the amount of lymphoid tissue in the spleen has no significance with regard to the phylogenetic position of the species examined, but may be correlated to some extent with seasonal changes in environmetal conditions.

That the lymphoid tissue enveloping the central artery constitutes an essential structural element of spleen in Anura, Reptilia and Aves, with the exception of Ophidia (snakes), seems to be indisputable. There are reported no different opinions concerning this point in the literature, except the claim of *Alder* and *Huber* (1923) who deny the presence of lymphocytes (mammalian type) in amphibians and reptiles.

2. *The Role of Spleen in Hemocytopoiesis*

In order to discuss the role of spleen in hemocytopoiesis in lower vertebrates, it is necessary to consider the variations in the chief sites of blood formation in different classes and orders or suborders of the vertebrate series.

As described in a preceding paper by *Kanesada* (1956), the spleen of cyclostomes, that is, the spiral fold of the intestine, is the sole hematopoietic organ which is chiefly concerned with granulocytopoiesis. In elasmobranchs, granulocytopoiesis

* It should be noticed here that lymphoid tissue of the spleen in lower vertebrates generally lacks germinal centers (secondary nodules), except in the case of some birds.

is to a great degree shifted to the stroma of gonads and to a lesser degree to the *Leydig*'s organ of the esophagus; and in the spleen only lymphoid tissue remains as organized blood-forming tissue. In teleost fishes, the chief loci of granulocytopoiesis are found in the interlobular spaces of kindey. In animals below teleost fishes, erythropoiesis seems to take place for the most part in the general circulation, because definite foci of erythrocyte production, comparable to those in the bone marrow of higher vertebrates, do not occur anywhere.* It is at the evolutional level of Urodela that the three hemocytopoietic tissues—erythrocytopoietic, granulocytopoietic and lymphcytopoietic tissues—become differentiated completely for the first time. At this level the erythrocytopoietic tissue occurs in the spleen, the granulocytopoietic tissue in the subcapsular layer of the liver, and the lymphocytopoietic tissue in the white pulp of spleen and in the intestinal mucosa. In the more advanced stages, represented by Anura, Reptilia and Aves, the bone marrow becomes differentiated, so that erythrocytopoiesis and granulocytopoiesis are confined to this region.

As outlined above, the chief sites of granulocytopoiesis are located outside of the spleen throughout the entire vertebrate series higher than Cyclostomata. Likewise, the spleen does not generally participate in erythrocytopoiesis, except in some species of Urodela and Teleostei.

The findings of the previous study by *Kanesada* (1956) were substantiated by the present comparative survey of the spleen in the submammalian vertebrate series. In view of the fact that the lymphoid tissue enveloping the central artery, though variable in amount and density, is of universal occurrence in the spleen throughout the vertebrate series higher than Elasmobranchii, it can be stated that the spleen is one of the chief sites of lymphocyte production not only in mammals but also in lower vertebrates.

This view is in sharp contrast with the opinion of *Jordan* and others, who maintain that in lower vertebrates the spleen is the chief site of production of erythrocytes. As regards thrombocytopoiesis, however, no detailed observations have been made in the present research except on the spleen of the sting-ray. It was revealed that among lymphoid cells, which amounted to 88.9% of all nucleated cells in the imprints of spleen, thromboblast-like cells were found only in as low percentage as below 1.0%, as reported previously by *Kanesada* (1956). This does not indicate that the spleen plays a more important role as a thrombocytopoietic organ than it does as a lymphocytopoietic organ. It should be noticed here that in lower vertebrates thrombocytopoiesis also probably takes place in the general circulation, as repeatedly claimed by previous investigators (cf. *Burret*, 1936 and *Jordan*, (1938).

* As an exception to this, it should be noticed that in some marine teleost fishes cell nests like erythrocytopoietic foci are found in the splenic red pulp.

In conclusion, it can be stated that the spleen of lower vertebrates is of minor importance with respect to erythrocytopoiesis and granulocytopoiesis, like mammalian spleen under normal physiological conditions. This is further corroborated by the splenectomy experiments in the newt, which indicated no remarkable influence upon the number of blood cells, the erythrocytes and granulocytes in particular (unpublished observations of the author). The finding of a preceding study (*Murata*, 1958) that the spleen weight is as low as below 1% of the body weight in the majority of lower vertebrates, should be emphasized in this connection.

SUMMARY

In an attempt to give a bird's-eye view concerning the minute structure of the spleen in lower vertebrates, the spleens of thirty species representing all the classes of the submammalian vertebrates, from Cyclostomata up to Aves, were examined, with special emphasis on the role of spleen in hemocytopoiesis. The chief findings are as follows:

1. An initial step in the evolution of spleen is seen in the spiral fold of the intestine of the larval lamprey, which shows the essential elements characteristic of spleen—the central artery, the periarterial lymphoid tissue, and the red pulp. However, the splenic tissue in the spiral fold of the intestine in Cyclostomata is very primitive in structure and arrangement and lacks the sheathed capillary (ellipsoid). Moreover, it disappears almost completely in the adult stage.

2. At the evolutional level of Elasmobranchii, the spleen has the three major elements—the periarterial lymphoid sheath, the ellipsoid, and the red pulp. Moreover, the periarterial lymphoid sheath and the ellipsoid are so well developed that the histological picture of the spleen closely resembles that of mammalian spleen.

3. At the next higher evolutional levels represented by Teleostei, Urodela and Anura, the periarterial lymphoid tissue in the spleen is greatly reduced in amount and shows less tendency to form definite nodules; whereas the ellipsoid is fairly well developed except in the spleen of Anura.

4. In Reptilia and Aves, both the periarterial lymphoid sheath and the ellipsoid in the spleen are again very well developed, except in snakes, in which the spleen shows a peculiar structure quite different from the spleen of other vertebrates.

5. In view of the fact that the lymphoid tissue enveloping the central artery, though variable in amount, is of universal occurrence in the spleens of lower vertebrates, it is evident that this organ is an important seat of lymphocytopoiesis throughout the entire vertebrate series, with the exception of Cyclostomata in which the spleen (spiral fold of the intestine) is chiefly concerned with granulocytopoiesis.

6. In the animals higher than Cyclostomata, that is, from Elasmobranchii up to Aves, the chief sites of granulocytopoiesis are located outside of the spleen. Likewise, the spleen does not generally participate in erythrocytopoiesis, except in some species of Teleostei and Urodela.

7. In lower vertebrates, erythrocytopoiesis and probably thrombocytopoiesis also seem to take place for the most part in the general circulation.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Transverse section through the spiral fold of the intestine of *Ammocetes barnchialis* (larval stage of *Lampetra planeri*), at the height between the liver and kidney. Hemalum and eosin azur II. × 20.

Fig. 2. Lymphoid tissue enveloping the central artery in the spleen of the sting-ray. Azan stain. × 50.

Fig. 3. Lymphoid cell aggregation enveloping the central artery in the spleen of the sting-ray. Hemalum and eosin azur II. × 200.

Fig. 4. Ramification of the central artery and the *Schweigger-Seidel*'s ellipsoids in the spleen of the sting-ray. Azan stain. × 50.

Fig. 5. *Schweigger-Seidel*'s ellipsoids in the spleen of *Mustelus manazo* (a kind of shark). Azan stain. × 20.

Fig. 6. *Schweigger-Seidel*'s sheathed capillary, opening directly into the spaces of the spongy pulp reticulum, in the spleen of the sting-ray. Azan stain. × 200.

Fig. 7. *Schweigger-Seidel*'s ellipsoids in the spleen of the sting-ray, 30 minutes after intracardial injection of a large amount of *India*-ink. Notice accumulation of cardon particules in the inner layer of the ellipsoids. Hemalum. × 200.

Fig. 8. *Schweigger-Seidel*'s ellipsoid in the spleen of the sting-ray. Silver impregnation of reticulum by *Gömöri*'s method. × 200.

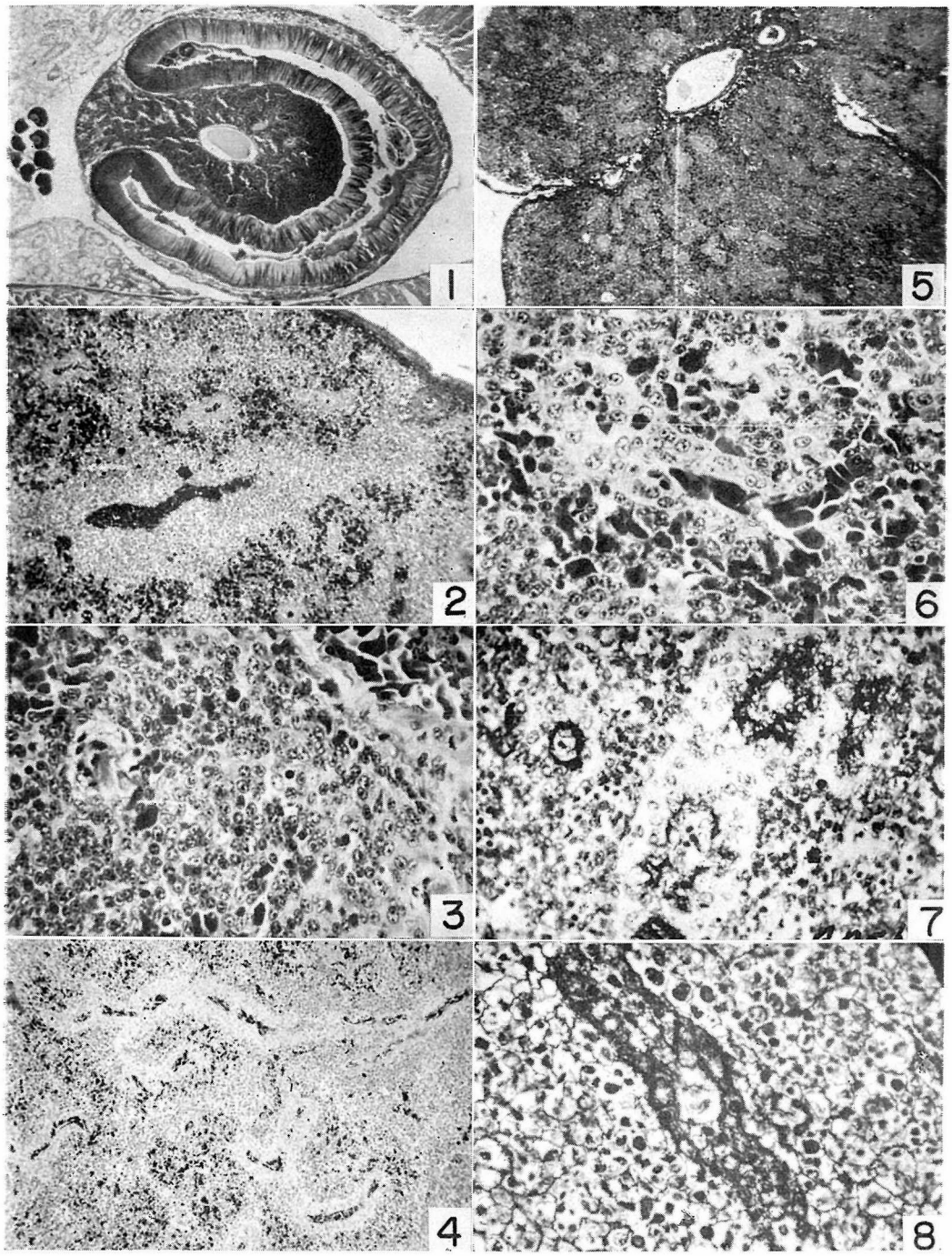
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PLATE II

Explanation of Figures

Fig. 9. *Schweigger-Seidel*'s ellipsoids in the spleen of *Sebastodes tokionio* (a teleost fish). Azan stain. × 20.

Eig. 10. Pigment nodules in the spleen of *Scomber japonicus* (a kind of mackerel). Hemalum and eosion. × 50.

Fig. 11. Spleen of the crusian carp, showing pancreatic tissue fragment incorporated within the spleen. Hemalum and eosin. × 50.

Fig. 12. Section through the liver (right), the peritoneum (center), and the spleen (left) of the crusian carp, repeatedly injected with trypan blue in order to demonstrated dye-storing cells. Notice that the liver lacks dye-storing cells, corresponding to the "stellate cell of *Kupffer*"; whereas in the spleen and peritoneum there occur cells which vigorously take up trypan blue (large dark-staining cells). Alum-carmine. × 200.

Fig. 13. Lymphoid tissue surrounding the central arteries and ellipsoids in the spleen of *Hynobius nigrescens*. Hemalum and eosin. × 50.

Fig. 14. *Schweigger-Seidel*'s ellipsoids in the spleen of the giant salamander (*Megalobatrachus japonicus*). Azan stain. × 200.

Fig. 15. Spleen of the newt (*Triturus pyrrhogaster*) in spring. Numerous dark-staining cells represent erythroblasts. Hemalum and eosin azur II. × 100.

Fig. 16. Network of reticulum fibers in the splenic pulp of the bullfrog (*Rana catesbiana*). Silver impregnation by *Gömöri*'s method. × 200.

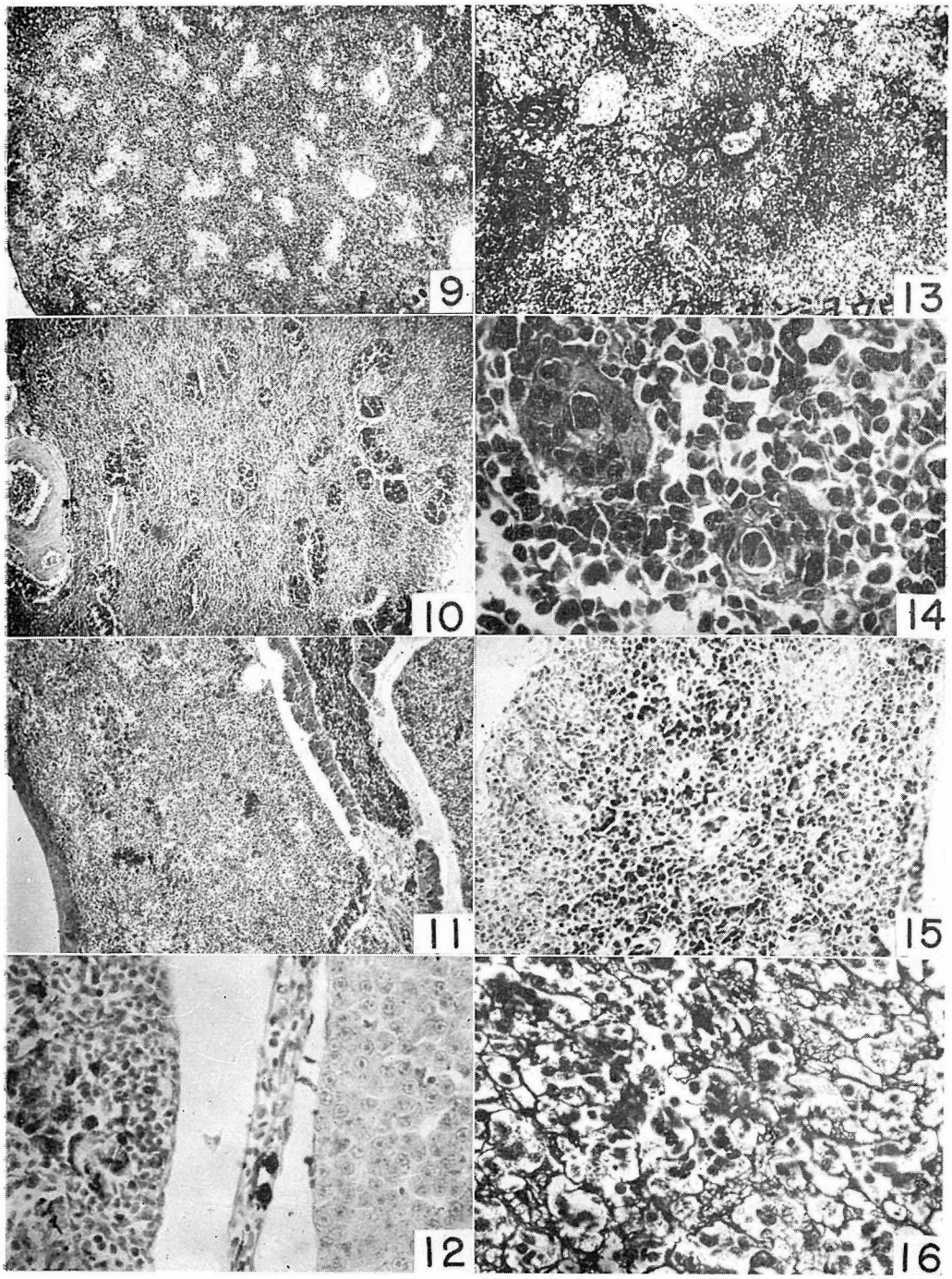
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PLATE III

Explanation of Figures

Fig. 17. Periarterial lymphoid sheath in the spleen of the bullfrog (*Rana catesbiana*), which is composed of densely aggregated lymphoid cells. Hemalum and eosin. ×200.

Fig. 18. Periarterial lymphoid sheath in the spleen of the tortoise (*Clemmys japonica*). Hemalum and eosin. ×200.

Fig. 19. Lymphoid cell aggregation around the *Schweigger-Seidel*'s ellipsoid (periellipsoidal lymphoid sheath) in the spleen of the tortoise (*Clemmys japonica*). Notice that the terminal portion of the sheathed capillary opens directly into the spaces of spongy pulp reticulum. Azan stain. ×200.

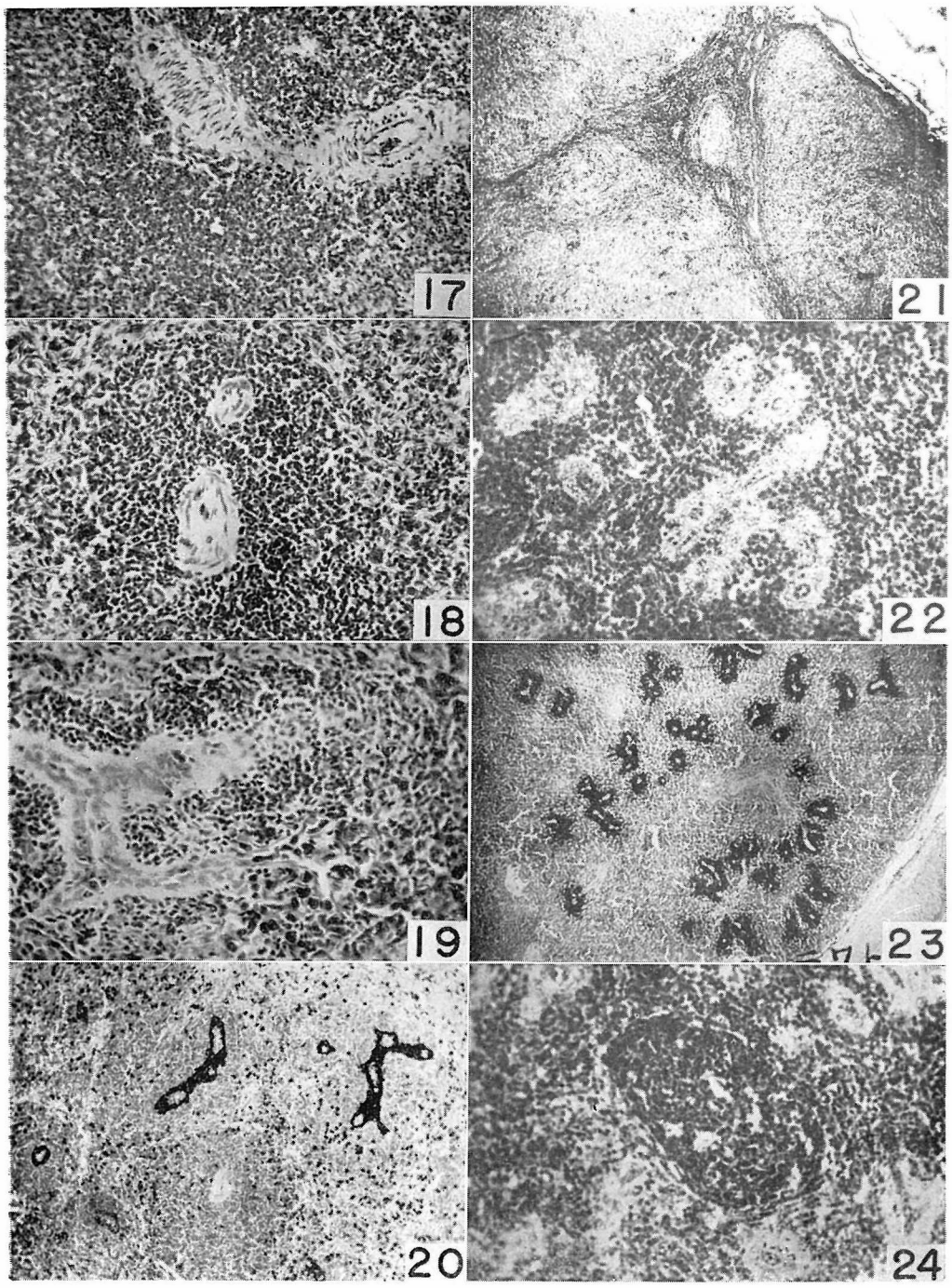
Fig. 20. *Schweigger-Seidel*'s ellipsoids in the spleen of the tortoise (*Clemmys japonica*), 30 minutes after intracardial injection of a large amount of *India*-ink. Notice striking accumulation of carbon particles in the ellipsoids. Also notice the well-developed periellipsoidal lmyphoid sheaths. Hemalum. ×50.

Fig. 21. Spleen of the snake (*Elaphe quadrivirgata*). Hemalum and eosin. ×50.

Fig. 22. *Schweigger-Seidel*'s ellipsoids in the spleen of the quail (*Coturnix coturnix japonica*). Hemalum and eosin azur II. ×200.

Fig. 23. *Schweigger-Seidel*'s ellipsoids in the spleen of the domestic fowl, 30 minutes after intravenous injection of a large amount of *India*-ink. Notice striking accumulation of carbon particules in the ellipsoids. Hemalum. ×50.

Fig. 24. Secondary nodule (germinal center) in the lymphoid tissue of the spleen of the quail (*Coturnix coturnix japonica*). Hemalum and eosin. ×200.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