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A SCANNING ELECTRON MICROSCOPY STUDY OF HUMAN SPLEEN: RELATIONSHIP BETWEEN THE MICROCIRCULATION AND FUNCTIONS

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Abstract

This paper reviews scanning electron microscope (SEM) observation of the circulatory system of the human spleen, based on our findings on freeze-cracked surfaces and vascular casts of the spleen.

Central arteries ran straight in the white pulp without branching out follicular arteries and became penicillar arteries in the red pulp. Some penicillar arteries returned to the marginal zone. Some of their branches ended by opening there, whereas others passed through the marginal zone, entered the white pulp and became follicular arteries. Some of them took the shape of an "arteriolar-capillary bundle". Most of the follicular capillaries ended opening into the marginal zone.

The penicillar arteries usually ran straight or gently curved among the sinuses of the red pulp and opened into cordal spaces. Occasional arteries formed a labyrinthine structure of arterial channels which directly connected with thin sinuses.

This study reveals that three different modes of arterial terminals are available in the human spleen: (1) arterial openings in the marginal zone which seem significant for presentation of antigens to the white pulp, (2) openings into the cords of Billroth which facilitate culling and pitting of blood cells, and (3) direct connections with sinuses (closed circulation) which account for the physiologically known quick blood flow through the spleen.

Key words: Spleen, white pulp, red pulp, marginal zone, open circulation, closed circulation, splenic function, immunology, vascular cast, circulatory regulation

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Introduction

The spleen has been said to be a mysterious organ and numerous questions remain unsolved until the present time. The splenic microcirculation is one of the most debated problems. Since Billroth (1862) introduced the concept of open and closed circulation in the human spleen, many researchers have been intensively studying the mode of arterial termination using various methods. However there has been a serious discrepancy between morphological and physiological data in this respect.

Light microscopic studies of the fixed spleens demonstrated that the open circulation occurred in the organ and suggested that it was the major route of the blood stream in the organ (Weidenreich, 1901; MacNeal et al., 1927; MacNeal, 1929; Snook, 1958). Only several researchers (Weidenreich, 1901) described that the closed circulation might also occur in the spleen. Intensive studies using transmission electron microscope of the fixed spleen confirmed the open circulation in the organ. However, they failed to demonstrate the closed circulation, i.e., the direct endothelial connection between arterioles and sinuses (Weiss, 1962, 1963; Blue and Weiss, 1981). Scanning electron microscopic (SEM) studies also confirmed the open circulation in the red pulp, however they could not find the evidence of closed circulation (Fujita, 1974; Irino et al., 1977, 1978; Suzuki et al., 1977; Fujita and Kashimura, 1981, 1983; Fujita et al., 1982, 1985).

Studies of the spleen in living state using various methods suggested that the closed circulation also occurred in this organ. Using translumination microscopy, Williams (1950) observed both fast and slow blood streams in the splenic tissue autotransplanted in the ear of rabbits. Knisely (1936) asserted that the closed circulation exclusively occurred in the unstimulated spleen. However his assertion was opposed by Mackenzie et al. (1941) who studied with the same method.

McNee (1931) and Chen (1978) injected microspheres, which passed through arterial capillaries but were not able to pass through the narrow slits of the sinus walls, into the vein. They counted the spheres in the sinuses and cords. McNee suggested that both open and closed circulation occurred in the spleen. Chen described that about 90 percent of the splenic blood flow passed through the open circulatory route and 10 percent through the closed route.

Splenic circulation determined by physiological methods such as radiolabelling blood cell kinetics in

the organ (Williams et al., 1968) and wash out kinetics of the blood cells from the organ (Levesque and Groom, 1981), showed that the majority of the red blood cells rapidly flowed out from the spleen.

These studies, except for morphological observation of the fixed organ, suggest that both open and closed circulation occur in the spleen and the majority of the intrasplenic blood flow is rapid. However, there is no evidence of morphological closed circulation which correspond to the rapid flow. Weiss (1983) proposed a concept of a physiological closed circulation. He observed that arterial capillaries ended opening in the vicinity of the sinus and that neighbouring reticulum cells and sinus endothelial cells possessed contractile elements in their cytoplasm. According to his hypothesis, when the elements contract the reticulum cells form a channel and the arterial orifice moves to the dilated slit of the sinus wall and arterial blood flows directly into the sinusal lumen as if the flow ran through a closed circulation.

Studies using vascular casts also demonstrated open circulation (Irino et al., 1977; Fujita et al., 1985). Schmidt et al. (1982) observed a small number of direct connections between arteries and sinuses in contracted dog spleen. Barnhart and Baechler (1974) and Barnhart and Lusher (1976) demonstrated that the casts of arterioles connected with those of sinuses. However, Weiss (1983) criticized that the method was not able to prove the morphological closed circulation, because physiological closed circulatory routes might take the same shape of the cast of the closed circulation.

We recently found a genuine closed circulatory system with a special structure in the red pulp of the human spleen (Kashimura, 1985). The present study further clarifies the peculiar structures representing the closed circulation sites in the human spleen by high resolution SEM observation of the tissue and resin vascular casting.

In this study we also demonstrate blood supply to the white pulp and the marginal zone. There are at least three modes of arterial termination in the human spleen. We mention the relationships between the three types of the microcirculation and splenic functions. Furthermore, we discuss a control mechanism of the splenic function.

Materials and Methods

Fifteen spleens removed from patients (both sexes, 39-75 in age) with gastric cancer could be used as organs with normal structure. Pathological spleens, including idiopathic portal hypertension or so-called Banti's syndrome (3 cases), hepatic cirrhosis (10 cases), hereditary spherocytosis (HS) (4 cases) and idiopathic thrombocytopenic purpura (ITP) (4 cases) have been observed. Pathological findings which seemed to express original functions or structures of the spleen are shown in this paper.

The human spleens were perfused with warmed Ringer solution through the splenic artery. Outflow of the perfusate was controlled by ligating the vessels at the hilus in order to keep the size of the spleen in a physiological state. Well perfused portions, where erythrocytes had been washed away, turned pale in colour and was suited for observation with SEM. Then the organ was gently perfused with 2 or 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, without monitoring the pressure because of some technical reasons. The tissue blocks were immersed in the same fixatives for several hours. The blocks were cut into small pieces measuring about 3x3x6mm and fixed according to the conduction staining method of Murakami (1974). Then they were freeze-cracked in isoamyl acetate (Tokunaga et al., 1974), critical point-dried and evaporation-coated with gold palladium.

Resin casts of the vascular bed of the spleen were formed by Murakami's method (1973). After perfusion with warmed Ringer solution, a methacrylic methyl ester mixture (Mercox CL-2R-5, Dainippon Ink Co., Ltd.) was gently injected into a branch of the splenic artery. The volume of the injected resin varied from a small to a large amount according to what vessel we wanted to observe. One of the spleens was perfused with 0.2% procaine hydrochloride just prior to injection of resin. After polymerization of the injected resin, a part of the organ was macerated with NaOH and rinsed in running water. The cast was microdissected under a stereo-microscope with a razor and was evaporation-coated with gold palladium.

Observations were made in a field emission SEM (HFS-2 Hitachi) or an HS-450 LB (Hitachi) under an accelerating voltage of 10kV. In order to visualize the three dimensional structure of the spleen or its vascular component, stereo-pairs of the SEM images were usually used.

Results

General view

The splenic pulp consists of the white pulp and the red pulp. A part of the red pulp surrounding the white pulp lacks splenic sinuses and is called the marginal zone. SEM observation at low magnification clearly visualizes the white pulp, the red pulp and the marginal zone as well as arteries supplying them on both freeze-cracked surfaces (Fig. 1) and vascular casts (Fig. 2) of the spleen.

White pulp

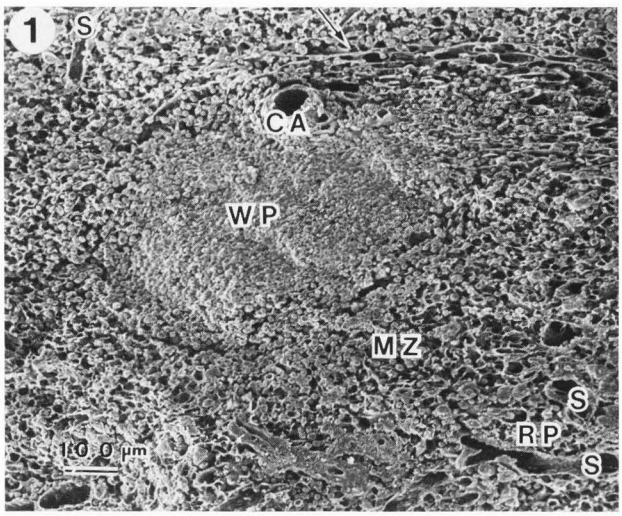
The white pulp is a lymphatic tissue composed of two parts: periarterial lymphatic sheath and lymph follicles. It is limited against the red pulp by a lamellar structure called the circumferential reticulum which comprises attenuated reticular cells and reticular fibers.

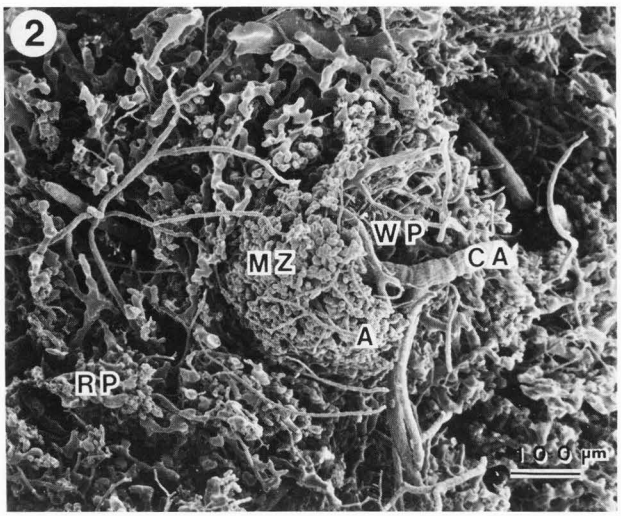
The circumferential reticulum concentrically surrounding the central artery seems to possess no pores in its wall. When follicles are developed, the central artery runs an eccentric course in the white pulp and the circumferential reticulum between the follicle and marginal zone takes the shape of meshwork and has lymphocytes attached to it (Figs. 3, 4).

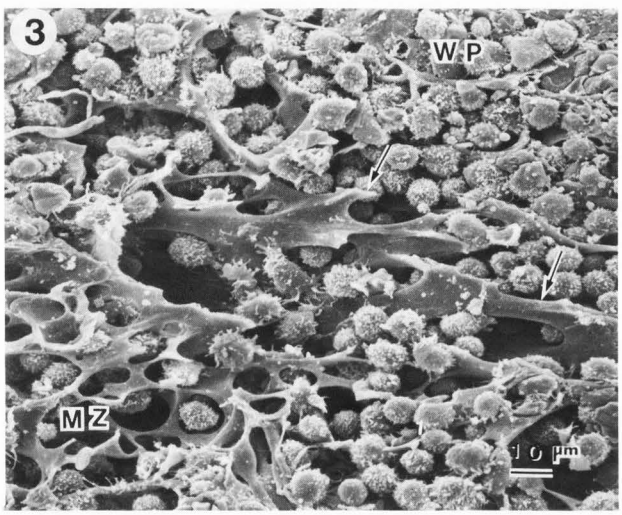
Inside of the circumferential reticulum, numerous lymphocytes are densely packed in the meshes of reticular cells. Arterioles and capillaries are supported by processes of the reticular cells. Occasionally several arterioles and capillaries are bound into a bundle by lamellar reticular cells, which seem to correspond to the "arteriolar-capillary bundle" reported by Snook (1975) (Figs. 5, 6).

Marginal zone

The white pulp, especially the follicle, is surrounded by a zonal area which is supported by reticular cells but contains very wide meshes. The reticular cells in this zone are continuous with those







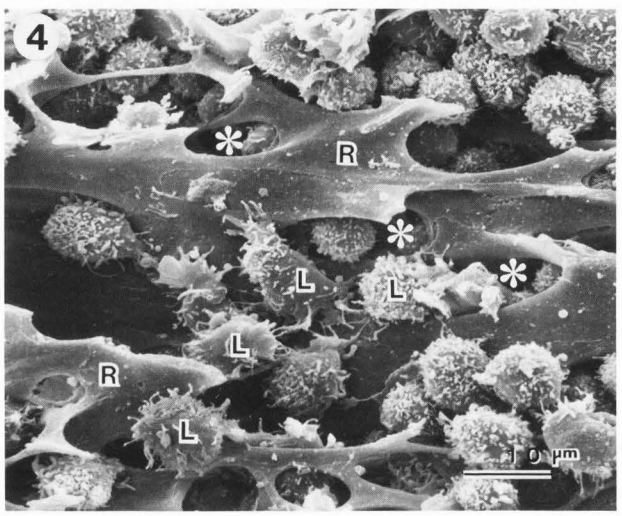


Figure 1: General view of the white pulp (WP), circumferential reticulum (arrow), central artery (CA), marginal zone (MZ) and red pulp (RP) with sinuses (S) in freeze-cracked surface of the human spleen.

Figure 2: General view of the white pulp (WP) with a central artery (CA), marginal zone (MZ) and red pulp (RP). Resin mass of the marginal zone (MZ) pushed out from a "Hof" artery (A) is seen.

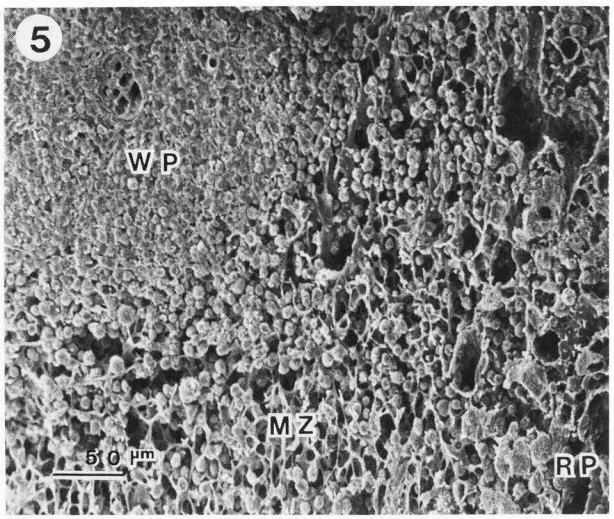
Figure 3: Several layers of the circumferential reticulum (arrows) extending between the white pulp (WP) and marginal zone (MZ).

Figure 4: Closer view of Figure 3. Lymphocytes (L) with small processes gathering to one pole are attached by the circumferential reticulum (R) which possess fenestrae (*) on their wall.

forming the circumferential reticulum. This marginal zone is characterized by occurrence of numerous free cells. Macrophages and lymphocytes of mainly medium size are especially common. As described below, many arteries open into the mesh spaces of this zone, which may be recognized in fractured tissue specimens.

Central artery

In most mammals, the central artery has been reported to branch out thin follicular arteries at right angles. The follicular arteries have been supposed to skim off plasm rich fractions from the external layer of the lamellar flow of the central arteries. As pointed out by Snook (1975), human spleens seem to differ markedly from those in most mammals in the pattern of arterial supply to the white pulp. Our observation of the vascular casts of human spleen with SEM revealed that the central artery ran straight; a larger one occasionally bifurcated into two central arteries but it did not branch out any follicular artery (Fig. 7). After leaving the white pulp, the central artery divided several times



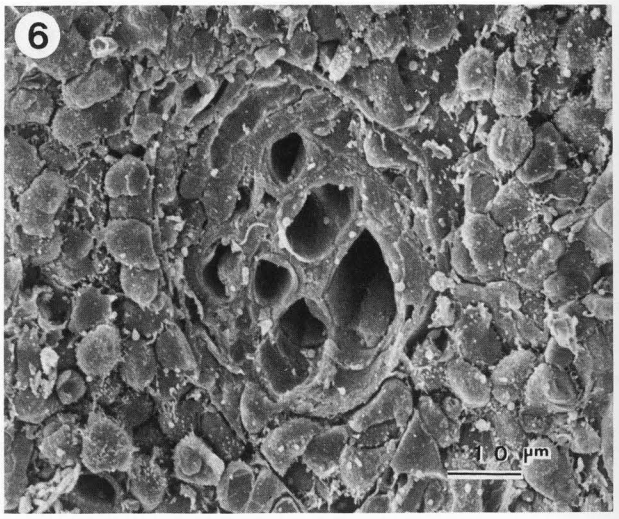


Figure 5: General view of the white pulp (WP), marginal zone (MZ) and red pulp (RP). Low magnification of the area containing the structures shown in Figures 3 and 4. An arteriolar-capillary bundle is seen at left upper corner.

Figure 6: Closer view of the arteriolar-capillary bundle shown in Figure 5.

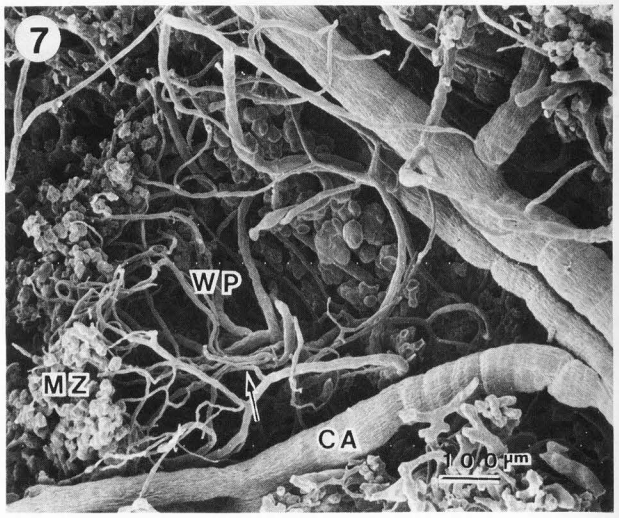
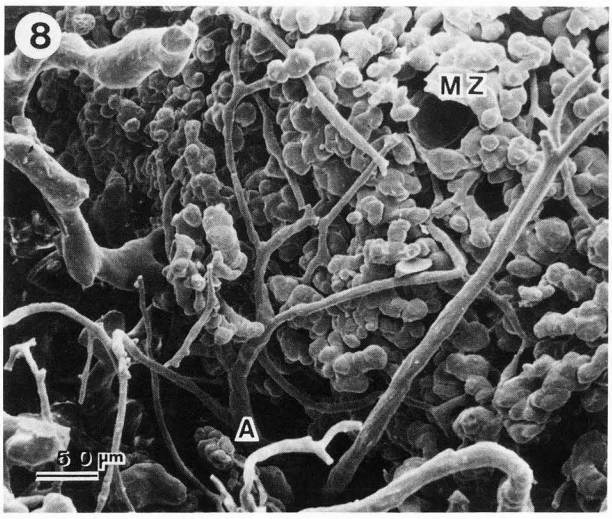
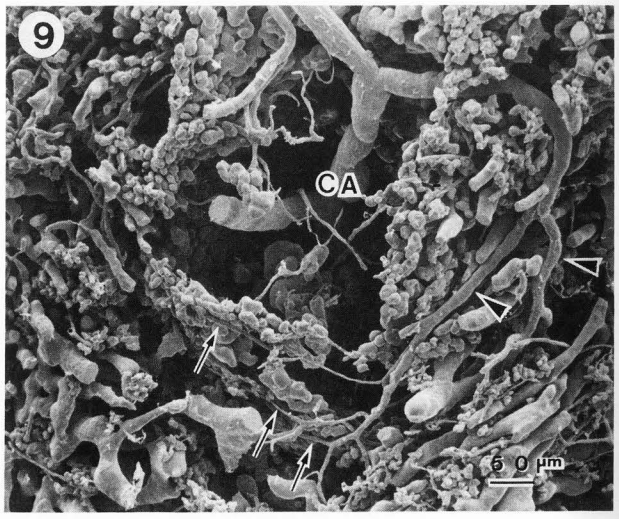


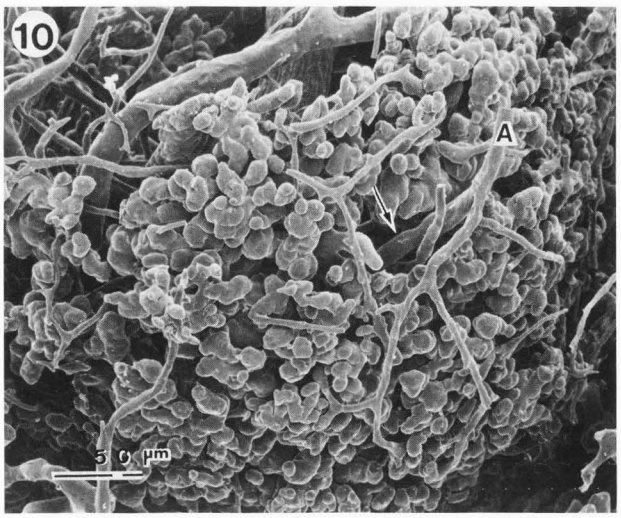
Figure 7: General view of the central artery (CA), marginal zone (MZ) and white pulp (WP). Several follicular arterioles gather and run in parallel to each other (arrow). They seem to correspond to the arteriolar-capillary bundle.

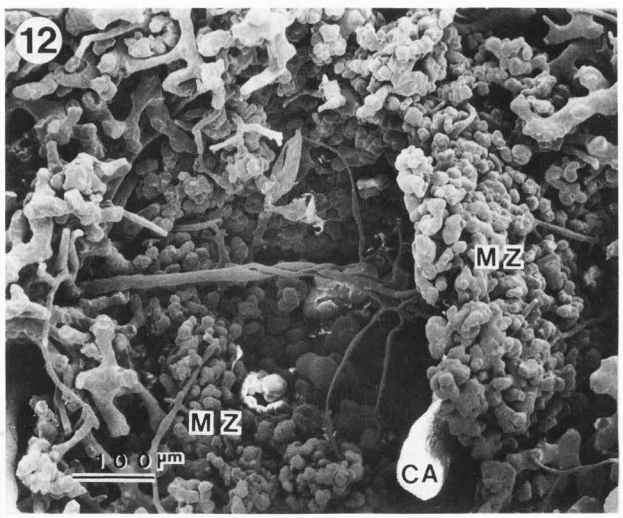
Figure 8: A "Hof" artery (A) bifurcates several times while running along the resin mass filling the spaces in the marginal zone (MZ).

Figure 9: Twigs (arrows) of penicillar arteries (arrowheads) run along the resin mass of the marginal zone and terminate there. CA: central artery.









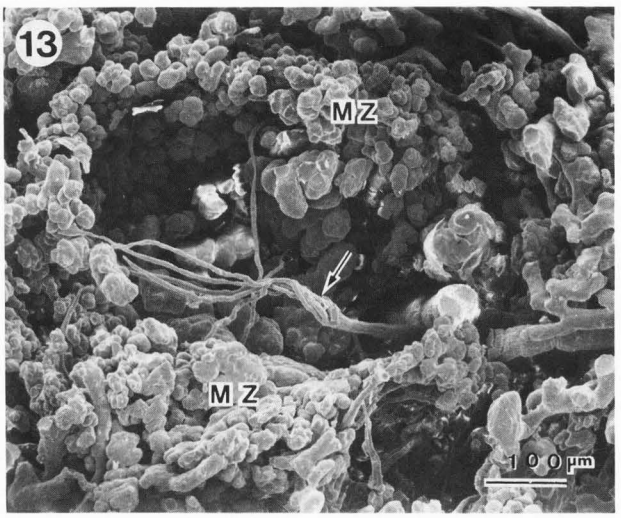


Figure 10: Closer view of Figure 2. A branch (arrow) of a "Hof" artery (A) passes through the marginal zone and enters the white pulp. Other branches end in the marginal zone.

Figure 11: Closer view of Figure 10. Terminal twigs of a "Hof" artery in the shape of funnels open in the direction to the white pulp. They never open in the direction to the red pulp.

Figure 12: Thin branches of follicular artery run intertwining to each other and radiate against the marginal zone (MZ). CA: central artery.

Figure 13: Thin branches of a follicular artery (arrow) intertwines to each other and run together. Then they radiate toward the marginal zone (MZ).

in the red pulp and became penicillar arterioles.

Blood supply to the white pulp and marginal zone

SEM observation of vascular casts made clear the blood supply to the white pulp and marginal zone. Several penicillar arteries return to the marginal zone. Some penicilli change their direction just outside of the marginal zone and run along the marginal zone as they bifurcate several times (Figs. 8, 9). They are called "Hof" arteries. Others may come there after a long course through the red pulp (Fig. 2). They may even come from neighbouring white pulp systems. Terminal portions of their twigs take the shape of a funnel and open towards the direction of the white pulp (Figs. 10, 11). They are connected with the resin mass filling the spaces in the marginal zone.

Other twigs of the penicilli, after passing through the marginal zone (Fig. 10), enter the white pulp and became follicular arteries. Some follicular arteries divide several times and the resulting branches intertwine with each other and run toward the center of the white pulp (Figs. 7, 12, 13). These capillaries pursue radial courses toward the marginal zone where they terminate. This bundle of blood

vessels, represented in a cross-sectioned view in Fig. 6, corresponds to the "arteriolar-capillary bundle" in the spleen of human and monkey reported by Snook (1975, 1980).

The branches of follicular arterioles running into the marginal zone to open there (Fig. 14) and the branches of penicilli coming to this zone are separated by a very thin wall of resin measuring only 20-30 μm. Therefore, arterioles seem to open in a restricted space in the marginal zone.

Red pulp

The general part of the red pulp consists of splenic cord and splenic sinus. The former is composed of a meshwork of the reticulum cells which are asteroid cells with smooth surfaces and connect with each other by their cytoplasmic processes. Since the cytoplasm encloses reticular fibers, we are not able to observe the fibers with SEM except for cracked surfaces of the reticulum cells. This may be one of the reasons why blood does not begin to coagulate after contact with the reticular mesh. In the mesh numerous macrophages reside attaching to the reticulum cells and extending their filopodia in all directions.

The splenic sinus is a vessel unique to the spleen. The special structure closely relates to the open circulation in the spleen which does not exist in other organs. Endothelial cells of the sinus are long and flattened cells with swellings at their nuclear portions and are called rod cells. They periodically connect with each other by small side processes. Basement membrane takes a shape of belt and bundles the rod cells. Thus they are called ring fibers. On the wall of the sinus there are numerous narrow slits, which are made among the cytoplasms and side processes of the neighbouring rod cells. Blood which pushes out in the cord from the arterial capillary is believed to flow into the sinus through its narrow slits (Fig. 15).

Open circulation in the red pulp

SEM observation on the end portion of arteries gives evidence for the open circulation in the human spleen.

The penicillar arteries terminated in the cords, mostly in a funnel shape and partly in a saccular structure as reported previously (Fujita et al., 1982, 1985). SEM observations of vascular casts demonstrate that a thin penicillar artery runs straight or gently curved, and extends to end in a funnel shape. The cast of this funnel-shaped terminal usually continues to granular resin masses which correspond to the resin pushed out into the cordal spaces (Fig. 16). The saccular arterial ending is represented by a smooth-walled swelling of the endothelial lining which is provided with a few round perforations. Erythrocytes, conspicuously constricted, are found passing through the perforations. Resin cast also shows a round swelling at the arterial end with structures showing leakage of resin.

Closed circulatory system with peculiar labyrinth of arterioles

On the fractured surface of the red pulp, winding arterioles are occasionally found to be gathered in distinct regions of the red pulp in the vicinity of arteries and veins as well as the marginal zone (Fig. 17). The tubular and partly swollen channels are anastomosed to each other forming a labyrinth. Their walls are lined by smooth endothelial cells. A few round pores less then 1 μm in diameter are occasionally recognized in the endothelial cells. Most of the pores keep erythrocytes hanging in an intensely constricted form. Round windows, mostly 10 μm in diameter, were seen here and there leading into the deeper channels. At some places in the channel of the labyrinth thin trabeculae were found to span the lumen. These trabeculae are columnar in shape, 0.5 μm or more in thickness, and covered by endothelial cells (Fig. 18).

Since the labyrinth extends three-dimensionally repeat anastomosing, it is difficult to visualize its terminal portions. Careful observations of compensatory surfaces of fractured tissue blocks could confirm the occurrence of the direct connections of the labyrinth and sinuses. A narrowed channel of the labyrinth covered by flat endothelial cells continued to a funnel-shaped sinus lined by typical rod cells with intercellular slits (Fig. 19).

Resin casts of the closed circulatory system

A complicated network of twisted tubular casts with partial swellings is found in the vicinity of the casts of the pulp arteries and veins as well as the marginal zone (Fig. 20). The casts of the labyrinthine vessels are smooth in surface and clearly distinguished from those of the sinuses whose surfaces are indented by nuclear swellings of rod cells. Pits of various sizes on their surfaces are believed to be impressions of the luminal trabeculae. Some parts of the labyrinthine casts can be followed to the casts of narrowed sinuses, indicating the direct connections of both vessels (Figs. 20, 21).

Discussion

Blood flow into the marginal zone and its functional implications

The marginal zone is a structure characteristic of the spleen and not found in other lymphoid organs. In the red pulp, foreign substances in the

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Figure 14: Closer view of Figure 13. A follicular capillary swells at its terminal portion and ends in the marginal zone (MZ).

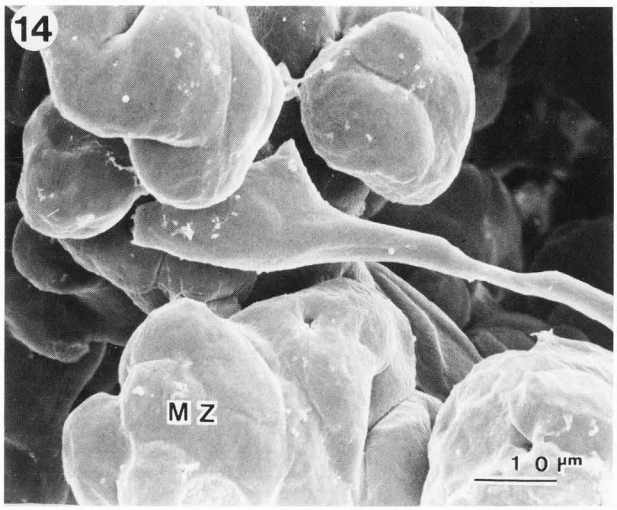
Figure 15: A small sinus and cord (C) of Billroth. Narrow slits (arrows) are seen on the wall of the sinus. Erythrocytes (E) hang in the wall.

Figure 16: Vascular cast of a small artery (A) ending open into the cord. Note that resin has been pushed out into the cordal spaces as an irregular-shaped clump. The large resin mass corresponds to a sinus (S). The longitudinal indentations (arrows) and three couples of small round indentations (arrowheads) correspond to rod cells and hanging erythrocytes, respectively.

Figure 17: An arteriolar labyrinth gathered in the vicinity of central arteries (CA) and periarterial lymphatic sheath (PLS). S: sinus T: trabecula.

Figure 18: Saccular swellings in an arteriolar labyrinth. Trabeculae (arrows) covered with endothelial cells span the lumen.

Figure 19: An artery (A) of a labyrinth directly connected with a sinus (S). C: cord.


14
MZ
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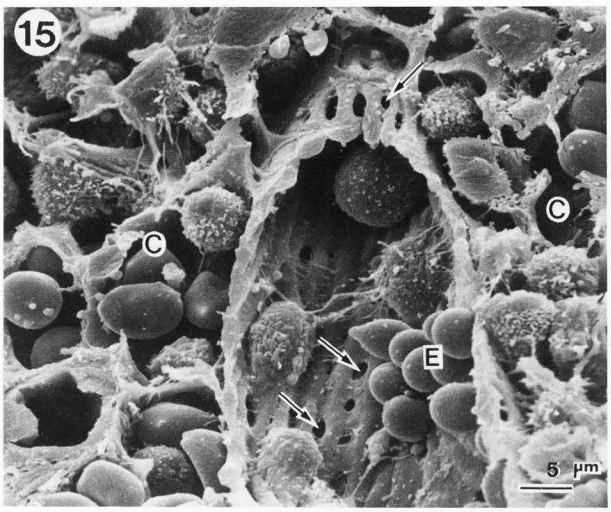
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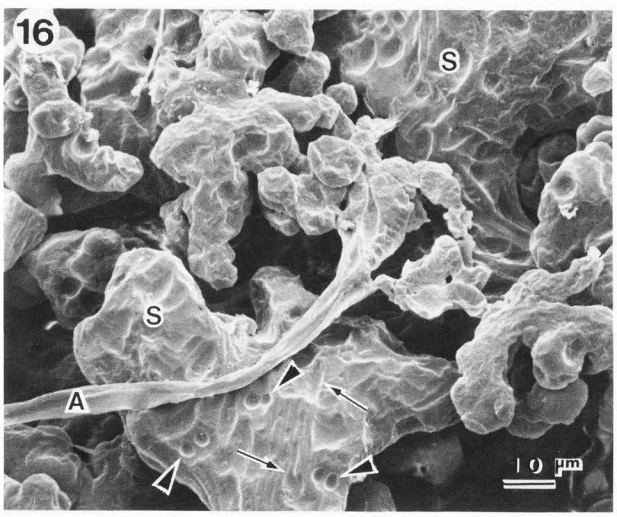
16
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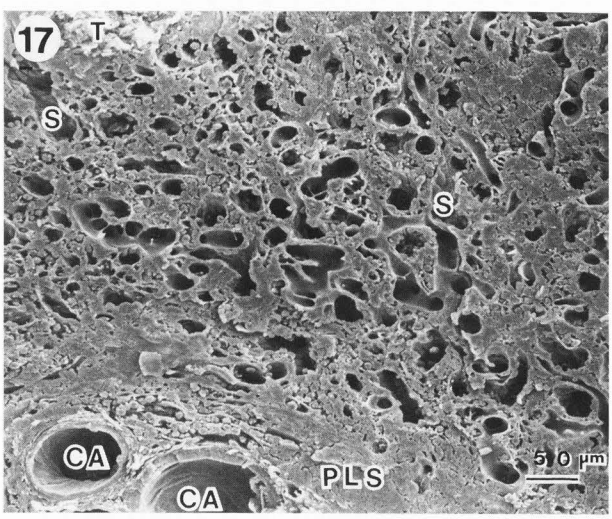
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CA
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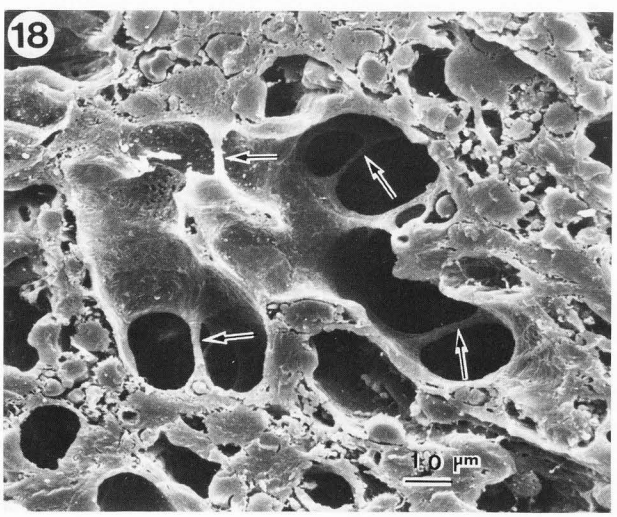
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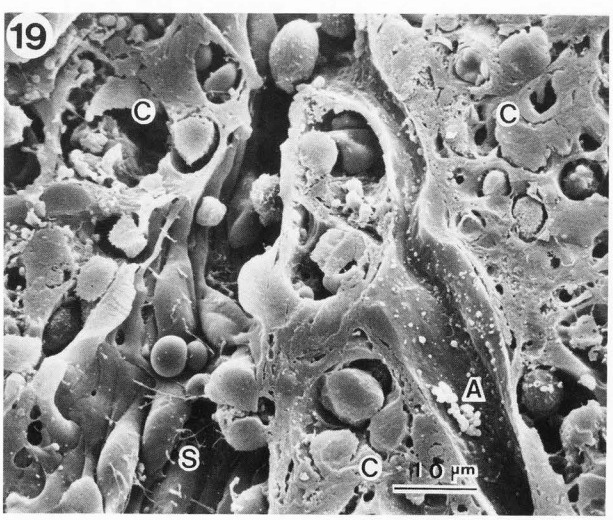
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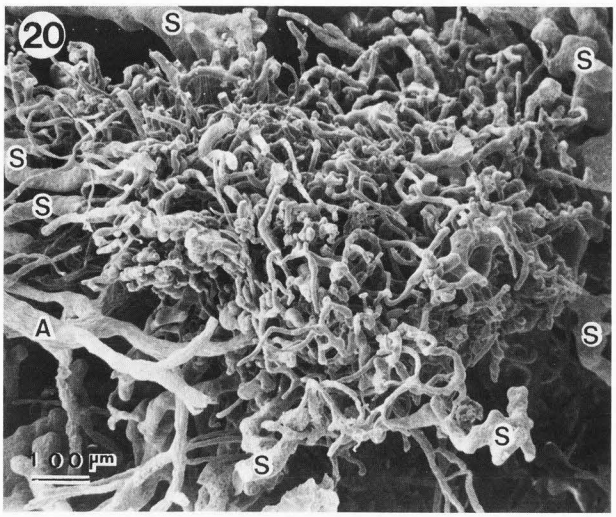

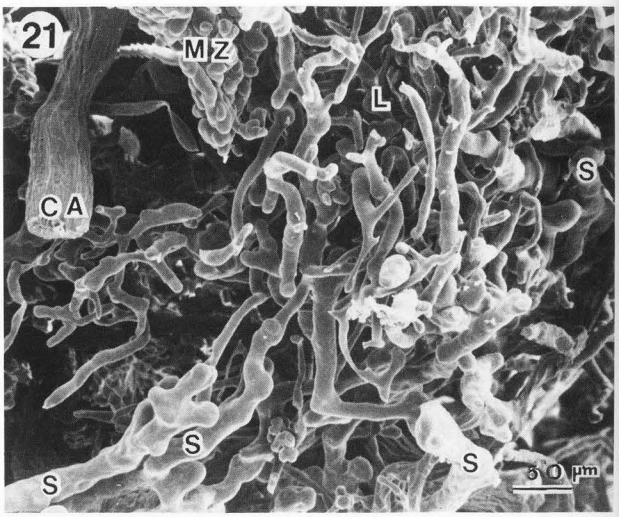

Figure 20: General view of a vascular cast of an arteriolar labyrinth. Terminal portions of arteriolar labyrinth connect with sinuses (S). A: afferent artery.

Figure 21: Vascular casts of a central artery (CA), marginal zone (MZ) and arteriolar labyrinth (L) which developed in the vicinity of the marginal zone. S: sinus.

blood are eliminated by the phagocytotic activity of macrophages, whereas the marginal zone is believed to be involved in transmitting the information concerning the foreign substances to the immunocompetent lymphocytes in the white pulp.

The human marginal zone is divided into inner and outer layers as described by Kamiyama and Saito (1979). The inner layers are rich in medium-sized lymphocytes of B cell nature, which, according to Timens and Poppema (1985), possess, as lymphocytes in the marginal zone of other mammals do (Gray et al., 1982; Kumararatne et al., 1981), surface IgM but lack surface IgD. The outer layers contain macrophages and various free blood cells. These layers are characterized by distribution of the twigs of penicillar and follicular arteries. Almost all these vessels terminate by opening in these layers of the marginal zone. The blood coming out of the arteries flows gently through the reticular meshes of the marginal zone and foreign substances in the blood are phagocytosed by macrophages. The antigenic portions of the foreign substances are, according to experiments in rats, carried by medium-sized lymphocytes, which are surface IgM positive and surface IgD negative, to follicular dendritic reticular cells (Gray et al., 1984). As the human marginal zone also possesses lymphocytes with the same features, the same mechanism as in the rat is postulated to occur in human. The marginal zone is regarded also as an important passageway for lymphocytes going in and out of the white pulp (Timens and Poppema, 1985).

It seems likely that the layer between the inner and outer layers of the marginal zone is most efficient for the disposal and transport of foreign substances. This intermediate layer is often represented by a wall of resin masses in cast preparations. Penicillar twigs terminating in this portion are never directed toward sinuses, but toward the white pulp. This finding supports the view that these marginal zone vessels do not carry blood to the red pulp as a mere foreign body scavenger, but serve transmission of immunological information toward the white pulp.

Open circulation: Pitting and culling functions

The open circulation in the spleen enables the blood to flow gently through the meshes of the spongy tissue of the red pulp. Numerous macrophages dwell in this tissue, extending filopodia which are chemo- and mechanosensitive. Degenerating blood cells and foreign substances, while they move through slowly, are detected by these marophages and phagocytosed. This is the procedure called the culling function of the spleen.

It has been known that blood cells coated with ample antibodies are captured by Kupffer cells in the liver, whereas those with a less amount of antibodies, are captured mainly by the spleen (Frank, 1977). This suggests that the macrophages in the spleen are more sensitive than Kupffer cells in the detection of abnormal and non-self elements. This sensitivity seems mainly due to the mechanism of the open circulation which facilitates cell to cell and cell to foreign body contact.

In the open circulation, formed elements of the blood must pass the slits of the sinusal lattice. Normal erythrocytes are flexible enough to do that, but abnormal erythrocytes containing rigid inclusions like a Howell-Jolly body or plasmodium gondii (Schnitzer et al., 1972) are stemmed by the lattice. Most of these erythrocytes manage to pass them, tearing a part of their body and leaving the inclusions in the cord. This process is called the pitting function of the spleen (Koyama et al., 1964; Rifkind, 1965; Fujita et al., 1982). Erythrocytes with increased surface area and with cytoplasmic vacuoles are known to appear after splenectomy. This effect may be accounted for by the fact that the spleen, if present in the normal state, eliminates vacuoles and excess plasma membranes of maturing reticulocytes. The sinusal lattice further stems rounded erythro-

cytes in hereditary spherocytosis and autoimmune hemolytic anemia; they are destroyed in the cord.

Closed circulation

Physiological studies have indicated that the blood flow through the human spleen is approximately 100 ml/min (Hughes Jones et al., 1957; Huchzermeyer et al., 1977). As the erythrocyte content of the normal human spleen is 50 ml, the average time of the erythrocyte passage through this organ is 30 sec. This quick passage of erythrocytes could not be accounted for by the mere open circulation of the spleen (Peters, 1983). Klemperer (1938), Weiss (1983) and Peters (1983) proposed that the arterial terminal portions, though "open" in structure, might function as if "closed" in nature, allowing the blood to flow quickly. During our study of numerous human spleens, we occasionally encountered structures which may deserve the name of "functionally closed" circulation. For example, a funnel-like arterial terminal was rarely juxtaposed to a sinusal lattice with widened slits. However, a direct end-to-end connection between arterial and sinusal vessels has never been encountered in the "ordinary portion" of the red pulp.

The very end-to-end connection can be found in particular "foci" in the red pulp, forming a peculiar labyrinth of arteriolar channels. The labyrinthine arterioles are characterized by (1) their large calibers, (2) occurrence of trabecules spanning their lumina, (3) ample anastomoses and (4) their connection with the venous sinuses. The intravascular trabeculae are unique for these arterioles, as no comparable structures have been known in other blood vessels. They may possibly serve to prevent an excess extension of the vessels. The labyrinth of anastomosing arterioles with a considerable volume is thought to be useful for quickly lowering the pressure of the flushing arterial blood before it is introduced to the venous sinuses with a delicate wall.

The problem as to how much volume of the blood passes this labyrinthine arteriole-sinus route belongs to future investigation. The quickly flowing fraction of the blood passing through the human spleen proposed by Harris et al. (1958) is presumed to pertain to this route.

In contrast to a simple arteriovenous shunt, this peculiar arteriole-sinus shunt can be involved in foreign substance elimination and immunological defence reactions, which may be less effective than those of the open circulation characterized by slow cordal blood flow. In the labyrinth-sinus route, the blood can be inspected by macrophages on the sinusal wall extending filopodia into the lumen. Furthermore, a considerable part of the blood passing the sinuses probably flows out into the cords, where it must interact with the macrophages. It is thus postulated that this route allows a quick blood flow through the spleen, and yet it retains the essential function of the spleen detecting and eliminating harmful bodies in the blood.

Nervous control

Numerous bundles of nerve fibers supply the spleen. They are almost all sympathetic in nature. They terminate on the smooth musculature of thicker arterial vessels and do not enter the white and red pulp (Heusermann and Stutte, 1977; Kudoh et al., 1979). Therefore no direct effect of the nerves to phagocytic and immunocompetent cells is conceivable. Yet the immunoreactions reactions in the spleen are conspicuously influenced by the nerves and neurotransmitters.

In their studies in the rat, Besedovsky et al. (1979, 1983) and Del Rey et al. (1981, 1982) demonstrated that the increase in antibody-producing cells induced by antigenic stimuli was significantly enhanced in the spleen after section of sympathetic nerves to the organ or after pharmacological blockage of noradrenaline. Antigenic stimulation caused decreased content of noradrenaline in the spleen (Besedovsky et al., 1979; Del Rey et al., 1981). Decreased noradrenaline, in turn, caused further increase in the immunocompetent cells; thus a positive feedback system seemed to exist between the nervous and immune systems in the spleen.

On the other hand, the role of nerves on the regulation of blood flow in the spleen has been widely accepted. McNee (1931) stimulated splenic nerves while perfusing the organ with small particles (yeast fungus) through arteries. When the spleen was contracting by nervous stimulation, the particles were present exclusively in the sinuses. When the organ was not stimulated, they were found in both sinuses and cords. McNee (1931) concluded that there were closed and open circulations in the spleen and they might be shunted by nerves. Levesque and Groom (1981) reported that in cat spleen, adminstration of noradrenaline caused rapid flow of erythrocytes and albumin via an arteriovenous pathway. Decrease in noradrenaline in the spleen, on the other hand, caused increased weight of the organ (Del Rey et al., 1982); the spleen with enhanced immune reactions showed increased weight and decreased blood flow per unit weight of the organ (Vaupel et al., 1977).

These data suggest that stimulation of the sympathetic nerve and administration of noradrenaline increase the blood flow through the closed circulatory system. Lowered activity of the nerve and noradrenaline, on the other hand, brings, the open circulation to the front. The amounts of blood flow into the cords, marginal zone and white pulp are enhanced and the incidence of contact of foreign elements in the blood with the phagocytic and antigen-producing cells in those areas is enhanced; the immunological reactions of the spleen are activated with an increased number of immunocompetent cells.

It remains to be investigated as to whether a true closed circulation system as demonstrated in the human in this study may also occur in animals. It must be also explored whether the above-mentioned hypothesis on the nervous shunting of open and closed circulation based on animal experiments might be an actual phenomenon in the human spleen.

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Discussion with Reviewers

J. Lusher: The elegant studies of Prof. Barnhart, I think, conclusively demonstrated that there exists a closed as well as an open circulation in the human spleen (Barnhart MI et al., (1976). Arteriovenous shunts in the human spleen. Am J Hemat 1:105-114).

However the authors state that the technique of vascular casting with resin was criticized (by Weiss, 1983) as unsuitable for documenting a closed circulation, and dismiss it at that. Do the authors believe this technique to be unsuitable? If so, why?
Authors: The continuity of the vascular cast between arteries and sinuses or veins demonstrated by Barnhart et al., and Schmidt (Schmidt et al., 1982) may possibly visualize the true morphological closed circulation. However, as mentioned in the Introduction, the physiological closed circulation proposed by Weiss may take the shape similar to those of morphological closed circulation. To finish this discussion, we have to demonstrate the endothelial continuity between arteries and sinuses.

S. Irino: Where does the arteriolar labyrinth with closed circulation distribute in the spleen?
Authors: The distribution of the arteriolar labyrinth (AL) is not exactly determined yet. The ALs are frequently observed in some tissue blocks, however, they are hardly found in others. Thus Als do not seem to distribute equally in the spleen. They possibly occur in the center of the organ.

S. Irino: Do you regard the lymphocytes attaching to the circumferential reticulum (Fig. 4) as the carrier cells for antigen from marginal zone to the white pulp?
Authors: This spleen was removed from the patient with idiopathic thrombocytopenic purpura. It may be possible that platelets as an antigen are always carried into the marginal zone, are phagocytized by macrophages and their antigenic portions are carried into the white pulp by the medium sized lymphocytes. Further examinations need to be studied to confirm this phenomenon.

S. Irino: In order to observe the arteriolar labyrinth and arteries in the white pulp and marginal zone, how much of the resin do you inject into the splenic artery?
Authors: After injection of a large amount of resin in the splenic artery, most sinuses were filled with resin and we were not able to observe the whole structure of the arterial system, especially the ALs. While it is difficult to evaluate arterial casts without sinusal casts or resin leaked into the cords. Thus we try to inject from a small to a large amount of resin according to the vessels which we want to observe.