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Vascular Arrangement of the Mammalian Spleen as Revealed by Injection Replica Scanning Electron Microscopy*)

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ABSTRACT

The three-dimensional vascular arrangement of the mammalian spleen was investigated by means of injection replica scanning electron microscopy.

The vascular arrangement of the white pulp and marginal zone in the mammalian spleen was divided into two groups: (1) the rat and mouse type, and (2) the human and dog type. In the rat and mouse, (a) the central artery gave rise to numerous follicular capillaries which usually terminated in the inner side of the marginal zone, (b) the follicular capillaries showed a small network in the germinal center, (c) the follicular capillaries occasionaly entered into the marginal zone and terminated in the intercellular space, and (d) some of the penicillar arteries showed a centripetal course and enter into the white pulp to open into the marginal zone. In the human and dog, (a) follicualr arteries gave off many arterial capillaries in the marginal zone shortly after penetration of the marginal zone, (b) recurrent branches of the penicillar artery entered into the marginal zone, (c) some of the recurrent branches entered into the follicle and supplied the follicular capillaries, (d) follicular capillaries orginated from vessels distributed in the marginal zone, recurrent branches of the penicillar arteries and follicular arteries, (e) the main sources of the follicular capillaries were from the vessels in the marginal zone, and (f) the follicular capillaries were chiefly distributed in the germinal center.

The terminal end of the penicillar artery in the pulp cord was mostly open type in the mammalian spleen.

INTRODUCTION

The exact manner of the splenic circulation is still a matter of controversy. It has not been known with certainty whether the terminations of the arterial capillaries open into the pulp cord or into the sinus. There are three major theories about intermediary splenic circulation. (1) the open circulation theory that the arterial capillaries open directly into the cordal meshwork of the red pulp and the blood then flow into the venous sinus (Robinson, 1926[19]; Hartmann, 1930[9]; Fleming and Parpart, 1959[6]), (2) the closed theory that the arterial capillaries are connected directly to the venous sinus (Helly, 1902[10]; Knisely, 1936[13]) and (3) the combined theory that both open and closed circulation exist in the spleen (Weidenreich, 1901[25]; MacNeal et al., 1927[15]; Snook, 1958[22]). However, these problems have been discussed

*) 梶原博毅，中上和彦，飯島宗一：鋳型走査電子顕微鏡による哺乳類脾臓の血管構築

chiefly on the terminations of the penicillar arteries in the red pulp cord. Recent advances of the immunology have called attention to the structure and function of the white pulp and marginal zone (Congdon, 1964[5]; Nossal et al., 1966[17]; Goldschneider, 1968[7]; Mitchell and Abbot, 1971[16]; Gutman et al., 1973[8]; Sasou et al., 1976[20]). As has been described by Snook (1950[21]), the circulation of the white pulp and marginal zone varies among different species. Jäger (1929)[12] previously described that in the human spleen branches of the central artery directly enter the marginal zone and are distributed like a hand grasping a ball. These arterial capillaries have no Schweiger-Seidel sheath and were named "Hofarterien". In the rat, on the other hand, follicular capillaries which are derived from follicular arteries directly enter the marginal sinuses (Snook, 1964[23]; Sasou et al., 1976[20]). Although many investigators have demonstrated the vascular arrangement of the spleen in various species by means of three-dimensional reconstruction methods of serial sections (MacNeal et al., 1929[15]; Jäger, 1929[12]; Snook, 1950[21]), the exact distribution of the splenic vessels, especially of the white pulp and marginal zone, is still unknown in detail. This study was carried out to investigate the three-dimensional architecture of the mammalian splenic vessels using a corrosion casting method and scanning electron microscopy.

MATERIALS AND METHODS

Five adult Wister rats, 5 adult C3H mice, 5 adult mongrel dogs and 6 human spleens were in this study. After ether anesthesia, rats and mice were perfused with a Ringer's solution through a polyethylene tube inserted into the ascending aorta or left ventricle. Thereafter, a dilute mixture of comercially available methacrylate medium (Mercox CL-2B-5, Dainippon ink Co., Ltd.) was injected through the tube until the inferior vena cava was filled with the injected medium. The injected resin was polymerized at room temperature for 1 h. The spleen was then removed and placed for 24 h in a warm bath (about 60°C) for further polymerization of resin. The dogs were anesthetized with an intramuscular injection of Ketaral (2-o-methylaminocyclohexanone-hydrochroride) and heparinized. After sacrifice, dog spleens were quickly removed. All human spleens were obtained as fresh surgical material from patients subjected to total gasterectomy and splenectomy for gastric cancer (41, 65 and 77 year-old males, and 48, 50 and 57 year-old females.) The human and dog spleens were perfused through a polyethylene tube inserted into a large branch of the splenic artery. Thereafter, methacrylate medium was injected throught the splenic artery until the splenic vein was filled with the injected medium. The injected resin was polymerized at room temperature for 1 h and then placed in warm bath for 24 h for further polymerization.

The splenic tissue of the experimental animals and human was removed by hydrolysis in repeated changes of a 20 to 25% NaOH aqueous solution for 24 h. Vascular samples were washed in tap water to remove remaining tissue, cut with razor blades into suitable blocks and washed again. The blocks of vascular replicas were dried in air and fixed on metal stubs. They were coated with gold in an ion coater (Eiko IB-3) and observed in a scanning electron microscope (Hitachi S-430) with an accelerating voltage of 20 kV.

RESULTS

1) Rat spleen

Vascular casts of the spleen afford good visualization of a threedimensional architecture in the scanning electron microscope. The white pulp or lymphoid sheath is represented by a cylindrical empty space which surrounds the central artery and which has side branches radiated outward (Fig. 1). The central artery gives rise to many arterial capillaries in the follicle, which repeatedly subdivide and form a network in the outer part of the follicle (Figs. 1 and 2). The terminal segments of these capillaries are usually dilated showing an ampular appearance and are connected with spaces of the marginal zone (Fig. 2). Some of these capillaries enter into the marginal zone and terminate at various depths therein (Fig. 2). A small network of the follicular capillaries is frequently observed in the follicle (Fig. 2). These capillaries are considered to lie within the germinal center. The marginal zone is revealed as a distinct area containing densely arranged irregular resin masses surrounding the lymphoid sheath, which occupy the intercellular space (Fig. 1). The penicillar artery enters the

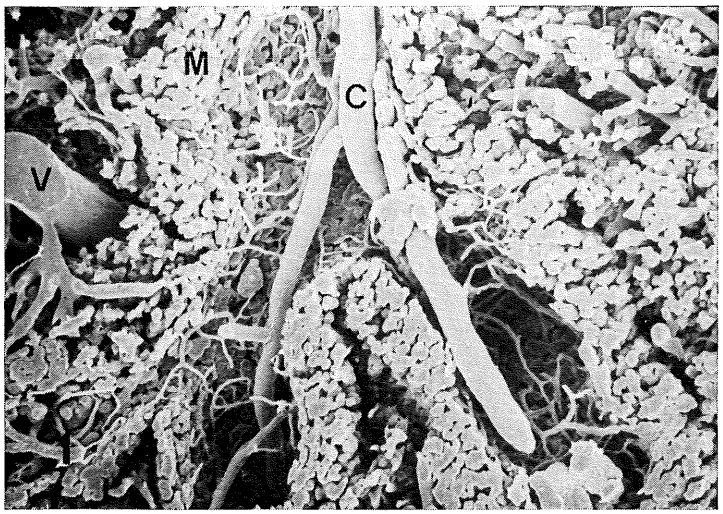

Fig. 1. A scanning electron micrograph of vascular casts of the rat spleen. The central artery (C) is eccentrically located in the follicle and gives rise to numerous follicular capillaries. M: Marginal zone. V: Collecting vein. ×160

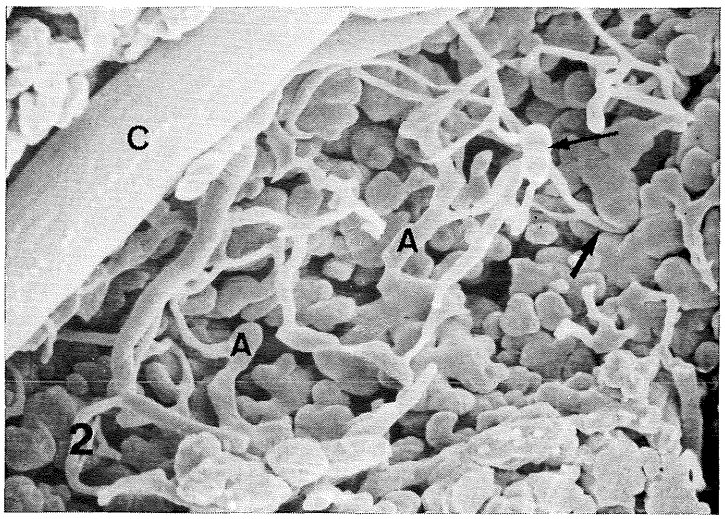

Fig. 2. Rat spleen. Higher magnification of the follicular capillaries which usually terminate in the inner side of the marginal zone. Terminal segments of the follicular capillaries show ampular dilatation (A). Some of these follicular capillaries enter into the marginal zone and terminate in this area (thick arrow). Follicular capillaries frequently form an anastomosing plexus in the germinal center (thin arrow). C: Central artery. ×500

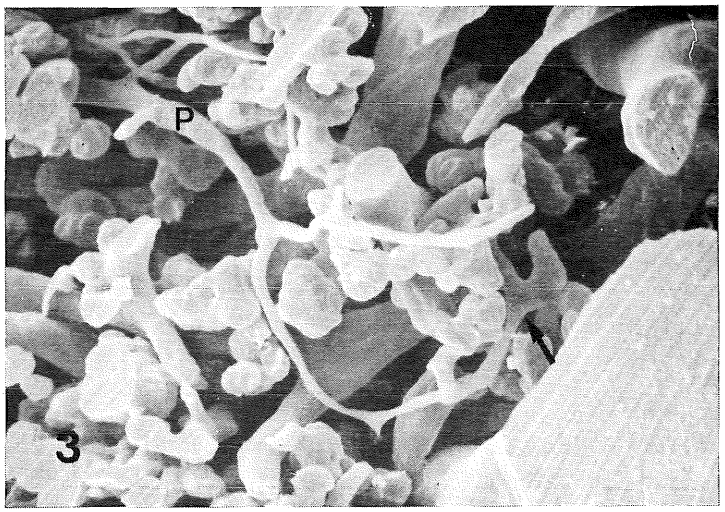

Fig. 3. Rat. spleen. Terminal segmens of the penicillar arteries (P) show frequently ampular dilatation (arrow). Injected resin leaks at these terminal segments. ×710

red pulp as the terminal branch of the central artery. A recurrent branch of the penicillar artery is rarely identified and terminates in the marginal zone. The penicillar arteries repeat bicornate divisions in the red pulp and the end of these arterial capillaries frequently shows ampullar dilatation (Fig. 3). The resin injected into the splenic artery usually leaks at the terminal end of the arterial capillaries. A large part of the injected resin passed through the marginal zone into the red pulp sinus. On the other hand, direct connection between terminal ends of the penicillar arteries and red pulp sinus is very difficult to be identified in the present study.

2) Mouse spleen

The vascular architecture of the mouse spleen resembles that of the rat. However, follicular capillaries are less numerous in the mouse as compared with the rat. The ramifying pattern and distribution of the follicular capillaries are also similar to those of the rat spleen (Fig. 4). These follicular capillaries usually terminate at the inner side of the marginal zone (Fig. 4). In contrast to the rat spleen, it is very difficult to find the capillaries which enter into the marginal zone. A small localized network of the follicular capillaries which are considered to lie in the germinal center is frequently observed in the mouse spleen (Fig. 4). The marginal zone is also a distinct structure as revealed in the rat spleen. The penicillar arteries also show bichornate divisions in the red pulp. However, they are shorter than those of the rat. Recur-

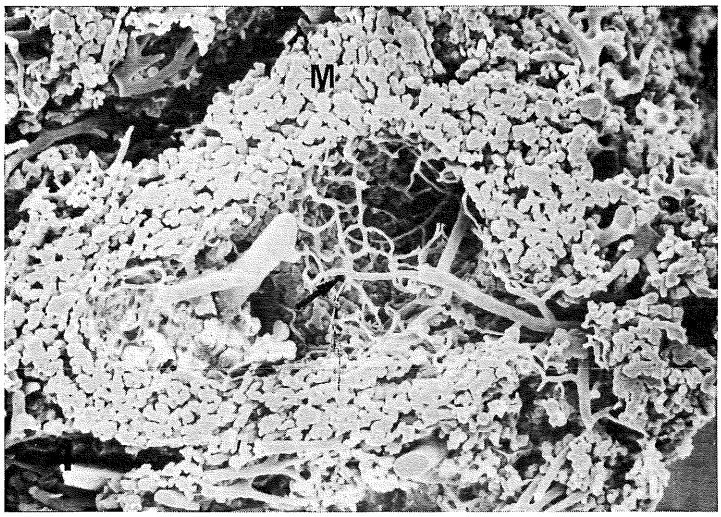

Fig. 4. Vascular casts of the mouse spleen. Follicular capillaries which originate from the central artery form a network in the follicle. A follicular artery gives off many follicular capillaries in the germinal center (arrow). M: Marginal zone. ×240

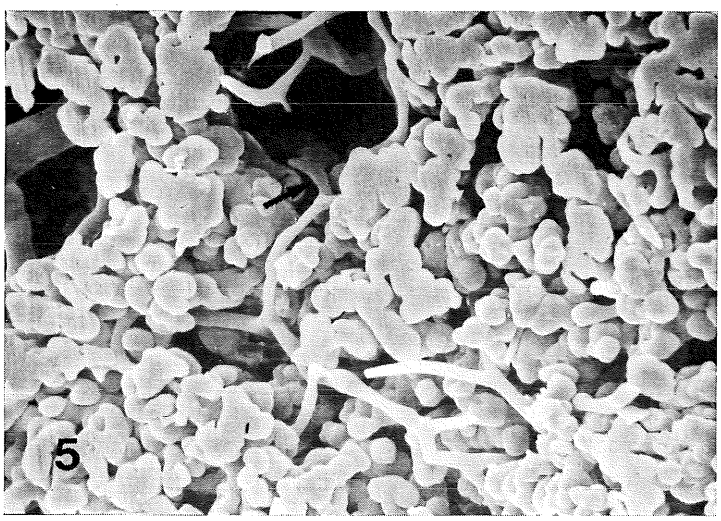

Fig. 5. Mouse spleen. Recurrent branches (arrow) of the penicillar arteries enter into the white pulp and terminate at the inner side of the marginal zone. ×720

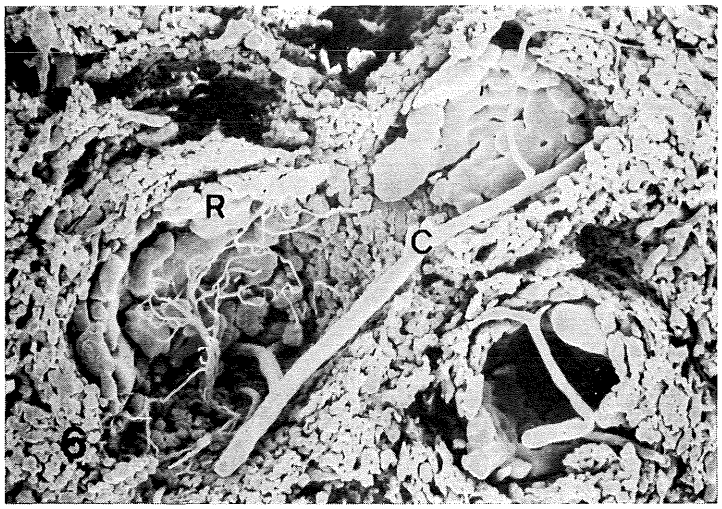

Fig. 6. Vascular casts of the human spleen. The central artery (C) gives rise to many follicular arteries which penetrate the marginal zone and transform into penicillar arteries. A plexus of the follicular capillaries is frequently observed in the follicle (arrow). Injected resin leaks from marginal zone into the follicular space and forms large masses (R) between follicle and marginal zone. ×120

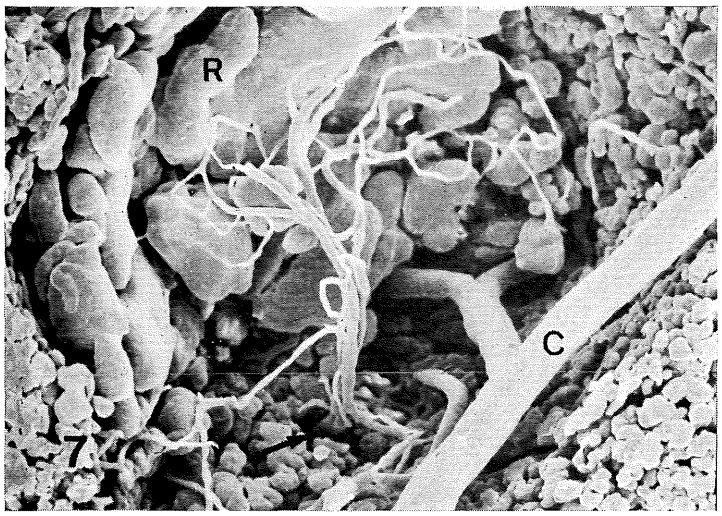

Fig. 7. Higher magnification of Fig. 6. Follicular capillaries orginate from the vessels of the marginal zone (arrow). C: Central artery. R: Leaked resin mass. ×300

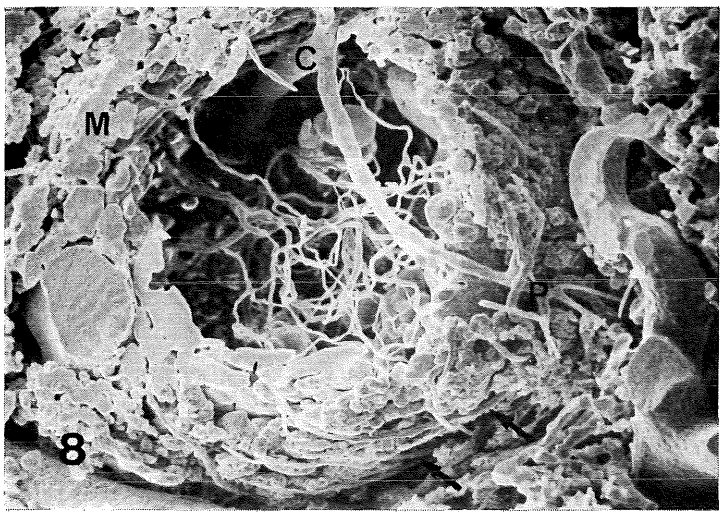

Fig. 8. Human spleen. Shortly after penetration of the marginal zone (M), the follicular artery gives off many arterial capillaries in the marginal zone. These arterial capillaries distribute like fingers grasping a ball (arrows). C: Central artery. P: Penicillar artery. ×200

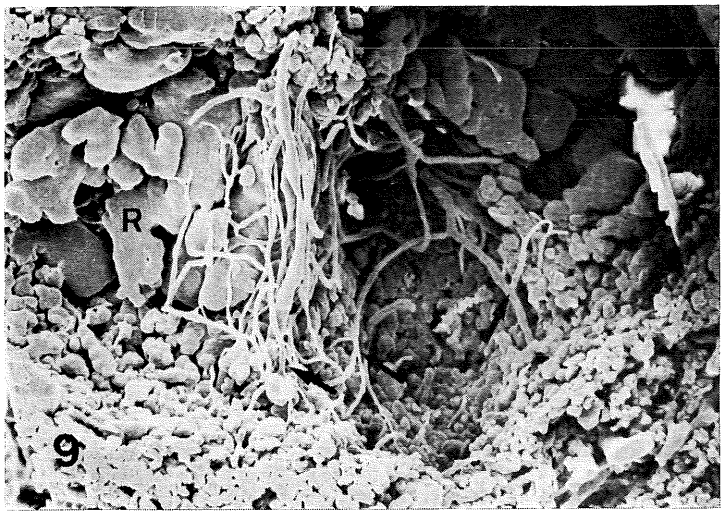

Fig. 9. Human spleen. Arterial capillaries in the marginal zone distribute closely and anastomose with the follicular capillaries (arrows). R: Leaked resin mass. ×160

Fig. 10. Human spleen. Recurrent branches of the penicillar arteries (arrow) enter into the white pulp and supply the follicular capillaries. M: Marginal zone. ×150

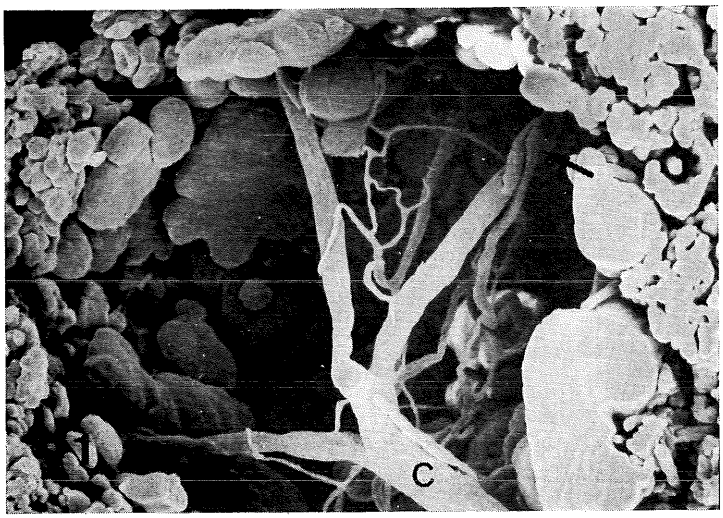

Fig. 11. Human spleen. Arterial branches of the central artery occasionally supply the follicular capillaries (arrow). C: Central artery. ×300

rent branches of the penicillar arteries are occasionally observed and terminate in the marginal zone (Fig. 5). The terminations of the penicillar arteries in the red pulp cord are also similar to those of the rat.

3) Human spleen

The central artery which is usually located in an eccentric position in the follicle gives rise to many follicular branch arteries (Figs. 6 and 8). These follicular arteries usually pass through the marginal zone and transform into penicillar arteries (Fig. 8). Shortly after penetration of the marginal zone, the follicular artery gives off many arterial capillaries in the marginal zone. These arterial capillaries are arranged like fingers grasping a ball and correspond to the "Hofarterien" named by Jäger (1929)[12] (Figs. 8 and 9). These arterial capillaries frequently anastomose with each other in the marginal zone (Fig. 9). A considerable number of the penicillar arteries return to the marginal zone and distribute in this area. In rare instances, recurrent branches of the penicillar arteries penetrate the marginal zone and supply the follicular capillaries (Fig. 10). Follicular capillaries are less numerous in human spleen as compared to those in the rat and mouse spleens (Fig. 6). These follicular capillaries usually originate from the branches distributed in the marginal zone (Figs. 6, 7 and 9). However, follicular arteries occasionally supply the follicular capillaries (Fig. 11). These follicular capillaries distribute chiefly in the germinal center, form an anastomosing network and termi-

Fig. 12. Vascular casts of the dog spleen. The central artery (C) gives rise to many follicular arteries which penetrate the marginal zone (M) and transform into penicillar arteries. Follicular capillaries are supplied from the vessels of the marginal zone (arrow). ×105

Fig. 13. Dog spleen. Arterial branches of the follicular arteries distribute in the marginal zone like fingers grasping a ball (arrows). ×120

nate in the marginal zone (Figs. 7 and 8). Large resin masses are frequently found between follicle and marginal zone (Figs. 6, 7, 9 and 11). These masses connect tightly with those of marginal zone. These findings indicate that the injected resin leaks easily from marginal zone into the follicular space. Injected resin also leaks at the terminal segment of the penicillar artery in the red pulp cord. It is very difficult to find a direct connection between the terminal end of the penicillar artery and the red pulp sinus.

4) Dog spleen

The vascular architecture of the dog spleen is very similar to that of human spleen. The central artery is located eccentrically in the follicle and gives off many follicular arteries (Fig. 12). These follicular arteries penetrate the marginal zone and enter into the red pulp cord. These follicular arteries give off many arterial capillaries in the marginal zone shortly after penetration of marginal zone. These arterial capillaries are distibuted like fingers grasping a ball (Figs. 13 and 14). Terminal ends of these arterial capillaries usually open in the marginal zone (Fig. 14). The sources of the follicular capillaries are classified into three groups similar to those of the human spleen: (1) originating from arterial capillaries distributed in the marginal zone, (2) from follicular arteries and (3) from penicillar arteries which enter directly into the white pulp. Most of the follicular capillaries belong to first category and few belong to the latter two. These follicular capil-

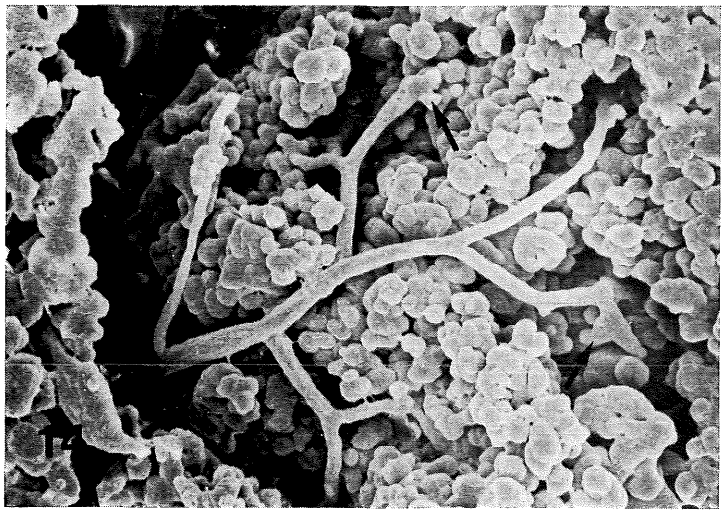

Fig. 14. Higher magnification of Fig. 13. Injected resin at the terminal segments of the vessels distributed in the marginal zone (arrows). ×340

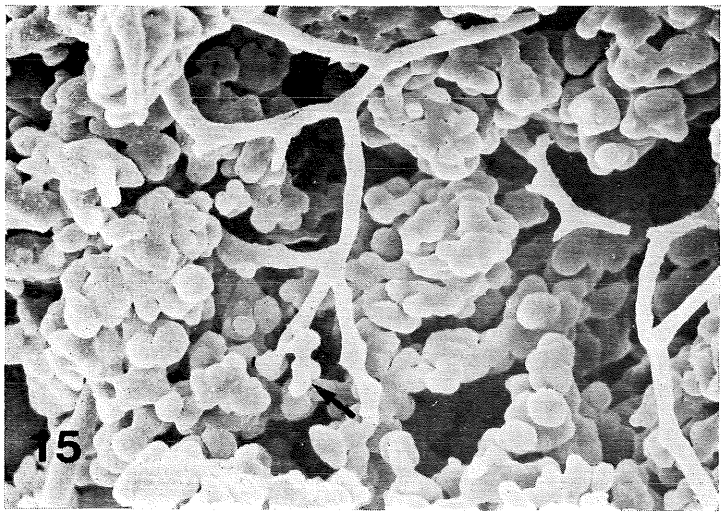

Fig. 15. Dog spleen. Injected resin leaks at the terminal segments of the penicillar arteries (arrow). ×530

laries are located chiefly in the germinal center and terminate in the marginal zone. The penicillar arteries usually enter into the red pulp cord. Direct connections between terminal segments of the penicillar arteries and the red pulp sinuses are also difficult to find in the dog spleen, while injected resin leaks at the terminal segments of the penicillar arteries (Fig. 15).

DISCUSSION

The scanning electron microscopic observations of the splenic vascular casts in the rat and mouse revealed that the central artery branched off many arterial capillaries in the lymphoid follicle. These follicular capillaries form a three-dimensional network and usually terminate in the marginal zone. In the germinal center, these capillaries show a focal anastomosing plexus. These findings support in principle the observation of Snook (1950)[21] who investigated the splenic vasculatures in different species of mammals by means of reconstruction from serial sections. The marginal zone of the rat and mouse is composed of a reticular fiber meshwork which supports a population of medium-sized lymphocytes (Krumbhaar, 1948[14]; Baillif, 1953[3]). Terminal segments of the follicular capillaries usually show ampullar dilatation at the inner side of the marginal zone and open in the marginal zone (Snook, 1964[23]; Sasou et al., 1976[20]). These dilated spaces between follicle and marginal zone were described under many terms such as intermediate sinus (Andrew, 1946[2]), perifollicular space (Altschul and Hummason, 1947[1]), marginal sinusoid (Baillif, 1953[3]) or marginal sinus (Snook, 1964[23]; Sasou et al., 1976[20]). In the present study, injected resin flowed through the follicular capillaries into the marginal zone and then into the red pulp sinus and the collecting vein. These findings indicate that a large part of the splenic circulation takes a pathway through the marginal zone. It is of particular interest that recurrent branches of the penicillar arteries are occasionally recognizable in the spleen of the rat and mouse. These recurrent branches terminate in the marginal sinus after a short centripetal course. Snook (1964)[23] previously reported three infrequent variations in the capillary pattern of the marginal zone in the rat spleen. The first is a capillary passing through the marginal zone from white pulp to red pulp. The second is a capillary passing through the marginal zone to open into the outer edge of the marginal sinus. The third is a capillary of penicillar origin entering into the white pulp to then open into the marginal sinus. The functional significance of these recurrent branches in the rat and mouse spleen remains obscure.

The vascular architecture of the human spleen has been repeatedly described by many investigators (MacNeal et al., 1927[15]; Jäger, 1929[12]; Ono, 1930[18]; Snook, 1950[21]). MacNeal et al. (1927)[15] described three types of arterial capillaries in the human spleen; (1) follicular arteries which come off in considerable numbers from follicular arteries and terminate in the marginal zone, (2) capillaries of the marginal zone which are given off from arterial branches running out of the follicle into the red pulp and have Schweiger-Seidel sheaths and (3) capillaries of the pulp cord, which are terminal branches of the penicillar arteries. Jäger (1929)[12] added another type of vessels distributed in the marginal zone and named as "Hofarterien". This type of capillaries originates from the follicular artery and has no Schweiger-Seidel sheath. Moreover, Snook (1975)[24] described infrequent branches of the penicillar arteries which return to the white pulp and supply the follicular capillaries. In our present study, it is comfirmed that the follicular capillaries in the human and dog spleen arise from three sources: (1) from arterial capillaries of the marginal zone, (2) from small arterial branches of the follicular arteries, and (3) from recurrent branches of the penicillar arteries entering into the follicle. The main source of the follicular capillaries is the first of these. The follicular capillaries are mainly related to the germinal center in the human and dog spleen.

On the basis of these findings, vascular arrangement of the white pulp and marginal zone in the mammalian spleen is divided into two groups (1) the rat and mouse type, in which the follicular arteries repeatedly divide and subdivide in the follicle and terminate in the marginal zone, and (2) the human and dog type, in which the follicular arteries and penicillar arteries give rise to many arterial capillaries in the marginal zone which supply the follicular capillaries.

The arterial capillaries in the pulp cord are derived from the penicillar arteries. The plastic resin injected leaks at the terminal segments of the cordal capillaries not only in the human and dog spleen but also in the rat and mouse. Similar observations have been reported in the human spleen by Irino et al. (1977)[11] who supported the open theory of the human splenic circulation. Moreover, Chen (1978)[4] intravenously injected plastic microspheres and histologically examined the spleen of the rabbit at intervals thereafter. He concluded that about 90% of the blood takes the open route in the normal unanesthetized rabbits. In the present study, direct connections between terminal ends of the penicillar artery and the red pulp sinuses are very difficult to identify in the mammalian spleen. These findings indicate that the splenic circulation of the mammalian is mostly open type.

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