Cubical Anatomy of Several Ducts and Vessels by Injection Method of Acrylic Resin

IV. On the vascular system of the spleen in rat and mouse

by

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with 32 figures in 3 plates

Preface

Previously, Ohta ('57) observed cubically on the arterial and the venous systems of the spleen in dog, cat, cattle, pig, goat, rabbit and human subject by injection method of acrylic resin. As a result of his comparative observations on vascular networks in the lymphatic follicle and the sheath, arterial termination and its recurrent branch, and ellipsoid of the arterial system, he described that arterial sinusoids were existent around the lymphatic follicle and the sheath, which could be filled with the injected material through the splenic artery. He further assorted morphologically the venous sinuses or venous origins in the spleen on their corrosions. Observations on rat and mouse, however, lack in his research, the authors observe on the vasculature of these species by same method, and add a supplement to his work.

Materials and Methods

The animals used for this research are 13 adult rats and 13 adult mice. The corrosion specimens are made, after injecting acrylic resin into their splenic artery and vein. The injection methods published by Taniguchi, Ohta and Tajiri ('52) and its supplement ('55), are adopted.

The animals are led to depletion death. The injection is done into the spleen *in situ* through the cannula inserted into the thoracic aorta after ligating the abdominal aorta at its caudal end. For the splenic vein, it is done through the portal vein, after ligating veins with which the portal vein joins, to centralize the injected main flow into the

splenic vein. The injection is performed at first through the artery and then the vein, the injecting pressure being below 0.6 kg/cm^2 (400 mm Hg) for the arteries and below 0.3 kg/cm^2 (200 mm Hg) for the veins.

On the other hand, part of the injected subject is fixed in 10 % formalin and made into the non-stained celloidin serial sections of 50μ without corroding, but some stained with the iron-hematoxylin. The observations are always proceeded in parallel with the corrosion specimens, the serial sections and simultaneously investigating specimens studied by Ohta under the binocular magnifier and the microscope.

Observations

A. Arterial system:

It is described about the lymph-sheathed artery, the follicular artery, the vascular networks within the sheath and the follicle, the arterial sinusoid and the penicillar artery, in rat and mouse, in order. But, as both species are destitute of the ellipsoid, its view is quite absent.

1. Rat: The lymphatic sheath is underdeveloped, while the follicles well-developed in close proximity one another (Fig. 3). The lymph-sheathed artery surrounded with lymphatic tissue runs through the centre of the sheath without tortuosity. Only one artery is observed in every case (Fig. 1), several arteries are never seen in one sheath, while the arteries are eccentric at the portion where they pass in a follicle (Figs. 3, 6, 7 & 9).

The capillary net in the lymphatic sheath is not so outstanding. The net consists of the capillaries diverged from the lymph-sheathed artery or from the stem out of it and the ones extended from the adjacent follicular capillaries (Fig. 1).

The capillary net in the follicle is developed densely, and constructed by the followings: capillaries diverged from the stem out of the lymph-sheathed artery (Fig. 4); capillaries from the stem ($13\text{--}30\mu$) which curves in arch in many cases soon after arising subrectangularly from the follicular (Figs. 6 & 8); capillaries from the arteriole originated from the stem above-described (Fig. 9). It is observed neither the artery called *centripetal*, as seen in dog, cattle, goat, pig and rabbit, nor the capillary diverged directly from the follicular artery. The capillaries in the sheath and the follicle spread radially and pour into arterial sinusoids in the periphery of the follicle (Figs. 5 & 12). Sometimes, the capillaries left the very stem pour into the sinusoids without participating in the construction of the capillary network in the

lymphatic tissue (Figs. 9 & 16).

The well-developed arterial sinusoids exist in the mantle and the halo of the sheath and the follicle (Figs. 3, 5, 9 & 12). Shape of the sinusoids is small drops ($10-50\mu$) as in dog. Accordingly, on the corrosions a tip of the capillary dissected out of the net shows a swollen end, corresponding to one of the sinusoidal lumina (Figs. 10 & 16). And in the periphery of the follicle the sinusoids are existent as congregations combined one another (Fig. 5). In the mantle and the halo where the arterial sinusoids exist, the venous sinus, which locates in periphery of them, is never seen, though observed the connection between both (Fig. 3).

The artery left the lymphatic tissue branches to the penicillar arteries, and they divide into several terminal capillaries soon or a little later. The branching aperture is relatively narrow, penicillar (Fig. 15) or cubical fan shape (Fig. 11) not spreading to octopus type. It can not be observed the terminal capillaries recur to the follicle to join with the capillary net in it, or to open on the arterial sinusoids.

The capillaries terminate blindly with ampulla (Fig. 14) or without (Fig. 15) in the red pulp, or connect with the venous sinuses (Fig. 13). The connections between the terminal capillaries and the venous sinuses are frequently seen both on the corrosions and the sections (Fig. 2). The capillaries connect with the venous sinuses from all directions at their ends.

2. Mouse: The development of the lymphatic sheath is not so well as in rat, but of the follicle as well as in it. The lymph-sheathed artery goes in the centre of the sheath.

The capillary net in the sheath is so poor that it is scarcely recognizable. Follicles are situated at the branching portions of the lymph-sheathed arteries, therefore the follicular arteries run eccentrically. It is rarely observed that a follicular artery divides into two branches equal in size in a follicle, and they run abreast in short distance (Fig. 19).

The capillary net in the follicle ($2.4-4.8\mu$) is constructed by: capillaries diverged from the lymph-sheathed artery (Fig. 18); capillaries from one or two follicular arteries ($17-27\mu$) (Figs. 17 & 20); capillaries from the stem ($10-27\mu$) which diverged subrectangularly from the follicular artery (Figs. 21, 22 & 23). Some of them are so short that they are lost (Fig. 23). Especially, in many cases the stem splits out soon after penetrating portion of the follicular and right before leaving portion (Fig. 22). These capillaries open on the arterial sinusoids, spreading to the outside of the follicle (Fig. 17, 18, 20, 21 & 23).

Arterial sinusoids, being located in the mantle and the halo around the sheath and the follicle, are developed as well as in rat. These individuals are chiefly in shape of small drops ($17-50\mu$) (Figs. 17, 18, 20, 21 & 23), without so much difference between the rat's and the mouse's about the development and the form of them. In mouse the venous sinuses also do not exist in these zones, and the arterial sinusoids communicate with the venous sinuses surrounding them.

The arteries left the lymphatic sheath diverge within the splenic pulp after several lengths, and then some of them connect directly with the venous sinuses (Figs. 25 & 26), others terminate blindly in the pulp without ampulla. It is observed largely in number that each terminal capillary ($2.9-4.8\mu$) is somewhat shorter comparing with the rat's. The branching shape is cubical fan or opening petal (Fig. 24). No centripetal artery is recognizable.

The terminal capillaries, as seen in rat, anastomose with the venous sinuses at their ends at various angles.

B. Venous system (especially on the origins):

1. Rat: The venous origins are very fine and complex (Fig. 27). Their appearances are suggestive of sinusoids of the dog's liver on the corruptions (cf. Figs. 27 & 32). Their end portions show round-pointed cylinder or short club (Figs. 27 & 28). Further, mutual anastomoses at their ends are never seen. The end is $5-28\mu$ in thickness.

2. Mouse: The venous origins are as fine and complex as the rat's (Fig. 29), and it seems there are no differences between both species. Almost of them show short cylinder (Fig. 30), or shorter club shape (Fig. 31) than the rat's. They never anastomose each other too. The thickness of the end portion is $8-25\mu$, not differing remarkably from the rat's.

Discussion

The lymphatic sheathes in rat and mouse are underdeveloped, so the capillary net in the sheath too. While the lymphatic follicles are comparatively well-developed, showing no differences between both species. In the comparative anatomical study on the structure of the spleen by Otsuka ('55), he has obtained similar view on the degree of the development of the follicle as the authors'. It also agrees with Kimura's observation ('52) that the lymph-sheathed artery passes through the centre of the sheath. Nor several lymph-sheathed arteries run parallel with one another in a sheath, as Ohta demonstrates in

cattle and goat, nor its crookedness and meandering as in cat, goat and pig, are seen in rat and mouse.

The follicular artery in the follicle developing at branching portion of the lymph-sheathed artery, runs eccentrically in its mantle in these species as Imai (T) ('38), Snook ('50), Kimura, Maximow ('53) state in other mammals. Therefore, the authors agree with the view considering to be unsuitable to call the artery *central* by Imai, Snook and Ohta. Mackenzie ('41) states by transillumination the follicular artery in mouse is 10–16 μ in thickness, the authors gain 17–28 μ . It is suspected the variation in both values may be accounted for by injection pressure.

The capillary net in the follicle is well-developed and finely dense, but it is inferior to the dog's and the rabbit's. On the net and its sources in various animals, it has been studied and discussed by many scholars. Hueck ('28, human subject), Jäger ('29, human subject) and Ono ('30, human fetus) describe that a branch of penicillar arteries recurs to the follicle and joins it to form the capillary net (penicillar arterial type by Kimura). Kimura, examining this problem in detail in rat by serial reconstruction, describes that capillaries participating in construction of a follicular capillary net are as follows: arteries divided from the follicular artery; capillaries split out of the above arteries; fine capillaries from the penicillar artery within the sheath (centripetal artery, modified penicillar). No artery to be called centripetal, however, is recognizable in rat and mouse by authors. The authors observe that the stem arises subrectangularly from the follicular artery curves in arch, branches to arterioles or capillaries, and stretches towards the periphery of the follicle. So rare are anastomoses among themselves as Kimura observes. In mouse, however, it is observed the capillaries derived directly from the folliculars join the net in the follicle, although unable to observe in rat.

The capillary net radiates towards its periphery and pours into the arterial sinusoids which situate in the mantle and the halo of the follicle, on which Ohta describes initiatively in dog, cat, cattle, goat, pig, rabbit and human subject. No capillary termination in the centre of the follicle as Kimura's description, is recognizable. Nishimaru and Steggerda ('32), Mackenzie state the end of the capillary net pours into the marginal zone, and Snook also states it opens on the space of the marginal zone. It baffles our understanding what portion of Jäger's *Mantel* or *Hof* corresponds to the *marginal zone* or *space*, or Ohta's *arterial sinusoid* existing in the halo, respectively. Then,

the authors regard them in the same light with Ohta's arterial sinusoids.

The existence of the connection between the arterial sinusoid and the venous sinus is comprehensible by the fact that the resin injected to the former through the artery penetrates into the latter and connects or mixes with the resin through the vein.

It is considered the form of the vasculature in the follicle presents various views as stated above, being affected by position of the follicle developed secondarily.

The penicillar artery left the sheath divides into the terminal capillaries after various lengths. Ohta classifies the branching types of the artery into the penicillar type, slightly separate one and more separate octopus type. As a result of the authors' observations in rat and mouse, the types are conformed to the penicillar or opening petal shape not so separate as the octopus type. Though Knisely ('36) states the existence of the anastomosis between the penicillar arteries by transillumination, the authors are unable to observe it.

On the aspect of the capillary termination, Mackenzie observes the ampulla ($6-12\mu$ in thickness) at its end, dilating in club shape, Snook also in mouse. The authors, on the corrosions, find the club or globe-shaped ampulla ($7.5-13.8\mu$) in rat, but none in mouse. It is considered that the globe-shaped may be formed by flowing out of the end of the ampulla into the reticular mesh. Accordingly, it is understandable the end may open clearly into the mesh under some physiological state.

On the connection between the arterial capillary and the venous origin, Knisely (white mouse, rat and cat), Mackenzie et al. (white mouse and rat), Nakata ('54, mouse) have observed by transillumination. Knisely describes some of the terminal capillaries connect with the venules *via* the sinus and sinus system, and others *via* the long straight capillary. Nakata also states the termination directly connects with the sinus, without opening on the pulp space. On the other hand, Blechschmidt ('37-'38, human subject), Mackenzie (mouse, rat, guinea-pig, rabbit and cat), Snook (mouse, rat, cat, mole and others), Otsuka (mouse and rat) state all the terminal capillaries open on the red pulp. Especially, it is described by Mackenzie that these capillaries open on the red pulp in funnel shape, or end blindly as ampulla, consequently these and the venous system being interposed by red pulp at the dilation phase, the both approaching and getting to appear the arterio-venous connection at the constriction phase.

On the author's corrosion specimens, nor so-called sinus system by Knisely, which is formed by ranged several sinuses is observed, nor the connection between the long straight capillary and the venule, except the direct connection between the terminal capillary and the sinus.

It is observed that the red-coloured resin through the artery and the blue-coloured through the vein show arterio-venous connections, joining each other completely in some corrosions, and that the end portion of the venous sinus is filled with the red-coloured, in other specimens (Fig. 2). Nevertheless, any view as the red-coloured one extravasates into the red pulp is not seen. From the fact, it is able to consider that the resin flows over to the venous origin through the arterial capillaries due to being injected in large quantity. Accordingly, if the whole terminal capillaries open on the red pulp, many indeterminate forms of the resin masses should be produced here and there in the red pulp. In conclusion, from the reason above-mentioned, the authors more ascertain that three kinds of the arterial terminal capillaries are recognizable in rat and mouse, *viz.* — capillaries opening on the red pulp, terminating blindly with or without ampulla, and besides connecting directly with the venous sinus largely in number.

On the venous origin, there are no differences between both species in their form and size, but its end portion in mouse is slightly shorter than the rat's. The thickness at its end is measured $4-16\mu$ by Mackenzie, $8-25\mu$ by authors. Considering the expansion of the thickness by injection pressure, the values approximately agree with each other. These sinuses, on the corrosions, are fine and complex, but no anastomoses among those origins are recognizable in both species. On the other hand, Knisely (mouse and rat) and Otsuka (mouse) announce the existence of anastomoses among the sinuses. Having the doubt whether the lack of the anastomosis on the corrosions is due to insufficiency of the injection or not, the authors have observed even the specimen injected in abundance. Nothing, however, is seen but the expansion of the caliver all for naught.

The venous sinuses have been classified in various ways for a long time: — Billroth (1861), Schumacher ('00) classify into two types, scarcely anastomosed one (horse, pig and cattle) and richly anastomosed (human subject and rabbit), besides Hoyer (1894) adds to them a compromised type (dog). Snook divides into anastomosing "sinusal" (rat, guinea-pig, rabbit, dog, human subject, skunk and squirrel) and not anastomosing simple "non-sinusal" (mouse, pig, cat, cattle,

mole, weasel, bat and horse), basing on the frequency of the anastomosis at these end portions. Ohta divides the venous origin, on the basis of the complexity, into the one, highly developed sinus (rabbit, human subject and dog), the one lacking it or not showing the sinusal feature (cattle, goat and pig), and besides a compromised type (cat).

As described above, there are two kinds of bases (exclusive of the histology of the sinusal wall) in the classification of the sinus, that is, frequency of the anastomosis and its complexity. The authors consider that the sinuses in rat and mouse should be placed under the category of well-developing, complex and not anastomosing.

Conclusions

1. Acrylic resin injection is done into the splenic artery and vein in rat and mouse, and it is studied cubically on their corrosions and sections.

2. In both species, the lymph-sheathed artery runs through the centre of the sheath, while the follicular artery eccentrically.

3. The lymphatic sheath and its capillary plexus are undergrown, but the follicle and capillary plexus in it well-developed.

4. The capillary plexus in the sheath, in rat, consists of the capillaries diverged from the lymph-sheathed artery or from the stem out of it and the ones extended from the follicular capillary. In mouse it is so undergrown that it is scarcely found.

5. In rat, capillary plexus in the follicle is constructed by capillaries diverged from the stem out of the lymph-sheathed artery, ones from the stem out of the follicular artery and ones from arterioles *via* the stem, and in mouse, by capillaries from the lymph-sheathed artery, ones from one or two follicular arteries and ones from the short stem out of the follicular.

6. These capillaries in the sheath and the follicle, spreading towards the outside of them, pour into the arterial sinusoids (Ohta) located in the mantle and the halo. Sometimes, the capillaries pour into the sinusoids without participating in the construction of the capillary net in the follicle.

7. The individual sinusoid is in shape of small drop ($10\text{--}50\mu$ in both species). These arterial sinusoids connect with the venous sinuses around them.

8. Branching types of the penicillar artery are penicillar or cubical fan shape in rat, and cubical fan or opening petal shape in mouse.

9. No arterial terminal branch recurs to the follicle in both species.
10. The terminal capillaries in mouse are somewhat shorter than the rat's. They terminate blindly in the splenic pulp with or without ampulla, or connect directly with the venous sinuses in rat, but no ampulla in mouse.
11. Splenic venous origins are fine and complex. The end portion shows round-pointed short cylinder or club shape, but mutual anastomosis at its end is never seen. Sinuses in both species are well-developing, complex and non-anastomosing.

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Explanation of Figures

Plate I.

- Fig. 1. Rat, cast. Lymphatic follicles (f) and lymphatic sheath. Lymph-sheathed artery (la) and follicular arteries (fa). Capillaries in the sheath extended from the follicular capillaries (✓). Penicillar arteries in shape of the cubical fan (→), and the blue-coloured back is venous sinuses. ca.×20.
- Fig. 2. Rat, section stained with iron-hem. The arterio-venous connections (↑) and arterial terminal capillaries (×). Red resin injected into the arterial system flows into the tips of the venous sinus. ca.×150.
- Fig. 3. Rat, section. Three lymphatic follicles developing in close proximity one another, eccentric follicular artery, arterial sinusoids (as) around the follicles, and the venous sinus (s). ca.×53.
- Fig. 4. Rat, cast. Arch-formed stem from the lymph-sheathed artery and the follicular capillary net out of the stem. ca.×140.
- Fig. 5. Rat, cast. The capillaries in the follicle pour radially into the arterial sinusoids (white in background) around the follicle. ca.×80.
- Fig. 6. Rat, section. Eccentric follicular artery (×), stems (arrow) curving in arch from it and capillaries out of the stem. ca.×94.
- Fig. 7. Rat, section. Capillaries from the arteriole out of the stem diverged from the follicular artery (×). ca.×75.
- Fig. 8. Rat, cast. Lymph-sheathed artery (la), arch-formed stem (↓) out of the follicular artery (↑), and the follicular capillary net. ca.×35.
- Fig. 9. Rat, section. Arterial sinusoids (as) around the follicle, and capillary, which diverged from the arteriole out of the follicular artery (fa), opens directly on them. ca.×183.

Plate II.

- Fig. 10. Rat, cast. Follicular capillaries open on the arterial sinusoids (the tips of the capillaries are swollen, ↓). ca.×81.
- Fig. 11. Rat, cast. Penicillar capillaries in shape of cubical fan. ca.×188.
- Fig. 12. Rat, section. Follicular capillary net, spreading radially, opens on the arterial sinusoids (as). ca.×77.
- Fig. 13. Rat, cast. Direct connection between arterial terminal capillary and the sinus (↑). ca.×50.

- Fig. 14. Rat, cast. Capillary terminations with ampullae. ca. $\times 267$.
Fig. 15. Rat, cast. Penicillar arteries in shape of penicillar, without ampulla. ca. $\times 83$.
Fig. 16. Rat, cast. Capillaries from the stem out of the follicular artery (fa), pour into the arterial sinusoids (as), without participating to form the net. ca. $\times 59$.
Figs. 17. & 20. Mouse, cast. Capillaries ($\uparrow$) directly from the follicular artery (fa), which pour into the arterial sinusoids (as). ca. $\times 75$.
Fig. 18. Mouse, cast. Capillaries ($\rightarrow$) directly from the lymph-sheathed artery (la). Arterial sinusoids (as). ca. $\times 38$.
Fig. 19. Mouse, cast. Two follicular arteries ($\uparrow$) equal in size, which run parallel with each other. ca. $\times 54$.
Fig. 21. Mouse, cast. Follicular capillaries out of the stem ($\uparrow$) which diverged subrectangularly from the follicular artery (fa). ca. $\times 71$.
Fig. 22. Mouse, cast. In two follicles (dotted line), the stems which split out soon after penetrating the follicle ($\uparrow$) and right before leaving it ($\rightarrow$, $\leftarrow$). ca. $\times 59$.
Fig. 23. Mouse, section. Follicular capillaries from the short stem out of the follicular artery (fa), open on the arterial sinusoids (as). ca. $\times 231$.

Plate III.

- Fig. 24. Mouse, cast. Penicillar arteries in shape of opening petal. ca. $\times 188$.
Figs. 25 & 26. Mouse, section. Direct connections between terminal capillary and sinus ($\downarrow$). ca. $\times 138$, 173.
Fig. 27. Rat, cast. General view of the venous sinuses. Those ends are fine and complex, non-anastomosing. ca. $\times 81$.
Fig. 28. Rat, cast. Magnified view of Fig. 27, cylinder and club-shaped end portions. ca. $\times 110$.
Figs. 29 & 30. Mouse, cast. General view of the venous sinuses. Shorter cylinder-shaped ends than the rat's. ca. $\times 71$, 109.
Fig. 31. Mouse, cast. Short club-shaped end portions of the sinuses. ca. $\times 244$.
Fig. 32. Dog, cast. Sinusoids of the liver. Richly anastomosing and complex. (Compare with Figs. 27, 28, 29, 30 & 31) ca. $\times 250$.