Splenic architecture reflected in the connective tissue structure of the human spleen

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The structure of the supporting tissues of the spleen is related to the organ's function. Two structures, macro- and micro-, can be distinguished. The macrostructure separates the lienal lobuli (Mall 1900) from each other, defines to a large degree the vascular organization within the organ and, as the *capsula lienis,* forms the organ's external boundary. The microstructure consists of lattice fibers and very thin elastic fibers. It is involved in the building-up of the *sinus lienales,* which form the splenic parenchyma wherein smooth muscular cells may also play a more or less important part. Whereas a number of definite statements can be made about the macrostructure, this is far less so when dealing with the microstructure. While many questions *re* the macrostructure still remain unanswered or have not yet been satisfactorily answered, there are even more open questions with respect to the spleen's microstructure. In the adult, the metabolic functions of the spleen are the most important, whereas in human foetuses the spleen is more important as a storage organ in the circulatory system. Comparative anatomy clearly demonstrates both extremes of these processes. The articles in the reference books by A. Hartmann[10], von Herrath[12] and Tischendorf[19] contribute extensive information on this subject. Throughout this paper, when we refer simply to 'the spleen', we are referring to the human spleen.

It is well-known from experience that an organ's fundamental structure is seen more easily in advanced foetuses than in adults; we have, therefore, examined spleens of a large number of different ages. Special attention was paid to examining perfused spleens. Faller[6] tested the tensile strength of the lattice fibers as to their tensile strength in perfused spleens that had not been fixed. As well as relatively simple methods, e.g. the description of slits ('Spaltliniensysteme') and the removal of parts of the *capsula lienalis* and the fibrillation of dry preparations according to Semper, we have made use of most of the available histologic specimen staining techniques and, above all, of polarization-optical examination. Together with two doctoral assistants, Martin[17] and Monney[18], we obtained two negative reconstructions in negocoll and hominit (linear enlargement: 300) by means of serial sections through the spleens of a new-born child and of a human foetus (18 mm crown-coccyx length). Relevant technical details can be found in the dissertations just referred to. We limited ourselves strictly to the reconstruction of the tension-proof connective-tissue structures, disregarding the lienal microstructure, i.e. the lattice-fiber structures of the red and the white pulpae as well as their respective continuations. The picture which we gained by these methods of studying the lienal structure is, therefore, by no means a final one; it leaves, we regret to say, considerable room for alternative interpretations of the findings.

After thirty years of research and teaching – again and again returning to the problem of the structure of the human spleen – we ourselves, are all too conscious of this shortcoming. In the words of Ramon y Cajal, there are no exhausted problems; there are only people exhausted by problems. Often it is wiser to lay a problem aside for a while to allow our understanding of it to ripen gently in our subconscious. Things can then be seen in their proper perspective.

Lienal mantle and lienal center

The fact that the spleen connective-tissue structure does not stem exclusively from either the lienal capsule or the trabeculae provides us with a ready basis for classification. On the one hand, we are dealing with the connective tissue which develops into the lineal capsule and, towards the inside, into the radial trabeculae. These trabeculae combine, secondarily, with the branching group of the arbor trabecularis which enters the organ at the hilus. In this way splenic capsule and zona subcapsularis are seen as a unit which surrounds, *qua* 'lienal mantle', the 'lienal centre' on all sides. This centre, in its turn, is arranged around the main trabeculae. In the *hilus* region the structures of mantle and center merge. The mantle is a pressure compensation zone which completely surrounds the central group of trabeculae, which causes lobulation. A structure is thus obtained which permits rapid enlargement, up to a certain limit (acute splenomegaly). The active force involved is the interior pressure of the organ itself. This pressure is stored up, so to speak, in the stretched-out elastic elements which, in their turn, tend to re-establish the initial situation and, in this manner, to reduce again, passively, the spleen's volume while smooth muscle cells, actively, work towards the same end.

The lienal capsule and the radial trabeculae ('Speichentrabekel') are, first and foremost, pressure-absorbing structures whose elements, e.g. the connective-tissue fibers and the lattice fibers, are tension-proof, whereas the organ as a whole is provided with a fair degree of structural elasticity. As far as the collagenous fibers are concerned, this fact is not disputed. With regard to the lattice fibers, however, there are two contradictory opinions. Benninghoff, in his manual of anatomy, describes the lattice fibers as 'fairly expansible and elastic'. Möllendorff, on the other hand, was sceptical; 'I shall not be conviced until I have extended such a fiber myself, and I consider that the collagenous substance of the reticulin is fundamentally made of the same kind of material. The collagenous fibers define the stretch limit in the micro-macroscopic region, whereas the lattice-fibers are found whereever a stable mechanical structure has to be maintained in a cellular area'. We considered

that the simultaneous use of two structural materials of the same nature would be meaningless if both had the same properties. This appears to be logical. However, logical argument is not decisive in this case; what matters is observation of behavior. Levi (1931) experimented with a micromanipulator on fresh rat liver as well as on rat spleen. He came to the conclusion that 'Although they are very fine, stroma fibers of the liver and of the spleen have a high tensile strength'. Bairati (1940) experimented with frozen sections of the spleen of various small animals and came to the conclusion: 'It would seem that the elementary fibers do not suffer a noticeable change under tension and that, therefore, they have a marked functional solidity'. The present writer, Faller[6], experimented with human spleens which had been perfused unfixed post mortem. Frozen sections, between 20 and 30 µm thick, were observed in a dark field. If thin glass hooklets are fitted in the micromanipulator, and used to draw out a loop, that loop narrows in its diameter when looked at perpendicularly, and is finally torn asunder when pulling continues. The spleen reticular fibers are obviously not stretchable in a rubber-like fashion even though they have a considerable tensile strength. Nevertheless, the structural elasticity achieved by the displacement of the fibers is not inconsiderable although the individual fibers do not stretch. The information obtained from experiments with lattice-fibers which are embedded in elastic sheaths, e.g. in a neurilemma, in a sarcolemma, or in capillary vessels, is quite different. The lattice-fiber net, which unfolds but does not stretch, is re-organized; the gel-like elastic fundamental substance easily creates an impression of real stretching.

Most of our preparations, however, were not made in order to study the fine structure but for the sake of a macro-microscopic study of the connective-tissue structure. The arsenal of methods that can be used in such an approach is extraordinarily well-stocked, but, unfortunately, most methods can only illustrate partial aspects; how these parts fit together very often remains a matter of opinion for the experimenter. This is true even of the very simple method of Dabelow[3] in which ca. 200-µm-thick sections are stained 'en bloc' and then cleared in xylol and examined under the stereomicroscope. Dabelow's method goes back to the very much older maceration method by means of which von Ebner[2] examined thick spleen sections. He describes them in vol. 3 of Kölliker's treatise on tissues, which was first published in 1900. Von Ebner made use of physiological saline solution. This analytic method using thick sections has seen a number of variations, of which no more than two especially ingenious ones will be mentioned. Romeis (1940) had the lienal pulpa eaten away by tadpoles; Schleicher (1941) in his turn making use of a 1% sodium carbonate solution at 40 °C in a thermostat, was able to diminish considerably the time of reaction. Very frequently, macertion was combined with a gentle, prudent massage of the thick sections (Kohira, 1958; Arinci, 1961; Vereby, 1943, et al.) Hofmann (1951) reverted to Semper's method for preparing dry preparations, from which he then tried to remove the fibers. Hartmann and Bennet[11] were 1927, to the knowledge of this writer, the first to introduce the wax disc method. Results, however, did not come up to expectation. This is why we tried out a negative reconstruction, such as had been recommended by Dankmeyer (1940). What is to be presented is cut out of preformed negocoll discs. The discs are then piled one on top of another; the cavities thus produced are then filled with molten hominit. Eventually the negocoll form, which can easily be cut, is removed. Martin[17] improved the method by reinforcing the hollow spacers, prior to filling them out, with thin steel wire; this gives the hollowed-out model greater stability. The operation is time-consuming and requires considerable skill.

Related to the problem of the structure of the spleen is the question of acute splenomegaly. Hueck[14] perfused, under pressure, 75 human spleens. He succeeded in obtaining an enlargement of the spleen's volume to approximately three times the normal size. Our experiments produced a little less than a doubling of the volume; this result agrees fairly well with those obtained by radiological examinations.

The lienal capsule

The capsule of the spleen is a coarse fibrous cover which is protected, with the exception of the hilus, by a serosa. As it is semitransparent it has a whitish tinge, which is the reason for its being called the *tunica albuginea*. According to Sobotta (1914) its thickness lies between 0.1 and 1.5 mm, with an increase in thickness toward the hilus. In Gighi's opinion (1933) the capsule's thickness hardly varies between 10 and 60 years. After 60 years it again begins to grow thicker because the connective-tissue grows coarser. The thickness values measured by Martin[17] in new-born infants are the same as those in the foetus of 18 mm crown-coccyx length examined by Monney (1980), i.e. 0.02–0.03 mm. A stained section prepared in the usual way shows, when the cut has been made in the appropriate direction, two layers which can be distinguished by the direction in

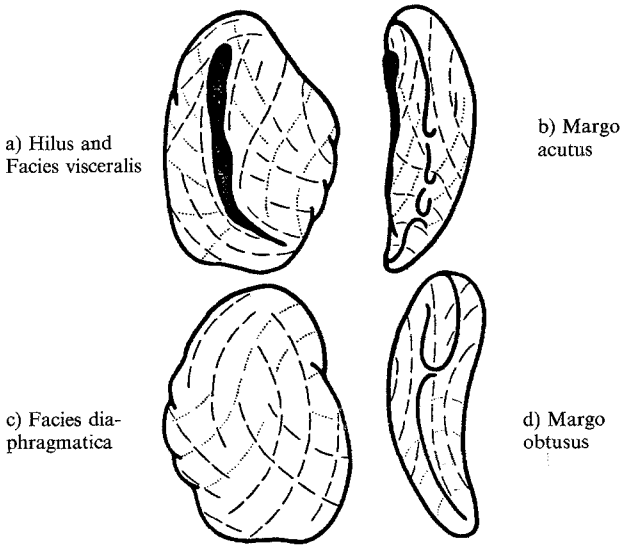

Figure 1. Schematic representation of the slit systems ('Spaltlinien') of the human lienic capsule. Dots show a less frequent type of course.

which their fibers run. Sometimes one layer is more strongly developed, sometimes the other. Toward the pulpa a third, loosely structured, zone can usually be observed. Occasionally such a zone, in which the fibers change direction, can also be seen between the two main layers. The collagenous fibers are of medium thickness and surprisingly uniform. In the transition zone towards the pulpa they become considerably finer and may even pass directly into the reticulum. A system of extremely fine elastic fibers is associated with the superficial connective-tissue layer. In the deep layer the elastic elements are much stronger. The collagenous fibers look as though they are threaded through the interstices of an elastic net. Not much is known about the spatial disposition of the structural elements. Vereby (1943), Baud[2] and Faller[6] published various papers dealing with the problem. The slits ('Spaltlinien') run tangentially in the direction of the hilus field (fig. 1). The fiber frame-work also disappears, to a large degree, in the radial trabeculae. The varied structures found seem to be dominated by two main types. In a full, taut spleen the linear systems intersect almost at right angles. The polarization microscope shows that the connective-tissue fibers in the capsule spread out and by no means radiate only into the deepest capsular layers.

The arbor trabecularis and the development of the lienal lobuli

There are different types of spleen trabeculae: vascular, venous, connecting, fixation, and radial. They will be described in greater detail when the *zona subcapsularis* is considered. The vascular trabeculae contain both arteries and veins. Their connective-tissue forms the stem as well as the major branches, from first to third order, of the *arbor trabecularis;* they limit what can be called the inner part of the lienic center (fig. 2). The lobuli are relatively large. The musculature of these arteries is totally independent of the trabecular muscular system, from which they can be distinguished because of their sharply outlined and loosely structured *adventitia.* The arteries are relatively more independent of the *arbor trabecularis* than the veins; the trabecular veins have a very thin *adventitia,* partly continuous with the trabecular tissue. The lienal venous branches follow the *arbor trabecularis* for a much longer distance. They form already at the outer part of the spleen center and approach more and more closely to the *arbor trabecularis.* Sometimes there are traces of them in the subcapsular net-work, which imperfectly separates the lienal center from the *zona subcapsularis.* On contraction of the trabecular muscles, the venous reflux is stimulated; on the other hand, in those parts where the veins completely disappear inside the trabeculae a kind of sphincter effect will develop. The connecting trabeculae contain no vessels; they serve to strengthen and to stabilize the *arbor trabecularis* and they assist in delimiting the lienic lobuli, Mall[16]. Each lobulus is extensively linked with the parenchyma of the neighbouring lobuli. Martin[17] and Monney[18] paid special attention to the development of the lienal lobuli. In human foetuses and in new-born infants the connecting trabeculae are roundish or slightly flattened. Only in adults are true septa found; these are often windowed. The angles between the individual connecting trabeculae are varied in the extreme. In foetuses or in new-born infants the connecting trabeculae are much more numerous than in adults. Hence it follows that some of the trabeculae must disappear in the course of a life-time. Mall[16] first mentioned lienal lobuli in 1898. Of utmost importance was the discovery by Jäger[15] (1931) and that their arterial supply is terminal, a fact hinted at as early as 1926 by Ward, McNeal, and Ravid. Around the inner part of the lienal center, an outer zone of smaller, characteristically polygonal lobuli is found.

The subcapsular zone

The *zona subcapsularis* lies between the inner side of the lienal capsule and the subcapsular network. The latter has also been called the tangential radial beam net ('Tangentialbalkennetz'). From the inner side of the capsule the radial or spoke trabeculae grow in the direction of the trabecular beam periphery forming, together with the latter, the tangential beam net ('Tangentialbalkennetz'). According to Orsós[19] the subcapsular zone lies like a buffer between the *arbor trabecularis* and the

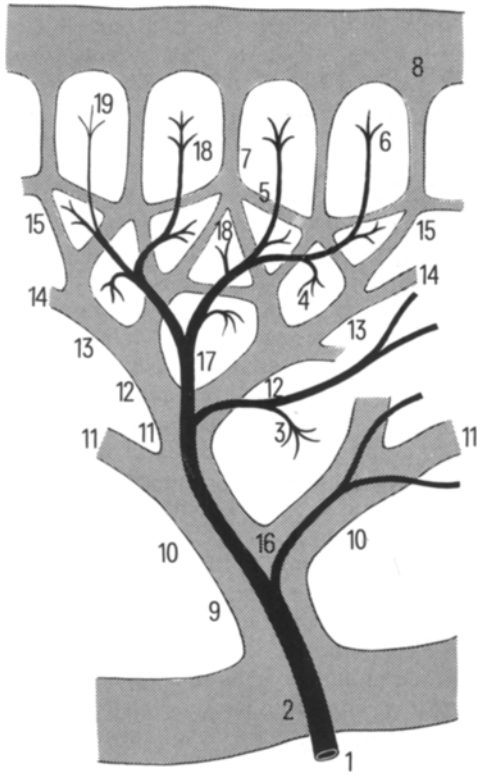

Figure 2. Diagram of the lienic structure and of the arterial vascular flow as obtained through the analysis of the connective-tissue structure of the spleen. 1) Hilus lienis. 2) Stem of the arteria lienalis. 3) Large lienic lobulus from the inner zone of the spleen center. 4) Small lobulus from the outer zone of the spleen center. 5) Subcapsular network. 6) Elongated lobulus of the spleen envelope. 7) Radial trabecula. 8) Spleen capsule. 9) Stem of the *arbor trabecularis.* 10) First-order branching. 11) Second-order branching. 12) Third-order branching. 13) Fourth-order branching. 14) Fifth-order branching. 15) Sixth-order branching. 16) Trabecular artery. 17) Pulpa artery. 18) Lobulus artery. 19) Penicillus.

lienic pulpa, on the one hand, and the lienic capsule, on the other. It is somewhat surprising that the subcapsular zone should contain no white pulpa at all.

Summary

An analysis of the connective-tissue structure of the human spleen can give us information about the basic architecture of the organ. The most important part of the spleen is the lienic center around which the subcapsular zone forms an envelope, like a mantle. This zone has but little depth and develops superficially. The tangential radial beam net ('Tangentialbalkennetz') is formed partly by the radial trabeculae of the capsule and partly by the outer branches of the *arbor trabecularis*. This *arbor* divides into 5–6 branching orders. The branches of orders 1 to 3 surround the parenchyma of the spleen center's inner layer. The lienic lobuli which are found between these branches are relatively large and are connected very extensively with their parenchyma. The branches of orders 4, 5, and 6 enclose the lienic lobuli of the outer layer of the spleen center. The splenic lobuli are defined by the vascular course. Mostly they are provided with one or two arterial influxes and, as a rule, with only one venous drain. Their mutual delimitation is more of a funcitional than of a morphological nature. This led von Herrath[12,13] to coin the term 'functional spleen lobuli'. The lienic envelope lies between the inside of the capsule and the outermost branchings of the *arbor trabecularis*. This *arbor* is subdivided, by the radial trabeculae, which never have any vessels, into elongated lobuli and serves first and foremost to regulate pressure. The lattice fibers are of high tensile strength and are extensions of the collagenous fibers seen at the microscopic level.

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Scanning electron microscopy and terminal circulation

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Key words. Spleen; terminal circulation; scanning electron microscopy; white pulp; marginal zone; red pulp; sinus endothelium; rod cells; cords of Billroth; macrophages; platelets.

Introduction

The spleen is one of the organs whose real, three-dimensional microfabric has been most enigmatic. Recent application of scanning electron microscopy (SEM) has contributed a great deal to the elucidation of its structure but many riddles still remain to be solved. During the last 13 years we have been engaged in fine-structural investigation of mammalian spleen mainly by means of SEM[13,16,17,18,20,32,33,61]. This paper reviews the results of this work, concentrating on the human spleen, and compares them with the reports issued by other research groups.