Virchows Arch [Pathol Anat] (1984) 403:27–40

Virchows Archiv A

Ellipsoids in the human spleen

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Summary. A study of ellipsoids (EL) in the human spleen was done on 25 surgically resected specimens in order to assess the number of EL, their structure, the nature of the cuff cells and the problem of lymphocyte traffic through their wall. The results show that an average spleen of 200 g contains 1.6×10^7 EL. They are localized in a constant segment of the vascular tree, just at the transition of the penicillary arterioles into capillaries and they consist of a capillary (not an arteriole) surrounded by a cuff of cells. These cells are neither endothelial nor smooth muscle cells, display lysosomal activity and are considered as fixed specialized pericapillary cells capable of cytoplasmic expansion. Since their number is inconstant in different individuals and in different species they are considered to be dynamic histological structures. Because of the constant presence of lymphocytes in their walls we consider the EL to be a transit system for lymphocytes and the splenic equivalent of the high endothelial venules in the lymph nodes.

Key words: Spleen – Ellipsoids – Morphometry – Electron microscopy – Lymphocyte traffic.

Ellipsoids in the human spleen

During the examination of resected spleens we were struck by the presence in the red pulp of groups of "histiocytes" centered around a small lumen sometimes containing an erythrocyte. In order to identify and localize the level of the vascular tree at which these cells accumulated we performed a graphic reconstruction of serial sections and realized that we were looking at the "Hülsenkapillaren", the sheathed capillaries or ellipsoids of Schweiger-Seidel (1863). Once recognized we could find them in all resected human spleens and noticed that they also contained scattered lymphocytes, a finding already reported by Hueck (1927), Solnitzky (1937) and Kellner (1962). This prompted an investigation aimed at:

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1. the assessment of the number of ellipsoids in the human spleen in standard conditions in a general pathology laboratory;

2. the study of the construction of the ellipsoids and the nature of the cuff cells;

3. the quantitative evaluation of lymphocyte traffic through the ellipsoids. We define the ellipsoids as a segment of a capillary surrounded by a cuff of large polygonal cells.

Material and methods

A graphic reconstruction of the ellipsoids, further referred to as EL, was done on a spleen of 490 g of a patient with congenital spherocytosis. We used the technique of Staubesand

Table 1

Accession number	Sex	Age	Spleen weight	Number of sheats
Group 1. Gastrectomy for adeno-Ca				
13762	♂	71	139	55
10721	♂	61	112	27
11874	♂	60	184	99
12602	♂	44	143	56
14008	♀	73	95	6
Group 2. Laparatomy for inflammatory conditions (e.g. pancreatitis)				
15970	♀	48	406	112
14486	♂	68	92	12
9658	♀	51	281	128
10519	♀	39	147	53
16746	♀	38	185	99
Group 3. Splenectomy for hematological diseases (ITP, spherocytosis)				
15614	♂	14	100	50
10605	♀	19	172	26
20063	♀	79	209	56
16726	♀	44	500	113
14016	♀	41	343	13
Group 4. Non involved spleens at staging for Hodgkin's Disease				
15528	♀	35	153	110
19537	♂	47	138	3
17809	♂	44	360	5
980	♂	21	135	37
1430	♂	21	360	41
Group 5. Traumatic rupture				
82.198	♂	13	100	94
80.2727	♀	9	60	65
80.2034	♂	20	190	63
82.13	♂	18	250	38
80.2069	♂	4	130	70

and Andres (1953). In short, serial sections of 5 mμ thick, stained by the Sirius red stain of Sweat et al. (1964) were photographed and projected at a final magnification of 562. The small arteries and the EL were drawn on transparent paper. These were superposed and projection lines from each sheet were marked on millimeter paper in a plane vertical to that of the sections in order to obtain an exact reconstruction on scale. This technique allows the choice of the most demonstrative unfolding plane of ramified structures.

Twenty-five resected spleens of 5 different categories of patients were studied (Table 1). Fixation of fresh tissue slices not exceeding a thickness of 3 mm was done in B5 mercuric chloride-formol solution with a fixation delay less than 3 h. Spleens ruptured by trauma were fixed in Bouin's fixative and the fixation delay could not be precisely determined but was less than 12 h. A quantitative study of the number of EL per unit surface of red pulp was obtained by counting the numbers of cross-, oblique or longitudinal sections of EL in a given surface limited by the largest square of the Zeiss Integrating Oculometer I. In each case 100 fields were scanned with a 40× objective, covering a total surface of 9 mm^2. The estimation of the total number of EL in a spleen was based on the following figures: mean spleen weight of 203 g, mean number of EL 57 for 9 mm^2 and the thickness of the tissue slice for which the 5 mμ section was representative two times the length of the longer EL, 2 × 100 mμ. A correction was made for the proportion of red pulp in the total volume according to Stutte (1970) and 80% taken as this proportion. With these figures it was possible to use Buffon's Needle Problem (Aherne and Dunnill 1982) and his formula $P = 2L/d\pi$ was applied: P is the probability of hitting structures of length L in a field of width d, provided d is greater than L.

In the cases of ruptured spleens only HE and Sirius red stains were done. In the other cases following techniques were used: HE, Hem. Safran Phloxine, Sirius red, PAS, methylgreen pyronine, iron, Gordon Sweets reticulin (1936), trichrome Masson stains.

Enzyme histochemistry on cryostat sections included: acid phosphatase, neutral and acid non specific esterases, betaglucuronidase, alkaline phosphatase, naphthol AS-D chloroacetate esterase. Immunohistochemistry was done on paraffin sections with the PAP technique of Sternberger (1974) and included antisera against IGA, IGG, IGM, Kappa, Lambda, muramidase and in a few cases alpha-1-antitrypsin (Nordic). In five recent cases cryostat sections were treated with monoclonal antibodies against peripheral T-lymphocytes, T-helper and T-suppressor cells, and cells with the HLA Dr antigen (Becton-Dickinson).

For electron microscopy, small tissue fragments were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer. Postfixation was performed with 1% OsO4 in veronal acetate buffer. Tissue fragments were then block-stained with 2% uranyl acetate in veronal acetate buffer, dehydrated through a graded series of acetons and embedded in Epon 812. Semi-thin sections were stained with 0.1% toluidine-blue.

Suitable parts containing EL were selected for ultra-thin sectioning with a diamond knife. Post-staining was performed with uranyl acetate and lead-citrate. Four EL from three patients were studied.

Results

Reconstruction

Two groups of EL, one in the vicinity of a lymphoid follicle and one in the middle of the red pulp, totalling 13 EL were reconstructed. Terminal arterial branches, devoid of further bifurcations, of a diameter between 12–15 mμ showed after a straight trajectory of about 100 mμ a transition into capillaries surrounded by one or more layers of pale polygonal cells. The capillaries, with or without cuffs, ramify irregularly. The EL generally form short segments of 50 to 100 mμ long with a diameter between 25 and 50 mμ. We observed one long segment of 214 mμ. Generally the capillary and its cuff end abruptly in a cord of the red pulp. Sometimes the

capillary runs farther without a cuff for a short distance of about 20 mμ (Fig. 1). The artery from which the group of EL surrounding the follicle originated was not the artery of this particular follicle but one from a distant ramification in the adjacent red pulp.

Quantitation

The figures for the numbers of EL are shown in Table 1. The figures less than 10 were counted 3 times in different fields. The values oscillate between 5 and 128, with a mean of 57 in a surface of 9 mm^2. Correction of this number for the possibility of hitting an EL in the 5 mμ section out of a tissue slice of 200 mμ thick: $P = 200/3.14 \times 200 = 0{,}31$. This means that of all the EL in the 200 mμ slice only 0,31 are hit, so that the counted EL represent this fraction. To get a P of 1, which means that each EL has been hit once, we must multiply the number by $1/0.31 = 3.2$. Thus the total number of EL in a tissue slice of 200 mμ amounts to $57 \times 3.2 = 182$.

These figures allow a quantitative estimation of the number of EL in an average spleen by calculating the number of tissue slices. Admitted that the specific gravity is 1, a spleen of 200 g has a volume of 200 cm^3. Of this 80% is composed of red pulp = 160 cm^3. The volume of a tissue slice is 9 $mm^2 \times 0.2$ mm = 1.8 mm^3. The number of slices is 160 cm^3 : 1.8 mm^3 = 88.000. Hence the total number of EL amounts to $88.000 \times 182 = 1.6 \times 10^7$.

Light microscopy

Examination of the histological structure of EL shows them to be composed of 1–3 concentric layers of cells with a fine granular pale cytoplasm (Figs. 2, 3). They generally lie in a circular position around the long axis of the capillary, although many variations may be found. The cell borders are indistinct in light microscopy, but the widest diameter in EM is 12 mμ. The nuclei are single, oval and sometimes indented. They have a loose chromatin network without nucleoli. These nuclei are larger than those of the endothelial cells in the capillary or in the arteriole. With the MGP stain the cytoplasm contains small pyroninophilic granules. In cases with phagocytosis of iron and PAS positive material by splenic histiocytes no accumulation of these substances was found in the cuff cells.

The lumen of the EL capillary is generally narrow and in many cross sections indistinguishable: however it can be traced in serial sections or by the presence of an erythrocyte. This is not an absolute criterion because the EL may contain erythrocytes in their cuffs. The lumen is often excentric, especially at the terminal segment of the EL because the cuffs do not end in a strictly transversel plane to the long axis of the capillary.

A striking finding is the presence in between the cuff cells of lymphocytes and to a lesser extent of polymorphs or erythrocytes. The number of lymphocytes is very variable but a minimum of one per EL is a rule and it is not exceptional to find 3–5 lymphocytes in a cross section. The application of monoclonal antibodies shows that the lymphocytes belong to both T-

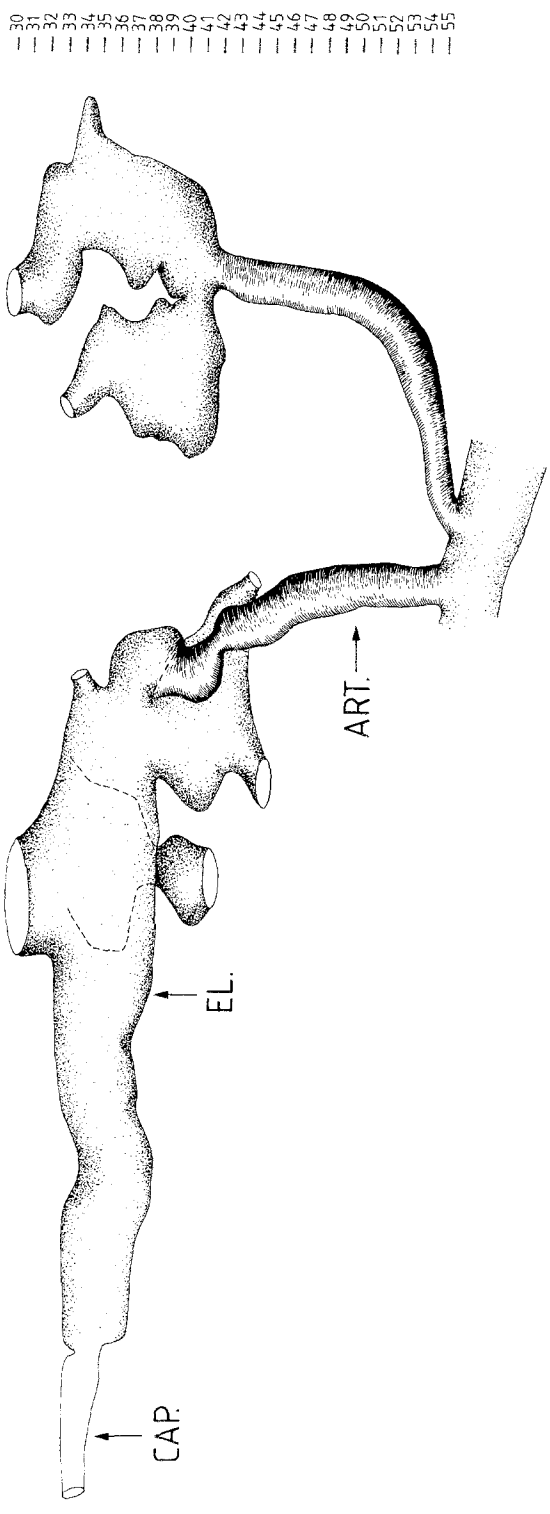

Fig. 1. Graphic reconstruction of a group of EL originating in two arterioles. The long cuff is 214 mμ long and the capillary to the left extends beyond it. The open endings of the EL correspond to the last section plane of the cuff cells and not to the capillary lumen which is too irregular to allow reconstruction. Original magnification 562 ×, reduced at 80%

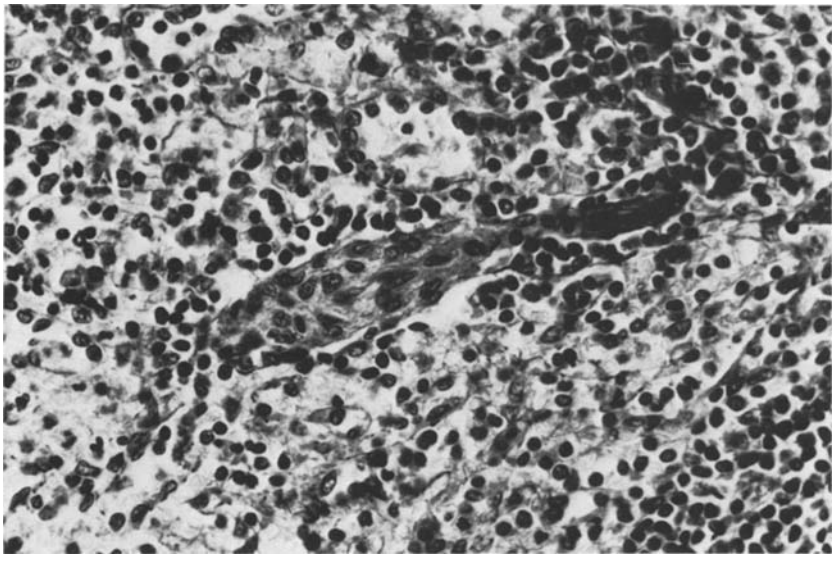

Fig. 2. Longitudinal section through an EL. The dense track to the right is the adventitia of the arteriole. The long nuclei in the center of the EL are from endothelial cells surrounding a narrow lumen. Between the cuff cells are a few lymphocytes. Sirius red $\times 300$

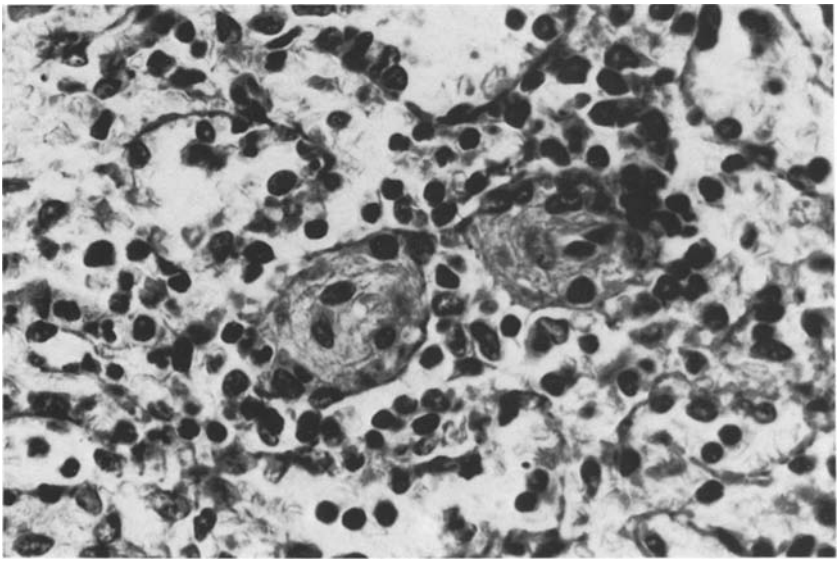

Fig. 3. Two cross sections of EL. In the center are narrow lumina. Lymphocytes are seen in the cuff. Sirius red $\times 450$

helper, T-suppressor and B cells. Generally the EL are not surrounded by lymphocytes. Sometimes when there are many lymphocytes in the pulp cords they may be more concentrated around EL. Exceptionally the lymphoid sheath of an arteriole continues around an EL forming 1–3 layers of small lymphocytes.

The cuff cells are entangled in a network of very fine fibers which can be readily demonstrated by the reticulin stain. These fibers are less conspicuous in the PAS stain. The medium sized and thicker fibers are seen with the Sirius red stain. We use the term fiber in the usual histological terminology, knowing that in fact we are dealing with a lamella or a ribbon. The stains show that the subendothelial basal membrane (BM) of the arteriole continues in the subendothelial BM of the capillary, throughout and beyond the EL. The adventitial fibers of the arteriole continue into the outer collagenous lamella of the EL, but they become gradually very thin and fenestrated. The cuff cells lie in a network of fine and thick fibers bound by the basal membrane and by the adventitial collagenous fibers. This network has very fine meshes distinct from all other reticulin networks in the spleen (Fig. 4).

Histochemistry

Enzymehistochemistry shows a strong coarse granular positivity of the cytoplasm of the cuff cells for acid phosphatase, acid and neutral non specific esterases. The endothelial cells of arteries and arterioles show a clear positivity for alkaline phosphatase, it is somewhat weaker and more diffuse in the capillaries. Immunohistochemistry shows a negative reaction of the cuff cells for muramidase, alpha-antitrypsin and for heavy and light chain immunoglobulins. Polymorphs in between the non stained cuff cells are readily identified by the PAS stain and the chloroacetate esterase activity.

The non EL capillaries have a wider lumen than the EL ones, the BM and the adventitial layer merge. The endothelial cells are identical to those of the EL segments.

Electron microscopy

The study of the electron micrographs shows that the lumen of the EL is narrow or slitlike (Figs. 5, 6). It is bound by endothelial cells displaying thight junctions. They contain Weibel-Palade bodies and are resting on a rather thin basal membrane. One or more layers of large irregular cells, 7–12 mμ in diameter, with many slender interdigitating cytoplasmic extensions are concentrically arranged around the endothelial cells. The cytoplasm contains some mitochondria, many profiles of smooth endoplasmic reticulum and a fair number of primary and secondary lysosomes (Fig. 7). The cells are incompletely surrounded by basal membrane-like material and collagen fibers which at the periphery of the EL form a dense lamina separating the EL from the other pulp cells and fibers.

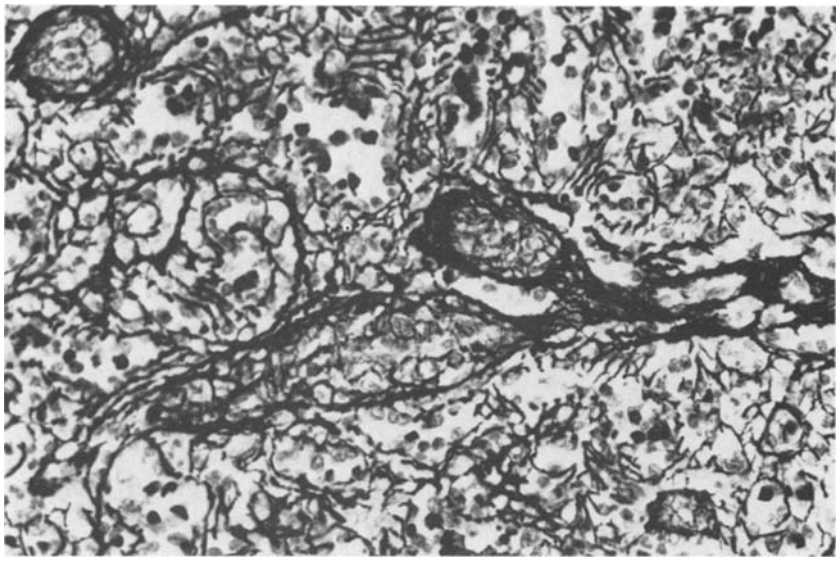

Fig. 4. One longitudinal, one oblique and in the upper left corner one cross section of EL. The fine and dense reticulum network and the peripheral limiting membrane are distinctive features. Gordon Sweets × 300

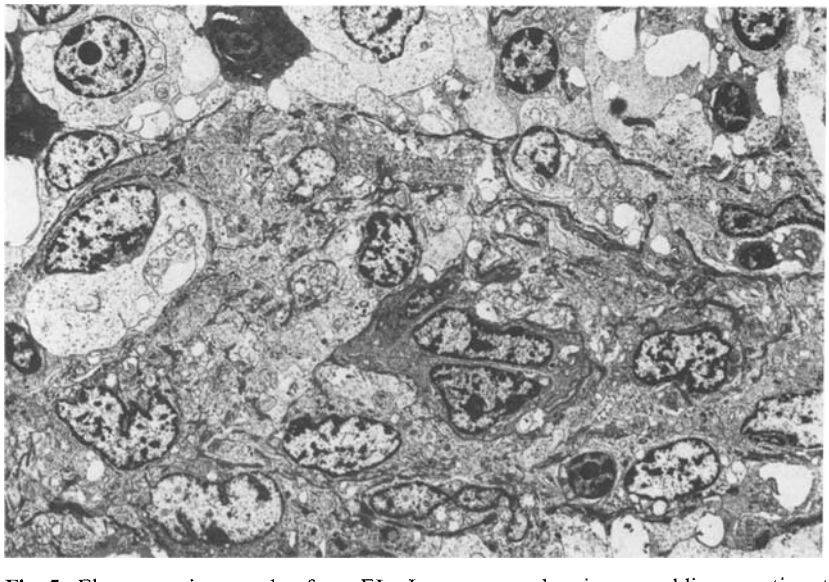

Fig. 5. Electron micrograph of an EL. Low power showing an oblique section through a complete EL. In the center is a slitlike lumen, hardly visible at this magnification surrounded by two endothelial cells. The cuff cells are surrounded by basal membrane-like material in continuity with the endothelial BM and the adventitial reticulin membrane. × 2,750

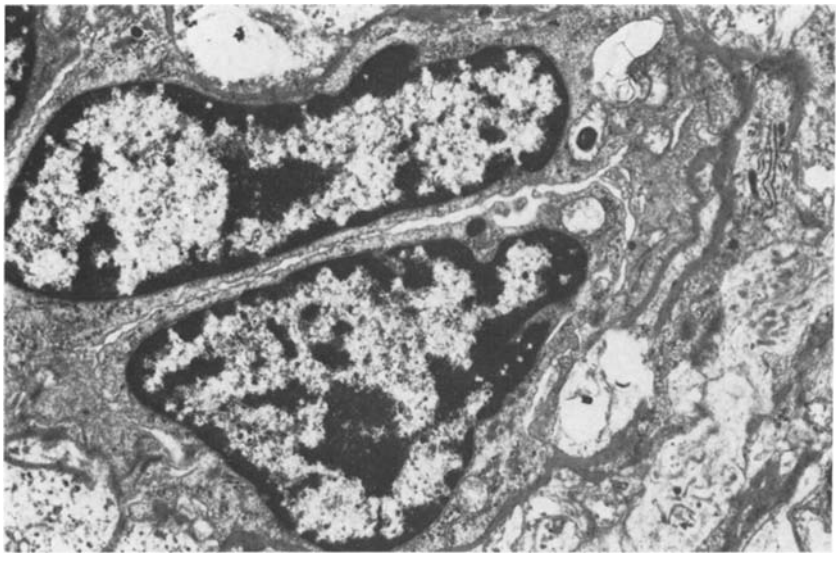

Fig. 6. Higher magnification of Fig. 5 showing the slitlike lumen and endothelial cells. ×9,000

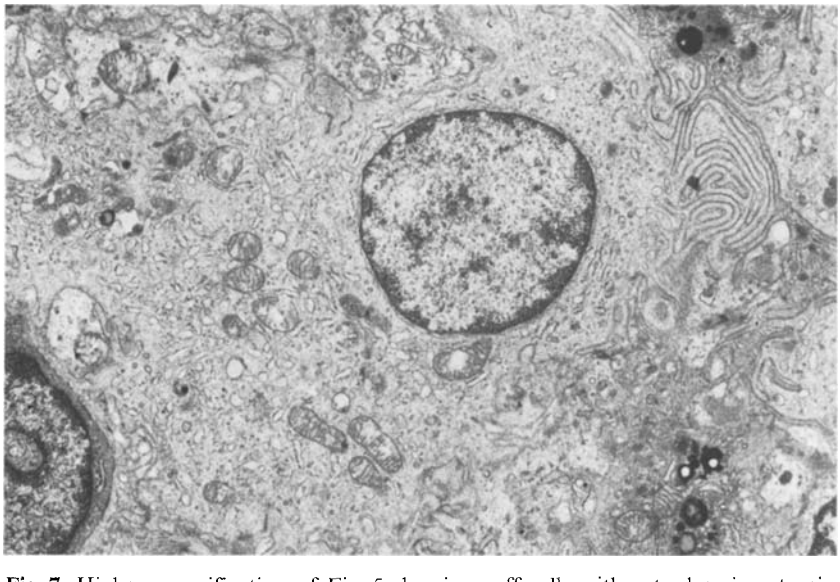

Fig. 7. Higher magnification of Fig. 5 showing cuff cells with cytoplasmic extensions, many profile of SER and lysosomes. ×9,000

In one EL we found adjacent to the endothelium a different cell: it has an elongated and irregular nucleus and a moderate amount of cytoplasm showing small irregular processess completely surrounding the endothelial BM. The cytoplasm contains many peripherally situated dense zones, some mitochondria, polyribosomes and a structure with a regular banded pattern

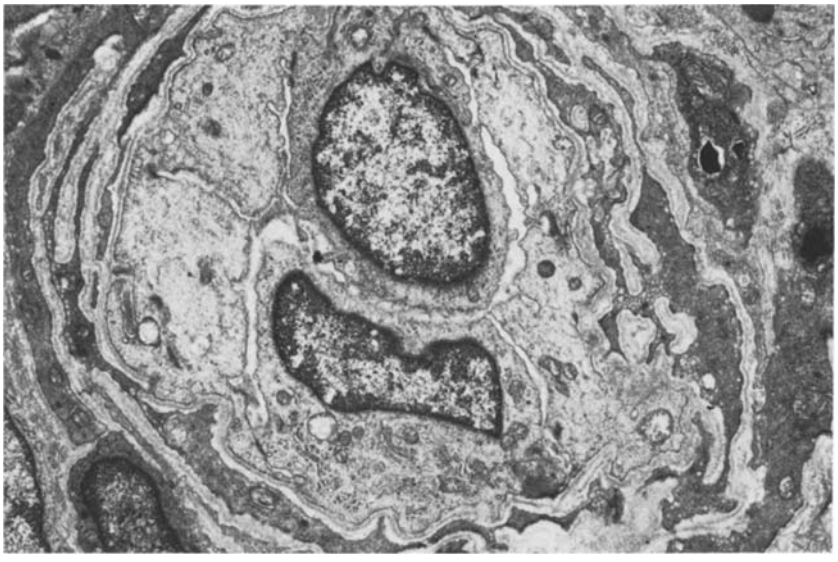

Fig. 8. Normal arteriole of the red pulp. The smooth muscle cells in the media allow the vessel to be recognized as an arteriole ×9,000

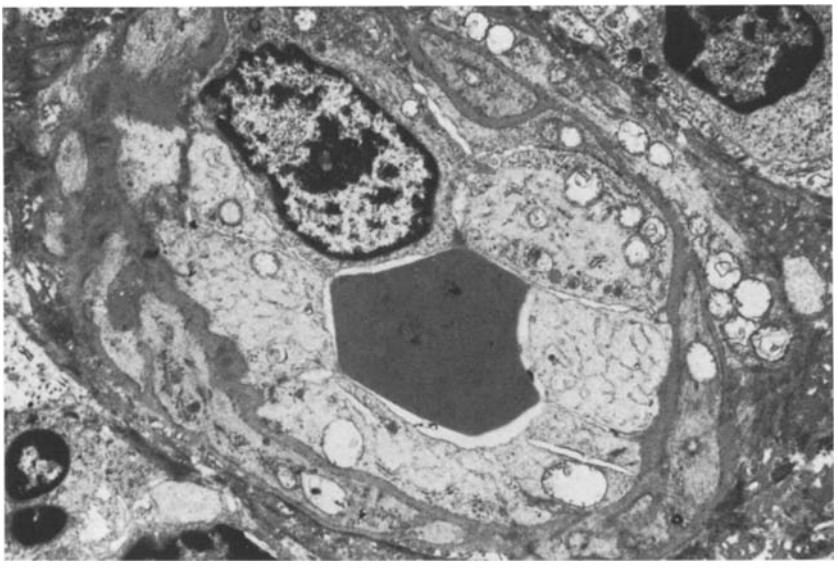

Fig. 9. Normal capillary of the red pulp. The lumen is wide and contains an erythrocyte ×9,000

of 30 nm width. A basal lamina completely surrounds this cell and the larger cells are lying outside of it. An arteriole and a capillary were also examined but they showed no differences with those of other organs. The difference with the EL however was clear (Figs. 8, 9).

Discussion

Nomenclature

Schweigger-Seidel in his first description in 1863 gave the name of "Hülsenkapillaren" to these structures. Although this name was pertinent, morphologists succeeded in creating confusion in the next 120 years by using the term "Hülsenarteriolen" or "Sheathed arterioles". EL are not arterioles when one accepts the definition that these are characterized by the presence of smooth muscle cells. On the other hand there are sheaths, formed by lymphocytes, around arteries and arterioles, and some authors confuse these two histologically distinct types of sheaths. The cells forming the EL are called "Hülsenzellen" by Kellner (1962) but from his description it appears that this term encompasses all large cells around the vessels, including periarterial, periarteriolar and pericapillary large transformed lymphocytes (immunoblasts), reticulum cells and histiocytes. Therefore the term ellipsoids, first used by Müller (1865), is the safest, although one must admit that the ellipsoid shape is best appreciated in a longitudinal section with a silver stain, showing to best advantage the peculiar reticulin network.

Number of EL

The current textbooks of histology describe EL as rare in the human spleen. Schweigger-Seidel himself wrote "I searched for a long time in vain in man, until at last I succeeded in finding them in the spleen of a 51 year old man deceased from a pneumonia" (p. 467).

Our results on standard material of a pathology laboratory show that the average number of EL in an average spleen is rather impressive and it should be stressed that these are minimum figures. The thickness of the tissue slice of 200 mμ accepted in the calculation of Buffon's formula is a maximum safety value. Terminal segments of EL may not have been counted. Table 1 shows no correlation between the number of EL, the age, sex, spleen weight and clinical condition.

Construction and cell type of EL

The reconstruction of serial sections is the only way to be sure of the localisation of the EL segment on the vascular tree. Snook (1950) and Jäger (1929), whose findings were also used by Hueck (1927), made schematic drawings of serial sections: these are not exact reconstructions on scale. Our results with the technique of Staubesand confirm the findings of Snook and Jäger, but some additional points must be stressed. EL generally form the terminal segment of capillaries ending in the red pulp, but the capillaries may continue beyond the EL. There are capillaries without EL, even in spleens with many EL. In spleens with only a few EL they are practically all free of cuffs.

Jäger (1929) describes that the EL which lie at the border of a follicle may stem from a distant artery not belonging to this follicle. We confirm this finding, which demonstrates that the EL are capillary and not follicle

dependent. Mc Neal (1929) mentioned that he was unable to find this arrangement.

The EL occupy a constant restricted segment of the vascular tree. They show a distinct dense network of reticulin and collagen fibers and as shown by EM also of BM material. The cuff cells of the EL are clearly different from endothelial or smooth muscle cells. They contain no filaments. They show a strong activity for acid phosphatase and non specific esterases and contain lysosomes: hence they are considered as histiocytes, phagocytes or reticulum cells (Grössner 1970; Stutte 1970; Weiss 1977). The number of histiocytes in the red pulp of the spleen as judged by the acid phosphatase reaction is high. They are randomly distributed in the red pulp. They have a very irregular form with many long projections. There is no morphological or topographical identity between these histiocytes and the cuff cells.

We did not observe phagocytosis. Solnitzky (1937) who made an in depth study of the EL, describes that animals injected intravenously with trypan blue or India ink showed many coloured granules in "sheath cells" 7–10 h after injection. His document however consists of a drawing showing granula containing cells around the sheath, which is normally the place were histiocytes are found. Dustin (1938) studied 50 autopsy spleens and found no signs of phagocytosis, especially of iron. In two children, aged 6 months, he described the accumulation of fat in the cuff cells: in our four EL studied by electron microscopy we did not disclose fat.

Kellner (1962) describes the uptake of Thorotrast by "sheath cells", but he pictures an arteriole surrounded by histiocytes and by a lymphocyte sheath.

One of the reasons why EL are reported to be rare in human spleens is probably that they are mistaken for histiocytes. They are seen but not recognized: their existence in man has been forgotten as testified by a recent paper (Bishop and Lansing 1982) and a recent book (Videbaek et al. 1983) which do not even mention them.

The exact nature of the cuff cells remains unclear. Histiocytes of the monocyte-macrophage system which accumulate on a fixed segment of the vascular tree and form basal membranes are not known in other organs. Reticulum cells, defined as stromal cells of the red pulp of the spleen and of the lymphoid system throughout the body are possible candidates, but do not form organoid structures elsewhere in the body. Ultrastructually the cuff cells are different from pericytes, but their localization is an argument in favour of them: the cuff cells could be regarded as "modified" pericytes. Finally we are left with the possibility of a specialized fixed pericapillary cell capable of cytoplasmic expansion.

Lymphocytes

The presence of lymphocytes in the EL in animals and man has been described by several authors, especially by Kellner (1962): he counted the

cell types in EL in man and found 93% large cells and 7% other cells, mostly lymphocytes, and he concluded that the presence of the lymphocytes in the EL can not be ignored. If we suppose that in a 5 mμ section we find one lymphocyte per EL (and there are more), and we accept an average length of 75 mμ, then we have 15 lymphocytes per EL. Multiplied by the total number of EL $1.6\ 10^7 = 2.4\ 10^8$. Studies with marked lymphocytes have shown that they leave very rapidly the arterial circulation and accumulate at the margin of the follicle within 30 min (Goldschneider and Mc Gregor 1968). De Sousa (1981) has demonstrated this pathway in the chicken. A short time after injection the lymphocytes were found around the EL.

It is interesting to note that Kellner (1962), who studied extensively the lymphatic intrasplenic system in man, places the beginning of the lymph flow in the EL where the lymph is drained further along small lymphspaces surrounding the arterioles and arteries. Many authors, among them Schweigger-Seidel himself, have noticed that injected fluids diffuse readily between the cells of the EL, supporting the view of Kellner.

The presence of the lymphocytes in the EL can not be considered as pure coincidence. It must have a meaning, and it is obvious that the most closely related structures with the same function are the high endothelial venules (HEV) of the lymphnodes. The spleen follicles have no HEV, and the venous system of the spleen has no postcapillary venules. The analogy of the HEV and the EL has already been suggested by Hueck (1927), although at the moment of his writings the transit of the lymphocytes was supposed to go in the opposite direction into the lumen of the venule.

A last feature of the EL is the enormous variation in number from individual to individual and in different species. This indicates that they are not constant histological structures, but functional adaptations of cells on a fixed place. In conclusion we consider the ellipsoids to be dynamic histological structures with a functional activity aimed at lymphocyte transport. They are the splenic counterpart of the HEV venules in lymph nodes.

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Accepted December 22, 1983