Pictures taken from the following websites, all accessed: 25.10.10:
http://education.vetmed.vt.edu/curriculum/vm8054/labs/Lab13/Lab13.htm#
http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artsep00/hucell1.html
http://faculty.une.edu/com/abell/histo/histolab3b.htm

Spleen

The spleen is really part of the circulatory system, but it's always described with the lymphatic organs because of the very large population of lymphocytes found in it. The spleen is a flaccid bag that serves as a **storage site for blood, a processing station for the scavenging of aged erythrocytes, and a few other things**. It's one of the "dispensable" organs, because mammals get along quite nicely without a spleen. In the case of a traumatic injury that ruptures the spleen, the easiest thing to do is to take it out, and a splenectomy is commonly done. It's easier to remove the spleen than to try to repair it and risk renewed hemorrhage.

"Red Pulp" and "White Pulp"

These are two of those troublesome terms that really applicable in gross anatomy, but with which the histologist has to deal as well. Cut a freshly-removed spleen across and it looks like a field of dark red material with white spots in it. On the basis of its *gross* appearance in fresh sections, the spleen is traditionally said to have the bulk of its parenchyma as red pulp, with isolated areas of white pulp interspersed through it. **The red pulp gets its appearance from the formed elements of the blood (mostly erythrocytes) it contains. The white pulp consists almost entirely of lymphocytes, in a peculiar association with the arterial blood supply** (see below). These two terms are quite logical when applied to gross specimens, but things become a little confusing when they're applied to microscopic sections. Briefly, in terms of microscopic sections, "white pulp" is equivalent to the lymphocyte population of the spleen, in the form of the periarteriolar lymphocyte sheath or PALS (see below). "Red pulp" is everything else, which means the splenic cords and the sinuses between them.

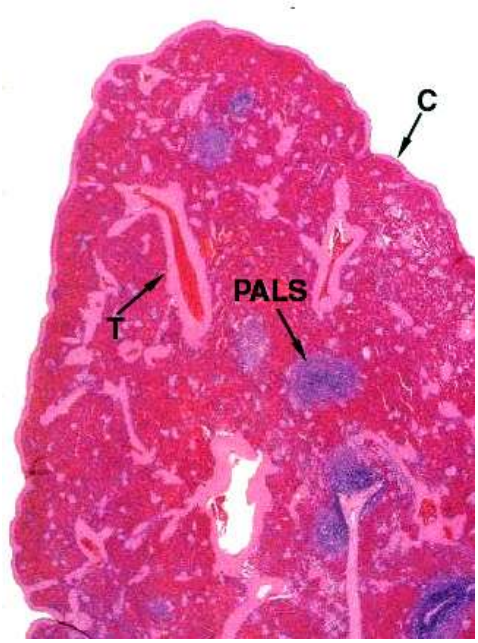


Figure 1. Slide 1023

This low magnification image from slide 1023 shows both the "red" and "white" pulp. "Red pulp" fills the bulk of the spleen's volume. White pulp is the blue stained areas visible within it. You're really seeing *aggregated masses of cells.* The pink stained strands running through the field are the connective tissue septae or trabeculae (**T**) that subdivide the spleen's interior volume. They arise from and are continuous with the capsule (**C**). The capsule is mainly collagenous fibrous CT, but there's a good bit of smooth muscle in it, too. This allows the spleen to contract and eject stored erythrocytes when needed. The magnification here is low enough (about 20x) that you can make out the red and white pulp pretty much as you would in a fresh gross preparation.

The "PALS" labeled here is the periarteriolar lymphocyte sheath, the characteristic association of lymphocytes and blood vessels in the spleen (see below). The lymphocytes are arranged along the arteries forming a sleeve or sheath. Collectively, the PALS *is* the "white pulp." The rest of the interior volume of the spleen is the splenic cords, collectively constituting the "red pulp," and the blood sinuses between them.

Spleen sections stained with Haematoxylin.

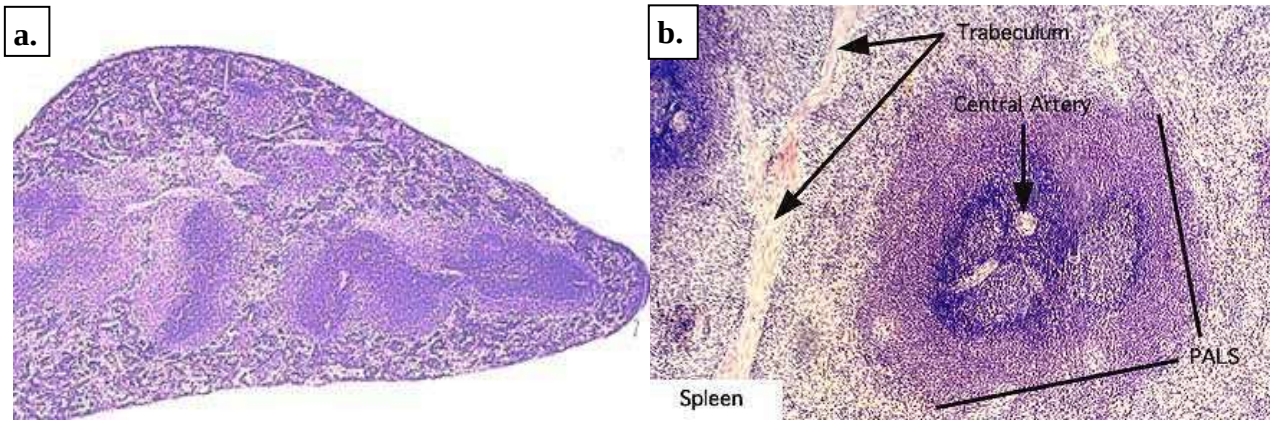


Figure 2a. A portion of a spleen of a BALB/c mouse, inbred for experimental studies. The dark areas are the lymphoid follicles. Note the different colour compared to Figure 1. This section has been stained with Haematoxylin (like we will have in the practical). With this staining the darkest bits are the white pulp and the lightest bits are the red pulp. If eosin is combined with Haematoxylin the red pulp is pink and the white pulp is purple.
Figure 2b. Close up of section of spleen. Showing PALS, central artery and associated lymphocytes, creating the area known as white pulp (purpley colour here).

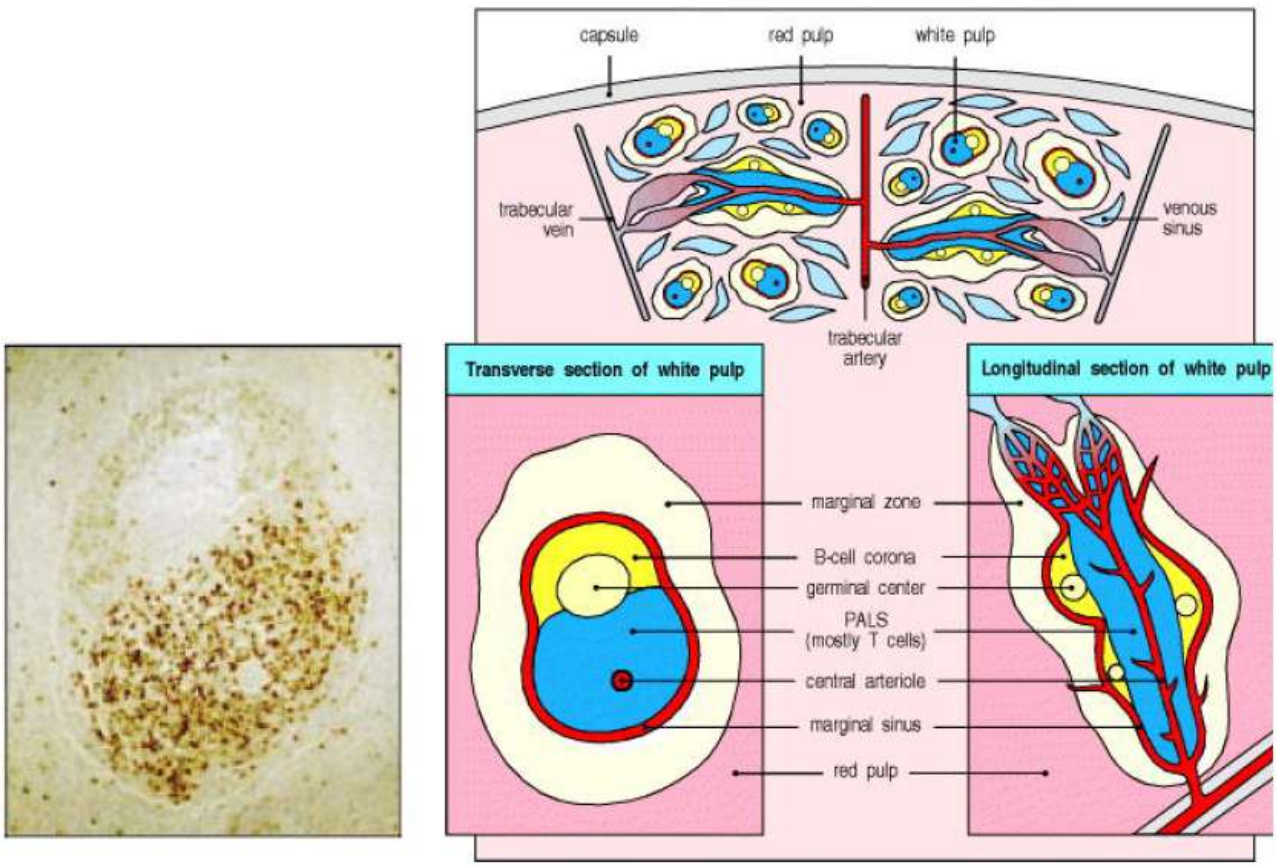


Figure 3. Picture from N. Rogers' "Primary and Secondary Lymphoid organs and tissues" lecture. From Janeway, Travers, Walport and Schlomchik. Immunobiology. 7th Ed

Spleen in disease.

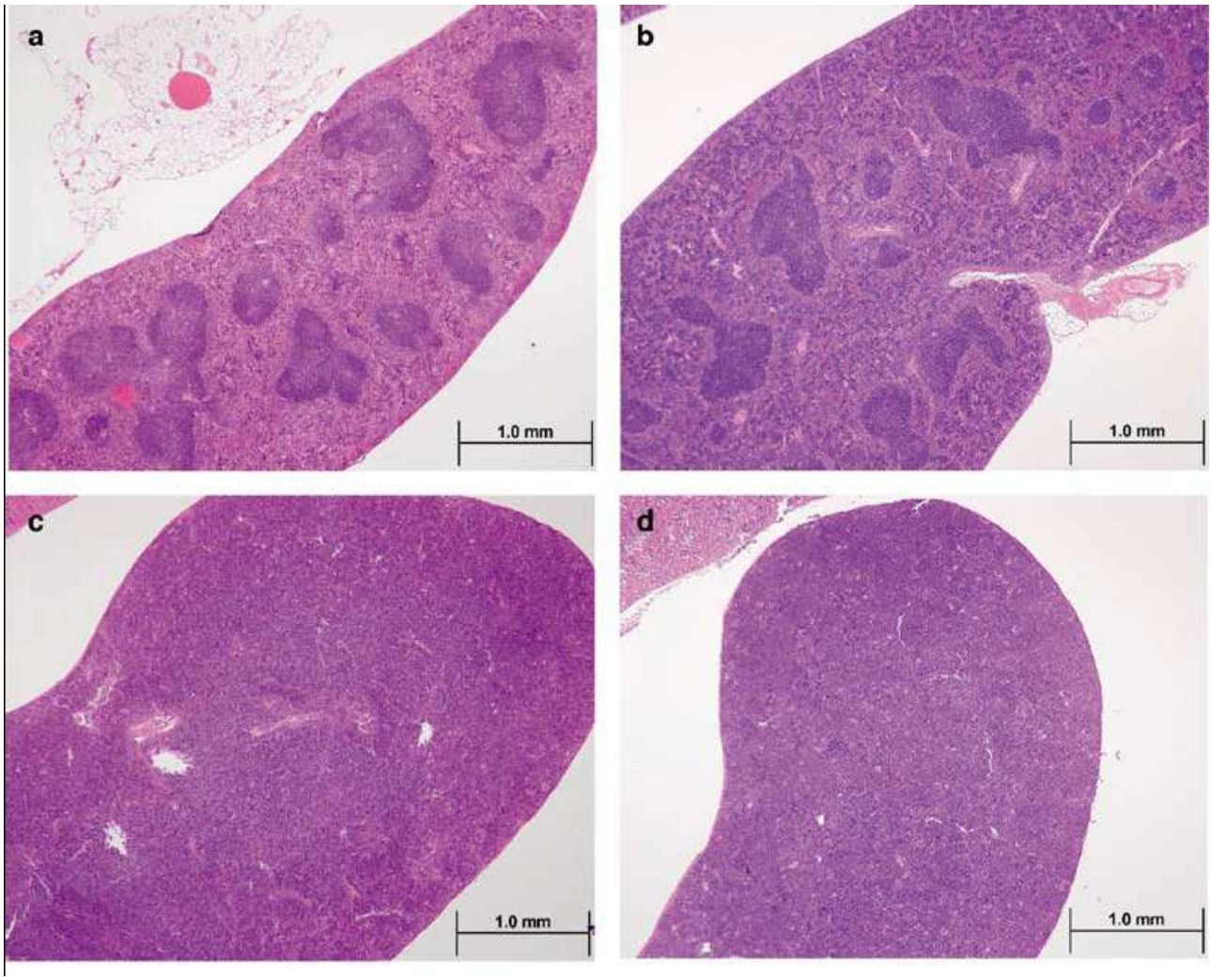
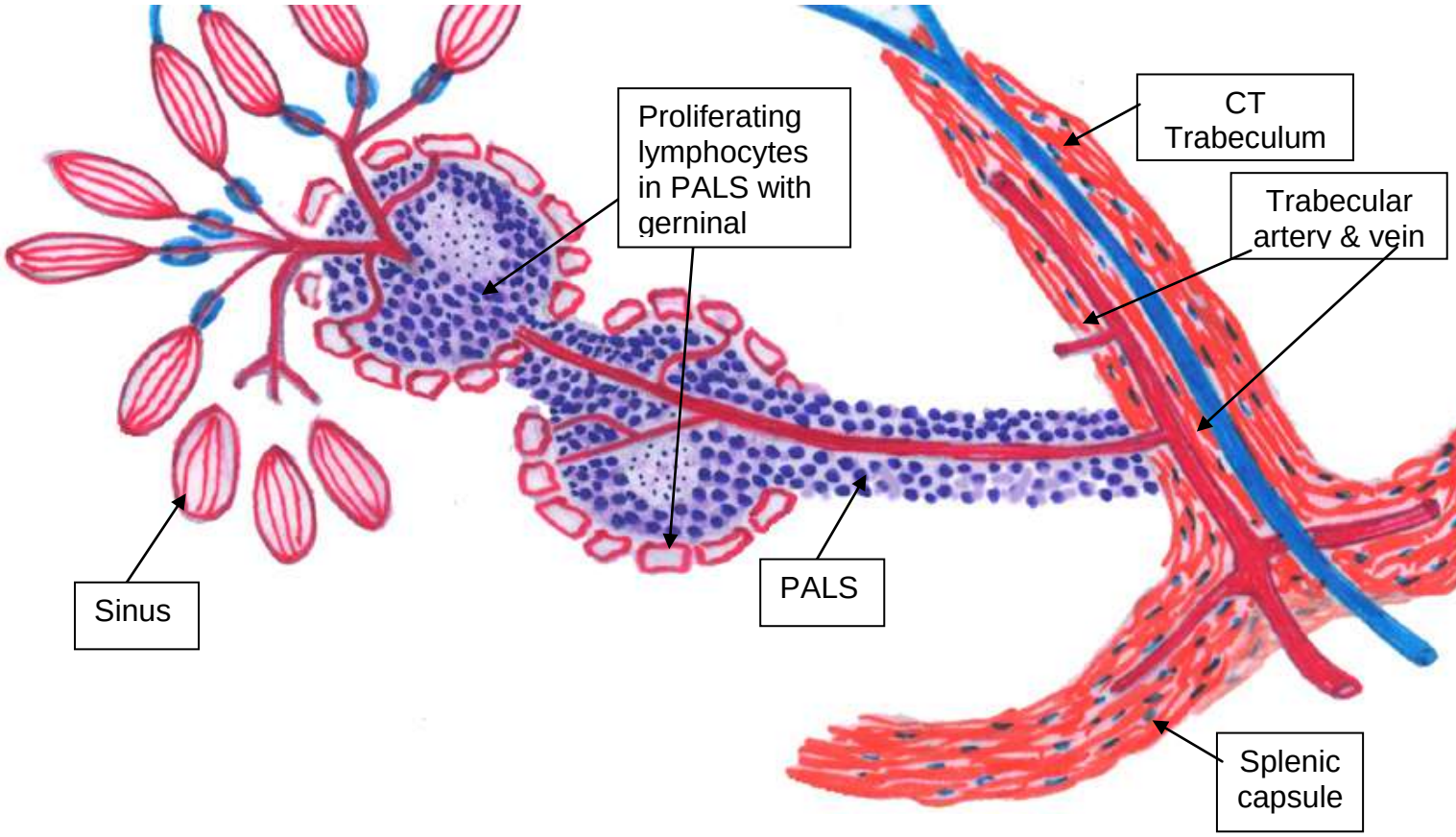


Figure 4. a: Normal spleen, b: slightly enlarged spleen, c: enlarged spleen with increased haematopoiesis and reduced lymphoid tissue and d: enlarged spleen with increased haematopoiesis and minimal lymphoid tissue. From: Oncogene (2007) 26, 6297–6306.
Tumor progression in Apc1638N mice with Exo1 and Fen1 deficiencies
M Kucherlapati1, A Nguyen1, M Kuraguchi1, K Yang2, K Fan2, R Bronson3,4, K Wei5, M Lipkin2, W Edelmann5 and R Kucherlapati1

Splenic Circulation



Any description of the spleen's histology must necessarily be centered around the arrangements of its blood vessels. The above sketch shows the layout of the blood supply to the spleen.

Blood enters from the splenic arterial supply, via the splenic capsule, at lower right; and the breakup of arteries into capsular and trabecular segments begins almost immediately. As is true in other organs, the angiogenic properties of the CT capsule are are needed to create the routes for the blood supply entering and leaving the spleen. The main input, the splenic artery, ramifies in the capsule and sends branches deeper and deeper. Blood leaving the organ is drained back through a series of veins in the septa and the capsule, eventually exiting via the splenic vein. Both the artery and vein are grossly located at the hilus.

As soon as a branch of the arterial input reaches the interior space of the spleen, it acquires the periarteriolar lymphocyte sheath, or PALS, which it retains almost to the smallest subdivisions. The PALS is a place in which the special conditions required for proliferation of B lymphocytes can be met, and at intervals there will be germinal centers along it. Germinal centers are individually transient and depend on the immune state of the animal, as they do in other parts of the immune system; but their presence is a feature of the spleen in all species to some extent. The arterial vessels that have been covered with lymphocytes are the central arteries**.**

Eventually the arteriolar supply subdivides to the point where the PALS becomes attenuated to a few cells, or even lost altogether. These small arteries tend to run in bundles and hence are termed penicillar arteries, from their resemblance to the hairs of a paintbrush (the Latin word for a paintbrush is *penicillum*). The role of the penicillar arteries is to deliver the incoming blood to the red pulp (which is not shown in this sketch) *i.e.,* the splenic cords and sinuses. They're sometimes called "arteries of the red pulp." Some segments of the penicillar arteries acquire a peculiar "sheath" of phagocytic cells in their walls, and have been termed sheathed arterioles in the short

regions where this occurs. The presence of a sheath is inconstant and there are species variations; its role is unclear, as well.

Beyond this point what happens to the blood is a matter of debate. Some authorities contend that the blood enters more or less directly into the sinuses of the red pulp. Others contend that there is no direct connection: that the arteriolar side simply ends and that erythrocytes enter the sinuses from the cords. These are, respectively the "closed" and "open" theories of splenic circulation. Whatever happens, though, the blood is eventually collected from the sinuses into actual veins and then routed back through the trabecular veins to the splenic vein and thence to the general circulation.

Splenic Capsule

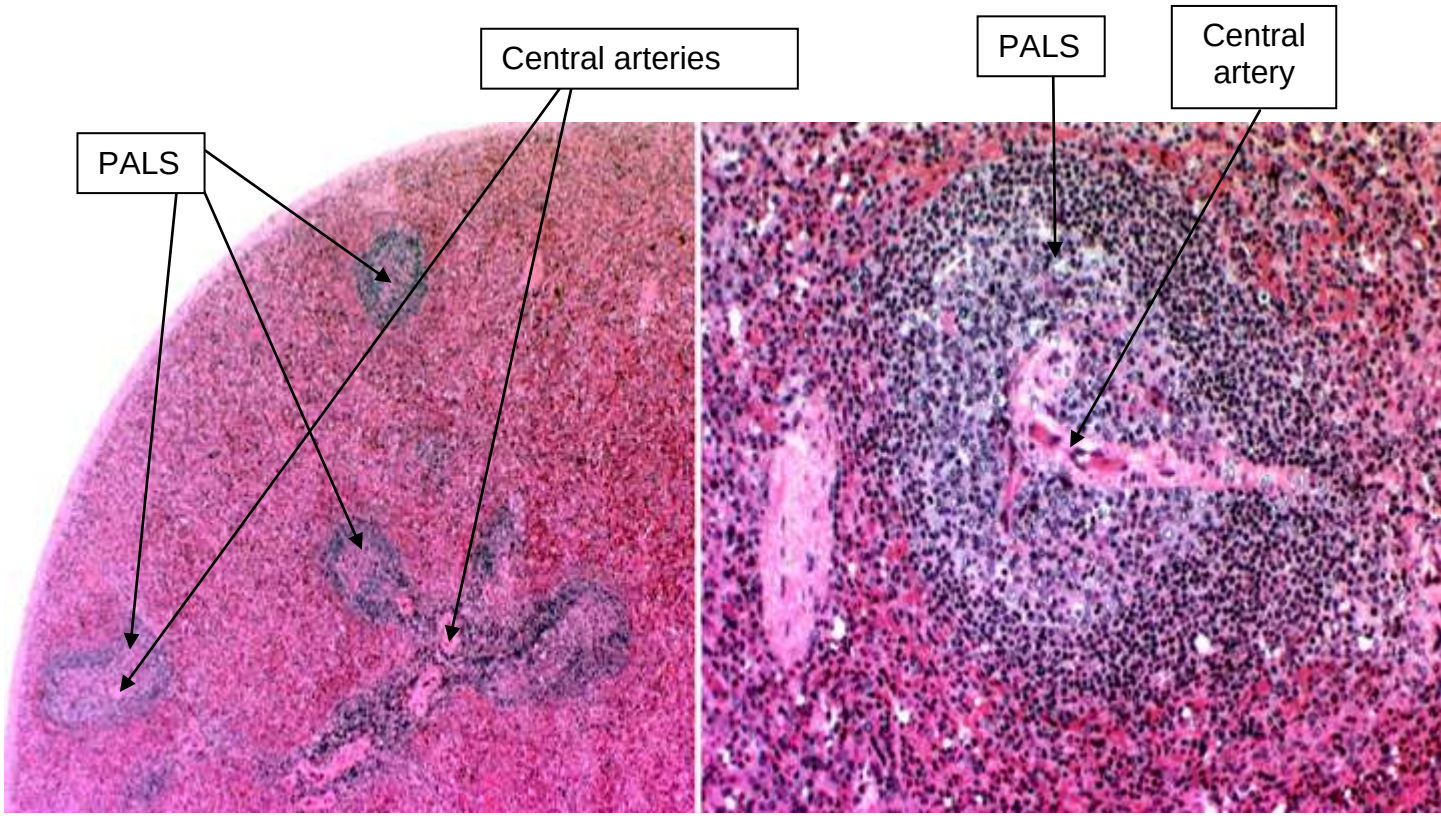
Returning now to slide 1023, you can see that there's a distinct capsule around the spleen. It's mostly collagenous and elastic connective tissue, interspersed with a fair amount of smooth muscle. One function of the spleen is to act as a reservoir of erythrocytes, and the contraction of the capsule expels these into circulation when needed. The admixture of muscle is particularly pronounced in ruminants and horses. Animals that depend on running for their survival tend to have rather muscular splenic capsules: they can store erythrocytes there and release them into the general circulation when needed for extra oxygen carrying capacity. "Blood doping" a racer by a transfusion just before the race gives him more erythrocytes; a practice that, properly done, is nearly undetectable. Animals evolved to live at low altitudes often have oxygen loading curves that depend on a high partial pressure of atmospheric oxygen. When moved to high elevations, they often generate extra erythrocytes to compensate for the thin air. These extra erythrocytes are stored in the spleen, to be released when needed to deal with exertion.

The capsule sends septae or trabeculae part way into the interior volume of the organ, and from these arise a delicate network of reticular fibers, to form a stromal network on which the lymphocytes sit. These are very difficult to see in H&E stained specimens, but with appropriate special stains you can see them.

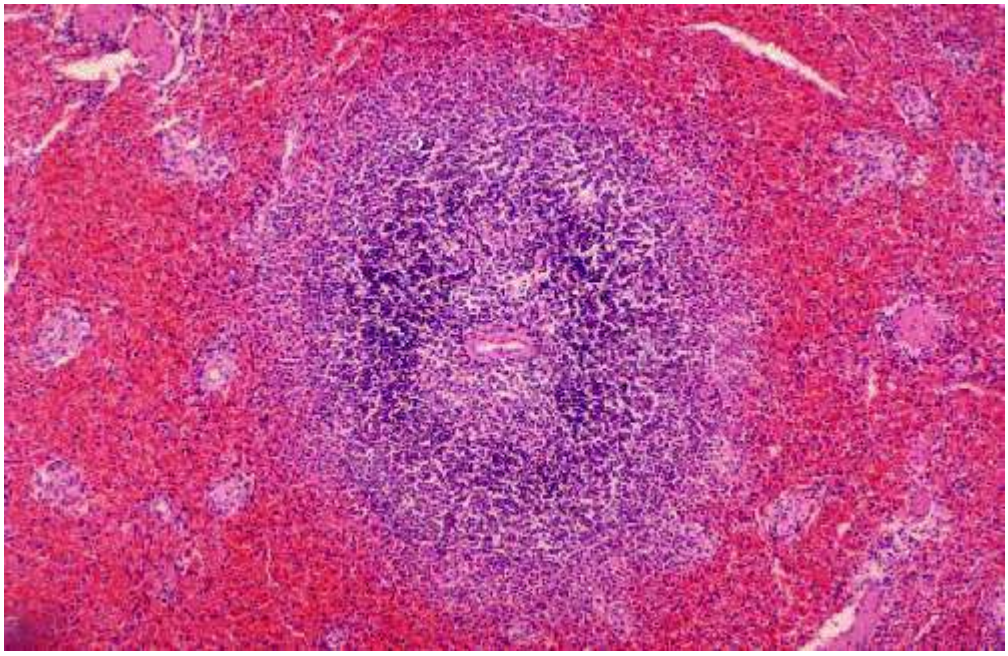
PALS: The Periarteriolar Lymphocyte Sheath

As an arteriole leaves a septum and enters the interior volume of the spleen it immediately acquires a continuous coating of lymphocytes. This "sleeve" of lymphocytes is the periarteriolar lymphocyte sheath, or PALS. You can see it on slide 1023 quite well as basophilic areas with small blood vessels running through them. These central arteries are the first part of the arterial input into the spleen. All of the PALS collectively constitute what the gross anatomist calls the spleen's "white pulp."

The PALS is a continuous feature of the arterial supply, almost to the point where it breaks up into capillaries. The main blood vessel you see running through most profiles of the PALS is the central artery, and it continues to branch. Eventually, the branching reaches the point where the PALS is reduced to only one or two cells on the smallest portions of the arteries formed by subdividing the central arteries.



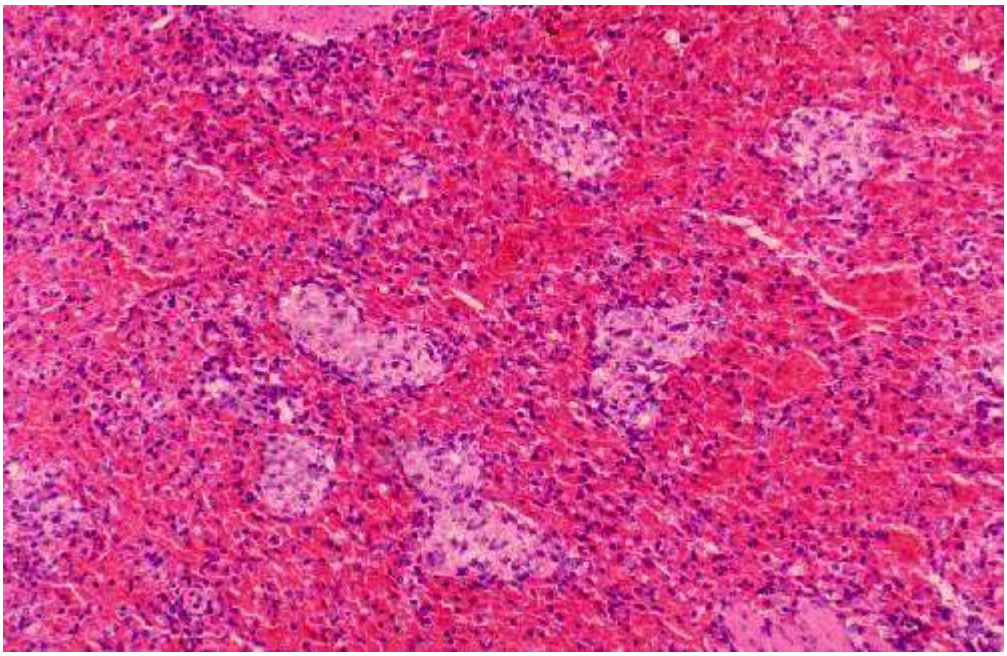
Here are two views from slide 1023, one at about 100x and another about 400x, to show the relationship of the PALS to the arterial supply. You can see the PALS in any orientation, from cross section (as if you were looking into a tube of lymphocytes, as at right) to longitudinal. The **central artery** is in the middle in either case. This PALS and the red pulp around it are easily differentiated.



The PALS is diffuse lymphatic tissue, but within it germinal centers frequently develop. Careful examination of the image above will show the dark "cap" of one of these. Now, the PALS, being diffuse, has a loose stromal framework of reticular fibers made by a variant for of fibroblast. The resident lymphocytes of the diffuse region of the PALS are supported by it, like birds sitting on telephone wires: this sort of arrangement is typical of all diffuse lymphatic tissue. Due to the dense packing of the lymphocytes in the PALS you will not be able to make out the fibers in an H&E preparation, even in the diffuse parts. But special staining for reticular fibers will reveal it pretty easily.

Within the germinal centers, however, things are different. The germinal center is a special place: it's a clone of B-lymphocytes, and it has to be isolated so that these cells can develop properly. Consequently the stroma in germinal centers isn't fibrillar in nature. Instead, special cells of a different cell line than the fibroblasts form a stroma within the germinal center and support the B cells in it. A similar non-fibrillar stroma is characteristic of the thymus.

Continuation of the Splenic Blood Supply Beyond the Central Arteries



The central arteries, like any self-respecting artery, break up into smaller ones eventually. Each one gives rise to a tuft of small vessels, the penicillar arteries. The PALS is almost wholly gone by the time this level of branching is reached. You're looking at them cut in cross section. These are very small vessels, and the PALS around them is almost nonexistent.

Sheathed Capillaries

Upon reaching the red pulp and finally losing the last of the PALS, some of the penicillar arteries individually break up into a peculiar form of capillary. These are the sheathed capillaries, the name referring to the presence of a specialized region of the capillary wall.

While most blood vessels are lined with simple squamous epithelium, the sheathed capillaries are an exception. The endothelium of the sheathed capillaries is composed of fusiform cells, oriented parallel to the long axis. These specialized capillaries are surrounded by a sheath of reticulocytes and macrophages, bound together by reticular fibers. Not all of the capillaries arising from the penicillar arteries have such a sheath. Those that do have special properties, and the cells of the wall are phagocytic. You can often find them by looking for lipofuscin in the walls of the sheathed portions.

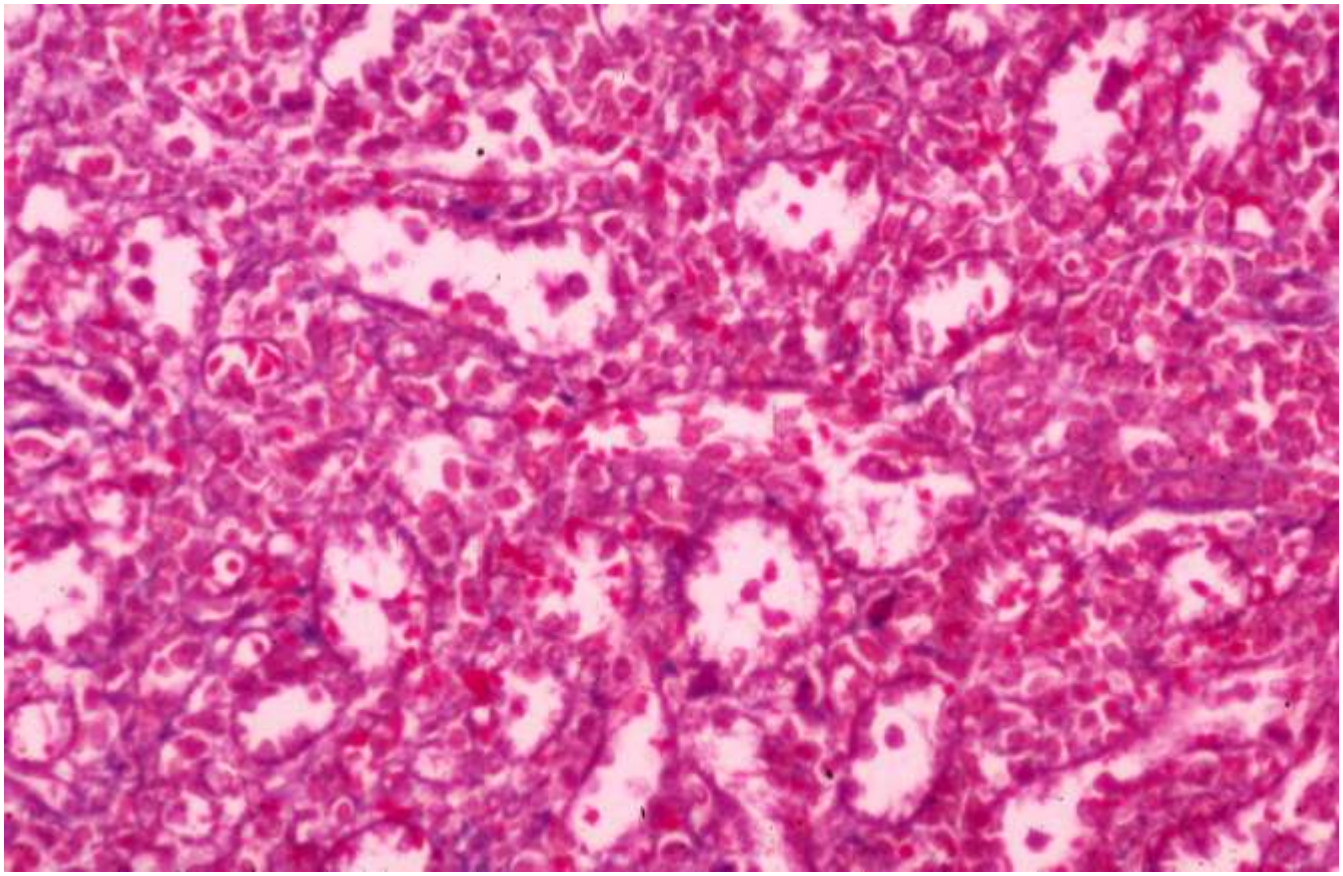
Past the sheath, the capillaries return to the normal configuration expected of these vessels. Not all species have this type of blood vessel.

We have now followed the arterial supply as far as we can, and it's time to look at the venous side of the system.

Splenic Cords and Sinuses

The red pulp constitutes the bulk of the splenic volume. The supporting reticulum of the red pulp is continuous with the septa. Again, it consists of reticular fibers invested by reticulocytes.

If you look carefully at the red pulp, it will become clear that it's arranged into anastomosing splenic cords, separated by venous sinuses. The sinuses are blood spaces. They're lined with endothelium and contain circulating blood cells. Hence most of what you find in them is erythrocytes.



The splenic cords are the masses of cells in between the sinuses. They contain a lot of erythrocytes, but they also contain many other cell types: macrophages most prominently. It's in the cords that the phagocytosis of the aged erythrocytes occurs, and consequently a stain for iron (such as that used on slide 674 below) will reveal the presence of hemosiderin in the cords very well.

This specimen shows the cord and sinus arrangement of the red pulp quite well. The sinuses contain a few erythrocytes and the normal circulating white cells of the blood; it is essentially a blood vessel. It can be difficult to see the sinuses clearly: often the spleen contracts on death (or anesthesia) and the sinuses get gorged with blood. Since there are so many erythrocytes in the cords, it can be hard to discern the boundary.

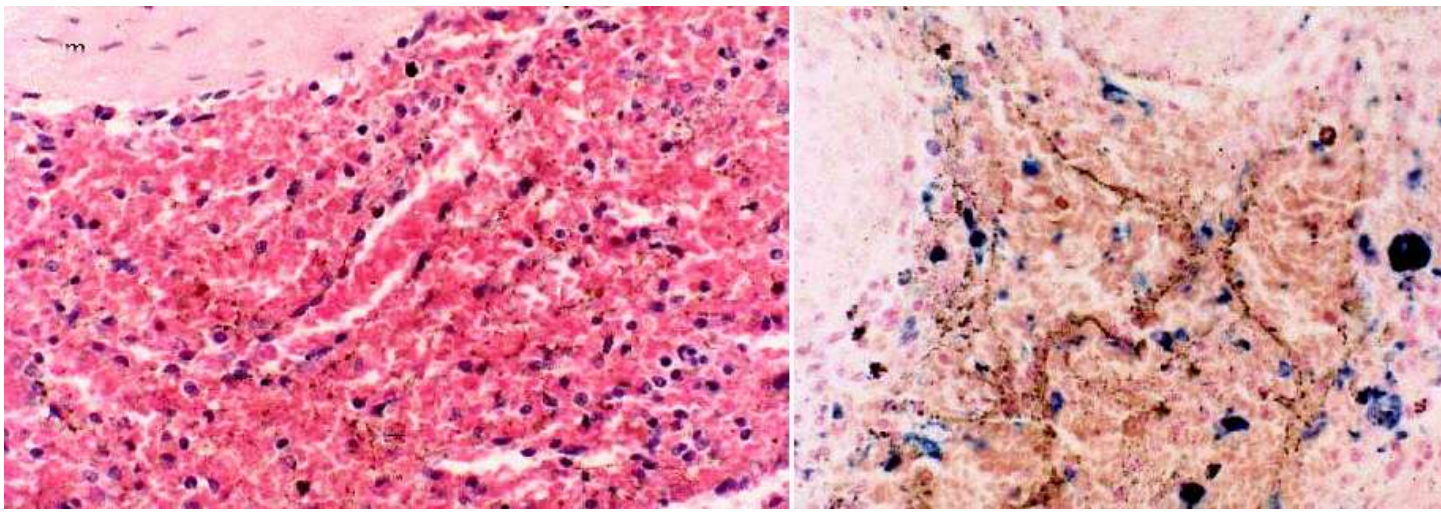


Figure 5. Slide 673 left, slide 674 right

However, like all blood vessels, the sinuses are lined by cells: In this case the cells in the lining are phagocytic. On slide 673 (left above) and 674 (right) you can make out bits of brownish material in them, defining the limits of the sinus. One of the functions of the spleen is the degradation of senescent erythrocytes: at the end of their useful lives (90 to 120 days) erythrocytes develop cell surface associated "senescence antigens" to mark them as the legitimate prey of the macrophages in the cords of the red pulp. They're seized, phagocytosed, and the salvageable components of their compounds recycled, like old cars being dismantled in a junkyard. The leftover part, hemosiderin can build up in the spleen considerably over long periods of time.

In slide 674, stained with Prussian Blue, the hemosiderin that's produced in the process of dismantling hemoglobin is seen as blue blotches. note that these are in the *cords.* The cords are the location of numerous splenic macrophages with this specific task. The phagocytic lining cells of the sinuses are less selective, and in them you'll see lipofuscin, the breakdown product of cellular scavenging.

In most spleen sections, this cord-and-sinus separation is very difficult to see, but if you can find an area of slide 1023 in which the density of cells is not too great, you should be able to decipher the cord-sinus-cord arrangement without too much trouble. One clue as to which is which on this slide is the ratio of erythrocytes to other cell types. In the sinuses, the erythrocytes are present in the same overwhelming proportion that they represent in the blood. In the cords, however, there are many more non-erythrocytes present, and the nuclear density is much higher than in the sinuses. Look also for the endothelium which separates cords from the adjacent sinuses.

Venous Drainage

Since the sinuses are blood vessels, they're lined by endothelium, the cells of which are fusiform in shape and oriented longitudinally with respect to the long axis of the sinus. The wall of the sinus, while it demarcates the cells in the cords from the blood flowing through the sinuses, is nevertheless compliant enough to permit passage of cells through it. Cells of the blood can and do move from a sinus to an adjacent cord, and *vice versa*, and the static impression given by microscopic sections is incorrect. The endothelium lining the splenic sinusoids is phagocytic. The sinusoids eventually join together to form veins of the red pulp, which in turn coalesce into larger veins in the septa. Drainage of the entire organ is through the large vein running parallel to the splenic artery at the hilus.

Connecting the Plumbing: Arterial-Venous Relationships

Having accounted for the arterial flow and the venous drainage, it's necessary to deal with the question of the connection between the two sides.

Open Theory

The termination of splenic capillaries is a matter of debate. It's believed by some investigators that the capillaries of the spleen end by opening right into the splenic cords, and that blood thus released passes through the cords and into the adjacent sinuses. This would mean that there is no direct connection between the arterial and venous sides, and that the cords in essence act as "filters" through which the total volume of blood has to pass before returning to the venous circulation. This is the open theory of splenic circulation.

Closed Theory

An alternative view is that the capillaries do in fact end by joining the sinuses directly, as is the case in other organs in which sinusoids are found. Self respecting arteries, after all, *should* end in capillary beds. Thus the circulatory loop is closed, and the cells in the splenic cords get there from the blood passing through the sinusoids. This is, of course, called the closed theory.

The arguments for and against both theories rest on theoretical and empirical grounds. The Open Theory proponents point out that if one cannulates the splenic artery, and pumps in dyes, the dyes will be found in both the cords *and* the sinusoids. But if one cannulates the vein, the dyes are only found in the sinusoids. Therefore, they argue, there is no direct connection, as otherwise the cords and the arteries leading into them would be full, too.

The adherents of the Closed Theory reply that there would be more or less constant hemorrhage if the Open Theory were correct, as the ending of arteries in parenchymal tissue could not result in anything else, in the absence of venous drainage capable of handling the large volumes of blood the spleen holds. To the evidence of the dyes, they respond by saying that splenic veins are so delicate that it is impossible to retro-perfuse them without damage, and that a failure to get dyes into the arteries simply means the connections between the two vessel types was broken by the pressure of perfusion.

Compromise Theory

As you probably expected, there is a third theory arguing that both types of ending exist simultaneously. That at times the circulation is closed, and others open; that in normal times the open system operates, but when there is need for those reserve erythrocytes, the connections between arteries and veins takes over, the system is closed, and the blood is pumped out.

You pays your money and takes your choice.