ON THE CIRCULATION THROUGH THE PULP OF THE DOG'S SPLEEN.

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WITH ONE PLATE AND ONE TEXT FIGURE.

Recent researches upon the blood-vessels of the spleen prove definitely that the arterial capillaries communicate quite freely with the pulp-spaces, but there is still a difference of opinion regarding the presence of distinct channels, independent of the pulp-spaces, connecting the arterial capillaries with the venous sinuses. It seems that the more the subject is investigated the more the two views regarding the circulation through the pulp approach each other. A system of capillaries completely closed does certainly not exist, but the pulp-spaces seem to have among them larger and more direct channels leading from the artery to the vein. These may be likened to larger and more direct holes punched through a sponge, which therefore communicate freely with one another.

A number of reseaches by Thoma and his pupils have brought up the spleen problem anew, and judging by the methods they have employed, these studies will probably lead to a final solution of the question.[1] The works of Sokoloff and of Wicklein deal mainly with the vascular walls in hyperaemia of the spleen and contain many valuable experiments, while those of Thoma deal with the relation of the blood-vessels to the surrounding tissues when injected with either fluid or with granular masses. Thoma says (1899, p. 281): "In den meisten Organen ergeben Injectionen der Blutbahn mit den genannten körnigen und gelössten Farbstoffen keine auffälligen Unterschiede. Die bei der Milzinjection hervortretenden Unterschiede müssen somit als bedeutungsvoll anerkannt werden. Die Injectionen mit körnigen Farbstoffen beweisen, dass die Milzarterien durch die Verbindungsstücke unmittel-

[1] Sokoloff, Virch. Arch., 112; Wicklein, Ibid., 124; Kalenkiewicz, Inaug. Diss., Dorpat, 1892; Golz, Inaug. Diss., Dorpat, 1893; Thoma, Dorpater Naturfor.-Gesellsch., 18; Thoma, Verhandl. der. anat. Gesellsch., 1895; and Thoma, Archiv für Anatomie, 1899.

bar in die Milzvenenplexus einmünden. Damit ist der Weg gegeben, welchen die zelligen Elemente des Blutes nehmen, wie auch die Erfahrungen bezüglich der venösen Hyperämie bestätigen. Es besteht somit auch in der Milz ein geschlossenes Gefässsystem. Die Ergebnisse der Injectionen gelösster Farbstoffe beweisen jedoch dass die Wandungen dieses Gefässsystems in höherem Grade durchlässig sind als die Wandungen anderer Gefässverzweigungen. Es ist zu schliessen, dass normaler Weisse während des Lebens ein Theil des Blutplasmas denselben Weg durch die Spalträume der Milzpulpa strömt, welchen bei der Injection die gelösten Farbstoffe nehmen."

My own work upon the spleen [2] has led me gradually to conclusions similar to Thoma's. In my preliminary communication, which appeared before Thoma's last paper, the conclusions are almost identical with his regarding the circulation through the pulp. "The microscopic anatomy shows that the ampullae and venous plexus have very porous walls which permit fluids to pass through with ease and granules only with difficulty. In life the plasma constantly flows through the intercellular spaces of the pulp cords, while the blood corpuscles keep within fixed channels." Later on I found it necessary to modify this view somewhat, making the walls of the capillaries of the pulp still more porous. "By studying numerous successful injections of the last third of the ampulla I find that its communication with the vein is not wide but is cut up with bridges of tissue passing across its lumen before it communicates with the vein (1900, p. 34). It appears as if in the neighborhood of the lymph follicles the walls of the ampullae are most porous (p. 35). Experiments show that if the muscle is paralyzed the blood discs enter the pulp-spaces, thus causing an hemorrhagic infarction (p. 36). It seems as if the pulp-spaces are in all cases filled through these openings in the walls of the veins. Yet I am unwilling to accept this explanation until further arguments are made to support it, but am rather inclined to the idea that the pulp is filled with blood passing through the openings in the walls of the ampullae (p. 39)."

I do not quote the instances in which I brought forward arguments in favor of a closed circulation, but only those in which the closed channel was doubted. The reason for this will become apparent when I discuss some new specimens and experiments I have made recently.

Two years ago Weidenreich [3] published an extensive and excellent criti-

[2] Mall, Johns Hopkins Hospital Bulletin, 1898; Zeit. für Morphol. u. Anthropol., Stuttgart, 1900; and Spleen, Reference Handbook of Medical Sciences, New York, 1903.

[3] Weidenreich, Arch. f. mik. Anat., LVIII, 1901.

cism of the literature upon the vascular system of the spleen, giving also a valuable experiment which throws light upon the circulation through the pulp. His conclusion regarding the arterial capillary is as follows: "Die arterielle Capillare geht aus der Hülsenarterie hervor und stellt ein dünnwandiges, leicht dehnbares Rohr dar von wechselnder Weite; ihre Wand besteht aus einer äusseren Schicht, welche aus stark in die Länge gezogenen Hülsenzellen und anscheinend auch wirklich durch eine Fortsetzung der Hülse selbst gebildet wird, und einer inneren Endothellage mit spärlichen, grossen Kernen. Diese Capillaren münden entweder unter spitzem Winkel direct in einen Milzsinus ein oder lösen such durch Auffaserung ihrer Wand in dem Reticulum des Milzparenchyms auf (p. 322)." Apparently Weidenreich has come to a conclusion practically identical with that of W. Müller,[4] although he made no direct injections (p. 340).

Shortly after Weidenreich, Helly[5] published two papers upon the spleen with conclusions practically the same as mine and Thoma's. He says: "Die Milz hat ein, überrall von einer regelmassigen Endothelschichte ausgekleidetes, daher geschlossenes Gefässsystems mit sehr durchlässigen Wandungen" (Vol. 61, p. 272). Helly repeated Weidenreich's transfusion experiments and confirms him regarding the presence of foreign red blood corpuscles in the spleen pulp. According to Weidenreich these foreign cells passed over into the pulp from the capillary artery, and according to Helly they entered the pulp backward through the capillary vein.

The quotations just given show that the recent authors are of one opinion regarding the large pores in the capillary walls. It appears that these openings are smallest according to Thoma, larger according to Helly, still larger according to Mall, and so large that they communicate most freely with the pulp-spaces according to Weidenreich. The problem is further complicated by W. Müller, Weidenreich and others, who find that both open and closed capillaries occur in the same spleen. When we consider the difficulties and the differences of opinion, it is not hard to understand that there should be different conclusions regarding this question. While I have found numerous direct capillary channels in the distended spleen, I have never seen one in the contracted spleen. Furthermore, I have never been able to follow anything like an endothelial lining from an artery to a vein. So while the interpretations of the results are apparently different in the publications of

[4] W. Müller, Feinerer Bau der Milz, Leipzig, 1865, p. 79.

[5] Helly, Arch. f. mik. Anat., LIX and LXI, 1902.

Thoma and Weidenreich, they have had in reality the same kind of specimens before them. Weidenreich's single complete capillary, which is extremely difficult to obtain when the spleen is not injected, is Thoma's *Zwischenstück,* quite easily found when hyperaemic spleens are injected with fine granular masses. On the other hand Thoma's extravasations, which are always present, represent the normal course according to Weidenreich. So it seems to me that all of the recent workers practically agree regarding the walls of the capillary vessels of the spleen, and the question formulates itself anew, *How complete is the capillary wall, and is it exactly the same in all portions of the spleen?* With this there is joined a second but most important question, *Do all of the blood corpuscles enter the pulp-space in passing from the artery to the vein?* Both of these questions must be answered by making experiments and injections. To attempt them through simple sections of the spleen, no matter how thin they may be, is practically a waste of time.

For purposes of description I have shown that it is well to divide the spleen into a number of lobules,[6] which are practically identical in arrangement with those of the liver. Each lobule is about a millimeter in diameter with its artery in the centre and its main veins and trabeculae on the periphery. There are about 80,000 lobules in the dog's spleen. Each lobule is broken up into terminal or histological units, one for each terminal artery or ampulla. Around this there is some spleen pulp which lies within one of the meshes of the venous plexus. This is well shown in Fig. 13 in my paper in the Zeitschrift für Morphologie und Anthropologie. The arteries of the lobule are covered with a lymphatic sheath continued from the Malpighian follicle, known as the ellipsoid sheath. This ellipsoid of Schweigger-Seidel[7] continues to the end of the artery as a small group of round cells (see figure) and marks the beginning of the ampulla. Assuming then that the ampulla communicates with the venous plexus, it may be divided into three parts. The first third, which is the ampulla proper of Thoma; the second third, which contains large side openings, and the third third, which is Thoma's *Zwischenstück.* For the present I shall speak of the ampulla in this way, even if at times it appears to contain neither cavity nor walls. The capillary veins or venous plexus flow together into intralobular collecting veins, which in turn empty into the interlobular veins lying within the trabeculae at the periphery of the lobule.

The division of the spleen into lobes and lobules is not new. Kyber[8]

[6] Mall, Zeit. f. Morphologie u. Anthropologie, 1900.
[7] Schweigger-Seidel, Virch. Arch., XXIII.
[8] Kyber, Arch. f. mik. Anat., Bd. VI, 547.

used the term lobule in a vague sense (p. 548), corresponding more with my histological unit. This division is also accepted by Hoyer.[9] Kyber's lobes correspond with the arterial branches which enter the spleen, and form about ten unequal subdivisions of the spleen substance. The lobules I have described are about a millimeter in diameter and can easily be seen on the surface of the organ, or in sections. They are not to be confounded with Kyber's lobules.

That the terminal artery and the ampulla are very porous is shown easily by injecting the artery with any fluid, such as carmine gelatin, which in all cases passes over into the pulp at once, filling all of its spaces and the veins evenly. Such specimens naturally lead one to conclude that the circulation through the spleen is open, and were it not for other facts all anatomists would be willing to accept this conclusion. And when the spleen is evenly injected with carmine gelatin it is impossible to find the end of an artery with any degree of certainty, and it is very exceptional to find one reaching to a vein.

If a spleen, made oedematous by injecting gelatin into either the vein or the artery, or by filling the pulp with blood by ligating the vein for half an hour, is injected through the artery with some fine granular mass like Prussian blue, it is found that at the end of each arterial capillary there are a number of ampullae which communicate with the pulp-spaces, with one another and with the veins. It seems as if the ampullae are only large holes within the spongy pulp. A picture showing this arrangement is given by me in my article in the Zeitschrift für Morphologie und Anthropologie, Vol. II, Fig. 12. It must be remembered, however, that in all such specimens a considerable quantity of the Prussian blue has passed over into the smaller pulp-spaces. If the injection is continued until the veins are well filled all the pulp-spaces are also filled with the blue. Free communications between the ampullae and veins have been seen from time to time by numerous investigators from W. Müller to Helly, but no one has ever been able to find them in great number nor free from extravasations. In fact, a recent investigator, who no doubt is strongly inclined towards a closed circulation, states expressly that he could not find a single communication between the artery and the vein.[10]

The first third of the ampulla is lined with spindle-shaped cells which are directly continuous with the endothelial cells of the artery. The second third branches and often communicates with neighboring

[9] Hoyer, Morph. Arbeiten, Bd. III, 267.

[10] Von Schumacher, Arch. f. mik. Anat., LV, 1899.

ampullae. The last third of the ampulla is difficult to demonstrate, but under certain conditions it can be injected, as has been shown by Thoma and by me. I find from numerous specimens that its communication with the vein is not wide, but is cut up by bridges of tissue passing across its lumen before it connects with the vein. This cutting up is so extensive that in uninjected specimens it has been impossible for me to find a single ampulla connecting with a vein. In other words, it may be better to state that the ampulla rarely reaches the vein, but is separated from it by a small band of spleen pulp. When the spleen is distended to its maximum and fine granules are injected into the artery they pass directly from the ampulla to the vein, as well as into the surrounding tissue, for they must pass somewhere. This condition is illustrated in Plate I.

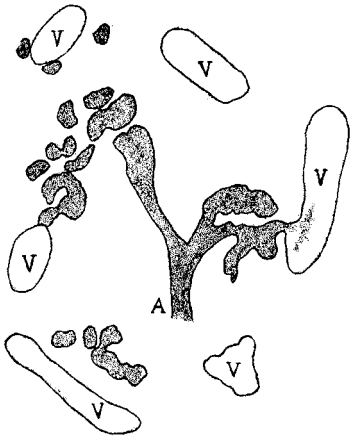
When cinnabar granules and gelatin are injected into the artery for a long time with a pulsating pressure most of the granules are found in the veins, but a great many are also scattered throughout the pulp. According to one's inclination this becomes an argument either for or against an open circulation. When either granules or foreign nucleated blood corpuscles are injected into the circulation of a living animal many of them are found between the cells of the spleen pulp, and they no doubt entered the pulp-spaces through the holes in the walls of the ampullae. Helly believes that the foreign red corpuscles pass from the artery to the veins and then through the "homogeneous membrane of v. Ebner" in the walls of the veins back into the pulp. That this roundabout way is the improbable course I shall show presently.

It is of decided advantage to inject an organ like the spleen with a fluid that will not mix easily with water, and I have tried a variety of mixtures of asphalt, turpentine and granules with great success. Hoyer[11] has already used this mixture in the contracted spleen, and what I have found in the oedematous spleen confirms and supplements his results. When the spleen is distended to its maximum with either blood or gelatin the relation of the terminal artery to the pulp is shown beautifully by injecting the turpentine-asphalt solution into the artery. Some granules of carmine should be added to the solution, for they lodge in the fine arterial branches and ampullae, and a few of them pass over into the pulp. It is well in making an artificial oedema of the spleen to inject the gelatin, to which ultramarine blue is added, into the veins. Finally the specimen is hardened in formalin and cut

[11] Hoyer, Internat. Monatschr. f. Anat. u. Physiol., 1887; and Morphol. Arbeiten, 1894, 276 and 284.

into sections, by the freezing method, 20 μ thick. The specimens are to be mounted in glycerine. In the sections of such specimens the veins are found filled with blue granules, the pulp with gelatin and some blue granules, the terminal arteries with some carmine granules and the ampullae with asphalt and some carmine granules. As there is no mixing of the fluid gelatin and the asphalt in the pulp, the asphalt must take the course of the least resistance from the artery to the vein. With Prussian blue this is often directly through Thoma's *Zwischenstück;* with asphalt the course is always through the pulp-spaces.

In specimens in which little of the asphalt reaches the veins it is found that the asphalt passes out into the pulp-spaces from the ampullae, then piles up, forming clusters like bunches of grapes (Text Fig. 1). Each of these "grapes" fills a pulp-space. As the clusters spread out the globules of asphalt often radiate, leaving intervening pulp-spaces free. Finally all the pulp-spaces are filled with the clusters of globules, then they encircle the veins and are continued into them as fine threads of asphalt. At other times some of the veins are surrounded with asphalt globules without many of them communicating with the veins. Sometimes the injection of the asphalt follows the reticulum in all directions, encircles the veins and enters them at many points. When the veins have first been plugged with ultramarine blue the asphalt which may enter the veins at points cannot spread far. When fine carmine granules are injected with the asphalt but few of them enter the veins, most of them lodging in the arteries and ampullae and some of them are scattered throughout the pulp. It appears then that the asphalt may push the aqueous fluid from the pulp-spaces and gradually pile up as does wax when injected into a lung. Or it may follow the reticulum, pushing the watery fluid to the centre of the pulp-spaces, as is again the case when the lung into which wax is injected contains much air. In this case the lung is filled with many small vesicles of wax full of air. The asphalt injections, therefore, give a most decided argument in favor of an open circulation.



TEXT FIG. 1. Outline of the terminal artery, ampulla and venous sinuses in a spleen made hemorrhagic by ligating the vein and then injecting the artery with asphalt and turpentine. The masses of asphalt reach from the artery to the vein and fill the large irregular spaces in the pulp. *A*, artery; *V*, vein.

Numerous experiments upon the dog's spleen show conclusively that the walls of the venous plexus are very porous. This can be shown well by injecting aqueous Prussian blue into the vein, which gradually passes through its walls at every point. If granules a little larger are injected (cinnabar), it will be found that they also pass through easily, but still larger granules (ultramarine blue) pass through the walls of the veins with greater difficulty. Yet often many of these granules pass through the vein-wall at all points, showing that it is very porous. Around the Malpighian follicles there is always an extensive "extravasation," showing that the openings at this point are very large and numerous. So constant is this "extravasation" found that it is out of the question to consider the openings around the follicles as artificial.

The nature of the wall of the vein has been well demonstrated by Höhl,[12] who gives an excellent picture of it (Fig. 10). It is composed of an extremely dense network of fibrils which withstand the action of pancreatin, and is therefore not elastic in nature. Höhl considers the fibrils as belonging to the reticular group, for they anastomose frequently and are directly continuous with the reticulum of the pulp. The fibrils around the vein were first isolated by Henle[13] by means of a diluted solution of potassium hydrate, and for this reason v. Ebner[14] considers them as elastic tissue, a conclusion which he supports with the Unna-Tänzer stain for elastic tissue. It is well known that reticulum fibrils as well as white fibrous tissue fibrils do become transparent in diluted solutions of potassium hydrate, but that their sharpness is again brought out in case they are stretched,[15] a condition easily obtained under the cover-glass by slight pressure upon it. Von Schumacher[16] repeated v. Ebner's tests for elastic tissue in the veins of the dog's spleen and sometimes found numerous elastic fibrils encircling them and at other times none at all. These tests have been repeated by Höhl[17] and by Hoyer,[18] who find that all or nearly all of the fibrils are reticular, which may be accompanied by some elastic fibers. In tests made with Weigert's elastic tissue stain I have been unable to find any elastic fibrils accompanying the reticulum fibers encircling the smaller veins. It is well when staining for elastic tissue not to stain too long,

[12] Höhl, Archiv für Anatomie, 1897.
[13] Henle, Anatomie, II, 1866.
[14] Von Ebner, Anat. Anz., XV, 1899.
[15] Mall, Abhandl. d. k. sächs. Gesellsch. d. Wiss., XIV, 1890.
[16] Von Schumacher, Arch. f. mik. Anat., LV, 1899.
[17] Höhl, Anat. Anz., XVII, 1900.
[18] Hoyer, Anat. Anz., XVII, 1900.

or else reticulum and even white fibers will take on some of the color and make them look like elastic fibers. Höhl has shown, however, that the reticulum fibers of the lymphatic gland, and even those of the spleen pulp, are accompanied in part by elastic fibers, a result which has been confirmed by Thomé [19] and by myself.[20] I found that the amount of elastic tissue accompanying the reticulum in lymph nodes varied very much indeed and that often the periphery of the follicle contained many elastic fibrils while the centre had no fibrils at all that would stain with Weigert's stain. This result corresponds well with that of Thomé. In the Malpighian follicle, however, the elastic fibers radiate from the artery and do not extend to the surrounding pulp.

It is apparent from the above that the fibrils around the veins are mostly reticulum, and that elastic fibrils may accompany them. The work of Hoyer, Thomé and myself points clearly towards this conclusion.

A discussion of the homogeneous elastic membrane of v. Ebner between the fibrils encircling the veins and the large spindle-shaped endothelial cells within is more difficult. If this membrane is present it is an additional argument in favor of closed circulation through the pulp and is much in the way of both Weidenreich and Helly in their discussions. Weidenreich finds the membrane present but accepts an open circulation, while Helly, who believes in a closed circulation, sees foreign blood corpuscles and leucocytes passing through it. According to Böhm [21] these veins have an elastic intima analogous to that of the artery. Von Schumacher found it inconstant in man and absent in the dog. Hoyer was unable to find it at all. Furthermore, in an extensive study upon the elastic tissue of the spleen by Lebrell,[22] no mention is made of this elastic membrane. Were it present he certainly should have seen it. Since it is not supposed to be present in the dog's spleen, my failure to find it is not remarkable, and from my experience with the Weigert elastic-tissue stain I am inclined to think that it really does not exist at all.[23] No doubt all stages of the development of reticulum, from the connective-tissue syncytium to the complete reticulum fibril with its accompanying elastic fiber, is found in the lymph follicle, judging by my own experience and by the works of Hoyer and Thomé. Recently Flint [24] has followed the development of the basement membranes in the

[19] Thomé, Jena. Zeit., XXXVII, 1902.

[20] Mall, Amer. Jour. Anat., I, 1902, p. 361.

[21] Böhm, Von Kupffer Festschrift, 1899.

[22] Lebrell, Internat. Monatschr. f. Anat. u. Physiol., XX, 1903.

[23] See also Kyber, Arch. f. mik. Anat., VI, 1870, p. 566.

[24] Flint, Amer. Jour. Anat., II, 1902.

submaxillary gland of the pig and found that they arise from a syncytium and are fibrillar (pp. 5 and 10), even if in transverse sections they appear homogeneous. If we could add to such preparations an excessive stain for elastic tissue it is easy to conclude, as v. Ebner did, that these basement membranes are also homogeneous and elastic. Since, however, the elastic membranes are not present in the veins of the dog's spleen they do not stand in the way of an open circulation in this animal.

Recently Weidenreich has asserted that there are numerous lymphatics in the spleen which empty directly into the veins of this organ. This is only another way of expressing what W. Müller stated a number of years ago. It is easy to state that the channels from the pulp to the veins are lymphatics, but in view of the work of Ranvier,[25] MacCallum [26] and Sabin [27] our conception of lymphatics has been greatly sharpened. According to Sabin all of the lymphatic channels arise from four points from the veins, which correspond with the lymph hearts, and then spread all over the whole body. In order to accept Müller's notion it must be shown that lymphatics have an independent origin the spleen, which is very improbable.

Judging by the structure of the vascular system of the spleen-pulp, it is not remarkable that numerous investigators have concluded that the circulation should be directly through the pulp-spaces. That this should be so appears very remarkable when the circulation through other organs and tissues is considered, where the capillary walls are lined by a complete layer of endothelial cells and their lumina are equal. No one admits, however, that such capillaries are within the spleen pulp, but because they are closed elsewhere it is concluded that they must also be closed within the spleen. If the ease with which "extravasations" take place from the capillaries of embryos, when the blood-vessels are injected, is recalled, one is struck with the similarity between them and those of the spleen-pulp. In fact I have frequently observed that in certain portions of the embryo the "extravasation" takes place with greater constancy than elsewhere. Recently this system of irregular capillaries has been more sharply defined by Minot,[28] who terms them sinusoids and shows that a sinusoidal circulation is present in many of the organs of the embryo and in some of the organs of the adult. He states that he does not consider it improbable that the circulation through the spleen will prove to be sinusoidal. That

[25] Ranvier, Archiv d'Anatomie, I, 1897.
[26] MacCallum, Archiv für Anatomie, 1902.
[27] Sabin, Amer. Jour. Anat., I, 1902.
[28] Minot, Proc. Bost. Soc. of Nat. History, XXIX, 1900.

this is true I think the recent work upon the spleen proves quite conclusively. Since sinusoidal or open circulation exists elsewhere, the old argument that the capillaries of the spleen are closed because the capillaries of the rest of the body are closed, is no longer of any value.

It remains still to be shown that in normal circulation through the spleen the solid elements of the blood are carried through the spleen sinusoids or pulp-spaces. It is well known that in higher animals but relatively few red blood discs are found in the pulp-spaces and that it is practically impossible to obtain natural injections of them. This fact, together with a mythical homogeneous membrane lining the capillary veins, is v. Ebner's strong argument in favor of a closed circulation in the spleen. Von Schumacher, who also believes in this elastic membrane, always finds blood in the pulp with the membrane intact

In case the blood passes through the pulp-spaces in its normal circulation, it should be possible to retain it in them by making the proper experiment. Sokoloff and Wicklein made numerous experiments which appeared to prove that after ligature of the splenic vein the blood first accumulated in the veins, then the pulp becomes oedematous, after which the blood elements pass over into the pulp. No doubt all of these observations are correct, but it appears to me that their reasoning is wrong, as Weidenreich has shown recently. Sokoloff states that as a rule the normal spleen contains no blood within it, and it is difficult to find it even in the blood-vessels (p. 211). This is due to the contraction of the muscle, which always takes place when the organ is removed and presses the blood out of the spleen (p. 213). " Wie wäre es denkbar, dass bei einer solchen Ueberfüllung der Venen, die Pulpa nach dem Tode ihr Blut in die Venen entleeren sollte. Wenn der Blutstrom unter normalen Verhältnisse aus der Arterie in die Milzpulpa und von da in die Venen sich ergiessen würde, musste hier effenbar die Pulpa mit Blut überladen erscheinen," etc. (p. 215).

In addition to this Sokoloff showed that the walls of the capillary veins are very porous, in fact wanting around the Malpighian follicles, and it was through these pores he thought that the blood passed into the pulp in case the vein was closed long enough.

Wicklein repeated the experiment of Sokoloff and found that if the vein was ligated long enough to produce an extensive infarction of the pulp this infarction would disappear in the course of time in case the ligature was removed. In all cases he ligated the vein for 30 minutes, a time sufficiently long to cause an extensive distention of the pulp with blood, then he removed the ligature and killed the animals in from 4 hours to 21 days, and in all cases found the pulp normal in every respect.

He concludes from these experiments that the blood first passed into the pulp backward through the veins, and then when the ligature was removed the blood passed into the veins again, by what mechanism he does not state.

I have repeated these experiments a number of times and find that in the course of half an hour after ligature of the veins the spleen is distended to its maximum and the pulp is always gorged with blood, as described by Sokoloff and by Wicklein. If now the spleen is removed from the body by cutting its attachments to open all the large vessels, it empties itself at once, and sections show that the pulp is as free from red corpuscles as is the normal spleen. There must, therefore, be some mechanism by which the blood which enters the pulp either from the artery or the vein may be rapidly expelled from the pulp. In case the ligature from the vein is simply removed in the living animal the spleen does not become entirely anaemic, but when it is removed from the body it contracts to its maximum and expresses every drop of blood from its pulp. An extremely instructive experiment is made by keeping the vein ligated until the spleen is blue, but not distended to its maximum. This takes from 10 to 15 minutes. Then the spleen is to be removed from the body, leaving the ligature intact. The contractions begin at once and pump the blood around under the capsule, thereby giving some large hemorrhagic spots in which the pulp is gorged with blood. At other points, however, the spleen is anaemic and practically normal. A portion of the spleen-pulp has been emptied of its blood, and due to the severe contraction it was passed to some other portion of the spleen to make it even more hemorrhagic than before. A double process has taken place, which can be observed with ease; the contraction has forced blood from the pulp into the vein in one portion of the spleen and from the vein into the pulp in another portion. At any rate one thing is definite—when the blood is free in the pulp contraction of the muscle has the power to squeeze it out at once and to force it over into the vein.

Miescher-Rüsch,[29] in one of the best among the many excellent papers upon the spleen, states that in the dog the blood is pressed out of the pulp by the muscular contraction, while in the salmon, which has no muscle fibers in the spleen, the blood stays in the pulp-spaces. In the salmon, at any rate, the blood corpuscles must be flowing constantly through the pulp-spaces, forced onward by the arterial pressure.

It is practically impossible to determine with certainty whether the blood circulates through the pulp-spaces of higher animals as it does in

[29] Miescher-Rüsch, Archiv f. Anatomie, 1881.

fishes unless the muscles of the trabeculae and capsule are paralyzed or are thrown out in some way. Jaschkowitz[30] found by cutting the nerves of the spleen that the muscle becomes paralyzed and the pulp fills with blood. I have repeated Jaschkowitz's experiment and have discussed it more fully elsewhere.[31] It appears to me that in view of the anatomy of the spleen and the effect of contraction of the muscle upon blood in the pulp it is rational to conclude that when the muscle is paralyzed by cutting the nerves the increased amount of blood in the pulp came directly from the arteries rather than back through the veins. The muscle is excluded and we have on one hand high arterial pressure and on the other hand low venous pressure. It appears to me that there is but one way by which this blood got into the pulp-spaces, i. e., through the holes in the ampullae.

In connection with the effect of contraction and relaxation of the muscle of the spleen and its effect upon the blood in the pulp we may consider Roy's[32] discovery of its rhythmic contraction. It was found that the contractions of the spleen are very regular, one a minute, and that the change of volume during each contraction may be as much as 18 per cent. In a dog's spleen of average size this is about 5 grams. Since about 5 cc. of blood flows from the veins of the spleen every minute,[33] there should be a variation of the outflow from the vein during this time. Roy concludes that the pulsation of the spleen acts as a pump by which the circulation through the spleen is aided. Without entering upon the discussion of this question it may be noted that this conclusion is doubted by Schäfer and Moore.[34] "The spleen volume is extremely responsive to all fluctuations in the general blood-pressure, and the circulation through the organ can be and probably is entirely brought about, as in other organs, by the difference between the arterial and venous pressure." Artificial circulation is carried on easier when the organ is distended, even after death of all the muscles, and the same seems to be the case in the living spleen. W. Müller has shown that it is impossible to inject the arteries of the spleen through the veins unless the spleen is pretty well distended (p. 85), and this should be the case judging by the arrangement of the ampullae. When the pulp is distended the reticulum fibrils pull the ampullae open and when it is, compressed the ampullae will be closed, making of them a kind of valve.

[30] Jaschkowitz, Virch. Arch., XI.

[31] Mall, Zeit. f. Morphol. u. Anthropol. II, 1900.

[32] Roy, Journal of Physiol., III, 1880-82.

[33] Mall, l. c., p. 36.

[34] Schäfer and Moore, Journal of Physiol., XX, 1896, p. 50.

It happens also that forcible distention of the spleen, or contraction of the muscle of a hyperaemic spleen pulls all of the veins open, even after they leave the trabeculae to enter the spleen lobule. So a contraction of the muscle of a hyperaemic spleen will tend to close the ampullae of the arteries and to open the veins. The effect of this of necessity must be to force the blood from the pulp into the veins, which is always the case. When it is once outside of the spleen the valves will prevent its return. And I have found that in certain cases the pressure in the splenic vein may exceed the arterial, a condition which can be brought about only in case the spleen contraction acts as a pump. In one case the pressure in this vein rose to 190 mm. Hg. with the artery closed, i. e., the arterial pressure practically at zero (l. c., p. 37).

Objection may be raised against Jaschkowitz's experiment as an argument in favor of an open circulation, for considerable time elapses between cutting the nerves and the following hyperaemia and infarction. It must still be shown that in the living animal the blood is constantly passing through the pulp, as is the case in the salmon's spleen. The influence of the contraction of the muscle upon the blood in the pulp must be removed in other ways.

In order to bring more evidence to bear upon this question I have made four kinds of successful experiments, which prove conclusively that practically all of the blood corpuscles pass through the pulp of the spleen.

In the first of these the dog was bled to death and the blood whipped. Cannulae were then tied into the splenic artery and vein, after which the anastomoses were tied and the spleen removed. The whipped blood and spleen were then kept at from 3° to 5° C. for 24 hours. Then the blood and spleen were warmed to 37° and the blood was injected into the artery with a pulsating pressure from 80 to 100 mm. Hg. In just one minute the blood began to flow from the vein. In 5 minutes the spleen began to swell a little, which continued slowly until the spleen was quite red and of the appearance it has in the living animal. Now the flow from the vein was at the rate of 5 cc. a minute, about the same as in the living animal. At the end of half an hour the spleen was placed carefully in strong formalin. During the whole time of artificial circulation the arterial blood was red and the venous blood blue, and at no time was there the faintest indication of the contraction of any muscle. Next day frozen sections were made and in all cases it was found that the pulp was filled with blood, extending freely over into the lobular veins, which were gorged. The interlobular veins were not so full, for during the whole experiment the vein was freely open, the

pressure in it being practically zero. That the injection into the pulp is not a backward injection is proved by ligating one of the arteries to the spleen while making the experiment. The veins of the pulp of the region supplied by the closed artery were injected through the venous anastomoses within the lobule, but in this region the pulp-spaces were all empty. It is natural to conclude from this experiment that the blood passed from the ampullae into the pulp-spaces, then through the pores in the walls of the veins to form columns of blood discs which are pushed from the smaller to the larger veins of the spleen.

The muscle of the fresh isolated spleen is easily paralyzed by injecting the artery with normal salt solution or even with ordinary aqueous solutions of gelatin. When fresh spleen is paralyzed in this way it is found by injecting gelatin and cinnabar granules, or by injecting whipped blood, specimens are obtained which are exactly identical with the experiments described above. I thought for a long time that the large number of cinnabar granules found in the lobular veins indicates that the granules pass in great part from the artery directly into the vein and in lesser part from the artery over into the pulp-spaces. But when the experiments are graded it is found that the granules do not enter the veins until the pulp-spaces are first filled. The same is true when artificial circulation is carried on with whipped blood. All the injections, especially those with asphalt and turpentine, confirm this view. In them numerous terminal arteries are found, around which the pulp spaces are injected, forming pictures much like bunches of grapes. From these the injection passes into the veins. Incomplete injections after the veins are plugged with granules are especially valuable for the study of the relation of the arteries to the pulp-spaces. It may be added that in all of these tests the greatest "extravasation" of blood, of granules or of coloring matter is always immediately around the Malpighian follicles, showing that the communication between the ampullae and pulp-spaces are there the freest.

The muscle of the spleen can be paralyzed in the living animal by injecting nitrites into it. In all of my experiments I injected nitrite of soda from one-half per cent to one per cent in strength. The latter solution paralyzes the muscle very quickly. In one experiment the upper, or larger end of the spleen, was washed out with a half per cent solution through the vein with the artery closed for a period of five minutes. At the end of this time the upper part of the spleen was totally paralyzed and flabby. The artery was then opened for 25 minutes, and to show that the circulation through the paralyzed portion was well established the blood was collected from the open vein, which was

9 cc. in 10 minutes. The vein was now closed for 15 minutes and then the animal was killed by cutting the aorta. During the time the vein was closed the spleen became very much distended with blood. The spleen was now cut out, leaving the veins and arteries freely open. During the 5 minutes which followed the normal end of the spleen contracted to a solid mass, the paralyzed end contracted slightly and the zone between contracted markedly. Careful examination showed that there were no clots in any of the veins. Frozen sections, after the spleen had been hardened in formalin, showed that the pulp of the paralyzed end of the spleen was filled with blood, that of the normal end was free from blood, and that in the intermediate zone there was some blood in the veins and some in the pulp. Experiments of this sort only show that the blood which enters the pulp after the vein is closed is not expelled in case the muscle of the spleen is paralyzed

A better test is made by injecting the nitrite through the artery. This can be done easily by inserting the cannula into one of the anastomoses to the stomach, pointing towards the spleen, and when the fluid is injected the main artery is to be clamped to prevent the fluid from escaping into the aorta. Of course all of the anastomoses are to be tied. If a spleen is injected in this way for ten minutes it is found that the muscle is paralyzed completely. Then by closing the cannula and opening the main artery the normal circulation is re-established. In case the first blood enters the pulp directly it should be found filled with red blood corpuscles. So at the end of one minute the spleen was cut out with one stroke of the scissors, leaving the main arteries and veins open. The paralyzed end of the spleen did not contract. The specimens were placed in strong formalin at once and frozen sections were made a few days later. They showed that the pulp was gorged with blood in the paralyzed portion of the spleen. It was practically impossible to find the line of demarcation between the pulp and the veins. As the end of the spleen which was not paralyzed was approached the pulp was found to contain a much smaller number of red blood corpuscles, but at no point was it full of them. Experiments of this kind show that when the muscle of the spleen is paralyzed with sodium nitrite the blood which enters the artery passes first into the pulp-spaces and remains there, for there is no muscular contraction to expel it.

Probably the most satisfactory experiment is made by paralyzing the muscle of the spleen so quickly that it has no time to contract and empty the pulp-spaces. This can be accomplished by making an interstitial injection with strong formalin and then removing the spleen at once and with one stroke of the scissors. The whole experiment can be performed

in 5 seconds, and it is usually found that the spleen does not show the faintest sign of contracting when it is laid upon a glass plate. When it is simply removed from the body without the injection of formalin it contracts during a period of several minutes and gradually expels all of the blood from the pulp and veins.

In order to make this experiment more definite I used the very hyperaemic spleen, found during active digestion. The animal was placed under the influence of morphine and the hyperaemic spleen drawn gently through an opening made in the abdominal wall. Next ten cubic centimeters of the strongest formalin were rapidly injected into several portions of the spleen with a hypodermic syringe and then the organ was severed from the body with one stroke of the scissors. After the spleen was observed for 5 minutes in order to see that it had been completely paralyzed it was hardened in formalin. The sections which were subsequently made showed that the whole pulp was filled with blood, just as is the case in the salmon's spleen or the dog's spleen when the muscle is paralyzed for a greater length of time. This differs from the rest of the experiments in one most important respect—the blood was caught in position in the pulp of the normal hyperaemic spleen. There can be but one interpretation of this experiment—the blood passes through the pulp-spaces in normal circulation, and due to the rapid fixation and paralysis of the muscle it is held there.

The crucial experiment is made by fixing the spleen in the living animal by injecting formalin directly into the carotid artery. For this purpose the dogs were fed four hours before the experiments in order that their spleens should be very hyperaemic. The cannula was introduced into the carotid artery pointing towards the heart and 2000 cc. of a 10 per cent solution of commercial formalin was injected at a pressure of 200 mm. Hg. This treatment coagulated immediately all of the tissues fixing definitely the chyle in the lacteals throughout their extent as well as all of the blood in the spleen. All of the muscles were immediately and completely paralyzed. The spleen remained very hyperaemic and showed no indication whatever to contract. Frozen sections showed that there are a great mass of red discs in the pulp especially around the Malpighian follicles. The distribution of the blood corresponds in every respect with that of cinnabar granules when injected either into the artery or the vein. At many points the veins are well filled with blood and at others the pulp and veins are so evenly filled with blood that the line between them cannot be seen. In general the spleen is nearly as full of blood as is the case when the vein has been ligated for half an hour. The effect of the formalin upon the

spleen when it is injected into the artery of a live animal must be practically instantaneous, paralyzing the muscle and fixing the blood corpuscles in their natural channels. That the pulp of the spleen is found gorged with blood in this experiment is conclusive evidence in favor of an open circulation.

The forces which drive the blood through the pulp are undoubtedly the arterial pressure, the elasticity of the reticulum and the contraction of the muscle of the spleen. Judging from the arrangement of the trabeculae and veins in distended spleens it is very apparent that the contraction of the trabeculae have a decided influence in pulling the veins open. So when the muscle contracts it exerts a pressure upon the pulp more than it does upon the contents of the larger veins; in other words, the arrangement is such that the contraction of the muscle forces the flow of blood from the intralobular veins to the interlobular veins. When the pulp is compressed, however, the elasticity of the reticulum (l. c., p. 29) acts more upon the pulp than upon the capillary veins, again favoring the circulation from the pulp-spaces into the capillary veins. Therefore the anatomical and physiological arrangement is such that the contraction of the muscles of the trabeculae and capsule will press blood from the pulp into the veins without the aid of the arterial pressure. On the other hand it is through the arterial pressure that the pulp-spaces are filled when the spleen is relaxed, for it alone can overcome the elasticity of the reticulum. The arterial pressure itself is sufficient to carry on a complete circulation through the spleen, but the pulp-spaces can be fully emptied only by the contraction of the splenic muscle. The contraction of the splenic muscle has a tendency to close the capillary arteries while on the other hand with the aid of the elastic reticulum the capillary veins are pulled open. Therefore this contraction forces blood in one direction only.

The conclusion to be drawn from this study is that the course of the red blood corpuscles is always through the pulp-spaces in passing from from the artery to the vein. I have been unable to gather any good evidence in favor of some closed capillaries, as suggested by W. Müller, Miescher, Weidenreich and others. Thoma's *Zwischenstück* represents only the more direct pulp-space between the ampulla and the vein, which is best demonstrated when the rest of the pulp-spaces are gorged with blood.

EXPLANATION OF PLATE I.

Section of a spleen made oedematous by injecting chrome yellow suspended in gelatin into the vein. The artery was then injected with an aqueous solution of Prussian blue. The specimen was cut on the freezing microtome, tinged with picric acid mounted in glycerine. Enlarged 250 diameters. *A*, artery; *a*, ampulla; *V*, vein.

THE CIRCULATION THROUGH THE PULP OF THE SPLEEN. PLATE I.

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