THE RÔLE OF THE SPLEEN IN BLOOD FORMATION.*

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Various authors[1] have described the appearance in the spleen in the course of chronic anemias of abundant cells which give evidence of the new formation of blood in that organ when blood formation is greatly needed by the body. These cells are erythroblasts and mononuclear cells of various sizes which become very abundant in the venous sinuses. The same cells are found in the capillaries of the liver and occasionally outside them in the periportal tissues, but not in great numbers in the circulating blood.

The information derived from attempting to estimate the change in the numbers of cells in such an organ as the spleen was felt to be unsatisfactory and a method of study other than the staining of sections of the fixed spleen was sought. Without any knowledge of previous efforts in this direction it was thought that accurate counts of the blood leaving the spleen by the splenic vein when compared with similar counts of that entering by the splenic artery should give a clear idea of the activity of the spleen with respect to its contribution of cells to the blood. Since the completion of the work a few references to similar studies have been found, but it nevertheless seems that a detailed statement of the result of these counts would be of value, especially since the method which is so useful has been employed by hardly any modern workers.

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[1] Bizzozero, G., and Salvioli, G., *Arch. p. le sc. med.*, 1880, iv, 49. Meyer, E., and Heineke, A., *Verhandl. d. deutsch. path. Gesellsch.*, 1906, ix, 224; *Deutsch. Arch. f. klin. Med.*, 1907, lxxxviii, 435. Morris, R. S., *Bull. Johns Hopkins Hosp.*, 1907, xviii, 200. von Domarus, A., *Arch. f. exper. Path. u. Pharmakol.*, 1908, lviii, 319. Heinz, R., *Virchows Arch. f. path. Anat.*, 1902, clxviii, 501. Jolly, J., and Rossello, H., *Compt. rend. Soc. de biol.*, 1909, lxvi, 40. Schridde, H., *Centralbl. f. allg. Path. u. path. Anat.*, 1908, xix, 865. Naegeli, O., Ehrlich, P., and Lazarus, A., Die Anaemie, Vienna and Leipzig, 1909.

It has been known for a long time that the leucocytic count of the blood from the splenic vein exceeds that of the artery.

Vierordt[2] in four counts made on an executed man found the relation of leucocytes to red corpuscles in the splenic vein to be 1 : 49. Hirt[3] found that in the calf the relation was 1 : 60, while in the arterial blood it is 1 : 2,200. Funke[4] gives this relation in the splenic vein as 1 : 4, while Frey[5] calculated the proportion in an old man dead of pneumonia as 1 : 102. Rindfleisch[6] called attention to the richness of the splenic blood in leucocytes especially after a meal. Weidenreich[7] undertook counts from the sinuses of the spleen and obtained variable results inasmuch as some of them contained few or no leucocytes, while others were filled with them doubtless because those sinuses into which the arterial stream enters directly will contain predominantly red corpuscles while the reverse condition will obtain where the lymph channels enter. As a mean I found a proportion of 1 : 15. If we take the relation in the artery (following Hirt) as 1 : 2,200, and the average of the figures from the literature as 1 : 30 it is seen that the splenic vein contains about seventy times as many leucocytes as the efferent artery. Since special efferent lymphatic channels for the drainage of the lymphocytes of the spleen have been shown not to exist, the cells must be discharged into the vena lienalis which thus contains both blood and lymph.

Von Ebner[8] states that it has been shown that the blood of the splenic vein contains an uncommonly large number of white corpuscles and that the site of their formation is the splenic tissue itself from which they enter through the permeable walls of the venules. Loewit[9] studied the relation of leucocytes of arterial blood and splenic venous blood in the guinea pig and found 30 to 80 times as many leucocytes in the venous blood, the increased proportion being due to small and large mononuclear elements. This finding is not obtained in all animals probably because of the irregular or intermittent discharge of the cells. Certain negative results of Tarchanoff and Swaen[10] are probably due to this cause.

The cardinal idea, therefore, in the present study was to examine carefully the blood entering the spleen and to compare it with that emerging by the splenic vein. Any differences should

[2] Vierordt, *Arch. f. physiol. Heilk.*, 1854, xiii, 259, 408.

[3] Hirt, E., *Arch. f. Anat., Physiol., u. wissensch. Med.*, 1856, 174.

[4] Funke, O., Lehrbuch der Physiologie, Leipzig, 1863, i.

[5] Frey, H., Handbuch der Histologie und Histochemie des Menschen, 4th edition, Leipzig, 1874.

[6] Rindfleisch, G. E., Experimentelle Studien ueber die Histologie des Blutes, Leipzig, 1863.

[7] Weidenreich, F., *Arch. f. mikr. Anat.*, 1901, lviii, 247.

[8] von Ebner, V., *Köllikers Handbuch der Gewebelehre*, 1902, iii, 276.

[9] Loewit, M., *Folia Haematol.*, 1907, iv, 473.

[10] Tarchanoff, J., and Swaen, A., *Arch. de physiol. norm. et path.*, 1875, ii, series 2, 324.

represent the changes produced by the spleen. Counts were made with the Thoma-Zeiss apparatus with blood from the splenic artery, splenic vein, mesenteric vein, and, for purposes of control, from a peripheral systemic vein. Smears of blood were also made from each situation. Rabbits, cats, and dogs were studied, some in perfect health, others suffering from spontaneous or artificially induced disease. Thorough autopsies were made in each case and the organs studied microscopically. The counts were made in the usual way but with the most scrupulous attention to technical accuracy in every detail. For the white corpuscles 0.2 per cent. acetic acid was used and for the red cells fresh Toison's solution. Differential counts of the white cells were made in all cases for the blood from each situation.

In securing blood from the splenic artery and vein considerable care must be taken to have a dry field without great loss of blood in the earlier steps of the operation. An incision from the midline along the left costal margin to the axillary line was found to give the best exposure, after which the assistant could elevate the spleen without making traction upon the vessels, by lifting up the stomach and gently drawing the spleen forward so as to expose the hilum. Care was taken not to handle or squeeze the spleen so as to force out its contents artificially. The remaining viscera were protected and the spleen supported by warm cloths so that the circulation proceeded normally. The blood must be obtained from the splenic vein first, as interference with the arterial current will produce changes in that of the veins, while the venous anastomosis and collateral trunks are so numerous that occlusion of one produces no effect.

Some counts were made from blood allowed to spurt from a puncture in the vein, but since this hemorrhage makes it difficult to work later with the artery it was found advisable to put on two Carrel clamps and make the puncture between them. The latter is the more satisfactory procedure since a few drops only may be allowed to flow, and the counts do not differ appreciably from those made from the freely flowing blood.

Ehrlich's triacid stain after fixation by heat and Jenner's, Wright's, and Giemsa's stains after methyl alcohol fixation were

used for the differential counts. Prolonged staining with diluted Giemsa's stain gave good results.

The experiments showed that the blood of the splenic vein differs remarkably from that of the artery not only in the character of its cells but in their number, and this difference was found to exist also between the blood of the splenic vein and that of a peripheral systemic vein.

These differences may be summed up as follows:

(1) The number of red corpuscles per cubic millimeter in the blood of the splenic vein is greater than in that of the artery.

(2) Similarly the number of white corpuscles in the venous blood is greater than in the arterial blood.

(3) The character and proportion of the several varieties of white corpuscles is different in the splenic vein from what is found in the artery. Large mononuclear leucocytes appear in great excess there.

(4) The blood of the splenic vein contains more perfect and larger red corpuscles than that of the artery and they seem richer in hemoglobin.

(5) The blood of the inferior mesenteric veins differs from that of the splenic vein in being relatively richer in small mononuclear cells and poorer in the large mononuclears.

In the tabulated and detailed results given below (table I) these differences are more definitely brought out. It will be noted that the counts made from the ear vein and splenic artery of the same animal correspond very closely, as would be expected, while a marked difference was always found when blood was taken from the splenic veins.

It will be seen from table I that when the autopsy showed the animals operated on to be normal the results were invariably the same. There were always more red and white cells in the splenic veins than could be found in any of the other vessels. Frequently this excess of corpuscles rose to such a degree that there were twice as many red or white cells coming out as going into the spleen. That the excess of white cells should be mainly of the mononuclear variety was to be expected. But there occurred a further noteworthy fact, namely, that these mononuclears were mainly of the

TABLE I.

Animal.	Blood count.	Ear vein.	Splenic artery.	Splenic vein.	Inferior mesenteric vein.	Autopsy.
Rabbit 1	Red blood count	5,400,000	5,200,000	6,700,000		Small nodule of coccidiosis in liver, otherwise normal.
	White blood count	4,000	4,000	9,400		
	Polynuclears	60%	61%	32%		
	Mononuclears	40%	39%	68%		
Rabbit 2	Red blood count	5,900,000	5,700,000	8,600,000		All organs appear normal.
	White blood count	10,600	10,000	25,600		
	Polynuclears	47%	49%	20%		
	Mononuclears	53%	51%	80%		
Rabbit 3	Red blood count	4,600,000	4,560,000	5,360,000		Young rabbit, normal.
	White blood count	5,200	5,000	13,600		
	Polynuclears	30 to 32%	30 to 32%	18 to 20%		
	Mononuclears	68 to 70%	68 to 70%	80 to 82%		
Rabbit 4	Red blood count	Not done	5,280,000	6,800,000		Lactating rabbit. Marked coccidiosis in liver. Spleen small, shrunken, and dark red.
	White blood count	9,800	9,400	4,600	Portal vein	
	Polynuclears	38%	36%	10%	32%	
	Mononuclears	62%	64%	90%	68%	
Dog 1	Red blood count	5,280,000	5,280,000	6,640,000		Normal.
	White blood count	17,000	16,400	34,400	16,400	
	Polynuclears	90%	85%	70%		
	Mononuclears	10%	15%	30%		
Dog 2	Red blood count	4,500,000	4,320,000	6,600,000		Normal.
	White blood count	6,900	7,000	14,800	10,600	
	Polynuclears	61%	60%	44%	45%	
	Mononuclears	39%	40%	56%	55%	
	Large mononuclears			47%	10%	
	Small mononuclears			9%	45%	
Dog 3	Red blood count	5,900,000	5,800,000	7,240,000		All organs normal.
	White blood count	19,700	19,700	19,900		
Dog 4	Red blood count	4,200,000	4,100,000	6,500,000		All organs normal.
	White blood count	20,000	20,000	25,000		
	Polynuclears	80%	81%	70%		
	Mononuclears	20%	19%	30%		
	Large mononuclears	12%	12%	24%		
Cat 1	Red blood count		4,400,000	9,120,000		Apparently normal.
	White blood count		18,000	44,000		
	Polynuclears		75%	30%		
	Mononuclears		25%	70%		

TABLE I.—*Continued.*

Animal.	Blood count.	Ear vein.	Splenic artery.	Splenic vein.	Inferior mesenteric vein.	Autopsy.
Cat 2	Red blood count		9,600,000	13,104,000	9,440,000	All organs apparently normal except spleen which is enlarged to about 4 times its normal size and is very mottled on cross-section, due to tremendously enlarged Malpighian bodies. Two accessory spleens presenting same structure. *Microscopical Examination.*—Enormously enlarged follicles. Active mitoses. Huge mononuclear cells.
	White blood count		89,400	137,200	89,400	
	Polynuclears		75 %	74 %	75 %	
	Mononuclears		25 %	26 %	25 %	
	Large mononuclears		25 %	25 %	20 %	
	Small mononuclears		0	1 %	5 %	
Cat 3	Red blood count		7,280,000	11,200,000		Spleen appears normal in size, color, shape, and cross-section. Liver normal. All other organs except mesenteric lymph nodes are normal. Mesenteric lymph nodes especially in duodenal region are enormously enlarged, firm, and matted together in places. Dark red in color. *Microscopical Examination.*—Sections of lymph nodes show tremendous enlargement of germinal centers. Long rod-like bacilli in marginal sinuses. Sections of spleen and liver normal.
	White blood count		26,000	13,500	26,000	
	Polynuclears		18%	40%	19%	
	Mononuclears		82%	60%	81%	
	Large mononuclears		11%	50%	9%	
	Small mononuclears		71%	10%	72%	

TABLE I.—*Concluded.*

Animal.	Blood count.	Left ventricle.	Right auricle.	Splenic vein.	Portal vein.	Autopsy.
Received intraperitoneal inoculation of virulent paratyphoid culture 24 hrs. previously.						
Rabbit 5	Red blood count	4,300,000		6,240,000		Peritonitis. Focal necroses in liver. Large mitotic cells in spleen, especially at margins of follicles.
	White blood count	450		7,500		
	Polynuclears		40%	12%	88%	
	Mononuclears		60%	88%	12%	

large variety, whereas those coming from the adenoid tissue of the intestines and mesenteric nodes were mainly of the small variety. The significance of this was clearly shown in cat 3, where the white cells coming from the spleen were found to be only half as numerous as in the peripheral circulation and in the inferior mesenteric vein. The autopsy showed the reason for this. The spleen was unchanged and discharged its normal quota of large mononuclears into the blood stream; but the adenoid tissue in the mesentery was enormously hypertrophied and was discharging great numbers of small lymphocytes into the circulation. Microscopic sections showed the efferent vessels of these nodes to be packed with small mononuclears.

One of the most striking evidences of splenic blood formation was that of cat 2, in which 13,000,000 red cells and 137,000 white cells per cubic millimeter were found in the splenic vein as against about two thirds of that number in the peripheral circulation. The reason for this at once became apparent on examination of the spleen itself. There was an enormous hyperplasia of the organ, and the germinal centers on microscopical section showed very active production of large mononuclears. There were furthermore two accessory spleens. The organs elsewhere were normal and the cat seemed in good health.

Another marked instance of the difference between splenic and portal corpuscular content was seen in dog 2, in which the proportions of large and small mononuclears were almost exactly the reverse in the two veins.

Most interesting were the results in rabbit 5, which had been previously inoculated with a rather virulent strain of paratyphoid

bacillus. This strain in rabbits causes a marked leucopenia in the systemic vessels as a constant feature following inoculation. But the remarkable thing was that although this leucopenia had reached such a grade that the heart's blood contained only 450 white blood cells to the cubic centimeter the spleen still kept discharging leucocytes at a rate of 7,500 per cubic centimeter, a perfectly evident effort at compensation for the loss in the general blood stream.

These results all point to the inevitable conclusion that the spleen is a blood-forming organ of prime importance in the animal metabolism. The fact that the organ can be extirpated without causing death or even considerable detriment to the animal organism does not militate against this conclusion. Other organs (hemolymph nodes, bone marrow, and adenoid tissues in general) may assume part of the rôle of the spleen when this is absent, but only the severity of the blood-destroying agent and the individual resistance can determine whether the body can stand the strain when deprived of the spleen. Cases of death from removal of the malarial spleen indicate this strongly. And this same individual variation will doubtless account for the discrepancy in the results obtained by different observers regarding the importance of the spleen in the animal economy.

In closing, I desire to express my gratitude to Dr. William G. MacCallum for his interest in this work.