The Splenic Hemogram of the Unanesthetized Dog.* (28310)

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Despite the extensive literature pertaining to the relationship of the spleen and the formed elements of the blood, few direct studies have been made of the splenic content of those elements. Those that do exist are restricted in scope, being concerned either with the morphology of the tissue samples obtained by percutaneous needle aspirations of pathologically enlarged human spleens(1) or with the elevated red cell(2) or reticulocyte(3) levels of the splenic pulp of anesthetized experimental animals. In many animals anesthesia is known to exert a significant effect on the size of the spleen(4,5), with secondary alterations of the peripheral hemogram occurring. Some workers have avoided the use of anesthetic agents by studying mice which had been rapidly frozen in liquid nitrogen(6,7), or decapitated rats(8); believing that no marked terminal changes in splenic size occurred under those conditions. Confirmatory evidence of an elevated splenic hematocrit in anesthetized dogs has been obtained by the use of radioactive isotopes(2,9,10).

As the large expansile spleen of the dog is known to be a significant reservoir both of red cells(2,11,12) and reticulocytes(3,8), it was desired to assay its possible reservoir function for the other formed elements as well under more physiologic circumstances than may be found in anesthetized or recently sacrificed animals.

The technic employed was that of Swisher and Dale(13) involving subcutaneous placement of much of the spleen; its vascular supply being kept essentially intact. Their splenic aspirates, composed largely of the white pulp, were obtained in unanesthetized dogs several weeks following recovery from the surgical procedure. Their published cytologic observations were essentially confined to differential white cell counts. This paper is both a comparison of the splenic red pulp and peripheral venous blood in 5 dogs and an evaluation of the physiologic status of the subcutaneously located spleen.

Materials and methods. In 5 dogs 4 to 6 complete blood counts were done during the 2 to 3 weeks preceding surgery. Additional venous samples were obtained both before and after administration of intravenous sodium pentobarbital on the day that a sterile laparotomy was performed. With a minimum of handling, a small incision was made in the spleen and a blood sample collected for a hemogram. Local pressure resulted in cessation of bleeding. The organ was then elevated from its normal intraabdominal location and the bulk of it placed in a subcutaneous pouch previously prepared by blunt dissection of the fascia overlying the left anterior abdominal muscles(13). A few sutures were placed to anchor the splenic mesentery to the abdominal wall, care being taken that the vascular supply was not compromised. The subcutaneous tissue and the skin were then closed. Blood loss was minimal. Despite the recommendations of Swisher and Dale(13), it was not found necessary to aspirate fluid from the pouch during the immediate postoperative period and sampling was performed with an 18 instead of a 20 gauge needle while the dog stood on a table.

At least one month postoperatively, after the blood count had returned to the preoperative range, blood specimens were obtained from the radial vein, followed quickly by aspiration of the subcutaneously placed spleen. 1-2 ml of blood were collected in tubes containing EDTA as an anticoagulant (0.08 ml of a 5% disodium salt solution evaporated to dryness). As the needle was manipulated until blood flowed rather freely when suction was applied, samples were believed to be composed largely of blood emanating from the red pulp of the spleen.

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Routine hematologic procedures were performed in duplicate; including microhematocrit determinations, leukocyte counts, and platelet counts using phase microscopy. One hundred cell differential leukocyte counts were carried out; as were reticulocyte counts using new methylene blue, the number per 4000 erythrocytes being determined.

Statistical analysis of the data was done using the t-test with limits of significance set at 0.05. Due to the small sample size, many differences although perhaps suggestive of a trend are not statistically significant.

Results and discussion. When the dogs were calm, the splenic pulp of all 5 had higher hematocrit, reticulocyte and lymphocyte values than did the venous specimens. In 4, total white cell count and percentage of neutrophils were less in the samples obtained from the spleen than in those from the peripheral circulation. The splenic blood, as noted by Swisher and Dale(13), often tended to clot so that it was not possible to get accurate platelet counts in all such samples. The 16% greater hematocrit in the spleen compared to the venous blood (48.3:41.6) (Table I) is much less than the 3:2 ratio noted by Barcroft and Poole(12) in unanesthetized dogs with extracutaneously placed spleens. In only one of the present dogs was that ratio nearly attained (60.0:43.0), suggesting either that the other 4 animals were more excited than they appeared to be or that the subcutaneously placed spleen is usually unable to concentrate erythrocytes as well as the extracutaneous preparation.

In contrast to the calm state, splenic contraction induced by excitement(14) caused a 6 point fall in the mean splenic hematocrit and a slight rise in venous hematocrit, so that the levels were comparable in both locations (Table I). This similarity had previously been noted by Barcroft and Poole in the extracutaneous spleen(12). The mean venous reticulocyte level was also increased. These findings might reflect the emission of relatively more reticulocytes than erythrocytes from an organ in which the former are at least 3 times more abundant than in the blood, but whose concentration of mature red cells is at

TABLE I. Effect of Spleen Size on Venous and Splenic Hemograms in 5 Dogs.

	Hematocrit, %	Reticulocytes, %	Platelets* per mm³	White cells per mm³	Neutrophils, %	Bands, %	Lymphocytes, %	Monocytes, %	Eosinophils, %
Spleen in subcutaneous pouch—									
Calm:									
Vein	41.6 ± 4.3	.36 ± .37	371113 ± 89339	11761 ± 2462	46 ±13	17 ± 5	27 ± 14	2 ± 3	7 ± 4
Spleen	48.3 ± 8.9	1.20 ±1.06	350000 ± 58275	8901 ± 2771	40 ± 7	16 ± 9	33 ± 15	3 ± 3	9 ± 5
Excited:									
Vein	43.3 ± 2.6	.86 ± .68	449330 ±126289	10163 ± 5488	46 ± 8	10 ± 3	38 ± 3	1 ± 1	4 ± 2
Spleen	42.2 ± 6.0	1.50 ± .87	—	10219 ± 2398	39 ± 4	11 ± 7	45 ± 10	0	5 ± 3
Intact—									
Calm:									
Vein	40.7 ± 2.3	.16 ± .13	386400 ± 81993	13260 ± 3536	51 ± 6	14 ± 6	23 ± 6	4 ± 4	8 ± 5
Excited:									
Vein	46.1 ± 3.7	.36 ± .26	420800 ± 63275	14883 ± 6547	51 ±14	17 ± 6	19 ± 7	4 ± 5	9 ±12
Pentobarbital:									
Spleen	61.5 ±12.2	.98 ± .23	369333 ±204003	7583 ± 3843	49 ± 9	12 ± 4	25 ± 15	6 ± 6	9 ± 5

* Based on 3 dogs.

Figures represent mean ± one standard deviation.

the most about half again as great. Lymphocyte count varied in the direction of the reticulocyte count, but during excitement their greater number in the subcutaneously located spleen might indicate a greater tendency to sample the white pulp in the contracted spleen having a decreased red pulp volume. An alternative is that the relocated spleen may preferentially emit other types of cells so that relatively more lymphocytes remain within the organ. However, their increased number in the venous blood in the presence of a slight decrease in leukocyte count and particularly in percentage of band neutrophils ($p < 0.05$) favors the former explanation.

To assay the capacity of the relocated spleen for sequestration of various formed elements, venous hemograms when the animals were calm or excited were compared with those obtained under similar circumstances from the same 5 dogs prior to surgery (Table I). At that time, excitement raised the venous hematocrit 5.4 points ($p < 0.05$) and more than doubled the reticulocyte count. The latter was not quite a significant difference despite the fact that all 5 intact dogs had increased reticulocyte levels. Following surgery, excitement caused only a 1.7 point rise in venous hematocrit; either suggesting that the relocated spleen could contract but one-third as well as normal or, more likely, that its content of red cells available for expulsion was less because its position between the relatively unyielding skin and abdominal musculature prevented its dilating to a normal size before the animal became excited. This would be analogous to constrictive pericarditis in which a smaller diastolic filling volume reduces stroke volume and hence cardiac output. As in intact dogs, the peripheral reticulocyte count doubled with emotion, so that the splenic release mechanism for those cells which may depend on their larger size and increased adhesiveness(8) seems to be unaffected by relocation of the organ. In excitement, the mean venous lymphocyte percentage in the intact dog is only half that noted following surgery ($p < 0.001$), perhaps reflecting greater expulsion of other types of leukocytes by the normal spleen.

In the calm dog, the spleen is dilated, as also occurs with barbiturate anesthesia(4,5). Yet, in the 5 dogs when intact the splenic hematocrit under pentobarbital was 13.2 points higher than postoperatively when those same animals were calm (Table I), suggesting that the subcutaneously placed spleen was unable to concentrate erythrocytes normally. However, that comparison was of intact anesthetized dogs with themselves following surgery when calm and unanesthetized. Therefore, in 3 of the operated dogs venous and splenic blood counts were obtained both before and after pentobarbital and compared with hemograms obtained under the same circumstances on the day of surgery when they were intact. Of course, at that time no spleen samples could be obtained prior to anesthesia (Table II).

The 3 unanesthetized dogs with subcutaneously located spleens were somewhat excited when the pre-anesthesia samples were obtained as evidenced by the similar hematocrit values of the vein and spleen. Despite that, the mean splenic reticulocyte content remained at twice the venous level. After pentobarbital, the venous hematocrit fell, the splenic hematocrit rose and the reticulocyte content of the latter organ decreased, perhaps because of dilution by mature erythrocytes entering from the circulation. Under anesthesia their venous blood had 3 times more reticulocytes ($p < 0.02$), fewer band forms, more lymphocytes ($p < 0.05$), and fewer monocytes ($p < 0.05$) than when intact. Their spleens had a 17 point lower hematocrit, fewer reticulocytes, fewer monocytes ($p < 0.05$), double the number of white cells and more lymphocytes than when intact. At that time, after pentobarbital, the splenic pulp had a 20.3 point higher hematocrit, a 6-fold concentration of reticulocytes ($p < 0.001$) and apparently more lymphocytes and fewer band neutrophils than the peripheral blood. These varied findings suggest that diverse splenic functions were impaired by the surgery. The much lower hematocrit and reticulocyte level of the relocated spleen whose sinuses have been reported to be compressed (13) indicate that its sequestering function

TABLE II. Effect of Anesthesia on Venous and Splenic Hemograms in 3 Dogs.

	Hematocrit, %	Reticulocytes, %	Platelets per mm³	White cells per mm³	Neutrophils, %	Bands, %	Lymphocytes, %	Monocytes, %	Eosinophils, %
Spleen in subcutaneous pouch—									
Before pentobarbital:									
Vein	45.3 ± 2.9	.50 ± .30	477000*	11358 ± 1363	50 ± 6	10 ± 5	33 ± 4	0	3 ± 3
Spleen	44.5 ± 7.1	1.00 ± .70	526000*	8915 ± 939	44 ± 4	16 ± 2	35 ± 4	0	5 ± 4
After pentobarbital:									
Vein	41.4 ± 4.1	.57 ± .15	518000*	10633 ± 810	54 ± 4	10 ± 5	30 ± 4	0	6 ± 6
Spleen	48.6 ± 6.6	.65 ± .54	400000*	12719 ± 5732	45 ± 13	11 ± 3	39 ± 14	0	5 ± 2
Intact—									
Before pentobarbital:									
Vein	47.7 ± 2.4	.17 ± .12	289000 ± 115966†	10083 ± 5201	48 ± 9	17 ± 8	14 ± 12	8 ± 6	13 ± 15
After pentobarbital:									
Vein	45.0 ± 3.6	.17 ± .12	340553 ± 127823†	8267 ± 3182	44 ± 17	18 ± 5	17 ± 6	12 ± 6	9 ± 13
Spleen	65.3 ± 13.4	1.03 ± .57	262000 ± 118793†	6217 ± 4188	48 ± 12	10 ± 3	25 ± 19	9 ± 6	8 ± 4

* 1 dog. † 2 dogs. Figures represent mean ± one standard deviation.

for erythrocytes and reticulocytes is diminished. This reduced cell content may also explain the smaller rise in venous hematocrit induced by excitement in the 5 dogs after they had been operated upon.

Summary. Splenic and venous blood samples were obtained in unanesthetized dogs whose spleens had previously been placed subcutaneously with their vascular supply essentially intact. Erythrocyte and reticulocyte levels are elevated in the spleen. Under pentobarbital, the relocated spleen has less of those cells than does the normal organ. Evidence was presented that diverse splenic functions such as sequestration of formed elements and distensibility are interfered with by placing the spleen between the relatively rigid skin and abdominal musculature of the dog.

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