glucose in the same specimen of capillary finger blood by means of a chemical procedure similar to that reported here.

Summary. Use of the anthrone reagent offers a sensitive and accurate method that can be applied for the estimation of microquantities of inulin. Steps are given to prevent interference by glucose through the means of controlling the temperature of reaction. Boiling in alkali cannot be used to eliminate glucose because of an irregular distribution between blood and urine of the alkali labile fraction of inulin.

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Received April 21, 1954. P.S.E.B.M., 1954, v86.

Comparison of Blood Cell Counts from Major Vessels in the Dog.* (21098)

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Some investigators(1-3) have reported impressive differences in the number of cells found in blood taken from various sources in laboratory animals. However, other workers (4-6) have failed to confirm this observation. The present investigation was carried out on both normal and splenectomized dogs. Comparisons were made between samples obtained simultaneously from the aorta, hepatic vein, caudal vena cava, superior vena cava, and/or right ventricle in unanesthetized dogs and dogs anesthetized with pentobarbital sodium.

Methods. The catheterization of major arterial and venous vessels was performed in unanesthetized dogs by inserting small polyethylene catheters through a 15-gauge needle placed in the vessel after procaine infiltration of the area. The catheter was positioned by fluoroscopic visualization, the stylet removed and the catheter filled with 0.5% heparin in sterile saline. Catheters were placed in the aorta and in either the right ventricle or the superior vena cava. In addition to the comparisons with unanesthetized animals, a series of experiments was performed on animals under pentobarbital anesthesia (30 mg/kg body weight). In these experiments the catheters were placed in the right ventricle, and in the aorta and caudal vena cava at the anterior margin of the pelvic girdle. Additional data were accumulated from experiments in which Cournand catheters were placed in various positions in anesthetized and heparinized (5 mg/kg) animals, and in which all the catheters were introduced by exposing and ligating the vessels. In this series, comparisons were made with samples from the aorta and hepatic vein, and from the right ventricle and peripheral or hepatic vein. For the comparisons reported in this investigation data from samples drawn after any experimental procedure beyond that concerned with placing the catheters have not been included. The erythrocyte and leukocyte counts were done in duplicate, using National Bureau of Standard certified pipettes. The duplicate counts were required to check within 10%. At least 200 cells were counted in the differentials and checks were required between the first and second halves of the counts. Lymphocytes and monocytes were grouped together as mononuclear cells and no differentiation was made as to nuclear configuration of neutrophiles.

* This work was supported by a grant from the Life Insurance Medical Research Fund.

TABLE I. Comparison of Blood Cell Distribution in Normal and Splenectomized Dogs in Anesthetized and Unanesthetized Dogs. W = total leukocytes, R = erythrocytes, E = eosinophiles, M = mononuclear cells, N = neutrophiles, S. E. = stand. error.

Effect of splenectomy															
Comparison	Aorta vs caudal vena cava					Right ventricle vs caudal vena cava					Aorta vs right ventricle				
No. of comparisons	26					26					37				
Cell type	W	R	E	M	N	W	R	E	M	N	W	R	E	M	N
$\bar{X}_n-\bar{X}_s$*	106	142	53	71	135	130	88	309	131	295	248	16	16	636	262
S. E.	174	80	98	133	172	210	130	195	170	221	494	220	413	562	1064
"t" value	.60	1.78	.54	.53	.78	.62	.68	1.58	.77	1.33	.50	.07	.04	1.13	.24

Effect of pentobarbital										
Comparison	Aorta vs 'unmixed venous'					Aorta vs right ventricle				
No. of comparisons	26					37				
Cell type	W	R	E	M	N	W	R	E	M	N
$\bar{X}_u-\bar{X}_a$	113	20	367	618	199	290	27	163	628	386
S. E.	308	170	247	329	523	495	190	413	562	1066
"t" value	.37	.12	1.48	1.88	.38	.58	.14	.39	1.12	.36

* Leukocyte difference in cells/mm³, erythrocyte difference in thousands of cells/mm³.

Results. Statistical analyses were performed on the difference in the distribution pattern of blood cells in the anesthetized and unanesthetized animals. Student "t" values were calculated for the differences in the comparisons of leukocytes, erythrocytes, eosinophiles, mononuclear cells and neutrophiles, and in all cases the levels of significance were above 5% (Table I). Similar comparisons were made for the relationship between the distribution of blood cells in normal and splenectomized dogs. No significant difference (at the 5% level of significance) was observed in any blood cell type when normal and splenectomized dogs were compared (Table I). Thus, statistically significant differences did not exist in experiments from anesthetized and unanesthetized animals, nor in experiments from normal and splenectomized dogs. The experiments, therefore, could be grouped with reference only to the source of the samples, in so far as a study of the comparisons of blood cell counts from various regions was concerned.

Studies were made of the cellular distribution between the arterial and venous sides of the circulation. Comparisons were made between the aorta and hepatic vein, aorta and superior vena cava, aorta and caudal vena cava, and the aorta and right ventricle. The studies demonstrated no significant differences in the erythrocytes nor in any leukocyte cell type from arterial and venous samples drawn simultaneously (Table II). Additional studies were carried out on the distribution of circulating blood cells in the major venous vessels. They consisted of cell count comparisons between blood withdrawn simultaneously from the right ventricle and hepatic vein, peripheral vein and hepatic vein, right ventricle and caudal vena cava, and the right ventricle and peripheral vein. No significant differences were demonstrated in any blood cell type (Table II).

Discussion. One of the primary considerations in the distribution of blood cells within the vascular system is that the sample should be a valid representation of blood flowing in the vessels. A blood sample obtained by puncturing a large vein probably gives a reliable estimate of the cell content in that vein.

TABLE II. Comparisons of Blood Cell Counts in Samples Drawn Simultaneously from 2 Major Vessels in Dogs. W = total leukocytes, R = erythrocytes, E = eosinophiles, M = mononuclear cells, N = neutrophiles.

Arterial and venous comparisons										
Comparison	Aorta vs hepatic vein					Aorta vs right ventricle				
No. of comparisons	18					40				
Cell type	W	R	E	M	N	W	R	E	M	N
Mean diff.* $\frac{\Sigma(A-B)}{n}$	223	176	18	64	213	113	6	13	13	41
"t" value	1.20	1.18	.64	1.28	1.17	1.02	1.17	.13	.05	.36
Comparison	Aorta vs superior vena cava					Aorta vs caudal vena cava				
No. of comparisons	10					29				
Cell type	W	R	E	M	N	W	R	E	M	N
Mean diff.* $\frac{\Sigma(A-B)}{n}$	178	190	324	614	348	47	109	43	9	146
"t" value	.70	1.31	1.42	2.04	.71	.71	1.81	1.07	.19	1.66
Comparisons of venous samples from different sources										
Comparison	Right ventricle vs hepatic vein					Right ventricle vs caudal vena cava				
No. of comparisons	7					29				
Cell type	W	R	E	M	N	W	R	E	M	N
Mean diff.* $\frac{\Sigma(A-B)}{n}$	334	13	27	152	148	58	80	123	63	91
"t" value	.79	.10	.30	1.37	.24	.64	1.43	1.17	.91	.92
Comparison	Right ventricle vs peripheral vein					Peripheral vein vs hepatic vein				
No. of comparisons	7					7				
Cell type	W	R	E	M	N	W	R	E	M	N
Mean diff.* $\frac{\Sigma(A-B)}{n}$	1347	197	28	26	1177	1009	318	55	120	1028
"t" value	1.66	1.89	.22	.22	1.58	.94	1.54	.58	.97	.93

* Leukocyte difference in cells/mm³, erythrocyte difference in thousands of cells/mm³.

However, investigations carried out on blood drawn from punctures of the skin, commonly referred to as capillary blood, may be unreliable unless sufficient precautions are instituted to insure that misinterpretations are not made. Another important consideration in studies of the intravascular distribution of blood cells is that comparisons should be made only on samples drawn under the same experimental conditions. If direct comparisons are to be made, the samples should be drawn simultaneously.

Investigations of the distribution of erythrocytes have suggested that arterial and venous or peripheral blood have the same circulating erythrocyte content(1,6,9). One study(3) described experiments on the guinea pig which were interpreted as indicating a lower erythrocyte level in the heart than in the periphery. However, the experimental procedure consisted of collecting peripheral blood from a nick in the ear, then anesthetizing the animals with pentobarbital and collecting heart blood by direct punctures. It has been demonstrated(7,8) that pentobarbital administration results in an erythrocyte decrease of approximately the same magnitude as the difference reported in that study. Because of the design of their experiment, it would appear that those workers measured the effect of the anesthetic agent on the erythrocyte count and interpreted it as an effect of comparing the 2 sampling sites. The experiments being reported indicated that no erythrocyte count differences were found when arterial and venous blood samples were collected simultaneously. The data also suggested that there were no differences in various parts of the venous system, and that neither splenectomy nor anesthetization with pentobarbital affected the distribution of circulating erythrocytes in the major vessels of the dog.

Most of the disagreement regarding the distribution of blood cells is concerned with the leukocyte count. A difference was reported for tail blood and heart blood on rats which were stunned by a blow on the head after collecting the sample from the tail and before the heart blood was collected(1). The animals were not in comparable experimental conditions at the time the samples were collected. When these workers(2) compared the results of blood counts drawn simultaneously from anesthetized animals, they found no difference following ether anesthesia, while there was a significantly lower leukocyte count in blood from the heart than from the tail when pentobarbital was used as the anesthetic agent. They also reported a significant difference in unanesthetized rats which were immobilized while a cardiac puncture was performed. Other investigators(5) demonstrated no difference between tail and heart blood from rats anesthetized with ether or from rats killed with a blow on the head. Blood from the left heart, periphery or venous drainage in unanesthetized dogs showed no significant cell count differences(6). In an excellent study of the distribution of leukocytes in the vascular system, the importance of correctly interpreting results from peripheral blood samples was demonstrated(10). It was shown that the first drop of blood from a lancet-puncture had a significantly higher leukocyte count than the succeeding blood. Only by conscientiously attempting to collect the first drop of blood flowing from the puncture was it possible to demonstrate a leukocyte count different from that in the major vessels. It was suggested that the increased leukocyte count seen in the first drop of blood does not represent the leukocyte concentration in the capillary blood, but reflects a flushing out of leukocytes which have adhered to the endothelium of the para-capillary veins. This observation may account for some of the reports that peripheral blood has a higher leukocyte count that heart blood.

Summary. Blood cell counts were compared in samples drawn simultaneously from the aorta, right ventricle, superior vena cava, caudal vena cava, hepatic vein, and/or peripheral veins in dogs. The study was done on normal and splenectomized dogs, either unanesthetized or anesthetized with pentobarbital. No differences in erythrocyte or leukocyte distributions were demonstrated as a result of anesthetization or splenectomy. The erythrocyte and leukocyte counts were the same in arterial and venous blood samples and in venous samples from different major vessels. Some factors are discussed which may be responsible for apparently contradictory reports concerning cell count distribution in circulating blood.

The authors wish to express their appreciation to G. B. Spurr, B. K. Hutt, and Mrs. Doris W. Knapp for their assistance.

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Received May 7, 1954. P.S.E.B.M., 1954, v86.