of compact bundles of fibrils. This finding is comparable to the observation reported by Jakus and Hall (*2*), who found "ultramicroscopic fibrils" in the cilia of *Paramecium*. The cilia of *Paramecium* and *Spirostomum* may be regarded as having a complex type of organization, perhaps approaching that which characterizes contractile elements of more highly specialized organisms.

The accompanying figures and numerous original micrographs indicate that the methods mentioned above may be applied to the study of protozoa. Fine detail which has not been resolved by ordinary light microscopy has been photographed from sections prepared as indicated here. A more detailed account of this work will be published elsewhere.

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A Comparison of the Number of Circulating Blood Cells in Different Parts of the Circulatory System[1]

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Conflicting statements have appeared in recent literature concerning the differences in the number of circulating blood elements in heart and peripheral blood, Roofe *et al.* (*1*), Quimby, Saxon, and Goff (*2*), and Law and Heston (*3*) have shown what they consider to be significant differences in the blood count of different parts of the circulatory system of guinea pigs, rats, and albino mice, respectively. Nevertheless, Nichols and Miller (*4*) reported no significant differences in either erythrocyte or leucocyte counts done on heart and peripheral blood of rats.

Several investigators have found that anesthesia exerts an influence upon the number of circulating blood cells. Hahn, Bale, and Bonner (*5*) reported that 30% of the normal amount of circulating cells of dogs are found in the spleen after sodium pentobarbital (Nembutal) anesthesia, and that spleens removed under Nembutal anesthesia were four times the size of those removed under ether. Similarly, Hausner, Essex, and Mann (*6*) found that anesthesia with barbituric acid derivatives produces an enlargement of the spleen, with a subsequent decrease in the number of circulating erythrocytes. Ether anesthesia has an opposite effect, producing constriction of the spleen and an increase in the number of erythrocytes found in the blood stream. In male cats, Nembutal causes a 10% increase in plasma volume (*7*). The animals of Nichols and Miller (*4*) were either anesthetized with ether or stunned by a blow on the head in order to obtain heart blood. One author (*1*) administered anesthesia after taking the peripheral counts and before doing the heart counts.

Because of the contradictory reports of these several authors, and because various techniques were followed, some of which have been shown to influence the number of erythrocytes in central and peripheral parts of the vascular system, we judged it profitable to review the problem, avoiding the use of anesthesia. It is the purpose of this paper to report our studies on the number of erythrocytes and leucocytes in heart, venous, and peripheral blood in unanesthetized dogs.

Twenty female and 12 male mongrel dogs were used in this study. All animals were fasted for 12 hr before blood was drawn. Without anesthesia and with a minimum amount of struggle the dogs were fastened in a supine position to an animal board, and blood was drawn in the following manner: (1) Peripheral blood was obtained by carefully removing the hair from an area of the ear and then nicking the ear with a sharp razor blade. Counts were made in the standard manner, using Thoma pipettes certified by the National Bureau of Standards and hemocytometers with improved Neubauer rulings. (2) Venous blood was obtained by inserting a 20-gauge needle, with as little trauma as possible, into the large vein just above the lateral malleolus. The vein was distended by pressure upon the femoral vessels. The needles were previously coated with a solution composed of 2 parts of xylol and 1 part Dri-film 9987.[2] Thoma pipettes were filled with blood as it flowed freely from the hub of the needle. Smears for differential counts were also made from this freely flowing blood. Some of the blood was collected in oxalated tubes and placed in Wintrobe hematocrit tubes. Hematocrit determinations were made by contrifuging these tubes at 2,500 rpm for 30 min. (3) Heart blood was obtained during heart puncture by inserting an 18-gauge needle, also treated with xylol and Dri-film, into the thorax through the tenth intercostal space just to the left of the mid-line. In a similar manner to the method used for venous blood, blood was obtained from, presumably, the left ventricle. It was assumed that vigorously spurting blood indicated that the needle was in the ventricle.

Erythrocyte counts made upon peripheral blood ranged from 5,320,000 to 7,840,000 cells/mm^3, with the exception of one animal that had a count of 3,540,000 cells/mm^3. This animal appeared to be in excellent condition, and the anemia could not be anticipated from its external physical appearance. Although the counts of this animal will not be included in the following ranges for erythrocyte counts, it will be included in the statistical analysis reported in Tables 1 and 2. Venous blood erythrocyte content ranged from 5,100,000 cells/mm^3 to 8,330,000 cells/mm^3. That of the heart blood ranged from 5,170,000 to 8,040,000 cells/mm^3.

[1] Done in accordance with a contract between the Office of the Surgeon General, U. S. Army, and the University of Kansas.

[2] The Dri-film was obtained from the General Electric Co., Schenectady, N. Y.

The leucocyte counts for all three types of blood fell within the limits of 5,000–20,000 cells/mm³, except for peripheral blood, in which one count went as high as 27,500 cells/mm³. The results are summarized in Table 1.

The average for the hematocrit determinations on venous blood was 43.8% and that done on heart blood was 42.9%.

The significant differences of the various counts in regard to each other were determined statistically by the Student t formula.[3] In order to be significant for the number of cases employed, t should be 1.65 or greater.

TABLE 1

Source of blood	Average and standard deviation	Coefficient of variation and probable error
	Red cells (in millions)	
Peripheral	6.653 ± 0.953	14.33 ± 1.27
Venous	6.693 ± 0.964	13.79 ± 1.18
Heart	6.426 ± 0.871	13.55 ± 1.18
	White cells (in thousands)	
Peripheral	15.39 ± 4.12	26.78 ± 3.60
Venous	13.12 ± 3.16	24.05 ± 3.06
Heart	14.44 ± 4.64	32.10 ± 5.15
	Lymphocytes (%)	
Peripheral	26.24 ± 9.30	35.44 ± 3.51
Venous	20.75 ± 15.50	74.69 ± 9.59
Heart	23.73 ± 9.74	41.04 ± 4.15
	Polymorphonuclear (%)	
Peripheral	69.27 ± 10.30	14.88 ± 1.34
Venous	69.77 ± 14.10	20.57 ± 1.96
Heart	71.03 ± 11.10	15.62 ± 1.42

The averages for the erythrocyte counts are slightly lower than those reported by Wintrobe (*8*) and in a survey of several hundred counts done by a good number of investigators (*9*). The leucocyte count averages determined by us are slightly higher than those reported in this survey. However, the ranges for erythrocyte and leucocyte counts fit within the ranges elicited. The wide range in leucocyte counts is not significant, since normal animals (dogs) may show as much as 100% variability (*9*). The percentage of polymorphonuclear cells in our animals agrees with the figures reported in the survey of Scarborough (*9*), but we found the total lymphocyte count of peripheral blood to be 6% above the reported average. This increase may possibly be due to the effects of the emotional stress of being bound down. Although most of the animals submitted to restraint without much struggle, a few animals did make efforts to free themselves. These animals soon became quiescent, however, and were then allowed to rest for a short period before the counts were taken. Emotional stimuli do result in a lymphocytosis (*10, 11*).

TABLE 2

	t
Erythrocytes	
Peripheral-venous	0.16
Peripheral-heart	0.95
Venous-heart	1.19
Leucocytes	
Peripheral-venous	0.708
Peripheral-heart	0.255
Venous-heart	0.400
Polymorphonuclears	
Peripheral-venous	0.046
Peripheral-heart	0.195
Venous-heart	0.114
Lymphocytes	
Peripheral-venous	0.510
Peripheral-heart	0.315
Venous-heart	0.276

Previous investigators have not fully explained their methods of drawing heart blood, and it seems possible that there may be some error in counts resulting from the settling and adherence of cells if the cells are drawn up into a syringe before being counted. Our method of drawing blood with silicone-treated needles prevents clotting and adherence of the cells to the needle, and settling of the cells was prevented by taking the blood as it flowed freely from the hub of the needle. This method simulates as closely as possible the procurement of the actual number of cells circulating in the blood vessels.

In dogs, we found no significant differences between heart, venous, and peripheral blood with regard to the total number either of erythrocytes or of leucocytes per unit volume. However, studies in progress on rats may reveal that certain species do possess different numbers of cells, particularly leucocytes, in heart and peripheral blood.

[3] $t = (M_1 - M_2) / \sqrt{\left[\frac{\Sigma d_1^2 + \Sigma d_2^2}{(N_1 + N_2) - 2}\right]\left[\frac{1}{N_1} + \frac{1}{N_2}\right]}$, where M = mean, N = number of cases, and d = difference from mean.

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