A STUDY OF FROG BLOOD IN RED LEG DISEASE

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Red leg disease in frogs has been studied by several investigators, with regard to the nature and properties of the causative organism (*Pseudomonas hemophila*), the transmission of the disease among frogs and other animals, and the methods of prevention and treatment. Our knowledge of transmission and treatment is still uncertain and confused.

Because of the prominent blood-vascular changes externally visible in affected frogs, a study of the blood in such animals was thought advisable. Although certain hematologic data are known for normal frogs, there are no available data for physiologic changes occurring in the blood of frogs with red leg, except for descriptive studies of smears (1).

Material and Methods

Only the species *Rana pipiens* was used. Animals were definitely considered to have red leg disease when they showed gross signs and bodily symptoms of a hemorrhagic septicemia, and when the blood smear revealed the bacteria (fig. 1).

In connection with other studies here dealing with transmission and treatment, bacterial cultures on agar plates and on broth were made from the blood of some of the animals to prove the identity of *Pseudomonas hemophila* and to show its infectivity by injection into uninfected frogs and fishes. Through such cultures red leg was produced at will in the present study.

The animals were often found parasitized with other varieties of parasites, but these never produced the bodily changes associated with red leg. Such changes occurred only when the rods of *Pseudomonas hemophila* were seen in the blood smears.

Frogs which showed no characteristic evidence of red leg infection, as determined externally and by blood smears, were used as controls. Although much normal hematologic data may be found in the literature (2, 3, 4, 5) the facts are incomplete and it was thought preferable to establish our own data. There are, in this regard, certain factors which may appreciably change the normal blood picture. First, in some animals, blood counts may vary between the heart and the periphery (6). From preliminary tests run here on the frog no such differences have appeared, but the possibility should not yet be disregarded. Most blood samples in the present study were drawn from the ventricle. Secondly, the blood count, both red and white, varies with the seasons (3, 4, 7), the most active renewal of blood cells occurring in the late spring and early summer. We did not rigidly control this factor except to analyze normal and diseased samples in the same seasons. Thirdly, it was thought that sex differences might

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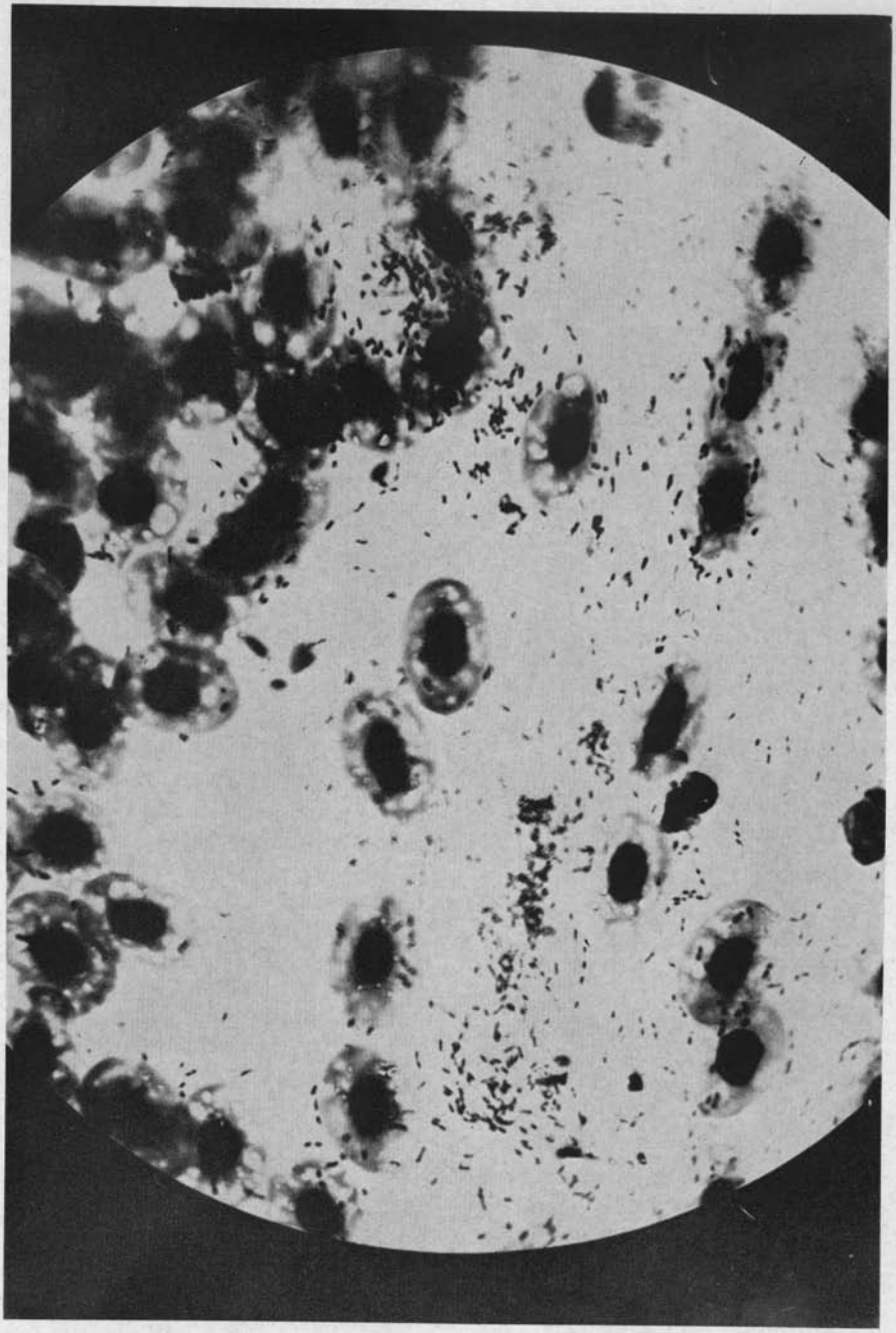

Fig. 1.—Blood smear treated with Wright's stain to show bacterial invasion of red blood cells. Rod-shaped bacteria are abundant. Red cells are vacuolated and swollen. About 680 x.

TABLE 1.—COMPARISON OF BLOOD CONSTITUENTS BETWEEN NORMAL MALE FROGS AND RED LEG INFECTED MALE FROGS

Factor studied	Number of animals	Sample mean	Standard deviation of sample mean	t value
Red blood cell count per c. mm. of blood	Normal 50	480,000	23,000	
	Diseased 50	440,000	15,000	10.2
Platelet count per c. mm. of blood	Normal 50	890,000	21,000	
	Diseased 50	792,000	36,000	16.4
White blood cell count per c. mm. of blood	Normal 50	16,134	1,240	
	Diseased 50	13,700	4,180	3.9
Coagulation time in seconds	Normal 50	102	15.6	
	Diseased 50	117	21.8	3.9
Hemoglobin in percentage	Normal 41	82	10	
	Diseased 25	61	14.7	2.15
Sedimentation rate in mm. (after 15 and 60 minutes)	Normal 50	(2.9-15 min.	1.98	0.7
		(9.38-60 min.	2.2	(15 min.)
	Diseased 50	(3.2-15 min.	2.43	4.4
		(11.9-60 min.	3.3	(60 min.)
Size of red cell (long diameter in microns)	Normal 30	19.79	0.22	58.1
	Diseased 30	24.52	0.375	

be present, so that the data were collected according to sex.

The variables considered included the red cell count, red cell size, white cell count, hemoglobin content of the red cell, sedimentation rate, platelet count, and coagulation time. All cell counts were made on a hemacytometer platform. Coagulation time was obtained by the capillary method with glass coagulation tubes. The hemoglobin percentage was found with the Sahli hemometer, and the human standard of 17 gm. per 100 cc. was used as the equivalent of 100 percent. Sedimentation rate was determined with the Landau-Adams microsedimentation apparatus (8, 9). Blood smears stained with Wright's stain were made in all instances. These were examined qualitatively for size, shape and structure, particularly of red cells. This kind of observation has been made elsewhere (1). The red cell diameter (using only the long axis as an index of cell size) was determined quantitatively in the smears using a micrometer ocular which had been previously calibrated in microns with a stage micrometer. Some distortion in size of red cells occurs in this technic using dry stained films, but this is equal for both normal and red leg preparations.

In the computations, color indices were computed from the mean values of hemoglobin and red cells, by dividing the percentage of hemoglobin by the percentage of red cells.

TABLE 2.—COMPARISON OF BLOOD CONSTITUENTS BETWEEN NORMAL FEMALE FROGS AND RED LEG INFECTED FEMALE FROGS

Factor studied	Number of animals	Sample mean	Standard deviation of sample mean	t value
Red blood cell count per c. mm. of blood	Normal 50 Diseased 50	512,000 466,000	37,000 56,000	4.8
Platelet count per c. mm. of blood	Normal 50 Diseased 50	874,800 769,800	23,600 92,300	7.7
White blood cell count per c. mm. of blood	Normal 50 Diseased 50	14,161 12,300	3,091 3,860	2.6
Coagulation time in seconds	Normal 50 Diseased 50	103 117	15.5 22.3	3.6
Hemoglobin in percentage	Normal 50 Diseased 20	76 50.7	9.8 19.0	7.3
Sedimentation rate in mm. (after 15 and 60 minutes)	Normal 50 Diseased 50 	(3.2-15 min. (10.7-60 min. (3.3-15 min. (12.3-60 min.	2.0 3.0 2.1 3.4	0.24 (15 min.) 2.5 (60 min.)
Size of red cell (long diameter in microns)	Normal 30 Diseased 30	19.83 Data unnecessary; see text	0.13	

All quantitative results were subjected to statistical methods of comparison, t-tests of significance being run. The number of animals included in each sample studied varied from 20 to 50, these facts being listed in tables 1, 2, 3 and 4.

The variables chosen for study were believed to be adequate to define and characterize the physiologic blood picture in red leg disease.

EXPERIMENTAL DATA

In the collection of data the possibility of sex differences was considered and all data were taken with this in mind. Significant sex differences were computed (tables 3 and 4) in both normal and diseased frogs for red blood cell count and hemoglobin percentage. No sex differences were found for the other factors. This is analogous to the situation in human blood. All previous normal counts in the literature have been reported without regard to sex.

For most of the factors studied, the variability in the measurements was greater in the diseased than in the normal animals. This is because the diseased frogs had red leg in different degrees of severity with consequent gradations in the blood picture.

All the factors under analysis showed significant variation between the normal and the diseased animals. The level of significance for the number of cases employed, using t-tables, was considered to be $t = 2$ (one possibility in 20 that this level of results could occur by chance); t was computed from the equation:

$$t = (M_1 - M_2) \Big/ \sqrt{\left[\frac{\epsilon\, d_1^2 + \epsilon\, d_2^2}{(N_1 + N_2) - 2}\right] \times \left[\frac{1}{N_1} + \frac{1}{N_2}\right]}$$

where M = mean, N = number of cases, and d = difference from mean.

The red blood cells were markedly affected in structure by the bacterial invasion. In the smears they were seen to be deformed, swollen in size, and vacuolated (fig. 1). This agrees with the observations of others (1).

Data on quantitative size are illustrative. A definitely significant average increase of almost 5 microns in the long diameter was found.

The difference in cell size between the mean values for normal males (19.79) and females (19.83) was so insignificant that only the more conveniently available male value was presented. This omission is noted in table 2.

The numbers of red cells in disease were decreased to a significant degree. The average decrease for males and females was 43,000 cells per c. mm.

The hemoglobin percentage fell significantly in disease. Whereas the average normal value for males and females was 79 percent (using the human Sahli value of 17 gm. = 100 percent), the average value in disease fell to 55.9 percent.

TABLE 3.—EVALUATION OF SEX DIFFERENCES BETWEEN NORMAL FROGS

Factor studied	Number of animals	Sample mean	Standard deviation of sample mean	t value
Red blood cell count per c. mm. of blood	Male 50 Female 50	480,000 512,000	23,000 37,000	5.1
Platelet count per c. mm. of blood	Male 50 Female 50	890,000 874,800	21,000 23,600	1.0
White blood cell count per c. mm. of blood	Male 50 Female 50	16,134 14,161	1,240 3,091	0.13
Coagulation time in seconds	Male 50 Female 50	102 103	15.6 15.5	0.32
Hemoglobin in percentage	Male 41 Female 50	82 76	10.0 9.8	2.85
Sedimentation rate in mm. (after 15 and 60 minutes)	Male 50 Female 50	2.9-15 min. 9.38-60 min. 3.2-15 min. 10.7-60 min.	1.98 2.2 2.0 3.0	0.74 (15 min.) 0.87 (60 min.)
Size of red cell (long diameter in microns)	Male 50 Female 50	19.79 19.83	0.22 0.13	1.0

TABLE 4.—EVALUATION OF SEX DIFFERENCES BETWEEN FROGS WITH RED LEG

Factor studied	Number of animals	Sample mean	Standard deviation of sample mean	t value
Red blood cell count per c. mm. of blood	Male 50 Female 50	440,000 466,000	15,000 56,000	3.1
Platelet count per c. mm. of blood	Male 50 Female 50	792,000 769,800	36,000 92,300	1.5
White blood cell count per c. mm. of blood	Male 50 Female 50	13,700 12,300	4,180 3,860	0.067
Coagulation time in seconds	Male 50 Female 50	117 117	21.8 22.3	0.0
Hemoglobin in percentage	Male 25 Female 20	61 50.7	14.7 19.0	2.0
Sedimentation rate in mm. (after 15 and 60 minutes)	Male 50 Female 50 	3 2-15 min. 11.9-60 min. 3.3-15 min. 12.3-60 min.	2.43 3.3 2.1 3.4	0.22 (15 min.) 0.21 (60 min.)

The color index, or ratio of percent of hemoglobin to percent of red cells, showed in disease a definite decrease. Taking our own figures for the normal as 100 percent (79 percent for hemoglobin and 496,000 cells per c. mm. for the red count), then the hemoglobin percent was 55.9/79 x 100 = 70.77, and the red cell percent was 453,000/496,000 x 100 or 91.33 percent; the color index was 70.77/91.33 or 0.77. The amount of hemoglobin per red cell was thus decreased, indicating a hypochromic anemia.

The white cell count was reduced to a significant degree in disease. The average decrease in both sexes was 2,148 cells per c. mm.

The platelet count and clotting time were done to reveal changes in the clotting mechanism of the blood. The platelet count in disease showed a significant decrease of about 100,000 cells per c. mm. The clotting time significantly increased to about 14 percent of the normal value.

The sedimentation rate showed in disease a significant increase of about 2 mm. in 60 minutes.

SUMMARY

Male and female frogs with red leg showed significant changes in all factors studied in the blood. There was a reduction in red cell, white cell, and platelet count as well as in hemoglobin percentage. The clotting time, sedimentation rate, and red blood cell size were increased. The red blood cells were especially affected qualitatively, showing marked changes in shape and structure.

Significant sex differences were found in normal and diseased ani-

mals for red blood cell count and hemoglobin percentage.

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