

VARIATIONS IN WHITE BLOOD CELLS BETWEEN NORMAL AND RED LEG FROGS

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The total and differential white-cell counts of normal frog blood are known (1). The writer (2) has shown that no sex difference exists in the total white-cell count in normal frogs, but that a significant reduction occurs in this count in frogs with red leg disease (table 1). Because of the importance of white cells, a further analysis of the white-cell picture was made by statistical comparisons of the differential white-cell count in normal frogs with those in red leg disease.

MATERIAL AND METHODS

Rana pipiens and at times *Rana clamitans* were used. No species difference in blood picture was noted. Ninety-three normal frogs were compared to 80 animals which presented fullblown signs of red leg disease. In addition, 40 animals in the terminal stages of the condition were studied. In the early experiments an attempt was made to use only frogs which had acquired red leg in nature. Since frogs appeared to develop a natural resistance at certain seasons, the disease was produced in most cases by injections of *Pseudomonas hemophila* cultures. These were obtained from the American Type Culture Company and were maintained by alternate growth on agar plates and broth. Artificially

and naturally infected animals had essentially similar blood pictures.

Recently we have used another more satisfactory method to grow red leg cultures. *Pseudomonas hemophila* is grown on a heart-brain agar slant and incubated 48 hours at 37° C. The slant is transferred every 24 hours. A virulent slant is washed with 10 cc. of physiological saline. This saline suspension is diluted 1/10 to 1/10,000 of its suspension volume. 1 cc. of each one of the dilutions is injected into frogs until a dilution which produces the disease without quickly killing the animal is obtained. Control frogs are injected with saline alone. Sterile technic is used.

The total white counts had been determined previously (2) on a hemacytometer stage. The blood for the smears was taken only from the ventricle of the heart to rule out possible differences between central and peripheral blood. The smears were stained in Wright's or Giemsa's solution and studied under oil immersion.

Only four varieties of white cells were tabulated. These included mature lymphocytes, basophiles, eosinophiles, and neutrophiles. Because of the questionable nature of monocytes and their rarity in the frog (3), these were classified as large lymphocytes and included in the lymphocyte count. This procedure has been followed elsewhere (4).

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TABLE 1.—COMPARISON OF TOTAL WHITE BLOOD COUNTS BETWEEN NORMAL AND DISEASED LEG FROGS FOR BOTH SEXES.

No. of animals	Sample mean	Deviation of sample mean	t value
Male			
Normal 50.....	16,134	1,240	3.9
Diseased 50.....	13,700	4,180	
Female			
Normal 50.....	14,161	3,091	2.6
Diseased 50.....	12,300	3,860	

The results were subjected to statistical analysis, using the Student *t* equation. The level of significance was chosen as 2.0.

EXPERIMENTAL DATA

The total white count had been found to be reduced significantly in disease. The possibility of sex difference in both normal and diseased frogs had been considered but no such difference was observed; the *t* value between normal males and females equals 0.13 and that between diseased animals equals 0.07. These facts are summarized or derived from data in table 1.

In the differential count the data were collected without regard to sex because of the absence of sex difference as noted above. In the acute stage, a statistically significant rise in lymphocytes was observed. This was offset, however, by a significant decrease in the number of circulating neutrophils together with a slight decrease in eosinophiles. Except for

the terminal stages there was no change in basophiles. These observations are summarized in table 2.

To check for changes in terminal stages of the infection, differential counts were done on 40 moribund frogs. The mean percentages and standard deviations were: lymphocytes, 87.59 ± 5.56 ; neutrophils, 5.98 ± 3.85 ; basophiles, 4.34 ± 1.92 ; and eosinophiles, 2.08 ± 1.74 . The granulocytes have been depressed to an even greater degree.

Some observers (5) have stated that a marked leukocytosis occurs in human beings under conditions of cold and pregnancy. Because the frogs were generally stored for one to three weeks in a refrigerator and also because gravid females were occasionally used, control experiments with non-gravid females kept at room temperature were run to see whether such conditions had any noticeable effect on the experimental data. Neither the total nor the differential counts were appreciably altered by

TABLE 2.—COMPARISON OF DIFFERENTIAL WHITE COUNTS BETWEEN NORMAL AND RED LEG INFECTED FROGS.

Variety of white cells	Number of animals	Mean percentage	Standard deviation of mean	t value
Basophiles	Normal 93	6.74	3.35	0.60
	Diseased 80	7.14	2.73	
Neutrophiles	Normal 93	20.75	10.60	6.54
	Diseased 80	7.36	4.11	
Eosinophiles	Normal 93	8.03	5.98	1.93
	Diseased 80	6.30	2.23	
Lymphocytes	Normal 93	64.48	10.63	7.12
	Diseased 80	79.20	4.91	

the environmental temperature or by the gravid state of the animal.

The changes in the blood picture are characteristic in red leg disease. Figure 1 shows a bacteremia with attendant vacuolization of infected red cells and a predominance of lymphocytes which everywhere crowd out the granulocytes. Figure 2 in contrast shows a haemogregarine infection, but most of the red cells appear normal and the granulocyte line was found not to be depressed.

An explanation of the decrease in the total leucocyte count was sought in the findings in the differential counts. This was done (table 3) by converting the mean percentage of each variety of white cell into the numbers per cubic mm. in both normal and diseased frogs and comparing the changes in these numbers between health and disease.

The analysis of results in the acute phase show that in spite of a relative lymphocytosis (for every 100 cells) as seen on smears, there is no substantial increase per cubic

mm. (except for terminal stages) because of a large drop in the total number of circulating cells. On the other hand, in the acute phase, the neutrophiles decrease greatly and the eosinophiles mildly. The basophiles may not significantly change. In the terminal stages all granulocytes decrease more sharply. The neutropenia in particular more than offsets any lymphocytosis, which apparently explains the drop in the total white count.

DISCUSSION

Red leg disease is produced by *Pseudomonas hemophila* and is infectious for several species. The characteristic effect is a hemorrhagic septicemia (6). The writer has described in this paper and elsewhere (2) some of the quantitative hematologic changes involved. It is important to know the nature of this usually fatal disease as an aid to control in an animal so extensively used as the frog.

TABLE 3.—CONVERSION OF MEAN PERCENTAGES OF WHITE CELLS INTO NUMBERS PER C. MM. IN NORMAL AND DISEASED FROGS.

Normal frogs			Diseased frogs				
Total count per c. mm. (average of all males and females)	Differential count Mean percentage	Number in total count per c. mm.	Total count per c. mm. (average of all males and females)	Differential count Mean percentage		Number in total count per c. mm.	
				Acute stage	Terminal stage	Acute stage	Terminal stage
15,148	Lymphocyte	9767.43	13,000	Lymphocyte	79.20	10,296.00	11,386.70
	Neutrophile	3143.21		Neutrophile	7.36	956.80	777.40
	Eosinophile	1216.88		Eosinophile	6.30	829.00	270.40
	Basophile	1020.96		Basophile	7.14	928.20	564.20

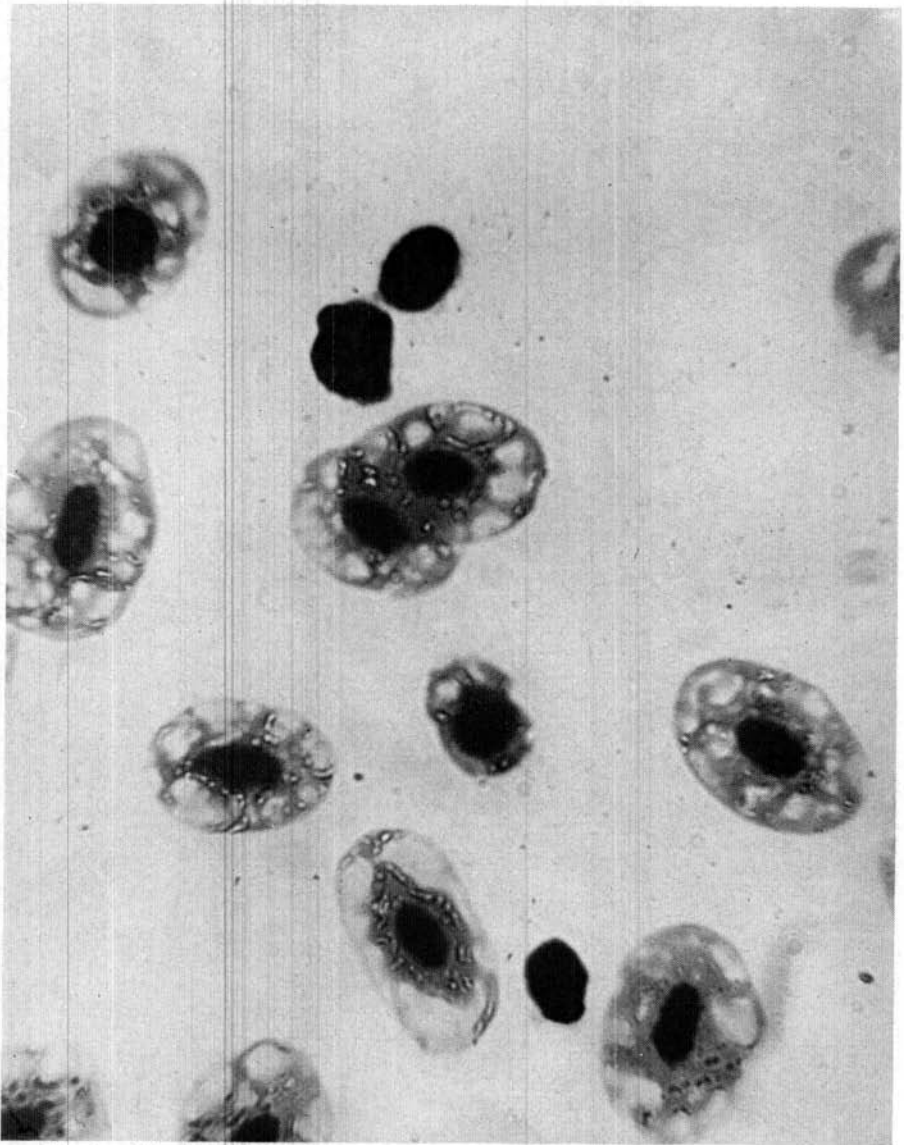


FIG. 1.—Blood smear treated with Wright's stain showing *Pseudomonas* invasion with vacuolization of red cells and predominance of lymphocytes. A typical field. About x 600.

The differential white count in the infectious diseases among animals and man varies with the specific disease. Leucocyte changes are among

the most conspicuous of the pathological signs of infection and a differential count can help determine the nature of the infection. In red

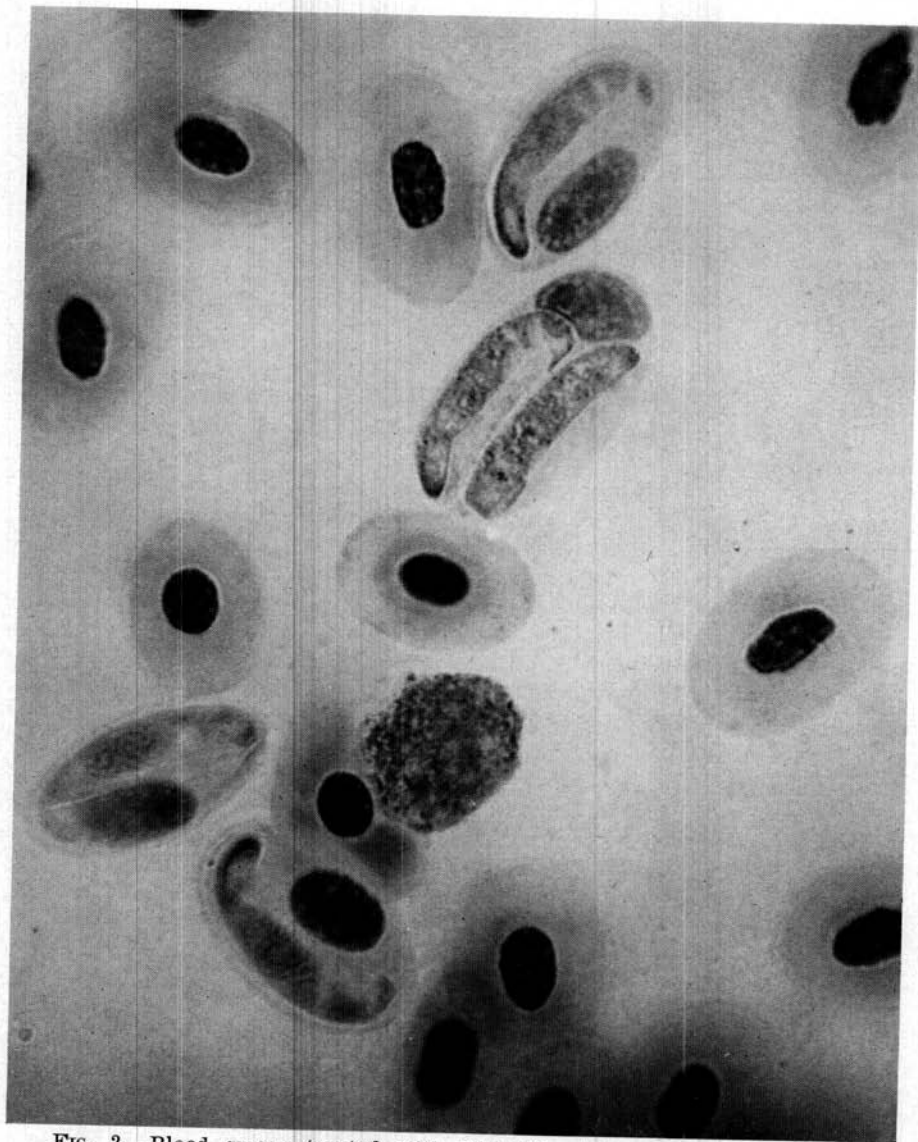


FIG. 2.—Blood smear treated with Wright's stain showing infection with *Haemogregarina* sp. Most red cells appear normal and the white count is not markedly changed. About $\times 600$.

leg disease there appears to be a leucopenia, involving the granulocytes and predominantly the neutrophils. In any human disease where the effects of the infection are

overwhelming there may similarly occur a leucopenia which is predominantly a neutropenia (7). In the diseased frog a sharp relative increase in lymphocytes occurs, even

when the disease is in the early stages, and it becomes more marked in the terminal stages. This is not unusual since there are many instances of lymphocytosis during acute infections (8).

In the frog the lymphocyte is said to be the usual progenitor of all other cells in the peripheral blood. Directly after hibernation the blood cells are produced in the bone marrow, and later in the spleen and intestinal submucosa. These sites of blood cell production are apparently not depressed in function since the stem cell has been found in this study to increase in percentage and perhaps in concentration at all times of year in response to *Pseudomonas* infection. Its derivatives, the granulocytes, tend to be destroyed, probably in attacking the invading organism. The resulting leucopenia is of grave import, since it indicates a depression of vital function which is always fatal within our observations.

The increase in lymphocytic stem cells appears to be a compensation for destruction of adult granulocytes and may represent a "shift to the left." Transitional or immature cells not found in healthy animals appear. This perhaps indicates re-

generation in hematopoietic areas.

Normal values for the types of white cells, although given in the literature (1), were redetermined in this paper because a more immediate set of standards was required for statistical comparison with values in disease under comparable conditions. Also, our results are based upon a larger sample than that used elsewhere to obtain normal values.

SUMMARY

Normal values for the types of white cells in frog blood were determined. The total white-cell count of frogs was significantly reduced in red leg disease. Differential counts showed a significant decrease in neutrophiles and a slight drop in eosinophiles with a concomitantly significant rise in the number of lymphocytes. In the acute stage there was no change in basophiles. The suppression of all granulocytes became more evident in terminal stages of the disease. Conversion of cell numbers from percentages to numbers per cubic mm. indicated that the granulocytopenia more than compensated for any increase in lymphocytes, thus apparently explaining the observed total leucopenia.

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