HEMATOLOGY OF *SIGMODON HISPIDUS*: AVERAGE PARAMETERS COMPARED WITH THOSE UNDER INDUCED STRESSES

By

Joseph Leland Hepworth

Bachelor of Science
Utah State University
Logan, Utah
1950

Master of Science
University of Idaho
Moscow, Idaho
1962

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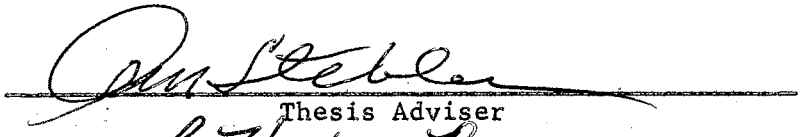
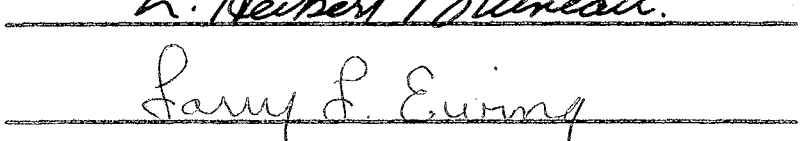
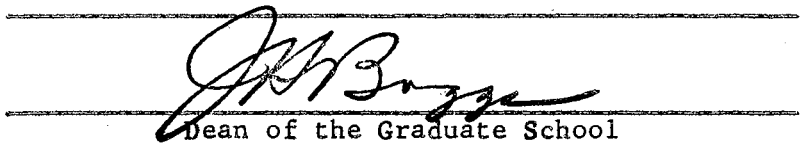


Thesis Adviser





Dean of the Graduate School

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TABLE OF CONTENTS

LIST OF TABLES

Table | Page

CHAPTER I

INTRODUCTION

The usefulness of blood values as an aid to the diagnosis of conditions deviating from a state of health in man and other animals has long been recognized. The major objective of this investigation, therefore, was to develop blood values for the hispid cotton rat, Sigmodon hispidus texianus, under average conditions and under conditions of induced stress. Emotional stress is believed to elaborate the pituitary adrenocorticotrophic hormone (ACTH) and this substance leads to an increase of circulating neutrophils and a decrease of circulating lymphocytes and eosinophils in man (Hills, Forsham and Finch, 1948).

Data were collected from wild, conditioned populations and from stressed, confined populations. Having observed rapid declines in wild microtine and blacktail jackrabbit populations due to causes that appeared not to be pathogenic or nutritional in origin, it would appear that some factor or factors of an emotional nature might induce these so-called "crashes" or rapid declines of populations. Others have studied the hormonal response to stress. An investigation of the blood picture of a wild mammal such as Sigmodon hispidus should be worthy of consideration. Stress may well be an important factor in the self-regulation of numbers in animal population including man. The stress of crowding may limit population increment and prevent a

population or populations from exhausting the environment to an almost irremediable state.

Green (1964) in his study of population density of Sigmodon hispidus refers to the rodent as sigmodon. This usage will be followed for simplicity in this study of the same species. By so doing the confusion that comes to mind brought about by the similarity of the names cotton rat and common rat will be avoided. Also, this usage will circumvent the onus associated with the disrepute of the commensal rat; plus the fact that there is precedent with drosophila and lynx, e.g.

There is a relative paucity of hematological data for wildlife species in the literature. This is especially true relative to the smaller mammals.

As for the sigmodon, the blood values reported in the literature seem to be for peripheral blood obtained by tail, toe or ear clipping and these data are limited. No hematological values including differential leukocytic counts were found. Most of the published blood data concern erythrocyte numbers and hemoglobin and hematocrit parameters.

Dunaway et al. (1962) reported on erythrocyte totals, leukocyte totals, and hematocrit for 23 penned sigmodon as a part of their study of radiation effects on blood. Hemoglobin values have been reported by Larimer (1959) for sigmodon.

Before changes in the blood picture can be recognized due to stress, average or standard values must be established.

All of the data were gathered in Payne County, Oklahoma. Blood samples were obtained between December 19, 1964 and September 28, 1965.

During this period of time, 337 blood samples were taken from sexually mature animals. It is recognized that in some instances the data are weak and this is due largely to the scarcity of the rodent at this particular time and place.

It is suggested that blood parameters may be found useful as diagnostic and prognostic indicators in the study and management of some aspects of wildlife populations.

CHAPTER II

METHODS

Conditioning Methods

The Havahart Humane Animal trap No. 0, which measures 3 by 3 by 10 inches was used for most of the trapping. Being curious as to the possible influence that trap size might have on the blood picture of an active rodent, 15 sigmodon were trapped the final time in the Havahart Humane Animal trap No. 1, which measures 5 by 5 by 18 inches. Otherwise the two traps are similar. Rolled or crimped oats served as bait in and around the traps. In both the field and the enclosures a handful of oats was utilized not only to attract the sigmodon but to feed them as well.

Sigmodon were trapped and released at the conditioning trapping area until they became accustomed to the traps and to handling. Only after several weeks of this procedure were they sampled. Traps within the enclosures were only checked in the mornings as all of the blood-sampling was done at that time. They were not re-set again until evening, but were left unset and in place during the day.

Blood Collecting and Analysis

The sigmodon to be sampled were quickly removed from the livetrap to a 10 by 18 inch cloth bag. These bags were carried in the top of the rolled oats container so the captives would be able to associate

with a familiar food odor. The bagged animal was wrapped up into a lower corner of the bag so it could not struggle. There it was firmly grasped by the nape of the neck and held. It was uncovered immediately to the point of exposing the ventral region, and pentobarbital sodium was injected intraperitoneally in the mid-abdominal region. A tuberculin type 1 cc syringe with a one-half inch 27-gauge needle was used. The dosage amount was established largely by trial and error. It was found that .10 ml per 100 grams of body weight gave adequate anesthesia. The sigmodon lost consciousness in from five to 11 minutes and remained insensible for at least 30 minutes. After the administration of the anesthesia, the animal was once again wrapped in the cloth sack to avoid struggling on its part while losing consciousness.

An animal holding board with four spring-tensioned foot clamps fastened to the board with rubber surgical tubing held the anesthetized sigmodon in an abdomen-up position for blood-sampling.

Blood samples were obtained by direct cardiac puncture. The area to be punctured was clipped of hair with scissors and the heart was located by palpation with the thumb of my left hand. Considerable experimentation was done with collecting syringes of various kinds and sizes. The one found to be most suitable was a 2.5 cc disposable type, with a 23-gauge, one inch needle. Approximately 1 cc of blood was collected from each live sigmodon.

The following hematological values were determined by standard laboratory procedures: hematocrit, hemoglobin, erythrocyte (RBC), leukocyte (WBC), and differential leukocyte or white blood cell count. MacGregor et al. (1940) indicated that the cover-slip blood films are preferred for differential counts. Personal experience is that the

leukocytes are more evenly distributed on cover-slip blood films than on full-slide films. Therefore, they were used. For the differential count the band neutrophils, segmented neutrophils, eosinophils, basophils, lymphocytes and monocytes were enumerated. Except for the differential count, all of the blood parameters were determined by a competent hematological technician. Differential counts and microscopic examination of the stained blood smears were done by the investigator in accordance with standard hematological procedures (Cartwright, 1958; Lynch et al., 1963; Wintrobe, 1961).

Stressors

It is assumed that the sigmodon were stressed due to their exposure to certain more or less controlled situations. Although stress is a rather ambiguous term it does have standing.

Crowding

The population of 100 of the west enclosure area was livetrapped and counted on April 1, 1965. At that time the animals within the enclosures appeared to be very irritable and were often observed in antagonistic posturing towards each other. Not infrequently they would engage in sporadic combat. The livetraps had been left inside the enclosure at the same station and were rebaited regularly. The livetrapped sigmodon had been trapped and released and retrapped for about two weeks to accustom them to being trapped and handled. On April 4, 1965, the first sigmodon from a crowded population were blood-sampled. The population was kept under conditions of high density and additional groups were sampled at a later date.

Food Insufficiency

Preparation for this type of stress began on June 11, 1965 with the transporting of the sigmodon from the enclosure to an avian flight cage. There the rodents were confined on a concrete floor with mesh wire walls so they would have no access to naturally occurring vegetation. They were provided with water, food and cover and allowed to become adjusted to new surroundings. Ten days later food was removed. They were still allowed water and cover. Groups so deprived were then blood-sampled after 24-, 48-, and 96-hour periods.

Lack of Cover

Sigmodon were released into the flight cage and allowed a week to become accustomed to the surroundings. Food, water, and cover were provided for them. After the period of adjustment, the cover, in the form of small cardboard boxes, one for each sigmodon, was removed. The rodents were then blood-sampled in groups having been without cover for 24-, 48-, and 72-hour periods. An additional group was held without cover for 30 days and then sampled.

Continuous Light and Lack of Cover

The test groups were allowed to become adapted to their environment and then subjected to continuous illumination. Cover was removed at the same time the flood lights were turned on. The sigmodon were on a concrete floor and, therefore, were unable to burrow to find cover or protection from the light. A group was sampled after 24 hours, another after 48 hours and the third after a 72-hour interval.

Continuous Foreign Sound

After becoming accustomed to their surroundings the sigmodon were subjected to the constant, high-pitched, loud whistle of a portable wireless interphone set. Although they were not crowded, none could get more than 30 feet away from the source of the sound. The intensity of the sound was such that it was distinctly audible by human ear 125 yards away over and above the ambient sounds of rustling leaves. Groups to be sampled were subjected to this noise for periods of four hours, eight hours, and 24 hours.

CHAPTER III

RESULTS

Blood Values for Sigmodon

All of the animals used in this study had lost their juvenile appearance. Guard hairs were plainly evident and the hispidness of adulthood were present. They ranged in weight from 49 to 280 grams and appeared in a state of apparent good health prior to sampling or stressing and sampling. All of the sampled rodents were given cursory checks for both external and internal parasites. Those few found to be infested by ticks or tapeworms were not sampled. No blood parasites were seen while observing the fluid blood or while examining the stained blood films.

Erythrocytes

The erythrocyte of the sigmodon was typically mammalian, a round, non-nucleated, biconcave disk. By comparison, they are slightly smaller than those of the laboratory rat and slightly larger than those of the laboratory mouse. Erythrocyte diameters vary considerably in an individual sigmodon. Fifty cells were randomly selected and their diameters ranged from 5.0 to 8.4 micra, with a mean diameter of 6.6 micra. Polychromasia was a common feature. This blue-gray staining of the entire erythrocyte was found in 57% of the stained blood smears. Poikilocytosis was not seen in any of the sigmodon

blood smears. Reticulocytes were seen in the blood of only one of the animals, a 188 gram female, and these constituted less than one per cent of the individual's total erythrocyte count. In contrast to the polychromatic condition, the reticulocytes were stained blue-gray only in what seemed to be centrally located nuclear remnants.

Leukocytes

The neutrophil nucleus in the segmented stage showed two or more lobes connected by bands or threads of chromatin, and was made up of condensed masses of light to dark purple-staining chromatin. The cytoplasm was pink to lilac. Granules, if present, were fine and had the color of the nucleus.

The band form neutrophil showed a nucleus that was not segmented and it usually appeared to be less condensed. Otherwise, the characteristics were similar to those of the segmented neutrophil. In a study of human neutrophils, Fliedner et al. (1964) found that contrary to widely accepted opinion there is no correlation between neutrophil age and the number of nuclear segments present. Band forms entered the blood from the bone marrow about 24 hours earlier than segmented forms but remained band neutrophils throughout their life span in the blood. No sex chromosome lobe was seen on any neutrophil nucleus of the sigmodon.

The eosinophil nucleus, usually bilobed or banded, stains about the same as the neutrophil nucleus. The cytoplasm was usually lilac to blue, but was very difficult to see due to the obscuring influence of the granules. The granules were much larger than those seen in the neutrophil. They were a light to bright orange-red and very spherical

with a sharply defined outline.

Details of the nucleus were partly obscured in all of the basophils seen. From what could be seen of it, the nucleus seemed to be slightly lobulated. The cytoplasm was usually lilac to blue. Large distinct blue-black granules nearly filled or masked the cytoplasm.

The small lymphocyte was the smallest leukocyte in sigmodon and was only slightly larger than the erythrocyte. The deep purple-staining nucleus was round or only slightly indented. It often almost filled the entire cell leaving only a thin crescent edge of cytoplasm unobscured. No distinct internal structure was seen. The periphery of the nucleus was usually defined rather sharply. Most often the cytoplasm was clear light blue, but in heavily stained smears the color was rather dark. The cytoplasm never formed more than a narrow rim around the nucleus.

The large lymphocyte may be as large as a monocyte. The nucleus was usually somewhat larger than in the small lymphocyte and was often slightly indented or nearly kidney-shaped. The cytoplasm was a clear light blue and was much greater in proportion to the nucleus than in the small lymphocyte. Occasionally red-purple granules may be unevenly scattered through the cytoplasm.

Many workers make no distinction between large and small lymphocytes. However, a leukocyte differential of the Alaskan ground squirrel gave the following parameters: large lymphocyte 16.2%, small lymphocyte 44.2% and monocyte 10.2% (Lieb and Wilber, 1954).

What was apparently a young lymphocyte occurred occasionally in sigmodon blood. This cell was about the same size as the large

lymphocyte, but the nucleus seemed less condensed and filled a greater proportion of the cell. No granules were seen in the deep blue cytoplasm.

The monocyte nucleus was indented or lobulated, often presenting an irregular ridge effect. The nucleus stained a lighter purple than most of the other leukocytes. The cytoplasm was gray-blue. Granulation varied from none to a fairly abundant stippling of fine granules. These usually appeared to be about the same color as the nucleus. Vacuoles were often seen in the cytoplasm, which tended to give it a characteristic lacy appearance. It was extremely difficult to differentiate the large lymphocytes from the monocytes in some blood smears.

Of the leukocytes, the monocytes seemed to be the most plastic or easily indented by erythrocytes and it was common to see this in the stained blood smears.

Disintegrated or degenerated leukocytes (Schalm, 1965) were observed in four per cent of the stained blood smears from the conditioned rodents. By way of comparison, 16% of the stained blood smears from the stressed animals contained disintegrated or degenerated cells.

An attempt was made to record thrombocyte (platelet) data when making the differential count of the leukocytes. In some stained blood smears, the thrombocytes appeared quite distinct and numerous. Other smears showed no recognizable thrombocytes at all. It was impossible to discern between minute fragments and thrombocytes; therefore, no thrombocytic comparisons can be made.

The following blood parameters are reported from the sigmodon sampled: hematocrit or packed cell volume, expressed in volume per cent; hemoglobin, expressed in grams per 100 ml of blood; erythrocytes (RBC),

expressed in millions per cubic mm of blood; leukocytes (WBC), total count, expressed in thousands per cubic mm and leukocytes, differential count, expressed in per cent.

A total of 337 blood samples were obtained by way of intracardial puncture. Eleven of these were double check samples. Of the remaining 326 samples, 37 developed some clotting and were rejected. This amounts to approximately 11% of the total. Insufficient amounts of blood being withdrawn resulted in a further decrease of two samples. Thirteen other samples were eliminated on the basis of poor blood film staining. This study, then, is based upon the results from 274 samples. Of this number, 24 samples were from 12 sigmodon that were punctured twice with a 60-day interval between samplings. The remaining blood samples represent single samplings from 250 sigmodon.

The results are given in tabular form for each group or category considered. Each group of stressed or non-stressed sigmodon was given a number to identify that specific group. For each of these groups or a combination of groups both averages and ranges of the ten blood parameters are given. This conforms to the precedent established by Schalm (1965) and Youatt et al. (1961) who reported their hematologic data for small mammals by giving average and range for each parameter measured.

The hematological values of sigmodon (Table I) present a hemogram for Group 2, which consisted of 15 animals randomly livetrapped from December 17, 1964 to February 18, 1965. No attempt was made to accustom these animals to being handled. They were blood-sampled after their first experience with a livetrap and are therefore referred to as Winter unconditioned. It is assumed that they were cold-stressed

TABLE I

HEMATOLOGIC PARAMETERS FOR WILD SIGMODON

Group	N	HCT %	HGB gm.%	RBC million/ c.mm.	WBC thousand/ c.mm.	Neutrophils		Differentials in percentages			
						Band.	Segmented	Eos.	Bas.	Lym.	Mono.
Winter-Spring conditioned animals:											
3	33	40.35 (32.5-48)	12.8 (10-15)	4.19 (3.46-5.68)	4.3 (1.5-9.1)	0.7 (0-10)	39.4 (18-59)	4.1 (0-9)	0.2 (0-1)	50.3 (26-72)	5.4 (1-14)
Summer-Fall conditioned animals:											
22-28	71	40.6 (33.5-48)	12.4 (10-15)	3.90 (2.98-4.92)	7.6 (2.4-15.5)	0.3 (0-6)	26.2 (1-63)	6.7 (0-18)	0.014 (0-1)	63.1 (26-92)	3.7 (0-10)
All conditioned animals:											
	104	40.5 (32.5-48)	12.5 (10-15)	3.99 (2.98-5.68)	6.6 (1.5-15.5)	0.4 (0-10)	30.4 (1-63)	5.8 (0-18)	0.077 (0-1)	59.0 (26-92)	4.2 (0-14)
All stressed animals:											
	170	41.2 (25-53)	13.0 (8-16.5)	3.97 (2.8-5.22)	6.8 (1.6-22.4)	2.6 (0-14)	32.8 (4-69)	4.9 (0-36)	0.10 (0-2)	55.2 (23-88)	4.4 (0-18)
Winter unconditioned animals:											
2	15	40.5 (32.5-47)	13.0 (10.5-15)	3.95 (2.80-4.45)	4.6 (1.6-6.7)	1.4 (0-6)	23.0 (4-38)	1.0 (0-7)	0.5 (0-2)	69.3 (54-88)	4.7 (0-10)

Range is shown in parenthesis below average.

by the first experience of livetrapping and transportation. Also included in Table I is a hemogram for Group 3, the Winter-Spring conditioned sigmodon numbering 33 individuals. These animals were repeatedly livetrapped and released in their natural habitat for a period of 26 days prior to sampling. At the time of sampling, they had become quite docile and behaved as though accustomed to being handled. There is further included in Table I an aggregate of Groups 22 to 28 that consisted of 71 sigmodon conditioned by the trapping and handling procedure. A composite hemogram for all of the conditioned animals is given along with a total for all of the stressed sigmodon. The parameters for Group 2, the Winter unconditioned animals, are included in the composite for the stressed animals.

In Table II, hematological data for each Group from 22 to 28 are presented separately along with the number of sigmodon in each group. As the possibility of livetrap size influencing the total leukocyte count was suspected by the investigator, Group 25, 26 and 27 were trapped in part, on the sampling day only, with Havahart No. 1 traps as opposed to the Havahart No. 0 traps that were used consistently for the rest of the study. Separate total leukocyte (WBC) count data is presented in Table II for the animals taken in the larger traps.

After being cardiac punctured for Group 3, one dozen samplees were released into an enclosure and integrated as a part of the crowding stress studies. Eleven of these were retrapped and blood-sampled 60 days later and their blood values reported in Table III as Group 6a. Their original parameters are reported in the same table as Group 3a.

Table IV included blood values from the groups that were subjected

TABLE II

HEMATOLOGIC PARAMETERS FOR SUMMER-FALL CONDITIONED SIGMODON

Group	N	HCT %	HGB gm.%	RBC million/ c.mm.	WBC thousand/ c.mm.	Neutrophils		Differentials in percentages			
						Band.	Segmented	Eos.	Bas.	Lym.	Mono.
22	3	42.2 (40-44)	12.2 (11-13)	4.06 (3.96-4.21)	7.5 (6.0-10.3)	2.7 (0-6)	39.3 (31-50)	10.0 (6.17)	0.0 (0-0)	46.0 (33-55)	2.0 (0-4)
23	14	40.0 (37.5-44)	12.1 (11-13.5)	3.82 (3.61-4.22)	6.1 (3.6-22.5)	0.07 (0-1)	21.9 (10-33)	7.0 (2-18)	0.07 (0-1)	67.7 (55-80)	3.3 (0-10)
24	10	39.6 (37-43)	12.2 (11-13.5)	3.75 (3.50-4.20)	6.4 (3.5-12.0)	0.10 (0-1)	28.4 (10-42)	6.9 (4-17)	0.0 (0-0)	59.9 (42-77)	4.3 (0-7)
25	13	41.6 (33.5-46.5)	12.5 (10-14)	3.98 (2.98-4.44)	9.5[a] (4.0-15.6)	0.6 (0-2)	31.1 (9-63)	6.1 (0-13)	0.0 (0-0)	58.5 (26-76)	3.9 (1-7)
	8				7.7 (4.0-15.0)						
26	11	40.1 (37-44)	12.5 (11.5-13.5)	3.86 (3.59-4.21)	10.3[a] (5.0-14.0)	0.18 (0-2)	27.3 (20-32)	7.4 (3-13)	0.0 (0-0)	60.9 (53-69)	4.5 (2-10)
	4				7.0 (5.0-8.0)						
27	10	42.2 (36.5-48)	13.2 (11.5-15)	4.10 (3.46-4.92)	8.2[a] (4.2-15.5)	0.1 (0-1)	21.3 (1-39)	5.4 (1-14)	0.0 (0-0)	70.4 (46-92)	2.9 (1-5)
	7				6.1 (4.2-8.0)						
28	10	39.9 (37-44)	12.4 (11.5-13.5)	3.88 (3.50-4.27)	5.0 (2.5-8.0)	0.10 (0-1)	24.0 (8-41)	6.5 (1-12)	0.0 (0-0)	65.7 (46-89)	3.7 (2-6)

[a]Large trap effect.

TABLE III

HEMATOLOGIC PARAMETERS FOR SIGMODON ANIMALS FROM THE CONDITIONED GROUP, RESAMPLED AS A CROWDED GROUP SIXTY DAYS LATER

Group	N	HCT %	HGB gm.%	RBC million/ c.mm.	WBC thousand/ c.mm.	Neutrophils		Differentials in percentages			
						Band.	Segmented	Eos.	Bas.	Lym.	Mono.
Conditioned animals											
3a	11	40.1 (33.0-45.0)	12.8 (10.5-14.5)	4.31 (3.64-5.68)	4.3 (1.9-9.1)	0.4 (0-4)	35.3 (18-48)	5.1 (2-8)	0.4 (0-1)	52.8 (38-72)	6.1 (4-11)
Resamples stressed due to crowding											
6a	11	40.5 (37.0-46.0)	13.3 (11.5-15.0)	4.01 (3.42-4.64)	7.9 (4.2-11.3)	3.6 (0-7)	38.6 (19-55)	11.2 (2-33)	0.0 (0-0)	41.1 (30-63)	4.5 (1-12)
Difference between group means											
		0.4	0.5	0.30	3.6	3.3	3.3	6.2	0.4	11.7	1.6

TABLE IV

HEMATOLOGIC PARAMETERS FOR SIGMODON UNDER INDUCED STRESSES

Group	N	HCT %	HGB gm.%	RBC million/ c.mm.	WBC thousand/ c.mm.	Neutrophils		Differentials in percentages			
						Band.	Segmented	Eos.	Bas.	Lym.	Mono.
Stressed by the crowding of natural increase											
5	25	40.3 (30-46)	12.5 (10.5-14)	4.12 (3.41-5.22)	5.7 (3.0-9.0)	2.1 (0-8)	44.8 (23-64)	6.7 (0-15)	0.2 (0-2)	41.9 (25-62)	4.3 (1-11)
6	14	41.4 (37-46)	13.4 (11.5-15)	4.00 (3.42-4.64)	7.5 (3.8-10.0)	3.9 (0-7)	39.6 (19-55)	11.4 (2-33)	0.0 (0-0)	39.8 (30-63)	5.4 (1-12)
7	7	43.2 (34-46.5)	13.6 (11.5-15)	4.12 (2.98-4.86)	6.6 (3.9-10.5)	1.7 (0-8)	30.6 (14-57)	2.0 (0-5)	0.1 (0-1)	60.7 (42-80)	4.9 (1-9)
12	8	44.4 (38-53)	14.3 (12.5-16.5)	4.27 (3.61-5.18)	7.7 (4.5-11.0)	1.3 (0-4)	26.4 (14-41)	12.2 (2-36)	0.0 (0-0)	54.0 (29-78)	7.0 (1-14)
Total	54	40.8 (30-53)	12.9 (10.5-16.5)	4.04 (2.98-5.22)	6.5 (3.0-11.0)	2.4 (0-8)	38.2 (14-64)	7.9 (0-36)	0.1 (0-2)	44.8 (25-80)	5.3 (1-14)

TABLE IV (CONT)

HEMATOLOGIC PARAMETERS FOR SIGMODON UNDER INDUCED STRESSES

Group	N	HCT %	HGB gm.%	RBC millions/ c.mm.	WBC thousands/ c.mm.	Neutrophils		Differentials in percentages			
						Band.	Segmented	Eos.	Bas.	Lym.	Mono.
Stressed by lack of cover											
8[a]	10	40.9 (38-44.5)	13.3 (12-14.5)	3.99 (3.65-4.86)	6.0 (5.2-7.2)	2.7 (0-7)	24.7 (5-53)	4.3 (0-11)	0.0 (0-0)	61.5 (35-75)	7.0 (1-14)
9[b]	7	40.0 (35-44)	13.4 (12-14.5)	3.74 (2.98-4.36)	6.1 (5.2-7.5)	5.6 (2-12)	25.0 (14-34)	7.9 (1-21)	0.0 (0-0)	57.4 (50-72)	4.9 (1-11)
10[c]	10	41.6 (37-46)	13.5 (12-15.5)	3.96 (2.98-4.63)	6.1 (4.4-7.5)	3.4 (0-7)	36.3 (18-60)	3.7 (0-10)	0.0 (0-0)	52.6 (30-67)	4.0 (1-7)
15[d]	6	41.5 (38-44)	13.2 (12-14)	3.86 (3.53-4.28)	5.4 (4.3-7.2)	3.2 (1-5)	30.2 (5-52)	5.2 (1-10)	0.17 (0-1)	56.5 (39-74)	4.5 (0-9)
Stressed by lack of cover and continuous light											
16[a]	9	38.6 (32.5-43.5)	12.0 (9.5-13)	3.69 (2.98-4.25)	13.8 (8.2-22.4)	1.3 (0-3)	37.1 (15-69)	3.3 (0-8)	0.11 (0-1)	56.4 (23-79)	1.8 (0-5)
17[b]	10	38.9 (37-43.5)	11.9 (10.5-13)	3.61 (3.02-4.25)	8.6 (6.5-15.0)	1.8 (0-4)	39.4 (25-50)	2.7 (1-5)	0.0 (0-0)	54.1 (42-69)	2.0 (0-4)
18[c]	4	40.2 (37.5-43)	12.5 (11-13)	3.74 (3.50-3.98)	10.4 (8.5-12.0)	0.5 (0-1)	28.0 (22-30)	1.3 (0-4)	0.0 (0-0)	69.3 (63-77)	1.0 (0-2)

[a]Duration of test was 24 hours.
[b]Duration of test was 48 hours.
[c]Duration of test was 72 hours.
[d]Duration of test was 30 days.

TABLE IV (CONT)

HEMATOLOGIC PARAMETERS FOR SIGMODON UNDER INDUCED STRESSES

Group	N	HCT %	HGB gm.%	RBC million/ c.mm.	WBC thousand/ c.mm.	Neutrophils		Differentials in percentages			
						Band.	Segmented	Eos.	Bas.	Lym.	Mono.
Stressed by food insufficiency											
11[a]	7	43.0 (39-44)	13.8 (12.5-16)	3.99 (3.51-4.76)	6.3 (4.0-9.0)	5.6 (2-10)	34.0 (20-49)	5.3 (1-10)	0.0 (0-0)	49.0 (36-63)	6.1 (2-10)
13[b]	9	42.7 (35-48)	13.8 (11.5-15.5)	4.31 (3.72-2.91)	5.7 (2.5-8.0)	3.7 (0-14)	37.6 (17-55)	3.5 (0-15)	0.0 (0-0)	48.5 (31-75)	6.7 (2-18)
14[c]	8	42.2 (35-46)	13.5 (11.5-15)	3.93 (3.22-4.61)	5.0 (2.8-8.0)	2.9 (0-6)	29.0 (6-45)	1.9 (0-7)	0.25 (0-1)	60.2 (44-78)	5.9 (3-14)
Stressed by continuous foreign sound											
19[d]	5	38.6 (35-43)	11.9 (8-14)	3.73 (3.01-4.20)	8.1 (2.2-10.4)	1.6 (0-3)	25.0 (10-51)	5.2 (1-9)	0.0 (0-0)	64.4 (42-88)	3.8 (0-12)
20[e]	8	42.3 (38-45)	12.7 (11.5-13.5)	3.98 (3.51-4.36)	5.8 (3.5-11.3)	0.9 (0-3)	25.1 (8-47)	3.0 (0-7)	0.0 (0-0)	69.6 (52-86)	1.4 (0-3)
21[a]	9	43.1 (38-47)	12.9 (11.5-14)	4.03 (3.26-4.52)	8.2 (4.2-14.7)	1.8 (0-7)	26.0 (14-53)	2.4 (0-5)	0.0 (0-0)	66.3 (36-82)	3.4 (1-15)

[a]Duration of test was 24 hours.
[b]Duration of test was 72 hours.
[c]Duration of test was 120 hours.
[d]Duration of test was 4 hours.
[e]Duration of test was 8 hours.

to induced stress. Sigmodon stressed by crowding were as follows: Group 5, 25 sigmodon; Group 6, 14 sigmodon; Group 7, seven sigmodon; and Group 12, with eight sigmodon. A total hemogram for all the groups stressed by crowding is included. There were 11 fatalities prior to sampling from the starters of the crowding groups.

Sigmodon stressed by lack of cover, including Group 8, with 10 animals; Group 9, with seven sigmodon; Group 10, with 10 sigmodon; and Group 15, with six sigmodon have their hematological values reported in Table IV. It is noteworthy that 15 sigmodon began the 30 day lack of cover stress (Group 15). Of this number, all of the males with descended testes at the time of commencement succumbed prior to the end of the stressing period. They were seven in number. Two males survived and they had ascended testes at both the onset and terminus of the experiment. All of the females survived, but two were not sampled due to advanced pregnancy. The dead males had begun to putrefy prior to being found and gave no conclusive evidence as to cause of death other than strife among themselves. All of the carcasses bore scars and wounds. Table IV also includes Group 16, with nine sigmodon; Group 17, with 10 sigmodon; and Group 18, with four sigmodon that were stressed by lack of cover and continuous light. After putting these animals under stress, one male died from the original 10 of Group 16 before sampling time. The cause was a fall from the side of the flight pen. Of the 10 animals originally in Group 18, two fell to their deaths on the concrete floor and four died under the influence of anesthesia before they could be sampled.

The blood parameters for Group 11, with seven sigmodon; Group 13, with nine sigmodon; and Group 14, with eight sigmodon, that were stressed

by food insufficiency are tabulated in Table IV along with those of Group 19, with five sigmodon; Group 20, with eight sigmodon; and Group 21, with nine sigmodon, all of which were stressed by continuous foreign sound. The variance in numbers among the groups do not represent death loss, but instead poor trapping success.

Tables V and VI portray the hematological data for the sigmodon females and males respectively that were used in this study.

Behavioral Observations

The non-stressed or conditioned groups of sigmodon displayed no outward signs of anxiety. Presumably they went about making their living, reproducing, and resting with a minimum amount of observed strife. Scarring of their bodies was uncommon. Within the enclosures they were seen to posture and challenge one another occasionally while feeding, but most of the time they behaved in a well ordered manner. No combat was ever observed.

On the whole, the sigmodon from Group 3, the Winter-Spring conditioned population, seemed most tractable of all the groups. The Summer-Fall conditioned sigmodon composed of members from Groups 22-28 were gentler than any of the stressed groups; however, they were more excitable and harder to trap and handle than the Group 3 rodents.

The sigmodon stressed by crowding were the least docile. When livetrapped and handled they displayed great excitation. In the enclosures they frequently were sighted stalking one another in a manner comparable to a canine stalking prey. Intense and prolonged combat occurred throughout the enclosure with the victor pursuing the vanquished for several yards. The livetrapped ones, especially the males,

TABLE V

HEMATOLOGIC PARAMETERS FOR SIGMODON FEMALES

Group	N	HCT %	HGB gm.%	RBC million/ c.mm.	WBC thousand/ c.mm.	Neutrophils		Differentials in percentages			
						Band.	Segmented	Eos.	Bas.	Lym.	Mono.
Winter-Spring average values											
3	15	40.4 (32.5-45)	12.8 (10-14.5)	4.21 (3.46-5.68)	4.7 (1.5-9.1)	1.0 (0-10)	37.7 (24-54)	3.6 (0-8)	.26 (0-1)	51.9 (37-68)	5.7 (0-14)
Summer-Fall average values											
23-28	35	40.2 (33.5-48)	12.3 (10-15)	3.84 (2.98-4.50)	6.8[a] (2.5-15.0)	.29 (0-5)	24.1 (8-46)	6.8 (0-18)	.03 (0-1)	64.6 (42-89)	3.6 (0-10)
	31				6.2 (2.5-15.0)						
Total average values											
	50	40.3 (32.5-48)	12.4 (10-15)	3.95 (2.98-5.68)	6.2[a] (1.5-15.0)	.5 (0-10)	28.2 (8-54)	5.8 (-18)	.10 (0-1)	60.8 (37-89)	4.3 (0-14)
	46				5.7 (1.5-15.0)						
All stressed values											
	94	38.8 (32.5-46)	12.2 (9.5-15)	3.73 (2.98-4.61)	6.6 (2.8-16.8)	2.8 (0-12)	32.0 (5-60)	5.3 (0-36)	.10 (0-2)	52.6 (29-86)	3.9 (0-18)
All Sigmodon values											
	144	39.3 (32.5-48)	12.3 (9.5-15)	3.81 (2.98-5.68)	6.4 (2.8-16.8)	2.0 (0-12)	30.7 (5-60)	5.5 (0-38)	.10 (0-2)	55.5 (29-89)	4.1 (0-18)

[a]Large trap effect.

TABLE VI

HEMATOLOGIC PARAMETERS FOR SIGMODON MALES

Group	N	HCT %	HGB gm.%	RBC million/ c.mm.	WBC thousand/ c.mm.	Neutrophils		Differentials in percentages			
						Band.	Segmented	Eos.	Bas.	Lym.	Mono.
Winter-Spring average values											
3	18	38.6 (33-48)	12.8 (10-15)	4.16 (3.46-5.66)	4.0 (1.8-7.8)	0.4 (0-2)	40.8 (18-59)	4.6 (1-9)	.17 (0-1)	49.1 (26-62)	5.1 (2-11)
Summer-Fall average values											
22-28	36	41.0 (36.5-47.5)	12.6 (11-14.5)	3.97 (3.46-4.44)	8.4[a] (3.0-15.5)	0.5 (0-6)	27.6 (1-63)	6.6 (1-17)	.03 (0-1)	61.5 (33-92)	3.8 (0-10)
	25				6.5 (3.0-15.0)						
Total	54	40.2 (33-48)	12.6 (10-15)	4.03 (3.46-5.66)	6.9[a] (1.8-15.5)	0.5 (0-6)	32.0 (1-63)	5.9 (1-17)	.07 (0-1)	57.4 (26-92)	4.2 (0-11)
	43				5.5 (1.8-15.0)						
All stressed values											
	76	44.1 (25-53)	13.8 (8-16.5)	4.27 (2.80-5.22)	7.1 (1.6-22.4)	2.2 (0-14)	47.1 (4-57)	4.4 (0-27)	.09 (0-2)	58.4 (23-88)	5.1 (0-15)
All conditioned and stressed values											
	130	42.5 (25-53)	13.3 (8-16.5)	4.17 (2.80-5.66)	7.0 (1.6-22.4)	1.5 (0-14)	33.2 (1-63)	5.1 (0-27)	.08 (0-2)	58.0 (23-92)	4.7 (0-15)

[a]Large trap effect.

bore scars and roughly one-half of those livetrapped on a sampling day had to be rejected because they had unhealed wounds. Immature sigmodon were often killed by adults. This was especially in evidence when the very small ones strayed from their nests.

Population growth was not limited by a lack of food. Even so, the populations being stressed by natural crowding remained at a density of one sigmodon per two square feet of area during the time that the stress due to crowding investigation was ongoing. A similar phenomenon was observed in a confined house mouse population for a period of two years (Southwick, 1955).

The sigmodon that experienced food withdrawal did not appear to be antagonistic towards one another. They were moreover, less active than any other stressed group. Only when the traps were picked up for the purpose of removing the captives did they display signs of excitement.

Those groups that experienced lack of cover stress showed signs of being disturbed. Immediately after removal of the cover they would crowd together and try to hide under each other. This milling and shifting of position would continue for several minutes before the animals settled down. The least little noise or disturbance or some unrecognized causative, (at least unrecognized by the observer) would set them off in a flurry of excited activity. When isolated from the group, they would often flatten against the floor and remain as if stupified for lengthy periods of time. Stepping near or over them would elicit no reaction from them. They were very easily livetrapped for blood-sampling. Group 15, which had a stressing duration of 30 days started with 15 sigmodon. The seven large, sexually active males

appeared to be exceedingly agitated. They were very combative, and fought to exhaustion, and near death while under observation. During the times they were being observed they never seemed to be at ease with their surroundings.

Observations of the groups subjected to continuous light and lack of cover were similar to those experiencing only lack of cover with one important exception. Nine of the 23 sigmodon sampled had broken off their upper incisors. This probably happened at night, as only then were they observed attempting to chew their way out of confinement. Several tooth fragments were found near the mesh wire walls.

Those groups exposed to the continuous foreign sound did not appear to be disturbed. At times, both during daylight and dark they were seen resting near, or investigating the noise producing apparatus. They were difficult to livetrap.

CHAPTER IV

DISCUSSION

Average Blood Values for Sigmodon

In their study of sigmodon, Meyer and Meyer (1944) indicated that although this rodent adapts well to laboratory conditions that even after five generations in captivity they remained essentially wild animals that were still excitable and easily frightened. If this is true it is doubtful if blood values could be derived from excited, frightened animals. Youatt et al. (1961) presented blood values for the following eight species of wild mammals: opossum, skunk, raccoon, long-tailed weasel, woodchuck, fox squirrel, red squirrel, and cottontail rabbit. They concluded that the blood values reported were undoubtedly influenced by the stress of handling and possibly other undefined environmental stresses.

In their discussion of the alarm reaction, Constantinides and Carey (1949) emphasized the necessity of conditioning and careful handling of small mammals in order to prevent hematological changes. According to Dalton and Selye (1938), it was best to leave animals in the security of livetraps until just prior to anesthetizing and even then the conditioned animals must be removed carefully from the trap so as not to induce changes in the blood picture. These points tend to confirm the findings of Green (1964), namely: that there were no external signs to indicate that confinement to a livetrap was stressing to

the sigmodon. Green's observations further indicate that, for all practical purposes, the sigmodon used the trap sites as feeding stations and made no effort to avoid them.

Whether or not there is a seasonal variation of the standard blood values is a controversial subject. Among a study group of healthy human beings composed of 40 males and 29 females no seasonal variation in the blood count was observed (Engebraith-Holm and Videbaeck, 1948). In contrast, Grunsell (1955) found that blood values for the Scottish sheep varied seasonally. A summer decrease in the hemoglobin level of wild voles is apparent (Newson, 1962). Studies of two populations of wild voles (Newson and Chitty, 1962) indicate seasonal hemoglobin variation with no evidence of a physiological abnormality being present. Sigmodon, harvest mice, and both free-living and captive deer mice showed higher hemoglobin values in winter and spring than in summer (Sealander, 1962). Studies of healthy human beings (Watanabe, 1958) reveal that the packed cell volume or hematocrit rises in the winter reaching a peak in February through March and subsides in the summer to an annual low during the months of July, August, and September. Haurani and Tocantins (1964) found that changes in season and climate may cause a significant, although small, variation in the number of formed elements of the blood.

With these observations in view, reason dictates that standard blood values for sigmodon should be arrived at from both Winter-Spring and Summer-Fall conditioned or non-stressed populations.

The population here referred to as Group 3 was conditioned and sampled and the results for this group are found in Table I. In order to condition this population, great care was taken to eliminate

possible stressing agents from the habitat area. During the months of November and December of 1964, the livetraps were placed at likely sites in the study area. They were concealed under vegetation and baited. Other small mammals which were captured were removed from the area. Predators were in the area and in order that they would not disturb or eliminate the livetrapped sigmodon several skunks, opossums and feral house cats were removed.

The 33 sigmodon sampled represent the adults of the population which presumably were in a state of good health. The blood sample clotted from only one animal from this conditioned group. This may have been occasioned by faulty technique. Of further significance is the fact that no death loss occurred due to sampling in this group. These animals were field-sampled and after recovery from the anesthesia they were transported away from the study area. The hematologic ranges for Group 3 are considerable. Dunaway et al. (1962) noticed that sigmodon leukocyte total counts exhibited wide variations, and they report higher mean values for total RBC, WBC, and hematocrit. These were the only blood parameters they accumulated. It is suggested that the leukocyte values of Dunaway et al. may be higher than those of Group 3 due to stresses.

Onlookers who were familiar with sigmodon could quite easily see that those of Group 3 were quite docile and showed no outward signs of excitation. While trapping and releasing them in the field they gentled to the point of not being in a particular hurry to escape the vicinity of the trap. On several occasions upon release, they would explore around the trap site without evidencing any fear. This behavior is quite opposite from the earlier releases when they would

dart for cover at first opportunity.

Forman (1956) reported blood values from 17 sigmodon of Durham County, North Carolina. The mean erythrocyte count reported is extremely high in comparison to Group 3 (Table I). Forman reports 16.13* as compared to 4.19. The RBC mean value of 16.13 is higher than any of the values obtained for non-stressed or stressed sigmodon of this study. It is also a higher reading than any values reported by Schalm (1965) in his compilation of values of laboratory and fur-bearing animals. The hemoglobin and hematocrit average readings of Forman's report are: Hb 14.61 and Ht 47.63. Again these values are higher than those from Group 3 (Table I). This disparity may be due to any number of reasons, but differences in technique and possible stress may have been influences. In attempting to compare hematologic values of different studies the enigma of emotional and physiological differences is quite readily apparent. Hemoglobin values of 9.7 and 11.6 were reported by Larimer (1959) for sigmodon.

Erythrocyte totals for 465 sigmodon having a mean of 6.89 and hematocrit values for 436 sigmodon with a mean of 44 are reported from the Oak Ridge, Tennessee area (Dunaway and Lewis, 1965). Eleven sigmodon trapped in Georgia and shipped to Oak Ridge were sampled by the aforementioned researchers one week after being trapped and they revealed an erythrocyte mean of 7.08 and a hematocrit mean of 47. These values are much higher than those from Group 3. Additional values for

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*Re: Blood values or parameters
RBC (erythrocyte count) - millions/c.mm.
WBC (leukocyte count) - thousands/c.mm.
Hb (hemoglobin value) - %
Ht (hematocrit) - grams %

sigmodon (Dunaway and Lewis, 1965), list erythrocyte means of 6.59 and 7.01 and hematocrit means at 39 and 40. These hematocrit means are comparable to those of Group 3 although the erythrocyte values are higher. In addition to the explanation offered relative to the blood values for the sigmodon from North Carolina, regional and subspecific differences may contribute to these variations.

In a hematological study of the beaver, Kitts et al. (1958) report a rather complete hemogram. When comparisons are made with the standard values of Group 3 (Table I), the following interpretations seem plausible. Blood values for the beaver are not very different from those of non-stressed sigmodon in reference to hemoglobin, RBC, eosinophils, basophils and monocytes. Mean leukocyte values are much higher, 13.2 as compared to 4.3, for the sigmodon. It is recognized, of course, that values for different rodent species need not be the same. But a difference as great as this suggests the possibility of physiological leukocytosis (Garrey and Butler, 1929) where the leukocytes are elaborated into the circulating blood due to either the animal's physical activity or excitement.

Physiological leukocytosis would not necessarily change the leukocyte differential picture. Therefore, the beaver can be said to be more neutrophilic with its average figure of 63.4 than the sigmodon. The low lymphocytic reading of 29.8 for the beaver is to be expected as neutrophils and lymphocytes generally counterbalance each other. Most animals (Albritton, 1951, Schalm, 1965, and Wintrobe, 1961) are either classed as being neutrophilic or lymphocytic.

Of the more common laboratory mammals, the non-stressed sigmodon, as exemplified by Group 3 (Table I), more nearly resemble the laboratory

rat than others (Albritton, 1951, Gardner, 1947, Schalm, 1965, and Wintrobe, 1961). It is, if anything, slightly less lymphocytic than the laboratory rat.

Group 2 (Table I) has reference to a randomly livetrapped non-conditioned sample of sigmodon. As these animals were livetrapped and blood-sampled at a colder time of the winter and were transported in the back of a truck, they were subjected to greater cold than were those of Group 3. This may well have influenced their blood picture. The eosinophil count was considerably lower for Group 2 than for Group 3. This compares favorably with the results of Louch, Meyer, and Emlen (1953) where they report eosinopenia for mice stressed by reduced temperatures. In a study of the hematological changes in man during cold acclimatization, Bass et al. (1951) found the eosinophil count of the cold stressed were lower than controls on the fourth and eighth days of the test.

Hematocrit parameters for Group 2 and 3 are essentially equivalents; the same thing holds for RBC totals. This is not in agreement with the findings of Sutherland, Trapani and Campbell (1958) in their study of the blood response to cold stress in rabbits. The hemoglobin comparisons of the two groups show a sameness. Griffiths, Calaby and McIntosh (1960) found that cold treatment did not influence this blood value in rabbits. A study of both short and long exposure to cold brought about no significant changes in either the hemoglobin or the RBC values of mouse blood (Stullken and Hiestand, 1954). There was only a slight increase in the total WBC count of Group 2 over Group 3. Group 2 animals were not observed to move around within the livetrap, so physiological leukocytosis due to muscular activity as described by Garrey

and Butler (1929) was not suspected. Even though the Group 2 animals were transported in the back of an open truck they did not appear frightened. That they apparently were not in a high emotional state may be supported to some extent by experiments with cats, where excitement without exercise brought about an increased erythrocyte count (Lamson, 1915). The erythrocyte count of Group 2 was, in fact, slightly lower than that of Group 3, which were purported to be non-stressed animals and therefore acceptable as standards.

The overall averages for the Summer-Fall conditioned sigmodon are included in Table I. Due to vagaries of weather and other aspects of their conditioning they will be discussed as separate groups more extensively. In comparison the means for the combined groups, namely: Group 22 to 28, differ from those of Group 3 in the following manner. The total WBC count is considerably higher. Segmented neutrophil per cent of the leukocyte differential is roughly 13% lower and this is balanced by the lymphocyte count being about the same per cent higher. Eosinophil count is about 2% higher in the Summer-Fall aggregate than in Group 3 and this rise is counterbalanced by a 2% decrease in monocyte count.

A preliminary statement relative to the separate groups of Summer-Fall conditioned sigmodon (Table II) seems appropriate. During the early summer exploratory livetrapping was done in about all of the possible Sigmodon hispidus habitat within the environs of Payne County, Oklahoma. The sigmodon population declined abruptly in April of 1965 and a suitable non-captive population was not located. There seems to be a regular yearly drop in numbers of sigmodon, and they have an apparent cycle based on several years (Goertz, 1962, Green, 1964,

Komarek, 1937).

At the area where Group 3 (Table I) conditioned animals were trapped, a remnant population of about five animals was found. These were livetrapped and released for 10 days and only three were successfully blood-sampled. These are Group 22 (Table II) which represents the total of the Summer-Fall conditioned sigmodon taken directly from the field. The number being inadequate, there seems little advantage in making any comparisons of Group 22, except as it is a part of the overall Summer-Fall conditioned or non-stressed aggregate.

Lacking a population in the field to condition and sample, the alternative was to condition samples of populations that were in the enclosures. Sigmodon within the enclosures were either those that had been livetrapped and enclosed in 1964, or their offspring.

Group 23 (Table II) was a docile group with which to work. The weather changed little during August 1965, which was the conditioning period. The 14 animals sampled were all females except two, and none of the females had open vulvae nor did any appear to be pregnant. Both males had ascended testes and appeared to be sexually inactive. All of the animals weighed 80 grams or more and one of the males weighed 175 grams. During the conditioning period no mating behavior was evident. None of the members of the group exhibited any scars or bobbed tails as evidence of strife within the group. In comparing any of the groups conditioned for Summer-Fall with those conditioned for Winter-Spring, the most evident differences are higher total leukocyte counts and a more lymphocytic and less neutrophilic differential picture.

Group 24 (Table II) was from a population very similar to Group 23, sampled one day later on August 24, 1965. There were six males

and five females, but one male died after being anesthetized and was not sampled. This group, with even numbers relative to sex, had no females with open vulvae nor males with descended testes. As compared to Group 23, they were less lymphocytic.

The next population, Group 25, was the first one where the larger livetraps were used. They were sampled a day later than the previous group. It seems readily apparent that the "large trap effect" is real, based both on observation and hematologic data. Two of the larger traps were placed near the west side of the enclosure. For more than two hours before the sigmodon were removed from the livetraps they could be heard moving about in these larger traps. Every time the captive sigmodon would cross over the trap treadle pan a very distinct metallic sound was heard, suggesting moving about on the part of the captive. The sigmodon were also seen to be constantly moving around the interior of the trap. In direct opposition, those in the smaller traps huddled into one end of the trap and appeared to be resting. The blood pictures were markedly different only in total leukocyte count, suggesting physiological leukocytosis as described by Garrey and Butler (1929) and discussed further by Wintrobe (1961).

The conditioned Group 26 (Table II) was also made up in part of those sigmodon trapped in the larger traps. They were sampled about a month later than Group 25 and were unique in that on the evening before sampling the area experienced a very hard rain. Traps were not set that evening as the possibility of drowning sigmodon in the traps was quite high. After a night of violent storm, with very pronounced lightning and thunder, the rain ceased and the traps were re-baited and set at 4:00 AM. The animals captured in the large traps were very

active and this group shows the highest mean total leukocyte value of any of the groups sampled from the larger traps.

Group 27 (Table II) was one comparable to Groups 25 and 26 as some of the sigmodon were also livetrapped in the larger traps and the results were similar in regard to both behavior and the blood picture. It may be noteworthy that the mean and upper range hematocrit values were higher in this group than for any of the Summer-Fall conditioned groups. This correlated with higher hemoglobin and total erythrocyte mean values. Analyzing the leukocyte differential, one finds the lowest neutrophil and eosinophil counts and, as would be expected, the highest lymphocyte count.

The sigmodon manifesting the "large trap effect", 15 in number, certainly seemed to evidence physiological leukocytosis due to muscular activity. According to Cartwright, Athens and Wintrobe (1964), granulocyte movement is uni-directional from marrow to tissues, and they do not return to the blood stream from the tissues. Therefore, physiological leukocytosis seems explicable due to an increase in muscular activity. Leukocytes that have been sequestered within resting capillary beds then are flushed into the larger blood vessels (Schalm, 1965).

The recognition of the "large trap effect" may give reason for not using any of those groups as being strictly representative of standard values for the Summer-Fall period. The increase in leukocytes has little, if any, influence on the differential count (Schalm, 1965, Wintrobe, 1961). The utilization of the two sizes of livetraps and the concurrent blood picture association may serve to illuminate an apparent controversy. Golley (1961) observed that those sigmodon which had been trapped and released had larger adrenal glands than those which

had not been previously trapped. In his opinion, this enlargement was due to stress associated with livetrapping. Green (1964) gives observations to the contrary and suggests that livetrapping is not stressful. Perhaps in light of the findings reported in Table II, an assumption might be put forth that the size of the livetrap relative to the size of the individual sigmodon may determine to a large extent whether or not the livetrapping is a stressful experience to the rodent.

Group 28 (Table II) was conditioned in September and sampled on day 28 of that month. Observations by the author suggest that this group was the most successfully conditioned group of the Summer-Fall conditioned aggregate. This suggestion is thusly supported; none of the animals involved were captured in the larger traps, and the sigmodon were more easily trapped than those groups trapped a month earlier, for which the rolled oats seemed less of an attractant. In fact, avoidance of traps was observed in Groups 24 and 25. Apparent trapping success, combined with external signs of docility and non-excitation, along with previously mentioned factors, lead to the selection of Group 28 as the group with which those groups that were stressed during the summer might best be compared.

Resamples Stressed Due to Crowding

One dozen sigmodon were selected from the conditioned Group 3 to be a part of a stressing and resampling by crowding experiment. While under anesthesia these rodents were numbered by a toe-clip method and after a recovery period were released into an enclosure. There they became an integral part of the existing population being stressed by crowding. As the crowded groups were livetrapped and sampled, these

previously sampled sigmodon were resampled and their hematologic data grouped. They appeared healthy when resampled and until their toes were checked could not be singled out from the rodents which had not been previously blood-sampled. One of the original died during the cardiac puncture. The 11 survivors (Group 6a, Table II) when compared against their previous conditioned blood values (Group 3a, Table III) present values of some interest. Only slight differences are seen between the two groups when comparing hematocrit, hemoglobin and total erythrocyte parameters. Total leukocyte value shows an increase of about 46% for Group 6a over Group 3a. Differences in neutrophils show slight increase in the segmented forms and a great increase in the band forms. This lends itself to possible explanation. The band forms may represent a response to a stress, as Fliedner et al. (1964) found that band forms are apparently elaborated into the circulating blood about 24 hours earlier than segmented forms. The eosinophil count for Group 6a is more than twice that of Group 3a, which suggests a response to histamine or decomposition of tissue protein (Schalm, 1965). The sigmodon that were resampled without exception showed very scarred skin. This is a representative picture of all of the sigmodon which experienced crowding. This manifested eosinophilia may then, in part, have been due to the wounding by fighting and toe-clipping, since some of the tissue associated with the toe-clip appears to decompose before healing and this may also be true of the combat wounds.

There was a decrease in basophils from some in Group 3a to none in Group 6a. Selye (1965) reports that all severe stressors decrease the basophil count. The lymphocyte count of Group 6a is about 22% lower than Group 3a, suggesting a reaction to the stress of crowding

as DeGroot and Harris (1950) suggest lymphopenia as a common response in rabbits to emotional stress.

From this group of sigmodon supposedly free of disease and parasites, it can first be declared that the blood pictures of the same animals will change as emotional and environmental influences change with the passage of time.

Groups Stressed by Crowding

Sigmodon were subjected to the stress of crowding from April through June of 1965. Groups 5, 6, 7, and 12, whose hematologic parameters are given in Table IV, will be discussed as individual groups and compared to Group 3, as a standard for Winter-Spring animals.

When the sigmodon that constituted Group 5 were released into the enclosure, they were free from prominent scarring and none of them had lost a segment of their tail. During the month prior to sampling, these sigmodon were observed frequently to engage in combat. Without question some of this was associated with regular reproductive behavior, but the assumption seems valid that social crowding, as it is spoken of by Southwick and Bland (1959), was also a combat inducing factor. Of the 25 sigmodon constituting this group, only three large females weighing 160, 175, and 225 grams respectively, were unscarred. Five had bobbed tails, one had lost toes from a hind foot and another had lost the lower part of its left leg, the healed stump remaining. Torn ears and scars in the rump region were common. None of those sampled had unhealed wounds at the time of sampling. Many were live-trapped and released unsampled due to unhealed wounds. In comparing the blood picture of Group 5 with that of Group 3 an increase in

neutrophils, especially the band form, is most noticeable. This is accompanied by a decrease in lymphocytes. This is comparable to the general reaction of rabbits to emotional stress (Colfer, DeGroot and Harris, 1950). Of all of the crowded groups, Group 5 was crowded for the shortest duration.

Group 6 (Table IV) was sampled after having been subjected to natural crowding about two weeks longer than Group 5. The blood picture of this group when compared with Group 3 depicts an increase of about 43% in the total leukocyte count, almost a fourfold increase in band neutrophils and about a 20% decrease in lymphocytes. This shift in the blood picture is generally accepted as being indicative of stress. The eosinophil count of Group 6 is about three times greater than that of Group 3. This eosinophilia is in direct opposition to the eosinopenia usually associated with the increased production of ACTH and glucocorticoids (Selye, 1965). An alternate explanation comes from the reaction of C57BR mice to behavioral disturbances (Southwick, 1959). These mice would experience an eosinopenia of 80% four hours after being stressed. Eosinopenia remained at about the same level for two days and then gradually the eosinophil numbers increased. Possibly the sigmodon of Group 6 and those of others that have been subjected to stress for longer periods of time had experienced eosinopenia but at the time of sampling the eosinophils were either on the upswing or had reached a higher level of concentration in the circulating blood.

It appeared that as a whole, Group 7 sigmodon were younger and less aggressive than those of Group 6, although they were from the same enclosure and were sampled only a day later. The average weight

of the rodents in this group was 119 grams as compared to 147 grams for those of Group 6. This group is the only one subjected to crowding to have an eosinophil count lower than Group 3, the group which serves as the basis for standard values. They bore no scars and this perhaps supports the previous supposition that the eosinophilia of Groups 5 and 6 were related to the processes of wounding and healing. None of the males of Group 7 had descended testes. In performing the anesthetizing and intracardial puncturing it seemed that the skins of the sigmodon in Group 7 were more elastic and had the greater tendency to cling to the hypodermic needles. Even though these were mature sigmodon they certainly appeared to be younger and less aggressive. It is noteworthy and also unexplained that this group was the most lymphocytic of any of the groups exposed to crowding. Their lymphocytic count was even higher than that for Group 3.

Crowding stress was experienced by the Group 12 sigmodon for about one month longer than for Group 7, and for about three months longer than Group 5. If the stress of crowding for lengthier periods of time manifests itself in the blood picture and if a high hematocrit reading is a stress indicator, then this group was the most stressed, as they had the highest mean hematocrit reading of any group in the entire study. Baboons kept confined were found to have a higher hematocrit reading than those just coming in from the bush (Foy, Kondi and Mbaya, 1965). Group 12 also had the highest hemoglobin averages of any group sampled. This is contrary to the findings of Newson (1962) where hemoglobin values for wild voles decreased as the year progressed from spring into summer. Erythrocyte totals differed little from those for Group 3. In viewing the total leukocyte count, it is

approximately 45% higher than for the standard value group. The studies of Garrey and Butler (1929) indicate an associated leukocytosis accompanying psychic or emotional stress in man.

In contrast to Group 5 and 6, the sigmodon of Group 12 were unscarred except for scrotal scars on one male. The males of this group all had descended testes.

The differential leukocyte picture portrays the highest eosinophil count, namely: 12%, of any group stressed or conditioned. In healthy mice, mild stress produced a great decrease in the number of circulating eosinophils (Speirs and Meyer, 1949). Eosinopenia lasted for about seven hours and was followed by a period of eosinophilia. By increasing the amount of stress, a greater decrease in eosinophils occurred lasting for a longer period of time.

A high monocyte count is again without explanation. Wintrobe (1961) discusses a high monocyte count in relationship to a chronic inflammatory condition, but no evidence of chronic inflammation was observed in these sigmodon.

Groups Stressed by Lack of Cover

As these groups were stressed and sampled during the summer they will be compared to Group 28 of Table II, which will serve as a standard.

When livetrapping sigmodon in the field they have on occasion been taken to an area devoid of cover and released. This situation plainly causes them anxiety as they often "freeze" as soon as they contact the ground and remain "frozen" with eyes bulging until they are forced to move. Their other reaction is a panicked dash for the nearest cover. Stoddard (1931) found that sigmodon appear to be dependent for their

existence upon a thick screen of protective vegetation. Goertz (1964) provided information to support Stoddard's findings by way of measured values.

The animals to be stressed by lack of cover seemed to adapt readily to the small 6 by 10 by 10-inch coardboard boxes that were placed on the concrete floor of the pen in which they were released. A small opening that had been cut in the side of each box was readily discovered by the releasees and they entered and accepted the boxes as cover. After a few days they seemed to have adjusted to their new environment.

The boxes were removed and this disquieted the sigmdon. Group 8 (Table IV) contained only one male. Why a preponderance of females were livetrapped the first time remains obscure. The vulvae of the females were closed. During their 24 hours without cover, no combat was observed. This may have been due to the lack of sufficient density or to the loss of a home to defend. The noteworthy facet of the blood picture of Group 8 was the high band neutrophil count associated with a segmented neutrophil count quite similar to Group 28 (Table II). This along with slight eosinopenia and complete basopenia suggests that they were stressed (Selye, 1965). An unaccountably high monocytosis was also evident.

Group 9 sampled after 48 hours without cover revealed a blood picture wherein the band neutrophils had continued to increase extensively in the circulating blood. Eosinophils also increased to a level a little above the standard value. Basopenia, a sign of stress, is still evident. There is a reduction in monocyte count as compared to Group 8, which is not far removed from the standard.

After 72 hours without cover, the rodents labeled Group 10 were sampled. Observation of this group indicated that they were less mobile than they were when cover was first withdrawn. Although the hematocrit, hemoglobin, and erythrocyte values are slightly higher than those of Group 28, the standard, they vary from Groups 8 and 9 only slightly. Looking at the leukocyte differential, continued basopenia, an additional decrease in eosinophils and lymphocytes, as compared to the values for Groups 8 and 9, a stress reaction shift in the blood picture is suggested. Using lymphopenia as a criterion, the consistent decrease from Group 8 through Group 10 may also be indicative of stress.

The death loss that occurred, which eliminated the large males with descended testes from the originals of Group 15, certainly must have some significance. However, this study fails to bring any light to bear upon the failure to survive except that combat was observed. This agrees with the findings of Warnock (1965) in a study of meadow voles crowded and deprived of cover and water. Among females there was a low frequency of social conflict and a high survival time. Just the opposite was observed among males. Of the sigmodon that survived it can be surmised that they had been able to survive the alarm reaction period (Selye, 1950) as they compare more favorably with the standard group than those stressed for shorter periods of time.

Groups Stressed by Lack of Cover and Continuous Light

The blood picture comparison of Group 16 with the standard, Group 28, shows no great difference is erythrocyte parameters. Comparing the leukocyte totals points up extreme leukocytosis, in fact,

the highest average leukocyte value for any group in the study. The leukocytosis could have been due to the combined effects of mouth irritation, the constant muscular activity and emotional pressure. The differential blood picture is one of neutrophilia, slight eosinopenia and lymphopenia. The blood of two animals of this group clotted.

The activity of Group 17 sigmodon had decreased during the second night of illumination. Again the erythrocyte picture reveals little change. The leukocyte total count dropped considerably, but was still much higher than the standard value. The differential shows increased neutrophilia, eosinopenia, and lymphopenia accompanied by complete basopenia. If the blood picture shift is one of a reaction to stress, then the stress is even more evident in Group 17 than in Group 16. Blood clotted from only one sigmodon in this group.

Only four samples make up Group 18. Six additional sigmodon of this test group were livetrapped but clotting occurred while cardiac puncturing three of them and the drawn blood of the remaining three clotted before it could be analyzed. This may be greater evidence of stress than the blood parameters, as Selye (1950) indicates that emotional excitement greatly accelerates blood-clotting due to adrenalin influence. The blood picture of the four, who were all females, shows leukocytosis greater than Group 17 but less than Group 16. The neutrophil-lymphocyte relationship is nearly that of the standard, Group 28. However, eosinopenia is greater and basopenia complete. The monocyte count was lowest of the entire study and this is contradictory to a chronic inflammatory condition. For all three of these groups the daylight period seemed to be a time of less stress. Even though they were without cover they were much less active and spent considerable

time just huddled together. Then when night would come again they would try to escape to the darkness. The sigmodon of these groups were livetrapped without bait. They filled the open traps rapidly. This reinforces a previous assumption that the livetraps served as cover.

Groups Stressed by Food Insufficiency

The sigmodon of Groups 11, 13, and 14 (Table IV) from each of which food was withdrawn seemed little disturbed. They were observed drinking water quite often and were also seen gnawing at the watering troughs. Most of the time they spent inside their boxes. At morning and evening they foraged about unsuccessfully and then returned to cover. Strecker and Emlen (1953) suggest that for house mouse populations food shortage was a greater stressor than cold. The sigmodon of Group 11 showed increase in all of the erythrocyte values. This compares favorably with a study of Hazelwood and Wilson (1962) who found that rats subjected to food withdrawal experienced a hematocrit increase. The increase in the leukocyte total count is not sufficient to suggest physiological leukocytosis. A general increase in neutrophils suggests stress with the band neutrophil increase being especially high. Only Group 9 (Table IV) had as marked an increase. The means for this parameter were identical in Groups 9 and 11. The eosinophil count is less than that of the standard. Best and Samter (1951) indicate that in man, fasting subjects show a continuous eosinophil decrease during the first 10 hours of fasting, which was the limit of their test. The basopenia of Group 11 is complete and is associated with lymphopenia and monocytosis. Group 13 had a hemogram differing

little from Group 11 except that the neutrophilia, eosinopenia and lymphopenia continue in the direction suggesting stress. This follows the pattern of blood picture changes reported by Lawrence and Josey (1932) when they subjected guinea pigs to starvation. The skin was very loose on all of the animals of this group, and only four sigmodon survived the anesthesia and cardiac puncture.

Some readily apparent changes occurred in the time lapse of 48 hours that separated Group 14 from Group 13. The leukocyte total is identical to that of Group 28, the standard value group. This should not represent a return to normality but rather be indicative of a trend toward leukopenia. The eosinopenia has continued to decrease suggesting stress, but basophils occur for the first time and this is unexplainable, at least from the standpoint of the current study. The lymphocyte trend has reversed, but is not back to standard values. The reason for this is obscure.

In an attempt to establish correlation between this sigmodon study and other mammals, one finds a quandary in regard to hematologic studies of deer in relationship to starvation. At near starvation, hemoglobin and total RBC dropped (Rosen and Bischoff, 1952). In contrast, Kitts et al. (1958) found that near starvation did not affect hematocrit or hemoglobin values of the Columbian Black-tailed Deer. A leukocytosis in white-tailed deer fawns due to malnutrition is reported by Teeri et al. (1958) and this is more in agreement with the present sigmodon findings.

Group 14 was a most difficult group to sample. Two of the sigmodon expired under anesthesia prior to cardiac puncturing. Loose skin and what appeared to be low blood pressure made blood withdrawal

difficult. There were no survivors from this group. This was in contrast to several groups where all survived, especially among the conditioned or non-stressed groups. It was surprising that these animals lost weight and weakened so rapidly. Being indicative of a high metabolism, this may in part, explain the rapid decline in numbers of sigmodon that have been observed in the field, especially during late winter and early spring in this geographical region.

Groups Stressed by Continuous Foreign Sound

Sigmodon of Groups 19, 20, and 21 (Table IV) did not show any avoidance reaction to the source of sound. Often they were seen attempting to climb onto the apparatus, and when they would succeed they tried to gnaw through the wood and plastic protective cover. Three of the group had broken upper incisors.

The sound was of such quality that observation was a nerve wracking experience unless wax ear plugs were used. Only five animals are included in Group 19. Eleven sigmodon were livetrapped. The blood films of three of these individuals stained poorly, and were considered unreliable. The blood of three others clotted prior to being analyzed. The hematocrit and hemoglobin means for this group are low and this is largely due to the influence of one male sigmodon whose parameters, already mentioned, were the lowest of any animal in the study. His hematocrit reading of 25.0 and hemoglobin of 8.0 remain unexplained. Sealander (1961) found that botfly infection in deer mice significantly reduced hematocrit and hemoglobin values so this type of infestation could be suspicioned. This male weighed 113 grams, was healthy in appearance and no external or internal parasites

were observed. No evidence of hemorrhage or anemia was found. The total leukocyte count mean was slightly above that of the standard value of Group 28. The rest of the parameters varied little from those of the standard.

With the exception of erythrocyte and eosinophil values, Group 20 varied little from the standard values. However, with the erythrocyte values manifesting an increase, excitement may be a causative. Larimer (1915) found that excitement without strenuous exercise brought about high erythrocyte counts in cats. The eosinopenia was greater than in Group 19 and is indicative of stress (Selye, 1965). Again, as in the previous group, three animals had broken off their upper incisors. Whether or not this is evidence of being stressed is strictly speculative, but this evidence did not occur in any of the conditioned or non-stressed animals.

A further increase in erythrocyte values is seen in Group 21. The total leukocyte count also rises and the eosinophil count decreases. Therefore, Group 21, although not showing any spectacular changes, deviates from the standard values of Group 28 more than any group experiencing continuous foreign sound. Blood samples from two of the animals clotted prior to analysis. Contrary to the two preceeding groups no sigmodon of Group 21 had broken upper incisors.

Sex Comparison of Sigmodon Hematologic Parameters

Blood values for penned sigmodon in Tennessee depict higher mean erythrocyte and leukocyte totals and hematocrit values for males; no other parameters were reported (Dunaway et al., 1962).

Average or standard values for 50 females and 54 males are given

in Table V and VI. None of the females were sampled in advanced stages of pregnancy or while lactating. Difference in the blood pictures are practically nil, except for the leukocyte differential. The males have a slightly higher mean neutrophil count and the females a slightly higher lymphocyte mean value.

In the comparison of change of the blood pictures due to the stresses used in this study, certain possibilities exist. Male sigmodon experienced a marked hematocrit increase, which suggests that from the standpoint of this parameter they show a greater response to having been stressed or else they are actually more emotionally disturbed by the stressors than were the females. For all of the erythrocytic values, the males show an increase of stressed values over standard values. The females by comparison depict a slight decrease in their stressed erythrocytic values as compared to their standard values. One can surmise that they were either little stressed or that their blood picture response to stress is exactly opposite of males. The literature offers little to support these conjectures. Stressed values of the males for leukocyte total show a greater increase than for females. In viewing the leukocyte differential picture evidence of stress is apparent in the increase of band neutrophils. This parameter is higher for females than for males, indicating again a greater response to stressors. Eosinopenia occurs in both sexes in response to stress and is greater for the males. Basophil and monocyte relationships offer little for comparison or speculation. The females react to stress in the direction of lymphopenia much more than do the males. The males show very little change.

A unique hematological study of four bottlenose dolphins stressed by being out of water for 36 hours during transportation showed a neutrophilia and eosinopenia, without the usual lymphopenia; no noticeable sex differences were in the hemogram values (Medway and Geraci, 1964). The paucity of data from other studies makes comparisons with sigmodon and the interpretation of sigmodon hemogram results very indecisive.

Selye (1950) infers that hypercoagulability is stress related. In this study clotting and difficult blood withdrawal affected 15% of the males sampled as compared to 10.5% of the females.

Comparisons of Hematologic Parameters for Sigmodon Under Conditions of Stress and Non-Stress

Although the new evidence offered in this report is not overwhelming, the result of a comparison of all stressed with all conditioned sigmodon is in agreement with the general consensus of evidence from the literature. The blood picture for the stressed animals is as would be expected, with the exception of the basophil count. The basophil numbers are so few and the difference so slight that in this instance the parameter cannot be given much reliability. Otherwise, the lymphopenia, eosinopenia, and neutrophilia accompanied by a slight general leukocytosis is indicative of stress. This study further suggests the need to accumulate additional information relative to stress and hematologic reaction in wild animal populations.

Analysis of Blood Parameters by Duncan's Multiple-Range Test

The means of the ten blood parameters ascertained from sigmodon

were compared by Duncan's multiple-range test (Steel and Torrie, 1960). This test lends itself to the comparison of each sample mean with every other sample mean. Table VII through XVI in the Appendix show these analyses graphically. By observing the relationships of the means in the tables associated with the statistical analysis it appears that crowding was the most stressful condition experienced by the sigmodon. This observation is compatible with Christian's (1961) conclusion that among many mammals increased numbers are stressful. Southwick and Bland (1959) found that increased density was a greater stressor than fighting among mice when considered on the basis of adrenal weights. In his discussion of microtine cycles, Frank (1957) suggests that crowding helps to produce a readiness for population declines.

This statistical analysis indicates that there are differences among the means of the various hematologic parameters of all of the sigmodon groups in the study. When parameters of stressed groups were compared with like parameters of groups selected as standards, a difference indicates that induced environmental stresses did manifest themselves in the animal's average hemogram. In only a few instances was this difference statistically significant at the 95% level. This does not negate the obvious, namely: that in many of the groups the parameter inclination is in the direction indicative of stress. Until more highly refined methods are developed and a greater amount of stress-hematologic information is collected, it will be difficult to assay blood parameter variations with a high degree of specificity relative to so-called stress.

CHAPTER V

SUMMARY AND CONCLUSIONS

This study has principally been one of dual-purpose. First, it undertook to develop an array of hematologic parameters for Sigmodon hispidus texianus, and from these data to establish standard values locally for the species. Secondly, it compared hemograms of sigmodon subjected to induced environmental stresses with those hemograms of the same species that were conditioned or non-stressed. These goals were accomplished.

Overt behavior of the species being studied was also observed and reported. Sigmodon was selected as an experimental wild animal because it is large enough to produce a sufficient quantity of blood by intracardial puncture for a routine hemogram. In addition, this species is found throughout much of the south and southwest and is subject to dramatic population changes. The possibility that the hemogram might mirror the blood parameter response to an induced emotive aspect of the environment was investigated. Hematologic data were collected from two general categories of animals, the non-stressed and the stressed.

A total of 337 blood samples were obtained from mature animals whose weight range was from 49 to 280 grams. Different groups were subjected to the following stresses: exposure to cold weather, crowding, lack of cover, lack of cover and continuous light, food

insufficiency, and continuous noise. The hematologic response to livetraps of the same design, but of two different sizes, was investigated and found to be significantly different. Blood taken from males was more prone to clot than that from females. Survival of females under the influence of stress was higher than for males. Conflict and low survival were characterisitic of the males, especially those which were sexually active.

No blood parasites were seen. Polychromasia was common and poikilocytosis was not observed. Reticulocytes, commonly associated with anemia, were observed in the blood of only one individual. The proportion was less than one per cent of that animal's erythrocytes.

On the basis of the leukocyte differential, these animals tend to have more lymphocytes, by about 28%, than neutrophils when in a state of good health.

Of those groups subjected to specific stress, crowding due to natural increase, lack of cover, and lack of cover and continuous illumination led to a change in the banded neutrophil-lymphocyte balance favoring the former cells. These stresses also brought about changes in their overt behavior.

It is not outside the realm of practicability that blood-sampling in the field may apprise us not only of a possible pathogenic condition in a population, but of its emotional health as well. Sigmodon reflected stressful environmental conditions with changes in the hemogram. While more data are needed before patternicity of the leukocyte differential shift can be adequately analyzed, and response to induced environmental stresses reliably predicted and interpreted, an inverted

neutrophil-lymphocyte ratio, on the basis of this study, appears diagnostic of stress. Population decline may be expected as the prognosis of this condition. This study further supports the proposition that both high density and lack of cover are major population hazards.

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A P P E N D I X

TABLE VII

COMPARISONS OF HEMATOCRIT OR PACKED CELL VOLUME MEANS BY DUNCAN'S MULTIPLE-RANGE TEST[a]

Group	N	Means
19	5	38.600
16	9	38.611
17	10	38.950
24	10	39.550
28[b]	10	39.900
09	7	40.000
23	14	40.036
26	11	40.091
18	4	40.250
05	25	40.320
03[c]	21	40.405
02	15	40.500
08	10	40.900
15	6	41.500
10	9	41.611
25	13	41.615
27	10	42.150
22	3	42.167
14	8	42.188
20	8	42.313
13	9	42.722
11	7	43.000
21	9	43.167
07	7	43.214
12	8	44.375

[a]Any two means not sidescored by the same line are significantly different (d.f. = 223; $p < 0.05$). Any two means sidescored by the same line are not significantly different.

[b]Used as a Summer-Fall standard value group.

[c]Used as a Winter-Spring standard value group.

TABLE VIII

COMPARISONS OF HEMOGLOBIN MEANS BY DUNCAN'S MULTIPLE-RANGE TEST[a]

Group	N	Means
17	10	11.900
19	5	11.900
16	9	12.000
23	14	12.071
24	10	12.150
22	3	12.167
28[b]	10	12.400
26	11	12.455
25	13	12.462
05	25	12.500
18	4	12.500
20	8	12.688
03[c]	21	12.810
21	9	12.944
02	15	13.000
15	6	13.167
27	10	13.200
08	10	13.300
09	7	13.357
10	9	13.500
14	8	13.500
07	7	13.571
13	9	13.833
11	7	13.857
12	8	14.313

[a]Any two means not sidescored by the same line are significantly different (d.f. = 223; $p < 0.05$). Any two means sidescored by the same line are not significantly different.

[b]Used as a Summer-Fall standard value group.

[c]Used as a Winter-Spring standard value group.

TABLE IX

COMPARISONS OF ERYTHROCYTE (RBC) MEANS BY DUNCAN'S MULTIPLE-RANGE TEST[a]

Group	N	Means
17	10	3.608
16	9	3.688
19	5	3.728
18	4	3.737
09	7	3.742
24	10	3.752
23	14	3.827
15	6	3.855
26	11	3.856
28[b]	10	3.880
14	8	3.930
02	15	3.948
10	9	3.957
25	13	3.978
20	8	3.978
08	10	3.991
11	7	3.994
21	9	4.033
22	3	4.063
27	10	4.096
03[c]	21	4.115
07	7	4.122
05	25	4.125
12	8	4.268
13	9	4.308

[a]Any two means not sidescored by the same line are significantly different (d.f. = 223; $p < 0.05$). Any two means sidescored by the same line are not significantly different.

[b]Used as a Summer-Fall standard value group.

[c]Used as a Winter-Spring standard value group.

TABLE X

COMPARISONS OF LEUKOCYTE (WBC) MEANS BY DUNCAN'S MULTIPLE-RANGE TEST[a]

Group	N	Means
03[b]	21	4366.000
02	15	4613.000
28[c]	10	5000.000
14	8	5031.000
15	6	5358.000
13	9	5705.000
05	25	5746.000
20	8	5837.000
08	10	5995.000
09	7	6042.000
10	9	6066.000
23	14	6110.000
11	7	6271.000
24	10	6350.000
07	7	6571.000
22	3	7483.000
12	8	7693.000
19	5	8110.000
21	9	8188.000
27	10	8190.000
17	10	8640.000
25	13	9453.000
26	11	10300.000
18	4	10375.000
16	9	13816.000

[a]Any two means not sidescored by the same line are significantly different (d.f. = 223; $p < 0.05$). Any two means sidescored by the same line are not significantly different.

[b]Used as a Winter-Spring standard value group.

[c]Used as a Summer-Fall standard value group.

TABLE XI

COMPARISONS OF BAND NEUTROPHIL MEANS BY DUNCAN'S MULTIPLE-RANGE TEST[a]

Group	N	Means
23	14	.070
24	10	.100
27	10	.100
28[b]	10	.100
26	11	.180
18	4	.500
25	13	.620
03[c]	21	.810
20	8	.880
12	8	1.250
16	9	1.330
02	15	1.470
19	5	1.600
07	7	1.710
21	9	1.780
17	10	1.800
05	25	2.120
22	3	2.670
08	10	2.700
14	8	2.880
15	6	3.170
10	9	3.440
13	9	3.670
09	7	5.570
11	7	5.570

[a]Any two means not sidescored by the same line are significantly different (d.f. = 223; $p < 0.05$). Any two means sidescored by the same line are not significantly different.

[b]Used as a Summer-Fall standard value group.

[c]Used as a Winter-Spring standard value group.

TABLE XII

COMPARISONS OF SEGMENTED NEUTROPHIL MEANS BY DUNCAN'S MULTIPLE-RANGE TEST[a]

Group	N	Means
27	10	21.300
23	14	21.930
02	15	23.070
28[b]	10	24.000
08	10	24.700
09	7	25.000
19	5	25.000
20	8	25.130
21	9	26.000
12	8	26.380
26	11	27.270
18	4	28.000
24	10	28.400
14	8	29.000
15	6	30.170
07	7	30.570
25	13	31.080
11	7	34.000
10	9	36.330
16	9	37.110
13	9	37.560
22	3	39.330
17	10	39.400
03[c]	21	40.810
05	25	44.760

[a]Any two means not sidescored by the same line are significantly different (d.f. = 223; $p < 0.05$). Any two means sidescored by the same line are not significantly different.

[b]Used as a Summer-Fall standard value group.

[c]Used as a Winter-Spring standard value group.

TABLE XIII

COMPARISONS OF EOSINOPHIL MEANS BY DUNCAN'S MULTIPLE-RANGE TEST[a]

Group	N	Means
02	15	1.000
18	4	1.250
14	8	1.880
07	7	2.000
21	9	2.440
17	10	2.700
20	8	3.000
16	9	3.330
13	9	3.560
10	9	3.670
03[b]	21	3.760
08	10	4.300
15	6	5.170
19	5	5.200
11	7	5.290
27	10	5.400
25	13	6.080
28[c]	10	6.500
05	25	6.720
24	10	6.900
23	14	7.000
26	11	7.360
09	7	7.860
22	3	10.000
12	8	12.130

[a]Any two means not sidescored by the same line are significantly different (d.f. = 223; $p < 0.05$). Any two means sidescored by the same line are not significantly different.

[b]Used as a Winter-Spring standard value group.

[c]Used as a Summer-Fall standard value group.

TABLE XIV

COMPARISONS OF BASOPHIL MEANS BY DUNCAN'S MULTIPLE-RANGE TEST[a]

Group	N	Means
08	10	.000
09	7	.000
10	9	.000
11	7	.000
12	8	.000
13	9	.000
17	10	.000
18	4	.000
19	5	.000
20	8	.000
21	9	.000
22	3	.000
24	10	.000
25	13	.000
26	11	.000
27	10	.000
28[b]	10	.000
23	14	.070
16	9	.110
03[c]	21	.140
07	7	.140
15	6	.170
05	25	.200
14	8	.250
02	15	.470

[a]Any two means not sidescored by the same line are significantly different (d.f. = 223; $p < 0.05$). Any two means sidescored by the same line are not significantly different.

[b]Used as a Summer-Fall standard value group.

[c]Used as a Winter-Spring standard value group.

TABLE XV

COMPARISONS OF LYMPHOCYTE MEANS BY DUNCAN'S MULTIPLE-RANGE TEST[a]

Group	N	Means
05	25	41.920
22	3	46.000
13	9	48.560
11	7	49.000
03[b]	21	49.520
10	9	52.560
12	8	54.000
17	10	54.100
16	9	56.440
15	6	56.830
09	7	57.430
25	13	58.540
24	10	59.900
14	8	60.250
07	7	60.710
26	11	60.910
08	10	61.500
19	5	64.400
28[c]	10	65.700
21	9	66.330
23	14	67.710
18	4	69.250
02	15	69.330
20	8	69.630
27	10	70.400

[a]Any two means not sidescored by the same line are significantly different (d.f. = 223; $p < 0.05$). Any two means sidescored by the same line are not significantly different.

[b]Used as a Winter-Spring standard value group.

[c]Used as a Summer-Fall standard value group.

TABLE XVI

COMPARISONS OF MONOCYTE MEANS BY DUNCAN'S MULTIPLE-RANGE TEST[a]

Group	N	Means
18	4	1.000
20	8	1.380
16	9	1.780
17	10	2.000
22	3	2.000
27	10	2.900
23	14	3.290
21	9	3.440
28[b]	10	3.700
19	5	3.800
25	13	3.850
10	9	4.000
05	25	4.320
26	11	4.450
15	6	4.500
02	15	4.670
24	10	4.800
07	7	4.860
09	7	4.860
03[c]	21	5.050
14	8	5.880
12	8	6.130
11	7	6.140
13	9	6.670
08	10	7.000

[a]Any two means not sidescored by the same line are significantly different (d.f. = 223; $p < 0.05$). Any two means sidescored by the same line are not significantly different.

[b]Used as a Summer-Fall standard value group.

[c]Used as a Winter-Spring standard value group.

VITA

Joseph Leland Hepworth

Candidate for the Degree of

Doctor of Philosophy

Thesis: HEMATOLOGY OF SIGMODON HISPIDUS: AVERAGE PARAMETERS COMPARED WITH THOSE UNDER INDUCED STRESSES

Major Field: Zoology (Animal Ecology)

Biographical:

Personal Data: Born near Malta, Idaho, November 8, 1925, the son of Joseph and Lona Chandler Hepworth.

Education: Attended elementary school in Elba, Idaho; graduated from Raft River High School in 1942; received the Bachelor of Science degree from Utah State University, in June, 1950; received the Master of Science degree from the University of Idaho, in June, 1962; completed requirements for the Doctor of Philosophy degree in May, 1966.

Professional Experience: Enlisted in the Army Air Corps as an aviation cadet in 1943, and was honorably discharged in 1946; was a distinguished military graduate from A.F.R.O.T.C. in 1950; has taught biological sciences in high school from 1951 to 1962; was employed by the Bureau of Land Management (summers) 1955-1956; was advanced biology instructor and head of the science department at Minidoka County High School, Rupert, Idaho, from 1957-1962; National Science Foundation Academic Year Fellow at Oklahoma State University, 1962-1963; Graduate Teaching Assistant, Oklahoma State University, 1963-1964; Instructor, Oklahoma State University, 1964-1966, including summers; a charter member of the Idaho Academy of Sciences, and a member of the Oklahoma Academy of Sciences, the National Association of Biology Teachers, Phi Sigma Society, and Sigma Xi.