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HEMOGRAM AND BONE MARROW DIFFERENTIAL OF THE CHINCHILLA

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

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HEMOGRAM AND BONE MARROW DIFFERENTIAL OF THE CHINCHILLA

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FOREWORD
(Nontechnical summary)

The current work was initiated in an effort to provide normal blood values for the chinchilla -- a species of rodent whose popularity as a laboratory animal is increasing. Blood tests were performed on 41-male and 52-female chinchillas whose ages ranged from 1-8 years. The resulting values agree for the most part with those few found in the literature. Additional information on the chinchilla blood system was obtained by counting the number of cells per unit volume of marrow from an upper rear leg bone of 41-male and 41-female chinchillas and determining the relative abundance of different cell types in the marrow from 20 of the males and 20 of the females. No significant difference was found between the mean values reported for males and females for each of the blood and marrow parameters examined, with the exception of the peripheral red blood cell values.

ABSTRACT

Normal blood values are reported for 41-male and 52-female chinchillas of the Laniger strain whose ages ranged from 1-8 years. In addition, femoral bone marrow was characterized. Cells per unit volume of marrow, and relative abundance of different cell types were determined. No significant difference was noted between the mean values for each sex for any of the parameters determined except the peripheral RBC values.

I. INTRODUCTION

In recent years, interest in the chinchilla as a laboratory animal has increased. The availability and decreased cost of these animals as well as the ease of maintenance, desirable husbandry characteristics, and unusual physiological and anatomical features are responsible for this trend. With the use of a relatively new animal specie in scientific research its biochemical and physiological base-line values must be established. Detailed hematologic analyses are particularly important since they provide a readily accessible means of evaluating the health of the animal at any given time and may assist investigators in selecting the most appropriate species for meeting research objectives.

Six references to normal chinchilla hematology were found in the literature.[2-7] In general, these studies employed small numbers of animals or presented data for only part of the peripheral hemogram. The current study provides a more complete peripheral hemogram and characterizes the femoral marrow cellularity of a relatively large number of chinchillas.

II. MATERIALS AND METHODS

Adult chinchillas (1-8 years old) of the Laniger strain, which had served as control animals for a radiation lethality study, were used. Details of chinchilla conditioning and maintenance have been described.[9] The chinchillas (41 males and 52 females) were anesthetized with ether, their thoracic cavities opened and 5-ml blood samples obtained by cardiac puncture. Several drops of fresh blood were used to make differential smears and two drops were used to prepare smears for reticulocyte counts.

The remaining blood was immediately expressed into a test tube containing anticoagulant (approximately 6-8 mg of the dipotassium salt of ethylenediaminetetraacetic acid) and the tube gently agitated to effect thorough mixing of the contents.

The right femur was isolated from the 41 males and 41 of the females, split longitudinally, and the marrow removed with a 5-inch Gross ear curette. The marrow was transferred directly to a calibrated, conical-tipped, microcentrifuge tube containing fresh chinchilla serum. A homogeneous suspension of marrow cells was prepared by a method previously described.[8] Erythrocyte counts and total nucleated cell counts per mm^3 of marrow were made on all marrow suspensions. Smears of the marrow from randomly selected chinchillas (20 males and 20 females) were made for differential counts.

For histological determination of marrow cellularity, the left femurs were isolated, fixed in Formalin, decalcified, embedded in Tissuemat*, cut at 2 μm and stained with hematoxylin and eosin.

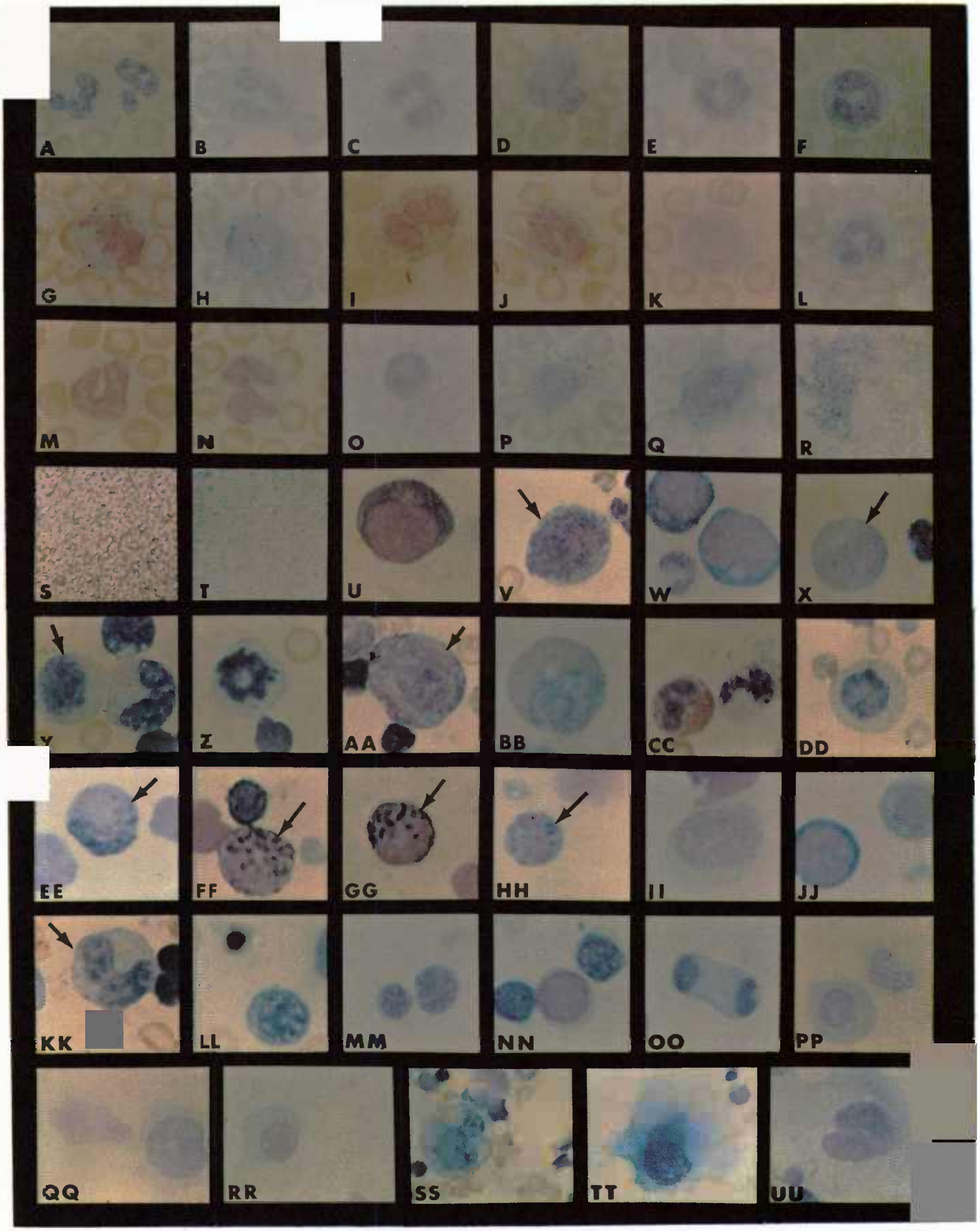
Identification of the peripheral blood and bone marrow cells was accomplished using the morphological characteristics described by Wintrobe.[12] The terminology used is that recommended by The Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs.[10,11] Representative cells of various types and stages of differentiation were photographed at 1000 X through a microscope with a Leitz Wetzlar Orthomat camera (some of the photomicrographs were enlarged 2 X during photographic processing). The substage lamp used 5.4

* Fisher Scientific Company, Silver Spring, Maryland

amperes of current. Kodak High Speed Ektachrome B film and Schott Didymium BG-20, Kodak Wratten #82, Kodak CC10R, or Kodak CC20R filters were used. Photomicrographs of the femur marrow sections and marrow-suspensions were made at 40 X and 100 X using the equipment described above. Representative cell types, marrow sections and suspensions are shown in Figure 1.

Figure 1. Photomicrographs of chinchilla peripheral blood and bone marrow cells

A, B. Neutrophilic segmented (2000 X)
C. Neutrophilic band (2000 X)
D. Eosinophilic segmented (2000 X)
E, F. Eosinophilic band (2000 X)
G, H, I. Basophilic segmented (2000 X)
J, K, L. Basophilic band (2000 X)
M, N. Monocyte (2000 X)
O. Small lymphocyte (2000 X)
P. Lymphocyte with granules (2000 X)
Q. Large lymphocyte (2000 X)
R. Platelets (1000 X)
S. Bone marrow section (40 X)
T. Smear of bone marrow suspension (100 X)
U. Myeloblast (2000 X)
V. Progranulocyte (2000 X)
W. Myeloblast, Neutrophilic myelocyte, Neutrophilic band (2000 X)
X. Neutrophilic myelocyte (2000 X)
Y. Neutrophilic metamyelocyte with cytoplasmic bridge (2000 X)
Z. Neutrophilic granulocyte in mitosis (2000 X)
AA. Eosinophilic myelocyte (2000 X)
BB. Eosinophilic metamyelocyte (2000 X)
CC. Eosinophilic segmented and Neutrophilic segmented (2000 X)
DD. Eosinophilic granulocyte in mitosis (2000 X)
EE. Basophilic myelocyte (2000 X)
FF. Basophilic metamyelocyte (2000 X)
GG. Basophilic band (2000 X)
HH. Basophilic segmented (2000 X)
II. Lymphoblast (2000 X)
JJ. Lymphocyte and lymphocyte in mitosis (2000 X)
KK. Monocyte (2000 X)
LL. Rubriblast and metarubricyte (2000 X)
MM. Rubricyte and prorubricyte (2000 X)
NN. Lymphocyte and prorubricytes (2000 X)
OO. Mitotic figure of erythrocytic series (2000 X)
PP. Plasmocyte and neutrophilic segmented (2000 X)
QQ. Hemocytoblast (1000 X)
RR. Histoblast (1000 X)
SS. Megakaryoblast (1000 X)
TT. Promegakaryocyte (1000 X)
UU. Megakaryocyte (1000 X)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A. The following hematological procedures were performed on peripheral blood:

Red blood cell count. Blood was diluted 1:50,000 with 0.9 percent NaCl solution and the cells in this dilution counted with an electronic cell counter*. Counts were corrected for coincidence losses.

White blood cell count. Blood was diluted 1:500 with 0.9 percent NaCl solution and sufficient 1 percent saponin solution (2 drops, or approximately 100 μl) was added to obtain a saponin concentration of 1:10,000. After a lapse of 25 minutes to permit lysis of the red cells, counts were performed using the electronic cell counter.

Platelet count. Method A as described by Bull et al.[1] was used. An aliquot of blood was allowed to settle for 1 hour in a plastic sedimentation tube placed at a 45-degree angle to speed separation. A 3-μl sample of plasma was removed, and diluted in 9 ml of a saline-potassium oxalate solution, counted in an electronic cell counter†, and the appropriate dilution and correction factors applied.

Hematocrit. Blood was drawn into a capillary tube and the tube was sealed at one end. The hematocrits were read after the tubes had been centrifuged for 5 minutes at 10,000 rpm in a microhematocrit centrifuge‡.

Hemoglobin. Blood was diluted 1:250 with Drabkin's solution. The optical density of the resulting cyanmethemoglobin was measured at 540 nm using a Coleman Junior spectrophotometer§. The optical density was converted to hemoglobin concentration using a previously constructed calibration curve.

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* Model B. Coulter Electronics, Hialeah, Florida
† Model B modified to improve signal to noise ratio
‡ Clay-Adams, Inc., New York, N. Y.
§ Coleman Instrument Company, Maywood, Illinois

Reticulocyte count. Two drops of blood were mixed with two drops of new methylene blue stain. Twenty minutes later a smear was prepared and the number of reticulated red cells per 1000 red cells was counted.

Differential white blood cell count. Blood smears were stained with Wright-Giemsa stain and 100 white cells on each of five different slides were differentiated per animal and the counts averaged.

Erythrocyte sedimentation rate. The uncorrected Wintrobe method was utilized. A Wintrobe hematocrit tube was filled with blood and placed in a vertical position. The number of millimeters the red cells had settled in 1 hour was recorded.

B. The chinchilla bone marrow suspension was gently agitated before sampling to assure uniform mixing since the cells tend to settle rapidly. The following hematological procedures were performed using this marrow cell suspension:

Erythrocyte count. The marrow RBC was estimated by the standard clinical technique using the Levy hemocytometer and Gower's diluting solution and by correcting for the amount of serum used to suspend the marrow specimen.

Total nucleated cell count. The identical procedure to that given for the marrow erythrocyte count was used, however, Turk's solution (3 percent acetic acid solution colored with gentian violet) was used as the diluent.

Differential nucleated cell count. Marrow smears were stained with Wright-Giemsa stain and 500 cells on each of five different slides were identified and counted per animal. The counts were averaged and expressed as percent of the nucleated cells.

Each of the hematological parameters reported in this study were statistically tested, using Student's "t" test, to determine whether the mean values reported for male and for female chinchillas differed significantly.

III. RESULTS AND DISCUSSION

The peripheral blood data for the chinchilla are summarized in Table I. The erythrocyte sedimentation rate data were not included in this table since zero values were recorded for all chinchillas except two females. Sedimentation rates of 1 and 5 mm/h were observed in these chinchillas. Rates of this magnitude are considered normal in humans and other animals and probably reflect a normal physiological state in the chinchilla. Nucleated red blood cells were seen in the peripheral blood of three-male and three-female chinchillas. In five of these animals 1 percent of the red blood cells was nucleated and in the remaining animal 2 percent. The presence of nucleated red cells in peripheral blood usually indicates an acute or chronic condition reflecting a temporary physiological problem or the early stages of a disease state. The gross pathology observed at the necropsy of the chinchillas exhibiting nucleated red cells indicated they were in good health.

Table I. Chinchilla Hemogram

Parameter	Units	Males (41)		Females (52)	
		Mean ± S.E.	Range	Mean ± S.E.	Range
Red blood cells (RBC)	millions/mm^3	7.3 ± 0.2	5.8 - 10.3	6.6 ± 0.1	5.2 - 9.9
Reticulocytes	percent of RBC	0.3 ± 0.1	0.0 - 2.8	0.2 ± 0.1	0.0 - 1.5
Hematocrit (Hct)	% of blood volume	38.7 ± 1.1	27.0 - 54.0	38.3 ± 0.8	25.0 - 52.0
Hemoglobin (Hgb)	grams/100 ml	11.7 ± 0.3	8.0 - 15.1	11.7 ± 0.2	8.8 - 15.4
Platelets	thousands/mm^3	254.0 ± 21.1	50.0 - 650.0	298.1 ± 20.6	45.0 - 704.0
White blood cells (WBC)	thousands/mm^3	7.6 ± 1.0	1.6 - 39.9	8.0 ± 0.9	2.2 - 45.1
Lymphocytes	percent of WBC	54.7 ± 2.8	19.0 - 86.0	53.6 ± 2.4	19.0 - 98.0
Neutrophils	percent of WBC	42.2 ± 3.0	9.0 - 75.0	44.6 ± 2.2	1.0 - 78.0
Eosinophils	percent of WBC	0.9 ± 0.3	0.0 - 7.0	0.5 ± 0.2	0.0 - 9.0
Basophils	percent of WBC	0.9 ± 0.3	0.0 - 10.0	0.4 ± 0.2	0.0 - 11.0
Monocytes	percent of WBC	1.3 ± 0.2	0.0 - 5.0	1.2 ± 0.2	0.0 - 5.0

Statistical evaluation of each of the listed peripheral blood parameters indicated that there was no significant difference between the mean values reported for males and females except for the red blood counts ($p < .001$).

The peripheral blood data obtained in this study are generally in agreement with values reported by other investigators (Table II), however, the WBC, hemoglobin, and platelet values are lower than those found by others. Many factors, i.e., site of blood sampling, method of analysis, age, environment, diet, etc., exert effects on each of these parameters, and it is suggested that some of these parameters played a role in the differences found. Results obtained from a very limited number of samples can be misleading. The 13.5 g/100 ml value reported by Kraft[6] for hemoglobin in female chinchillas is the mean of two observations of 16.2 and 10.8. The

Table II. Reported Chinchilla Blood Values

Parameters	References											
	Dougherty[4] *	Newberne[7] *	Casella[2] Males	Casella[2] Females	Casella[3] Males	Casella[3] Females	Kraft[6] Males	Kraft[6] Females	Johnson[5] Males	Johnson[5] Females	Present Study Males	Present Study Females
Number of animals	25	12	8†		90	38	10	5	10	5	41	52
Age (years)	.3-14		1	1	1	1	1.5-4	1.5-4			1-8	1-8
RBC x 10^6 per mm^3		6.93	8.75	7.69			9.45	10.67	4.94	5.99	7.25	6.60
WBC per mm^3	13,900	9,300	9,633	9,633			11,539	11,300			7,610	7,990
Percent of total WBC												
Neutrophils	30.5	45.0	30.0	23.0			27.3	37.1			42.2	44.6
Lymphocytes	60	51	64	73			68.5	59.0			54.7	53.6
Monocytes	3	1	4	2			1.6	1.4			1.3	1.2
Eosinophils	5	2	1	1			2.6	2.3			0.9	0.5
Basophils	1	0	1	1		.	none	none			0.9	0.4
Hemoglobin g/100 ml		13.2	13.0	13.0			12.8	13.5			11.7	11.7
Platelets thousands / mm^3	~375‡				491	499					254	298
Site of blood sampling	ear vein	ear vein			ear vein	ear vein	ear vein	ear vein			cardiac puncture	cardiac puncture

* Sex not stated
† Number of each sex not reported
‡ Mean value of 10 chinchillas

lower of these values falls in the range which was observed in the relatively large sample herein reported.

The femur marrow cell counts and the marrow differential data are summarized in Tables III and IV respectively. Again, no significant difference was found between the mean values of male and female chinchillas for each of the parameters investigated.

It was interesting to note that the cellularity of the chinchilla bone marrow appeared to remain rather constant throughout the life-span of the animal. All femur marrow sections were similar in appearance despite the fact that the sampling included young adult to 8-year old chinchillas. No sex difference in cellularity was noted. Photomicrographs of typical marrow sections are shown in Figure 1.

The marrow suspension technique was especially successful. As shown by the photomicrograph in Figure 1, a monocellular layer was achieved by making a smear of this suspension on a glass slide. Very few broken cells or clumps of cells were seen in these preparations. Under these conditions, identification of the various cells in bone marrow was facilitated. Some differences were noted in the morphological characteristics of various chinchilla marrow cells from those given by Wintrobe,[12] however, detailed descriptions of these differences are not included here.

Table III. Bone Marrow Cell Count Per Cubic Millimeter of Chinchilla Marrow

Cell Type	Males (41)		Females (41)	
	Mean $\pm$ S.E. ($\times 10^6$)	Range ($\times 10^6$)	Mean $\pm$ S.E. ($\times 10^6$)	Range ($\times 10^6$)
Total nucleated cells	1.16 ± 0.06	0.49-2.12	1.14 ± 0.07	0.53-2.19
Erythrocytes	1.81 ± 0.12	0.53-3.92	1.80 ± 0.12	0.43-3.73

Table IV. Bone Marrow Differential Expressed as a Percentage of the Total Nucleated Cells

Cell Type	Males (20)		Females (20)	
	Mean ± S.E.	Range	Mean ± S.E.	Range
Neutrophil Segmented	8.9 ± 1.1	2.0-24.5	9.7 ± 1.2	1.4-18.7
Band	11.0 ± 1.2	2.9-21.6	9.3 ± 0.8	2.5-15.1
Metamyelocyte	5.4 ± 0.6	1.9-11.3	4.5 ± 0.5	1.6-10.5
Myelocyte	3.2 ± 0.4	0.4- 7.2	3.0 ± 0.4	0.9- 6.3
Eosinophil Segmented	0.3 ± 0.1	0.0- 1.3	0.4 ± 0.1	0.0- 1.2
Band	0.9 ± 0.1	0.0- 2.3	0.8 ± 0.2	0.0- 2.1
Metamyelocyte	0.4 ± 0.1	0.0- 1.3	0.4 ± 0.1	0.0- 2.1
Myelocyte	0.1 ± <0.1	0.0- 0.4	< 0.1 ± <0.1	0.0- 0.2
Basophil Segmented	< 0.1 ± <0.1	0.0- 0.5	< 0.1 ± <0.1	0.0- 0.5
Band	< 0.1 ± <0.1	0.0- 0.2	< 0.1 ± <0.1	0.0- 0.3
Metamyelocyte	0.3 ± 0.1	0.0- 1.4	0.3 ± 0.1	0.0- 0.9
Myelocyte	0.3 ± 0.1	0.0- 1.2	0.1 ± <0.1	0.0- 0.4
Promyelocyte	0.4 ± <0.1	0.0- 1.1	0.3 ± <0.1	0.0- 1.0
Lymphocyte	26.6 ± 2.2	14.4-52.1	30.8 ± 2.5	17.1-63.2
Monocyte	< 0.1 ± <0.1	0.0- 0.3	< 0.1 ± <0.1	0.0- 0.3
Megakaryocyte	0.5 ± <0.1	0.0- 1.8	0.2 ± <0.1	0.0- 0.9
Plasmacyte	0.1 ± <0.1	0.0- 0.4	0.3 ± <0.1	0.0- 1.4
Nucleated Red Cell	3.7 ± 0.5	0.8-10.8	3.6 ± 0.4	0.6- 7.1
Metarubricyte	20.3 ± 2.1	6.0-42.3	19.2 ± 1.7	5.3-31.1
Rubricyte	11.6 ± 1.4	2.1-22.7	11.8 ± 1.3	2.7-23.6
Prorubricyte	3.7 ± 0.4	1.5- 7.9	3.7 ± 0.6	1.7-13.1
Rubriblast	0.5 ± 0.1	0.0- 1.6	0.4 ± 0.1	0.0- 3.0
Other	1.4 ± 0.2	0.1- 3.8	1.2 ± 0.2	0.3- 3.5

IV. SUMMARY

The peripheral hemogram for the chinchilla (41 males and 52 females) was reported and comparison made with the results of other investigators. Red cell and total nucleated cell counts were accomplished on the femoral marrow of 41-male and 41-female chinchillas and differential counts of the total nucleated marrow cells of 20-male and 20-female chinchillas.

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