US ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY 40121

REPORT NO. 755

CLINICAL HEMATOLOGICAL VALUES, ERYTHROCYTIC INDICES AND OSMOGRAMS OF CERCOCEBUS TORQUATUS ATYS AND PAPIO ANUBIS

(Interim Report)

by

Captain Robert W. Bull, VC
Captain Robert S. Dedrick, VC
and
Colonel Martin A. Ross, VC

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Pathology Division
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Fort Knox, Kentucky 40121

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ABSTRACT

CLINICAL HEMATOLOGICAL VALUES, ERYTHROCYTIC INDICES AND OSMOGRAMS OF CERCOCEBUS TORQUATUS ATYS AND PAPIO ANUBIS

OBJECTIVE

To determine the clinical hematological values and RBC osmotic fragilities of the subhuman primates Cercocebus torquatus atys (sooty mangabey) and Papio anubis (baboon).

METHODS

Standard clinical laboratory procedures were utilized in determining the total RBC and WBC counts, hematocrit, hemoglobin and leukocyte differential values. Erythrocyte fragility was determined by a continuous monitoring densitometric procedure.

SUMMARY

Study of the clinical hemograms of 73 sooty mangabeys and 18 baboons was carried out. The baboon erythrocyte was slightly larger and had more hemoglobin/cell. The hematocrit and MCHC were nearly identical. The WBC counts of the mangabeys were 3,000 cells/mm^3 greater than the baboons. The ratio of lymphocytes to neutrophils was 3 to 1 for the mangabeys and 2.5 to 1 for the baboons. The mangabey erythrocyte was more susceptible to osmotic lysis than the baboon and the male erythrocyte of both species was more fragile than the female.

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CLINICAL HEMATOLOGICAL VALUES, ERYTHROCYTIC INDICES AND OSMOGRAMS OF CERCOCEBUS TORQUATUS ATYS AND PAPIO ANUBIS

INTRODUCTION

The recognized similarities in human and subhuman primate physiology have long stimulated the use of the latter in biomedical research. Historically, the Macacca mulatta (rhesus monkey) has been the most widely used species and, therefore, a substantial amount is known about its normal physiology. In order to meet the need for more specialized research models, investigators have recently been using a wider variety of subhuman primates. A review of the pertinent scientific literature reveals a paucity of normal or baseline physiologic values for many of these species (1, 2, 3).

Two of these species, Cercocebus torquatus atys (sooty mangabey) and Papio anubis (dog faced baboon) have physiological or anatomical characteristics peculiarly advantageous to the mission of this laboratory and make up a substantial percentage of the animals within the primate colony. Therefore, it was imperative to establish the normal range of values for the clinical laboratory tests routinely used to monitor animal health and physiologic status. The hemogram, one of the most widely used tests to evaluate health of the mammalian organism, seemed a natural starting point.

MATERIALS AND METHODS

Animals. The data presented in this report resulted from a 2-year program of study on the primate residents of the USAMRL animal colony. The population has remained relatively static during the collection period, being composed of 73 sooty mangabeys, of which 37 were males and 36 were females, and 18 baboons, composed of 11 males and 7 females. The methods of age determination for these subhuman primates were not completely standardized so it can only be said that members of both species weighed from 4-15 pounds, and generally were not sexually mature. The animals were tuberculin tested quarterly and continuously monitored for intestinal parasites. They were housed either in individual cages or in a gang cage environment during the entire survey period. The diet consisted of free choice quantities of commercial primate biscuits and daily hand feeding of fresh fruits. All individuals of the population had normal weight gain during the entire period.

Sampling. The animals were bled periodically over the last 2 years. The blood was drawn in either heparin or EDTA anticoagulants from the femoral vein or artery in the fasting state. Each animal had multiple samples drawn, varying from 5 to 15, and the values for each animal were averaged to obtain an individual mean value which was then utilized to compare with the means of the other members of the population.

Parameters and Methods Employed.

Total Red Blood Cell Count. A Thoma red cell diluting pipette was used and the cells were counted in a hemacytometer with the improved Neubauer ruling. Cell counts will be reported as number of cells $(10^6)/mm^3$ of blood.

Total White Blood Cell Count. A Thoma white cell diluting pipette was utilized and the cells were counted in a hemacytometer counting chamber with the improved Neubauer ruling. Counts will be reported as number of cells/mm^3 of blood.

Hematocrit or Packed Cell Volume. The microhematocrit method was used with heparinized micropipettes and the International microhematocrit centrifuge. The values will be reported as the percentage of packed cells for the column of blood.

Hemoglobin. Determinations were made as grams of hemoglobin/100 ml of blood by the cyanmethemoglobin method. A Coleman Junior spectrophotometer was used with a 540 mμ wavelength.

Leukocyte Differential. Air dried smears were stained with the modified Wrights stain with buffer. One hundred cells were counted in each smear.

The Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) values were calculated for both subhuman primate species by standard formulae (6).

Erythrocyte Osmotic Fragilities. The method employed was that of Shields and Allen (4) and Shields, Allen and Fogel (5). This provides a continuous record of hemolysis of erythrocytes contained in a dialysis bag surrounded by distilled water. The rate of hemolysis was recorded by a densitometer which produces a sigmoid curve consisting of a high and low plateau with a sharp slope between them. By measuring the horizontal

distance from the start to selected points, the slope can be converted into a linear function of either distance or time to provide data for statistical comparison (4, 5).

RESULTS

Total Red Blood Cell Count. Tables 1 (below) and 2 (next page) present the average values obtained for the mangabeys and baboons as groups and by sex. The mangabey erythrocyte count was 4.7×10^6 cells/mm^3. There were no appreciable differences between the males and females in either species.

The mangabey WBC count was approximately 2,500 cells/mm^3 higher than the baboons, the former being 18,545, compared to 15,937 for the latter (Tables 1 and 2). Within the groups, there was very little sex difference.

Hematocrit and Hemoglobin Values. The hemoglobin values were 13.3 gm% and 12.9 gm% and the hematocrit values were 43.5 and 42.7 for the baboon and mangabey, respectively (Tables 1 and 2). There was no difference between the sexes in each species.

TABLE 1 - Mangabeys

Parameter	Number of Animals	Mean ± SD	Exp. Range
RBC's x 10^6			
Group	55	4.767± .641	2.720-6.240
Males	29	4.799± .583	2.980-6.240
Females	36	4.734± .699	2.720-5.850
WBC Count			
Group	73	18,545±4,454	8,550-30,040
Males	37	18,702±4,617	8,550-28,350
Females	36	18,383±4,273	9,220-30,040
Hematocrit (%)			
Group	73	42.69±3.25	31.5-49.0
Males	37	42.73±2.85	35.0-47.5
Females	26	42.65±3.62	31.5-49
Hemoglobin (gm/100 ml)			
Group	73	12.88±0.81	10.55-14.73
Males	37	12.76±0.89	10.55-14.73
Females	36	12.99±0.89	11.30-14.20

TABLE 2 - Baboons

Parameter	Number of Animals	Mean ± SD	Exp. Range
RBC x 10^6			
Group	18	4.350± .473	3.41-5.04
Males	11	4.346± .480	3.41-5.04
Females	7	4.359± .460	3.41-5.04
WBC Count			
Group	18	15,937±3,544	11,570-24,950
Males	11	15,586±2,035	11,570-18,650
Females	7	16,489±5,030	11,800-24,950
Hematocrit (%)			
Group	18	43.57±1.99	39.70-46.50
Males	11	43.15±1.56	40.40-46.0
Females	7	44.23±2.37	39.70-46.50
Hemoglobin (gm/100 ml)			
Group	18	13.38±0.68	12.0-14.7
Males	11	13.13±0.59	12.0-14.3
Females	7	13.77±0.61	12.9-14.7

Leukocyte Differential. Tables 3 (page 5) and 4 (page 6) present the differential cell counts for the two species. The ratio of lymphocytes to neutrophils in the mangabey was 3 to 1, whereas in the baboon, the ratio was approximately 2.5 to 1. The bands, eosinophils, monocytes and basophils occurred in similar proportions in both species.

Mean Corpuscular Volume (MCV). This parameter displayed the greatest difference between the two species. The mangabeys had a calculated MCV of 89.9 μ^3 whereas the baboons' average MCV was 103 μ^3 (Tables 5 and 6, page 7).

Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). Tables 5 and 6 list the calculated values for these two parameters. Comment on these values will be made in the Discussion.

Erythrocyte Osmotic Fragility. The utilization of the semipermeable dialysis bag in connection with the densitometer resulted in a rapid, but readily reproducible, osmotic fragility curve. The

TABLE 3 - Mangabeys
Differential

Parameter	Number of Animals	Mean ± SD	Exp. Range
Bands			
Groups	73	0.28± 0.52	0.1-2.7
Males	37	0.33± 0.48	0.2-1.7
Females	36	0.24± 0.54	0.1-2.7
Neutrophils			
Group	73	23.78±14.88	9.0-45.30
Males	37	22.97±10.59	9.0-45.30
Females	36	23.59± 8.59	11.3-44.0
Lymphocytes			
Group	73	73.46±10.19	48.70-89.00
Males	37	73.91±10.77	48.70-89.00
Females	36	73.00± 9.54	54.30-87.00
Eosinophils			
Group	73	1.02± 0.83	0.2-3.0
Males	37	1.16± 0.92	0.2-3.0
Females	36	0.87± 0.69	0.2-2.7
Monocytes			
Group	73	0.80± 0.77	0.1-3.7
Males	37	0.58± 0.60	0.1-3.0
Females	38	1.04± 0.86	0.3-3.7
Basophils			
Group	73	0.65± 0.88	0.3-6.0
Males	37	0.53± 0.74	0.4-3.5
Females	36	0.77± 0.99	0.3-6.0

TABLE 4 - Baboons
Differential

Parameter	Number of Animals	Mean ± SD	Exp. Range
Bands			
Group	18	1.17±0.86	0.2-3.5
Males	11	1.15±0.89	0.3-3.5
Females	7	1.18±0.85	0.2-2.5
Neutrophils			
Group	18	27.83±7.16	13.7-48.7
Males	11	28.64±7.13	22.4-48.7
Females	7	26.57±7.03	13.7-36.5
Lymphocytes			
Group	18	69.14±7.95	46.1-85.7
Males	11	68.46±7.46	46.8-74.1
Females	7	70.21±8.54	59.0-85.7
Eosinophils			
Group	18	0.94± .82	0.1-3.4
Males	11	0.85±0.60	0.3-2.0
Females	7	1.10±1.05	0.1-3.4
Monocytes			
Group	18	0.53±0.24	0.3-1.1
Males	11	0.50±0.23	0.3-0.8
Females	7	0.57±0.24	0.3-1.1
Basophils			
Group	18	0.33±0.26	0.1-1.0
Males	11	0.30±0.17	0.1-0.6
Females	7	0.38±0.34	0.2-1.0

TABLE 5 - Mangabeys

Parameter	Number of Animals	Mean ± SD	Exp. Range
MCV (μ^3)			
Group	55	89.78±11.87	72.11-147.55
Males	29	90.88±13.32	72.11-147.55
Females	26	88.55± 9.87	75.81-111.86
MCH (μμgm)			
Group	55	26.60± 4.10	20.35- 45.15
Males	29	26.75± 4.79	20.35- 45.12
Females	26	26.44± 3.15	21.01- 32.31
MCHC (%)			
Group	73	30.37± 2.65	27.19- 39.05
Males	37	30.04± 2.24	27.19- 37.31
Females	36	30.71± 2.98	27.20- 39.05

TABLE 6 - Baboons

Parameter	Number of Animals	Mean ± SD	Exp. Range
MCV (μ^3)			
Group	18	103.01±12.38	87- 130.1
Males	11	101.70±11.07	78.1-135.09
Females	7	105.07±12.79	87.1-130.1
MCH (μμgm)			
Group	18	32.12± 3.81	26.9- 41.79
Males	11	32.30± 3.36	26.9- 39.85
Females	7	31.99± 4.43	27.92-41.79
MCHC (%)			
Group	18	30.56± 0.86	28.33-32.01
Males	11	30.43± 0.58	29.59-31.78
Females	7	30.75± 1.14	28.33-32.01

interpretation of the resultant sigmoid curve indicated that the mangabey erythrocyte was more susceptible to osmotic lysis than the baboon erythrocyte. The male erythrocyte of both species was more osmotically fragile than the erythrocyte of the female (Table 7, below and Fig. 1A, page 9).

TABLE 7 - Osmograms
Heparin Anticoagulant

Species	Number	0%	50%	100%
Mangabeys				
Group	22	6.9±0.6	13.6±1.6	26.0±2.0
Males	16	6.9±0.5	13.6±1.7	25.8±2.2
Females	6	7.1±0.9	14.1±1.3	26.8±1.4
Baboon				
Group	18	7.6±2.8	16.2±1.3	30.1±1.8
Males	11	7.7±0.7	16.2±1.4	30.2±1.9
Females	7	7.6±0.2	16.4±1.2	30.1±2.0
Human 1	19	10.0	19.8±3.4	31.3±4.2

Values are expressed as cm from onset of hemolysis plus the standard deviation.

DISCUSSION

The utilization of laboratory animals as research models for biomedical investigation requires that the animals have known physiological parameters. If one attempts then to correlate the subhuman primate hematological values with the established values of the human, the 6-10 year old prepubescent human approximates the sexual maturity of this subhuman primate population.

The mangabey and prepubescent human have similar erythrocyte counts and hemoglobin values (4.7×10^6 cells/mm^3 and 12.9 gm%, respectively). The mangabey erythrocyte was slightly larger than the human (89.9 μ^3 versus 80 μ^3) (6). The MCH values were 26.6 and 27 $\mu\mu$gm for the mangabey and human, respectively (6). The baboon

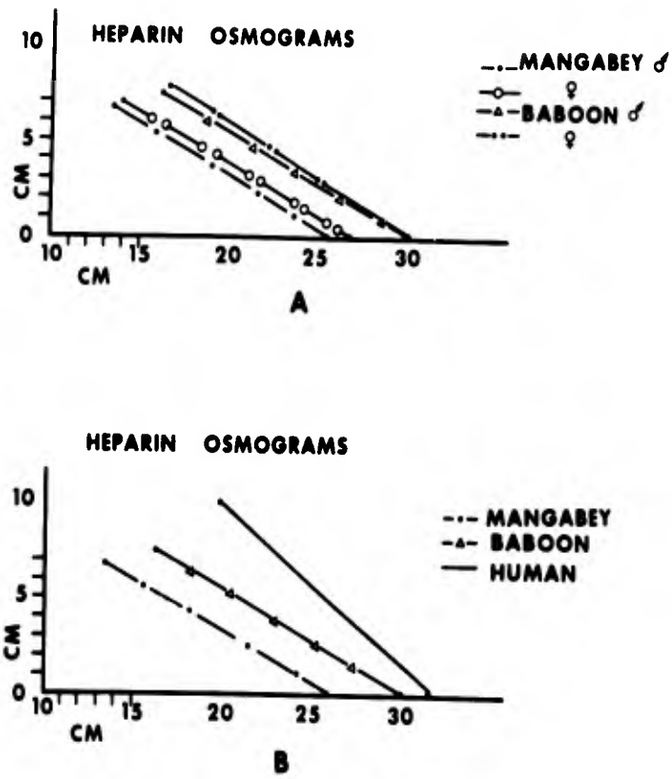

Fig. 1. Primate erythrocytes are more fragile than human erythrocytes (A); mangabey erythrocytes are more fragile than baboon erythrocytes (B).

differs even more from the young human in that the erythrocytes were even larger (103 μ^3 versus 80 μ^3) (6). The hematocrits of the primates were greater, being 42.7% for mangabeys and 43.6% for the baboons, compared to the young humans' hematocrit of 34% (6).

The primate total white counts were higher than the human, being 18,545 and 15,937 cells/mm^3 of blood for the mangabey and baboon, respectively, compared to the human WBC count of 7,000 cells/mm^3 (6). As can be seen in Tables 3 and 4, the primate differential distributions of the granulocytic and lymphocytic series were similar with the ratio being 1 to 3 and 1 to 2.5, respectively, for the mangabey and baboon. The normal human differential has a higher proportion of cells in the granulocytic series (6).

The morphological characteristics of the cells of the subhuman primate's lymphocytic and granulocytic series were quite similar to the human. The notable exceptions were that the lymphocytes of the

subhuman primates were larger and there was more frequent occurrence of eosinophilic granules in the cytoplasm of the lymphocytes and neutrophils than seen in the human (3, 7, 8). A most striking characteristic of the subhuman primate neutrophil was the hypersegmentation of the nuclei (3, 7, 8). This hypersegmentation was of the same magnitude as that which occurs in the neutrophils of humans suffering from Vitamin B_{12} and/or folic acid deficiency (9). These findings are presently being investigated to determine if the observed characteristic is normal or truly an indicator of nutritional deficiency in the primates.

The study of the erythrocyte osmotic fragility of the two species of primates indicated that the baboon and mangabey erythrocyte was more fragile than the adult human erythrocyte (Table 7, page 8, and Fig. 1B, page 9). These findings agree with the available data for the rhesus and baboon erythrocyte (3, 5). As can be seen from Figure 1A (page 9), the mangabey erythrocyte was more fragile than the baboon erythrocyte and the male erythrocyte of both species was more fragile than the female. The relationship of the hematologic data between these species must be recognized to properly interpret experimentation in which these animals are used.

SUMMARY

A study of the clinical hemograms of 73 sooty mangabeys and 18 baboons was accomplished. RBC counts of the primates were similar to each other and to the human of similar sexual maturity. The cells of subhuman primates tend to be larger and the hematocrits higher than the human. The WBC counts of the subhuman primates were greater than humans and the percentage of lymphocytes in the differential count was greater as well.

The subhuman primate RBC's were more susceptible to osmotic lysis than those of the human and the RBC's of the males of both subhuman primate species were more fragile than the females.

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13. ABSTRACT

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