

STUDIES ON COMPARATIVE HEMATOLOGY — I. HEMATOLOGIC
DATA OF *MYRMECOPHAGA T. TRIDACTYLA* L., 1758 (TAMANDUÁ-
BANDEIRA) AND *TAMANDUA T. TETRADACTYLA* L., 1758
(TAMANDUÁ-MIRIM) (*)

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By the study of some endemics, human epidemics and epizooties, we are frequently stricken by the scarcity of hematologic data, that would permit the interpretation of blood modifications in wild animals, either in those inoculated in laboratory or in those found in their natural conditions. On the other hand the knowledge of hematologic data of animals from the various points of the zoological scale, will probably facilitate or permit investigations about the physiology and physiopathology of many mechanisms like that of coagulation, phagocytosis, cytogenesis etc. that are till now obscure. These mechanisms will be perhaps more easily cleared when studied in some animals that for some peculiarity, demonstrate new facts or facilitate some experiences. As an example, the fact observed by Svihla and col. (9-10) in the squirrel and the hamster; when these animals hibernate, there is an extraordinary prolongation of the blood clotting time. This fact would permit the study of the mechanism of the thrombosis and endogenous anticoagulants, of great importance for the human physiology and physiopathology. With the deal to report the hematologic data of several animals it was done a study of the blood of anteaters occasionally sent to the Instituto Butantan.

6 specimens of *Myrmecophaga tridactyla tridactyla* L., 1758 (Tamanduá-Bandeira) coming, 5 from the State of Mato Grosso and 1 from the State

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MATERIAL AND METHODS

of S Paulo were examined. It was also examined 1 *Tamandua tetradactyla tetradactyla* L., 1758 (T. Mirim) coming from the State of Mato Grosso.

The blood for dosings and hematimetric determinations was taken from the cubital vein and heparinized with 1 mg/ml of blood. The specific counts of leucocytes, mean erythrocytic diameter and morphologic aspect were measured in venous blood smears.

It was considered the average of 2 counts of erythrocytes done in $1/5 \text{ mm}^2$, the dosing of hemoglobin was done in electrophotometer with green filter (525μ), the corpuscular volume was determined in Wintrobe's hematocrit centrifuged at 4.000 rpm during 20 minutes. Erythrocytes sedimentation rate was determined in Wintrobe's tube with heparinized blood and the reading was done after 60 minutes. All these determinations were done in duplicate. The reticulocytes were counted in smears by the brilliant cresil blue method, 1.000 erythrocytes were counted for each animal. The mean diameter was measured in 200 erythrocytes, for each animal. The differential count was done in 100 cells in smears stained by Rosenfeld's method (8).

The mean values were determined, being s the standard error of individual values and $s\bar{x}$ the standard deviation of the mean. Here are also included the variation coefficients C and $C\bar{x}$ respectively from the individual values and from the mean.

RESULTS

Erythrocytes — The mean number of erythrocytes was of 3.150.000 per mm^3 . Individual variations were small. The hemoglobin average was 13.28 gr % and varied mostly only in one animal (6227) that was infested with Nematodes in the stomach and Acantocephala in the intestine. Consequently the mean corpuscular concentration of hemoglobin in that animal was 23,6γγ, while in the others varied from 41.0 to 55.5γγ. That animal therefore presented a hipocromia but had no microcytosis, considering the volume or the mean diameter. Other numeric data about the erythrocytes can be observed in table 1. The erythrocytes were circular and biconcaves, presenting no polychromasy, anisocytosis or poikilocytosis.

Leucocytes — The mean number of leucocytes was 4.980 per mm^3 , being lower only in one animal (6245) that was suckling a young (6245A). With exception or nr. 6265, all presented a great number of stabform neutrophils, in spite of presenting no apparent infection. The number of eosinophils was

high in some animals, both in the infected by intestinal parasites as in those where the parasites were not found (Table 2).

The morphologic aspect of the leucocytes presented some special characteristics. The neutrophile granulations were very fine and scarcely visibles (fig. 1, 2, 3, 4) and these from the eosinophiles (fig. 5) were not as well individualized as they are in other mammals because they exist in a great number and become superposed. In the smashed cells (fig. 13) they are evident.

Lymphocytes, monocytes and blood platelets — The lymphocytes and monocytes presented sometimes azurophile granulations. The platelets had hyalomere and chromomere well visible but the last was very thin (fig. 12).

In a general way the morphologic aspect was not very different from the most part of the other mammals, calling attention, at first sight, the larger diameter of the erythrocytes, the almost absence of granulations in the neutrophiles and the small evidence of granulations in eosinophiles.

DISCUSSION

There are scarce and isolated data about blood elements of the *Myrmecophagidae*, but it was impossible to find a general hematologic picture. Gulliver (2) in an extense table of mean erythrocytic diameters from vertebrates, indicates for *Myrmecophaga jubata* (*M.t. tridactyla*) the value of 1/2769 inch (9.173 μ). He does not refer how many animals were used or the number of cells measured. However he informs that he measured a large number of erythrocytes in dry and thin smears. Peters (7) measuring 100 erythrocytes in a not specified number of animals found a mean diameter of 9.3 μ with a standard deviation of 0.67. Our data agree with those of these authors as we found a mean diameter of 9.398 μ with a standard error of 0.322 (Table 1 and graphs 1, 2, and 3).

In respect to the mean corpuscular volume Knoll (4) found 133-135 μ^3 but has inferred the volume from the erythrocytic shape, considering the cell as a cylinder and using as elements only the diameter and the height. As the cell has not that geometric form we understand that his measures are very different from our findings, that were 160.7 μ^3 . This value we found in 5 animals with methods utilised actually in hematologic routine, that is, considering the globular volume, hematocrit and the number of erythrocytes that occupy that volume. This method avoids errors introduced with individual measures of few elements.

Knoll refers in another work (3) that he found in *Myrmecophagus tridactylus* and in *Tamandua jubata* 4-8% of normoblasts in smears of the circulating blood. These results disagree with our findings. In 6 specimens of *M. tridactyla tridactyla* we found no normoblast in the smears of the peripheral

blood except rare ones in nr. 6227 and only 37 per mm^3 in a specimen of *T. tetradactyla* that was in very bad conditions of health and died some hours after the blood was taken. We attribute that finding of Knoll to the possibility that he worked with animals that were not in good conditions and probably had anemia. The ontogenic inductions done by the author about the great number of normoblasts found in the animal circulation are therefore unjustifiable. It is also possible that he confounded the small lymphocytes with erythroblasts, because these cells some times resemble erythroblasts (fig. 9, 10) although the difference does not present great difficulty (fig. 11). Knoll (3) refers that he studied one specimen of *Myrmecophagus tridactylus* and two *Tamandua jubata*, however, these two denominations are probably referent to a single species for *Tamandua jubata*, is probably *Myrmecophaga jubata* L., 1766, that is in the synonymy of *Myrmecophaga tridactyla tridactyla* L., 1758 (1-11).

About the percentages of leucocytes and their morphology Knoll (3) refers results found in 1 specimen of *M. tridactyla* and 2 of *Tamandua jubata*. Oria (5-6) presents results from 2 specimens of *T. tetradactyla*. There is no reference to the global count of leucocytes and differential count of neutrophiles. There is no divergence between these data and those found by us, because in so few animals it naturally occurs variations of the types of leucocytes and in respect to the morphology we only accentuate that the neutrophiles present very fine and little visible granulations and the eosinophiles have granulations not so individualized as in the man.

SUMMARY

Hematimetric data of 6 specimens of *Myrmecophaga tridactyla tridactyla* L., 1758 (Tamanduá bandeira) and 1 specimen of *Tamandua tetradactyla tetradactyla* L., 1758 (Tamanduá mirim) were studied. The mean values found were: 3.150.000 erythrocytes, per mm^3 , with a concentration of 43,12% of hemoglobin, a mean corpuscular volume of $160.6\mu^3$ and a diameter of 9.398μ . The erythrocytes are discoidal and biconcaves and their dimensions are great when compared with these from other mammals. The number of reticulocytes was 0.747 % and that of leucocytes 4.980 per mm^3 . Differential counts of leucocytes were done. The neutrophiles presented very thin granulations, and these of the eosinophiles were not very evident, because they exist in a great number and become superposed.

RESUMO

Foram estudados dados hematimétricos de 6 exemplares de *Myrmecophaga tridactyla tridactyla* L., 1758 (Tamanduá bandeira) e 1 do *Tamandua tetra-*

dactyla tetradactyla L., 1758 (Tamanduá mirim). A média de hemácias foi de 3.150.000 por mm^3 com uma concentração de 43,12% de hemoglobina, volume médio de $160,6\mu^3$ e diâmetro médio de $9,398\mu$. As hemácias que são discóides e bicôncavas apresentam portanto dimensões grandes para mamíferos. O número de reticulocitos era de 0,747% e o de leucocitos 4.980 por mm^3 . Foram feitas contagens diferenciais dos leucocitos, os neutrofilos apresentavam granações muito finas e as dos eosinofilos não eram muito evidentes por ficarem empilhadas devido ao seu elevado número.

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BIBLIOGRAPHY

1. Brehms, A. — Brehms Tierleben. Die Säugetiere, 1 Band: 527, 1912. Bibliographisches Institut Leipzig und Wien, 1 Band, pp. 527, 1912.
2. Gulliver, G. — On the red corpuscles of the blood of vertebrata, and on the zoological import of the nucleus, with plans of their structure form and size (on a uniform scale) in many of the different orders, *Proc. Zool. Soc. of London*: 91, 1862.
3. Knoll, W. — Das morphologische Blutbild der Säugetiere. I. Allgemeine und spezielle Morphologie der Kernhaltigen Blutzellen der Säugetiere, *Jahrb. Morph. u. Mikrosk. Anat. Abt. II. Zeitschr. Mikrosk. Anat. Forsch.* 30: 116, 1932.
4. Knoll, W. — Untersuchungen über die Morphologie des Säugetierblutes, *Folia Haematol.* 47: 201, 1932.
5. Oria, J. — Sobre os monocitos do sangue circulante nos *Xenarthra*, *Rev. Med. S. Paulo*, 13: 19, 1928.
6. Oria, J. — Sobre os elementos figurados no sangue circulante na preguiça, no tatu e no tamanduá. Tese inaugural, Faculdade de Medicina da Universidade de S. Paulo, Brasil, 1928.
7. Peters, N. — Das morphologische Blutbild der Säugetiere. II. Über die Grössenverhältnisse der Erythrocyten der Säugetiere, in *Jahrb. f. Morph. u. Mikrosk. Anat. Abt. II. Zeitschr. Mikrosk. Anat. Forsch.* 30: 151, 1932.
8. Rosenfeld, G. — Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só corante de emprego rápido, *Mem. Inst. Butantan*, 20: 329, 1947.
9. Szihla, A.; Bowman, H. & Pearson, R. — Prolongation of blood clotting time in the dormant hamster, *Science*, 115: 272, 1952.
10. Szihla, A.; Bowman, H. & Ritenour, R. — Prolongation of clotting time in dormant estivating mammals, *Science*, 114: 298, 1951.
11. Trouessart, E. L. — Catalogus Mammalium tam viventium quam fossilium, R.Friedländer & Sohn, Berolini, Suppl. pp. 803, 1898-1905.

TABLE 1

Myrmecophaga tridactyla tridactyla L., 1758

RED BLOOD CELLS

Animal Nr.	Red blood cells $\times 10^6/\text{mm}^3$	Hemoglobin gr %	Mean corpuscu- lar hemoglobin $\gamma \gamma$	Hematocrit %	Mean corpuscu- lar volume μ^3	Reticulocytes %	Mean diameter μ	Red blood cells sedimentation rate mm/60min.
6227	3,3	7,8	23,6	52,2	158	0,675	9,576	7,5
6226	3,4	16,7	49,0	55,5	162	0,657	9,050	2,25
6225	3,2	13,1	41,0	46,0	143	—	9,392	26,0
6245	2,7	15,0	55,5	50,5	187	0,778	9,707	21,75
6245A	—	—	—	—	—	—	9,221	—
6275	—	—	—	—	—	—	9,836	—
6265	3,0	13,9	46,5	46,0	153	0,879	9,004	29,0
Mean	3,15	13,28	43,12	50,04	160,6	0,747	9,398	17,28
s	0,28	3,33	12,09	4,10	16,37	0,1014	0,322	11,78
C %	8,8	25,0	28,0	8,2	10,12	10,3	0,34	10,06
s \bar{x}	0,124	1,48	5,4	1,83	7,32	0,05	0,130	5,26
C \bar{x} %	3,9	11,1	12,5	3,6	4,55	6,7	0,01	30,44

TABLE 2

Myrmecophaga tridactyla tridactyla L., 1758

LEUCOCYTES

Animal Nr.	Leucocytes x 10 ³ /mm ³	Differential count %						Procedence	Diseases or in- testations	Sex	Observations
		Neutrophiles		Eosinophiles	Basophiles	Lymphocytes	Monocytes				
		Stab	Segmented								
6227	6,25	14	33	19	3	27	4	Mato Grosso	Nematodes (sto- mach). Acantoce- phala (intestine).	Indet.	—
6226	7,40	14	48	6	—	23	9	Mato Grosso	—	♀	—
6225	4,85	16	43	1	1	30	9	Mato Grosso	—	♀	—
6245	2,35	25	42	17	—	12	4	Mato Grosso	—	♀	Animal was suck- ling a young.
6245 A	—	21	20	1	—	51	7	Mato Grosso	—	Indet.	Youngst of the animal above
6275	—	32	12	14	—	37	5	Mato Grosso	—	Indet.	—
6265	4,25	7	60	2	—	27	4	São Paulo	—	Indet.	—
Mean	4,98	18,42	36,85	8,57	0,57	29,57	6,0				
\bar{x} C %	1,947	8,26	16,5	7,89	1,13	12,10	2,3				
\bar{s} C %	39	42,6	44,7	92,0	198	40,9	38,3				
$\bar{s} \cdot \bar{x}$ C %	0,870	3,12	6,24	2,98	0,42	4,57	0,87				
$\bar{s} \cdot \bar{x}$ C %	17,4	16,9	16,9	34,3	73,6	15,4	14,5				

TABLE 3

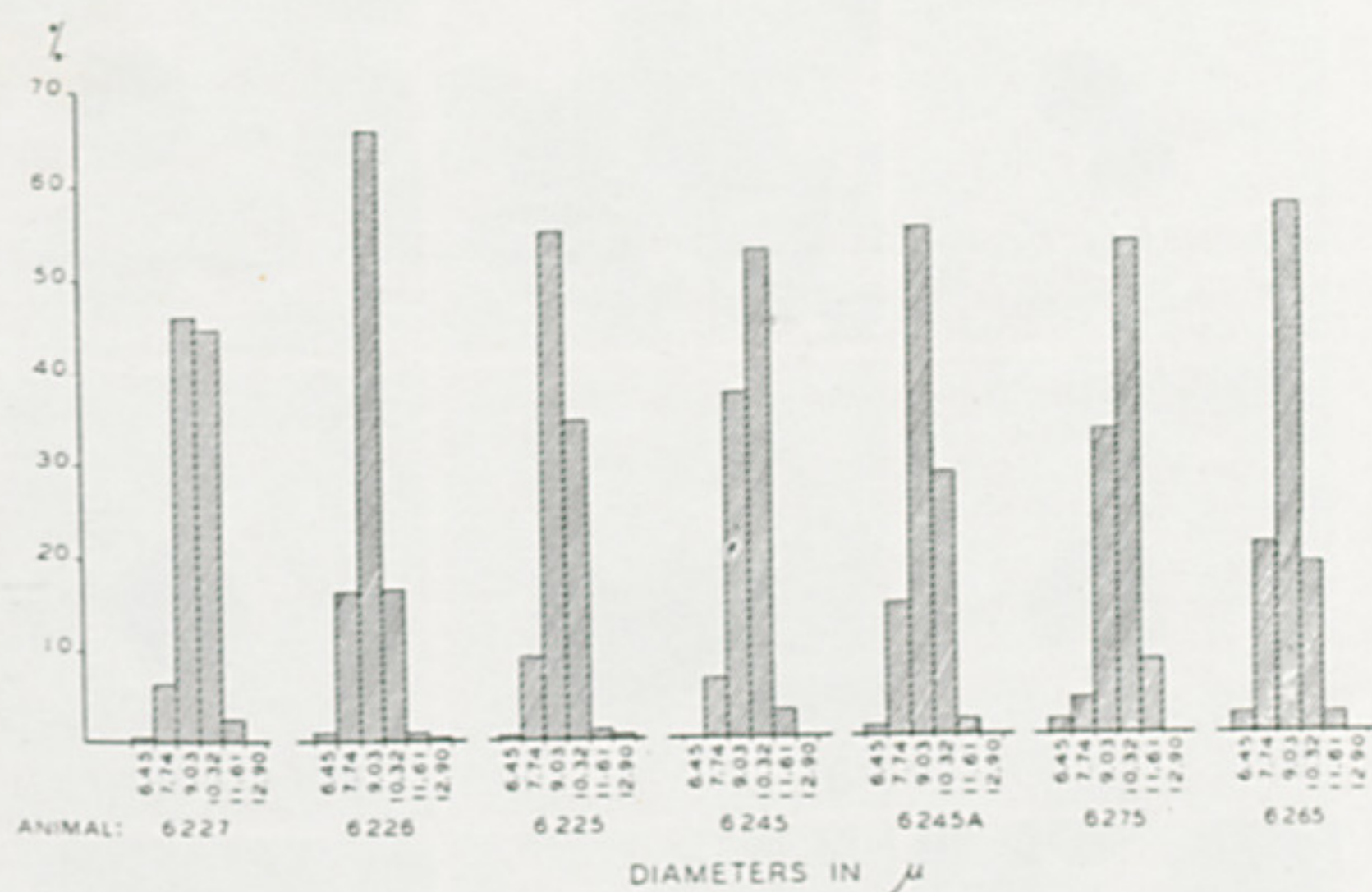
Tamandua tetradactyla tetradactyla L., 1758. Procecence Mato Grosso, sex indetermined, died some hours later.

RED BLOOD CELLS

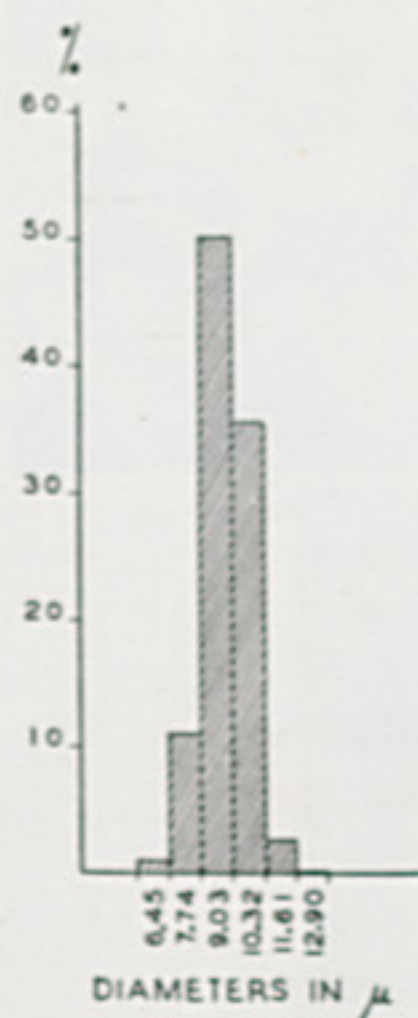
Red blood cells x 10 ⁶ /mm. ³	Hemoglobin gr. %	Mean corpusc. hemoglobin γγ	Hematocrit %	Mean corpusc. volume μ ³	Reticulocytes %	Mean diameter μ	Red blood cells se- dimentation rate mm/60 min.
2,8	11,3	40,3	37,0	132	0,167	8,947	20,25

LEUCOCYTES

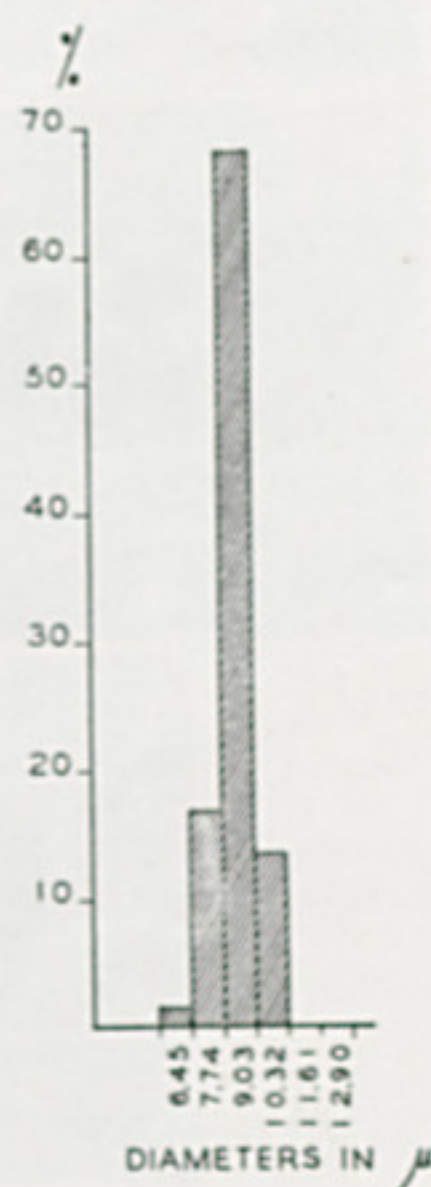
Leucocytes x 10 ³ /mm:3	Differential count %						Observation	
	Neutrophiles			Eosinophiles	Basophiles	Lymphocytes		Monocytes
	J	Stab	Segmented					
3,7	1	22	32	3	—	39	3	37 erythroblasts mm3



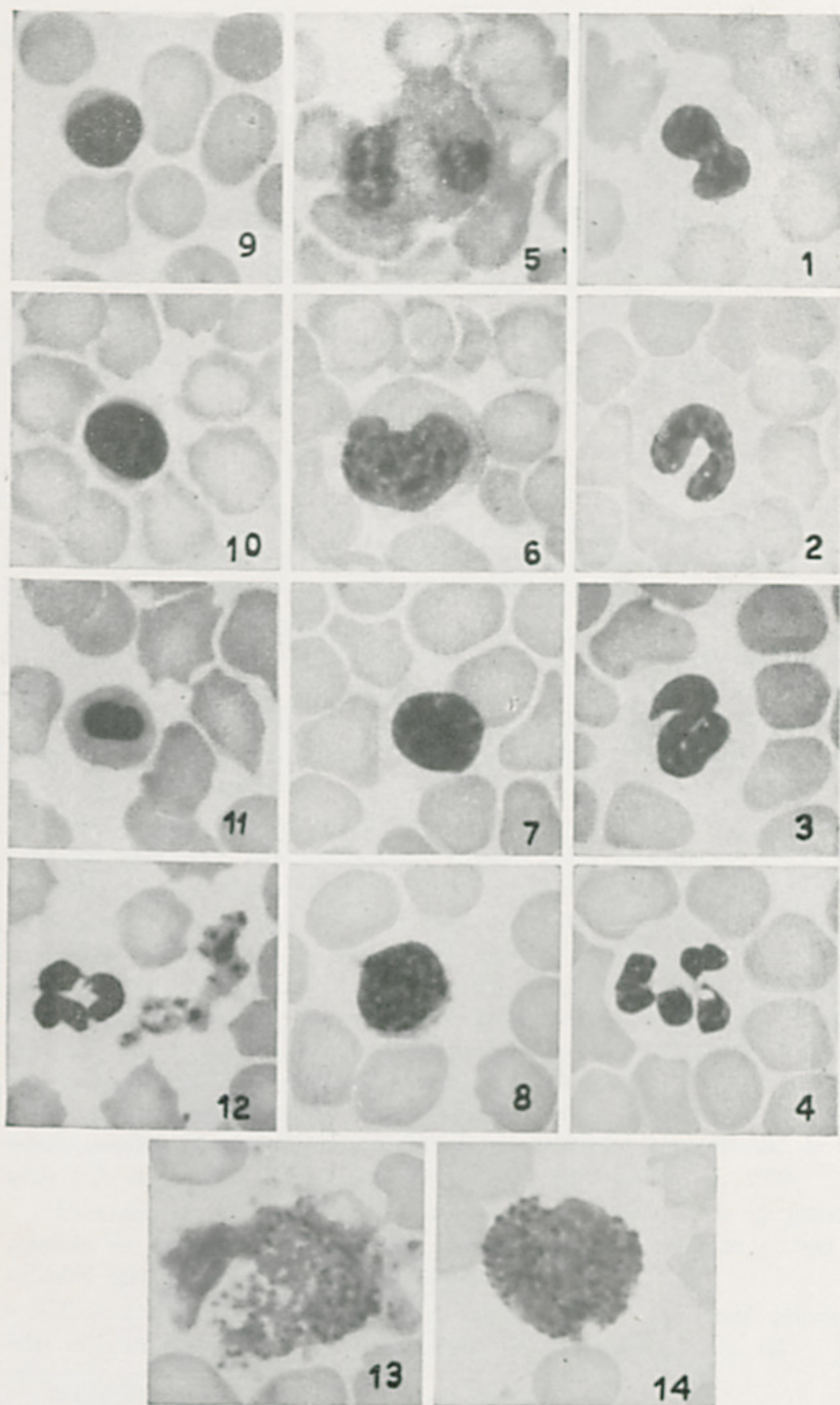
Graph 1 — *Myrmecophaga t. tridactyla*. Mean erythrocyte diameter of 6 animals.



Graph 2 — *Myrmecophaga t. tridactyla*. Mean erythrocyte diameter. Average of 6 animals.



Graph 3 — *Tamandua t. tetradactyla*. Mean erythrocyte diameter. 1 animal.



Photomicrographs of blood of *Myrmecophaga tridactyla* L., 1758. Rosenfeld stain. X 1,200
 1 — Neutrophile, juvenile; 2 — Neutrophile stab form; 3 — Neutrophile stab form; 4 — Neutrophile, segmented; 5 — Eosinophile; 6 — Monocyte; 7 — Large lymphocyte; 8 — Small lymphocyte; 9 — Small lymphocyte; 10 — Small lymphocyte; 11 — Erythroblast, orthochromatic; 12 — Platelets; 13 — Eosinophile, smashed; 14 — Basophile.

