Arq. Est. Biol. Mar Univ. Fed. Ceará, 8 (2) : 111 - 116
Dezembro, 1 9 6 8 — Fortaleza, Ceará, Brasil

HEMATOLOGY OF THE ATLANTIC THREAD HERRING, OPISTHONEMA OGLINUM (LE SUEUR)

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A great number of papers have been published on the hematology of fishes, but the majority of them is on freshwater species.

In 1965 we began a serial study on the blood parameters of marine fishes with the purpose of establishing methods that could be applyed to systematics. Soon gross hematological abnormalities were found in some specimens and due to the possible relationship to human blood diseases, a better knowledge of normal blood values was felt to be necessary.

In the present paper the data of blood cell counts, cytology and cytochemistry performed in 100 Atlantic thread herring, *Opisthonema oglinum* (Le Sueur), are presented.

MATERIAL AND METHODS

Blood samples were collected from 102 Atlantic thread herring captured through a beach net at Mucuripe Beach, in the basin of the port of Fortaleza (Ceará, Brazil). Only specimens showing good signs of vitality were included in the study.

Fork length was determined in all specimens; sex identification was not considered.

The data of 2 fishes showing marked leucocytosis were not included in this study, and were reported in a separated paper (Martins & Pitombeira, 1968).

The volume of 1.0 to 1.5 cc of blood was drawn from the gills, using heparin as an anticoagulant. The material was kept at 4°C for about 3 hours before blood counts were done. Hemolysed or clotted samples were not accepted for the study.

Red blood cell count was performed by the direct method in a Neubauer chamber, using Hayem solution as a diluent fluid. Hemoglobin was performed by Sahli method; it was necessary to wait 2 — 3 minutes before reading because the nuclei of the hemolysed erythrocytes could give some turbidity to the fluid before the sedimentation. Hematocrit was done in a limited number of samples due to the small amount of blood drawn; Wintrobe tube was used.

The white blood cell count was determined by indirect method, counting in a stained slide, with oil immersion lens, the number of leucocytes in relation to 5,000 red cells; usually we choose for the count the part of the peripheral smear in which the distribution of red cell was made evenly.

Peripheral blood smears were done with heparinized blood using the May-Grunwald-Giemsa stain. Two hundreds white blood cells were counted as a routine, but in leukopenic samples the counts were made in 100 cells. The nomenclature proposed by Jakowska (1956) was used with some limitations.

PAS reaction was performed according McManus technique (Hayhoe *et al.*, 1964). In many occasions the same cell was consecutively studied by Romanowski and PAS reaction (Hayhoe, 1960).

Thrombocytes were counted by Fonio's indirect method in relation to 5,000 red cells (Janini, 1959).

The cells were measured with a micrometric occular 6X and an oil immersion lens 90X; the correction factor previously determined was 1.44.

RESULTS

Red blood cells

The red blood cell count varied from 1,000,000 to 5,100,000 per cc with the mean

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value of 2,740,000 (table I) . A slight significative correlation was found between the red blood cell count and the fork length (figure 1) . There was a tendency for the red blood cells increase with the fork length up to 250 mm , when they began to fall.

Hemoglobin

Hemoglobin determination varied from 2.2 to 15.2 g% with the mean value of 8.6 (table I) . It was found a significative correlation between hemoglobin value and fork length (figure I) . It was also found a tendency for hemoglobin increase with fork length up to 250 mm , when a stabilization of the values was noted.

White blood cells

The leukocyte count varied from 600 to 22,260 per cc with the mean value of 4,182 (table I) . No correlation was found between the white blood cell count and the fork length (figure 1) . A tendency for the concentration of the leukocytes values between 1,000 to 7,000 per cc was noted. Nine fishes had their white blood cell counts outside of this range and 7 of them were young specimens (fork length smaller than 250 mm) .

The means values for the white blood cell differential counts performed in 100 specimens were: lymphocytes — 88.76% , monocytes (macrophages according to Jakowska, 1956) — 6.31% , neutrophilic granulocytes — 0.71% , eosinophilic granulocytes — 0.04% , basophilic granulocytes — 1.55% , and plasmocytoid cells — 2.63% (table II) .

CITOLOGY AND PAS REACTION

Red blood cells

The red blood cells are nucleated and elliptical-shaped (figure 2) . The cells have a mean larger diameter of 9.34 micra and the smaller of 6.93 micra ; for the nuclei the mean larger diameter is 3.84 micra and the smaller is 2.25 micra (table II) .

On Romanowski preparations they have an acidofilic cytoplasm and a discrete polychromatophilia may be seen in a small percentage of the cells. PAS reaction was negative.

Lymphocytes

Lymphocytes are small cells, round, with the mean diameter of 5.17 micra. The nucleus occupies almost all the cell volume; the mean diameter of the nucleus is 4.01 micra (figure 2 , table II) .

The cytoplasm is scant, basofilic, without vacuoles or cytoplasmic granules. The chromatin is compact, and no nucleoli are seen.

On PAS reaction the majority of the cells are negative; some of them may show a tinge positivity of the cytoplasm and other cells have fine granules with perinuclear crown arrangement. We did not find gross PAS granules and double crown distribution.

Neutrophilic granulocytes

The neutrophilic granulocytes are cells with the mean diameter of 7.24 micra , a slightly pink cytoplasm and fine granules. The nucleus is lobulated, with a mean diameter of 3.31 micra , and the chromatin is coarse (figure 3 , table II) .

A large amount of PAS positive granules was found in the cytoplasm of the majority of the examined cells.

Eosinophilic granulocytes

The eosinophilic granulocytes have the mean diameter of 9.49 micra and a small amount of elongated eosinophilic granules is found in the cytoplasm. The nucleus is round, excentric, with a fine chromatin network,

TABLE I

Hematological data of Atlantic thread herring, *Opisthonema oglinum* (Le Sueur).

Data	Number of fishes (n)	Range		Arithmetic mean ($\bar{x}$)	Standard deviation (s)	Coeficient of variation (C.V.)
Fork length (cm)	100	150	393	209	19	9
Red blood cells (10^5/mm^3)	100	1.0	5.1	2.7	0.8	31
Hemoglobin (g%)	100	2.2	15.2	8.6	2.6	30
Leukocytes/mm^3	100	600	22,260	4,182	3,748	89
— Limphocytes	100	560	16,416	3,711	3,060	82
— Neutrophils	100	0	444	30	79	264
— Eosinophils	100	0	62	2	1	48
— Basophils	100	0	667	65	68	104
— Monocytes	100	0	6,010	264	927	351
— Plasmocytoid cells	100	9	1,480	110	251	227
Thrombocytes/mm^3	100	216	39,720	5,442	7,423	136

Leukocyte differential count: limphocytes = 88.76% , neutrophils = 0.71% , eosinophils = 0.04% , basophils = 1.55% , monocytes = 6.31% , plasmocytoid cells = 2.63% .

TABLE II

Measurements made in blood cell types of Atlantic thread herring, *Opisthonema oglinum* (Le Sueur).

Blood cell types	Number of cells (n)	Range (u)		Arithmetic mean ($\bar{x}$)	Standard deviation (s)	Coeficient of variation (C.V.)
Red blood cells	500					
larger cell diameter		5.84	11.68	9.34	0.71	7.60
smaller cell diameter		4.38	10.22	6.93	0.70	10.10
larger nucleus diameter		2.92	5.84	3.84	0.36	9.37
smaller nucleus diameter		1.46	5.84	2.25	0.33	14.66
Limphocytes	200					
cell diameter		2.92	11.68	5.17	1.25	24.17
nucleus diameter		2.19	7.30	4.01	0.90	22.44
Neutrophils	13					
cell diameter		5.11	10.22	7.24	1.50	20.71
nucleus diameter		2.19	4.38	3.31	0.71	21.45
Eosinophils	7					
cell diameter		5.84	13.14	9.49	1.83	19.28
nucleus diameter		2.92	7.30	4.59	1.01	22.00
Basophils	60					
cell diameter		5.84	14.60	8.84	2.39	27.03
nucleus diameter		1.46	7.30	3.35	1.18	35.22
Monocytes	100					
cell diameter		6.57	16.06	11.29	2.45	21.70
nucleus diameter		2.92	11.68	6.03	1.42	23.54
Plasmocytoid cells	180					
cell diameter		5.84	18.98	10.85	2.17	20.00
nucleus diameter		1.46	7.30	3.57	1.19	33.33
Thrombocytes	200					
larger diameter		3.65	10.22	6.43	1.07	16.64
smaller diameter		1.46	4.38	2.68	0.54	20.14

without nucleoli, and the mean diameter is 4.59 micra (figure 4, table II).

On PAS reaction a small amount of slight stained positive material was found among the specific granules.

Basophilic granulocytes

The basophilic granulocytes are seen more frequently than the eosinophil's granulocytes. The mean diameter is 8.84 micra and the cytoplasm is full of gross dark colored granules. The nuclei are round, with the mean diameter of 3.35 micra, fine chromatin, without nucleoli and frequently, they can not be vizualized due to the large amount of basophilic granules (figure 5, table II).

On PAS reaction they show a small amount of positive color at the level of the specific granules.

Monocytes

The monocytes (macrophages according to Jakowska, 1956) have the mean diameter of 11.29 micra. The nucleus occupies 2/3 of the cell, has a fine reticulated chromatin, without nucleoli. The foamy cytoplasm is slightly basophilic and has azurophil granules. Some cells have a more marked degree of cytoplasm basophilia and gross granules. The nucleus has a mean diameter of 6.03 micra (figure 6, table II). The monocytes are seen more frequently in clumps.

On PAS reaction fine positive granules are found scattered in a tinge background.

Plasmocytoid cells

Plasmocytoid cells have the appearence of human plasma cells in the May-Grunwald--Giemsa preparation. They have the mean diameter of 10.85 micra. The cytoplasm is abundant, slightly basophilic, full of globules resembling Mott cells, with increased periglobular basophilia. The nucleus has an excentric position, and the mean diameter of 3.57 micra. The chromatin is made of delicate clumps and sometimes had the appearence of a fine net, like that seen in the monocytes. No nucleolus was seen in those cells (figure 7, table II).

On PAS reaction a homogeneous positive material was detected among the cytoplasm globules.

Thrombocytes

Thrombocytes are elliptical cells with the larger diameter of 6.43 micra and the smaller of 2.68 micra. They may be seen in small clumps but they are also found isolated. In the majority of the thrombocytes the cyto-

plasm is scant, acidophil and localized in one extremity of the cell. The nucleous has a compact chromatin and occupies most of the cell volume (figure 2, table II).

On PAS reaction some thrombocytes may show positive small granules located in the extremities of the cells or may have a tinge cytoplasm. We did not find granules in crown formation.

DISCUSSION

The analysis of our data shows that there is a great variation in the extreme values of the hemoglobin determinations, and in the counts of red blood cells, leukocytes and thrombocytes. Fork length can be, in part, responsible for these variations. Unfortunately we did not find other studies on the blood of the Atlantic thread herring to compare with our data.

Digestion process in fishes may increase the leukocyte count but only 2 to 5 times the fasting levels (Smirnova, 1965). In our studies we did not have data to correlate the white blood cell levels with digestion process. It was felt that fishes have a hematopoietic tissue and mechanism of the liberation in many aspects different from the mammals.

We found 2 fishes with a very high white blood cell count (900,000 and 1,000,000 per cc). The data of these fishes, not included in our study, were reported in a separated paper (Martins & Pitombeira, 1968), because it was felt that this leukocytosis could represent a blood fish pathology.

Unfortunately no pathologic examination was done in the fishes with abnormal white blood cell counts, because they were sold for the population before the blood studies were concluded. Eventhough no primitive cells were found in the peripheral smears we felt that these leukocytosis could represent instances of leukemic process in fishes.

More studies should be done in icthyological hematology. A better knowledge of normal levels and blood pathology is necessary, because a fish with a blood pathology may represent a potential danger of transmission of disease to the man.

SUMMARY

Red blood cell count, hemoglobin determination, white blood cell and differential counts, cytology and PAS reaction of the circulating cells were performed in 100 Atlantic thread herring, *Opisthonema oglinum* (Le Sueur), captured at Mucuripe Beach (Fortaleza, State of Ceará, Brazil).

Two fishes were found with a very high white blood cell count, suggesting the possibility of being a leukemic change.

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FIGURES

Figure 1 — Hematological data of 100 Atlantic thread herring, *Opisthonema oglinum* (Le Sueur), from Northeastern Brazil, correlated with total lengths.

Figures 2-7 — Blood cell types of Atlantic thread herring, *Opisthonema oglinum* (Le Sueur), from Northeastern Brazil.

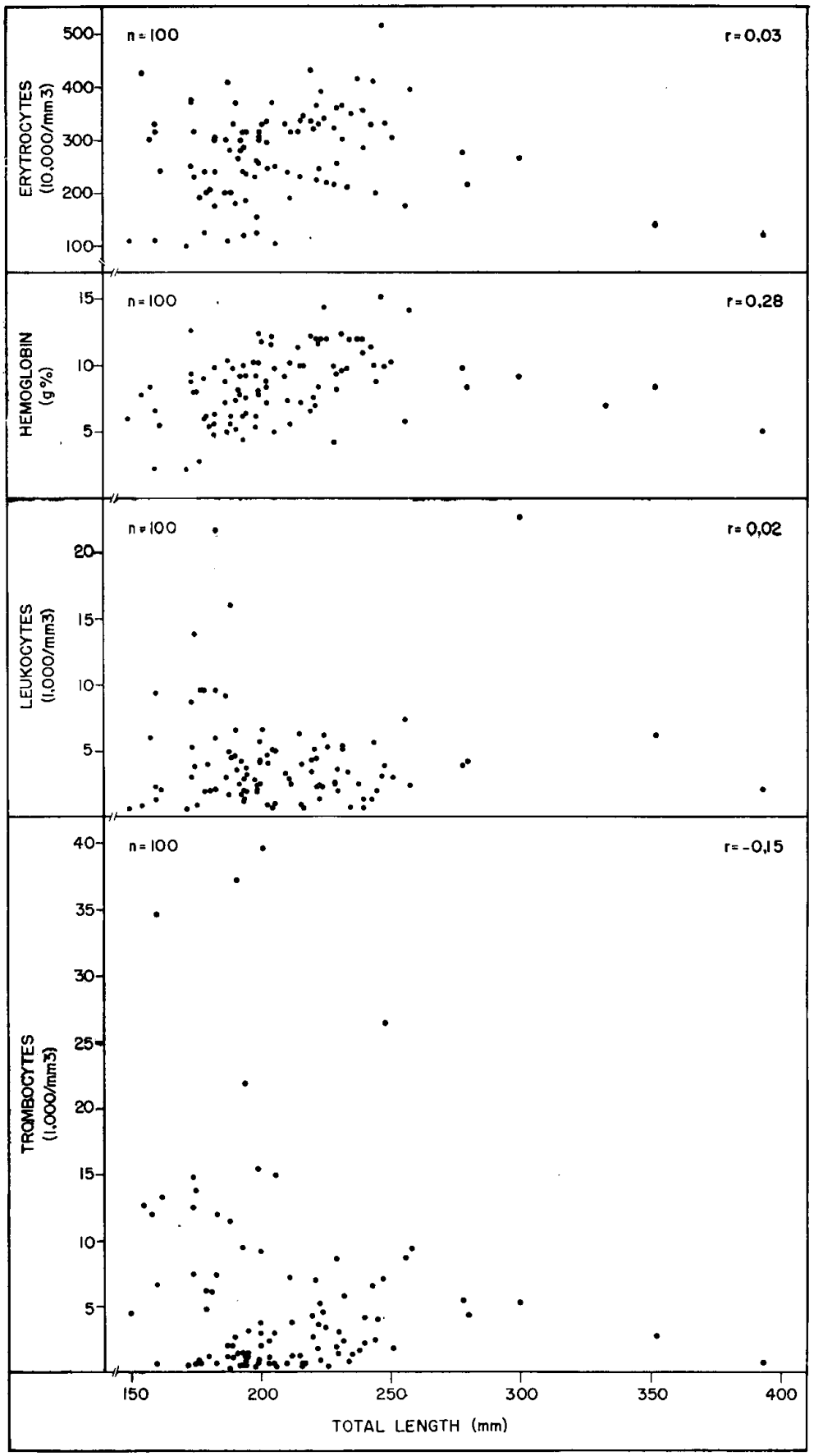
ERYTROCYTES (10.000/mm3)
n = 100
r = 0,03
HEMOGLOBIN (g %)
n = 100
r = 0,28
LEUKOCYTES (1.000/mm3)
n = 100
r = 0,02
TROMBOCYTES (1.000/mm3)
n = 100
r = -0,15
TOTAL LENGTH (mm)

Figure 1

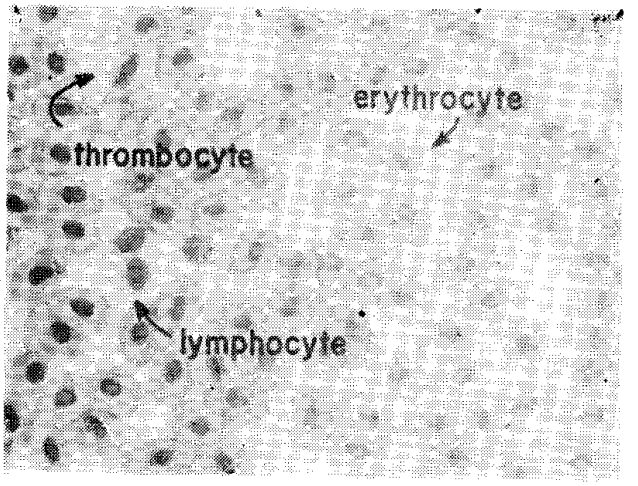

Figure 2

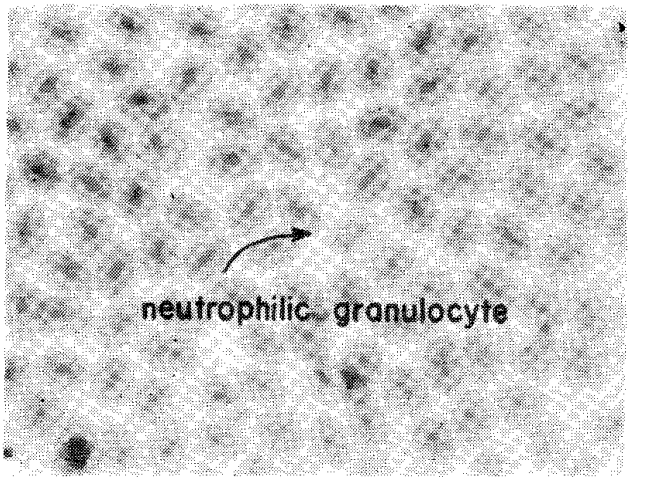

Figure 3

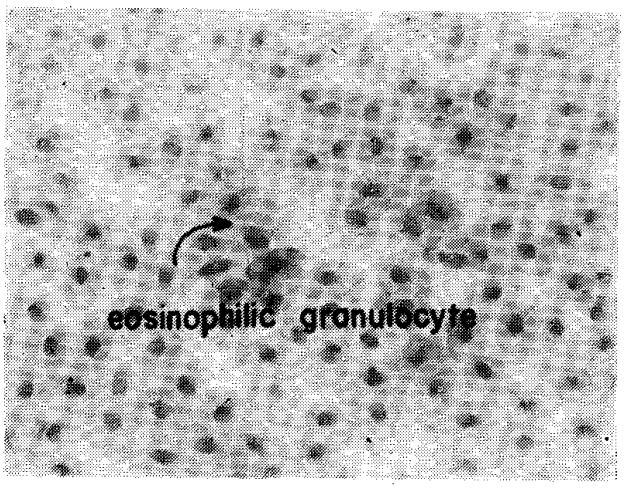

Figure 4

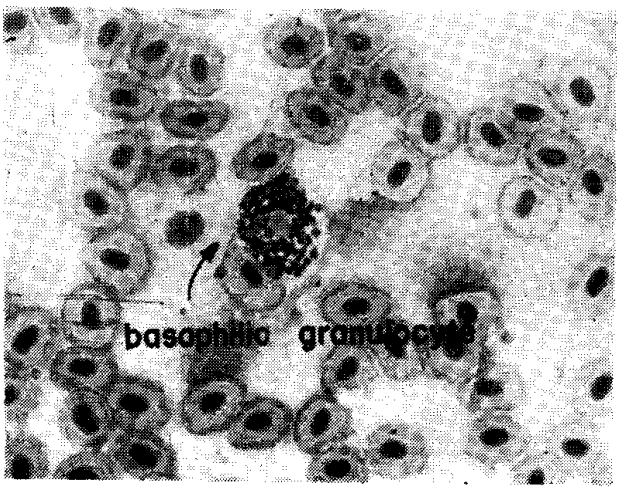

Figure 5

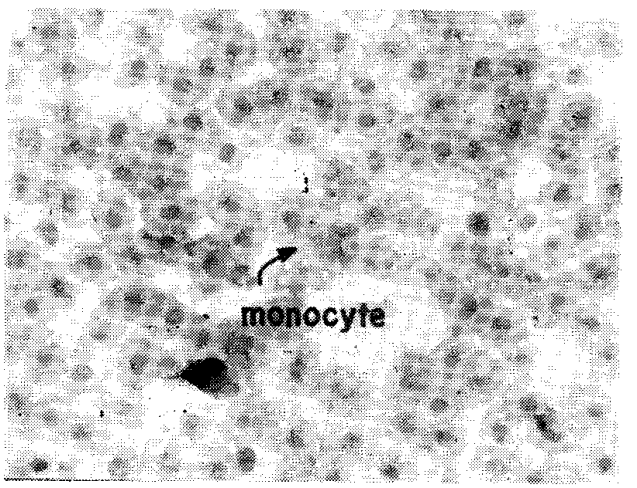

Figure 6

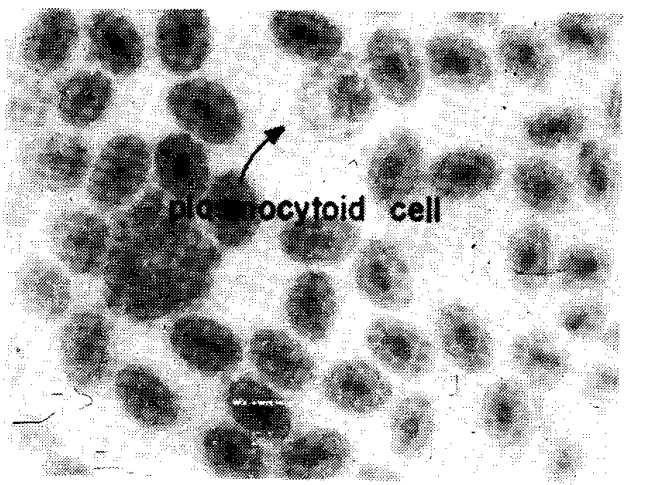

Figure 7