قسم : الباثولوجيـــــــــــــا .
كلية : الطب البيطرى ـ جامعة أسيوط.
رئيس القسم : أ.د / محمد ابراهيم الشرى .

فصل وحفظ كرات الدم البيضـــــاء من دم الكـــــلاب

محمود عبد الظاهر ، محمد خيرى ، عبد اللطيف بيومي

أجريت طريقة سهله ، سريعه ، ورخيصه لفصل وتركيز وحفظ كرات الدم البيضاء من دم الكلاب . أخذ يت العينات من عدد عشرة كلاب وأجريت الطريقه وأخذت عينات من الدم الكلي وكذا بعد ساعه ، ثمانية عشرة ، ثماني وأربعون ، اثنان وسبعون وسته وتسعون ساعه من الفصل .

فحصت العينات ميكروسكوبيا ودرست مورفولوجيه الخلايا وكذلك عدد هـــا وأجريت دراسة احصائيـــه .

أسفرت النتائج أنه يمكن الحصول على أعلى تركيز من كرات الدم البيضـــاء بعد ثمانية عشر ساعه من الفصل ، أما أعلى تركيز من الخلايا الليمفاويه يمكـــن الحصول عليه بعد ٧٢ ساعه من الفصـــل .

Dept. of Pathology,
Faculty of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. M.I. El-Sherry.

SEPARATION OF LEUCOCYTES FROM PERIPHERAL BLOOD OF DOGS

(With 1 Table & 5 Figs.)

By
A.Z. MAHMOUD; M.K. IBRAHIM and A.H. BAYOUMI

(Received at 21/5/1983)

SUMMARY

In the present study, an efficient method for separation, concentration and preservation of dog leucocytes from the peripheral blood were described. Statistical methods were carried out to postulate the changes in each test done. The most suitable time for maximum leucocytic concentration was eighteen hours after refrigration, however, nearly pure, Maximum lymophocytic concentration could be obtained 72 hour after preservation.

INTRODUCTION

WILDY and RIDLEY (1958) utelized the fact that, leucocytes had the ablity to adhere to glass to devise a technique for the separation of polymorphnuclear leucocytes from human blood. TOPP and CARLSON (1971) used the same fact for isolation of avian hetrophils.

TULIS (1953) used a basic salt and buffer solution for the preservation of human leucocytes. MAHMOUD (1979) used a hypotonic shook for separation of bovine lymohocytes in apparently healthy and leukemic cattle.

This study was initiated to investigate and establish and efficient method for separation, Concentration and preservation of dog leucocytes, from the peripheral blood, to facilitate their histochemical and ultrastructural studies in normal and pathologic conditions.

MATERIAL and METHODS

The present study is carried out on ten apparently healthy dogs. Fifty ml. blood was obtained from the jugular vein of each. One mg ethyl diamine tetraacetic acid per ml blood was used as anticoagulant. Before addition of the anticoagulant, blood smears were taken from the whole blood.

Haemolytic shock was conducted by addition of 150 ml. distilled water to each sample, while hypotonsity was restored by adding 50 ml. of 4.5% sodium chloride to each.

The samples were preserved in the refrigirator at 4°C and forty ml. from each sample were obtained 1, 18, 48, 72, 96 hours after preservation. The latter were taken after good homogenization, centrifugated for seven minutes at 800 R/M and smears were performed from the precipitate. The smears were fixed in ethanol alcohol, stained with Giemsa, haematoxylin and eosin and examined.

The leucocytes were examined morphologically as well as numerically in ten squares, each of 6.5 x 9 by 40 objective lens. The total number of leucocytes and their different types were calculated per square.

Statistical methods were carried out to postulate the changes given in each test. The mean values of each number were statistically analyed.

RESULTS

I. Micromorphological findings.

A. Whole blood smears:

The red blood cells were extremly abundant, however, the leucocytes were widely distributed here and there and not more than two to five cells could be observed in each square.

B. One hour after preservation:

The erthrocytes has undergone haemolysis, although remenants of haemoglobin could be seen. The leucocytic concentration was greatly increased; up to thirty to thirty five cells in each square were seen (Fig. 1). The staining affinity as well as their membranes could be good recognized. Neutrophils and lymphocytes were the predominating cells respectively.

C. Eighteen hours after preservation:

Maximum leucocytic concentration was observed in this group. up to forty, well stained leucocytes were observed in each square (Fig. 2). Neutrophils were the most prominent cells followed by the lymphocytes.

D. Forty-eight hours after preservation:

The erthrocytes were completely disappeared except for shades of haemoglobin could be seen. Although the maximum leucocytic concentration was observed (Fig. 3), as the previous group, some neutrophils showed degenerative changes represented in cytoplasmic vacuolation and nuclear condensation.

E. Seventy-two hours after preservation:

The erthrocytes nearly disappeared completely. The neutrophilic concentration was decreased and most of them showed degenerative changes. The maximum lymphocytic concentration was observed in this group (Fig. 4); their number overlaped the neutrophilic count in all squares.

F. Ninty-six hours after preservation:

The erthrocytes were completely disappeared. Most of neutrophils showed degenerative and necrotic changes. Lymphocytes constituted the main type of cells observed, associated with remenants of neutrophils.

Concerning the esionophils, they appeared in small proporation and they preserved their contour and staining affinity along the experiment.

II. Statistical analysis

Statistical analysis (Fig. 5) and Table (1) proved that the maximum concentration of leucocytes could be obtained 18 hrs. after separation, while the maximum lymphocytic concentration could be prepared 72 hr. after separation.

DISCUSSION

An efficient, simple, rapid and economically cheap method for separation, concentration and preservation of both leucocytes and lymphocytes from canine peripheral blood was established. Our study indicated that, the maximum concentration of leucocytes could be obtained 18 hours after separation, while the maximum lymphocytic concentration could be prepared 72 hours after separation; at which most neutrophils were destroyed.

Many investigators used the buffy coat layer technique for histopathological and ultrastructural studies, (BILKERMAN, et al. 1968; CUMONEN and AGACEN, 1968; DUNNE, et al. (1970), but leucocytes in this technique appeared to be admixed with amounts of primses; red blood corpscules and plasma; for this reason such leucocytes are not desirable for histochemical, immunohistochemical and ultrastructural studies. The present work yield a pure leucocytic concentration sufficient and suitable for such studies. The obtained purified leucocytic mass could be attributed to the haemolytic shock which resulted in erythrolysis and sedementation of leucocytes in refrigerator as well as the low speed centrifugation.

MENSHKOVA and KRIUKAN (1978), MAHMOUD (1979), stated that bovine neutropnils in short term tissue culture, showed degenerative changes after 24 hours and were completly destroyed at 48 hours, although in the present work, canine neutrophils manifested degenerative changes after 48 hours, while complete destruction occurred after 12 hours. The difference in time preservation and manifestation of degenerative changes between bovines and canines probably may be due to spiece difference as well as the number of leucocytes (ARCHER and JEFFCOTT, 1977).

Regarding the maximum lymphocytic concentration which could be obtained 72 and 96 hours after preservation, those obtained after 72 hours are preferable, younger and suitable for histopathologic studies.

REFERENCES

Archer, R.K. and Jeffcatt, L.B. (1977): Comparative clinical haematology. Blackwell scientific publ. oxford, London, Edinburgh, Melboubne pp. 365.

Bilkerman, B.E.; Sumurnov, A.N. and Eggrova, V.D. (1968): Ultrastructure of blood leucocytes from cattle. Bull. all union Expr. Vet., 7, 69-73.

Cumonen, G.A. and Agacen, A.B. (1968): Cytomorphological picture of blood from clinically healthy cattle and cattle with leucosis. Full. all union, exper. Vet. 5, 43-47.

Dunne, H.W.; Kmetz, M.; Schultz, R.D.; Zimmerer, R.P. and Yilmazer, S.K. (1970): Leucocytes as carriers in the transmission of bovine leukemia: Certain morphologic and functional characterstics of cultured leukocytes from normal and leukemic cattle. Am. J. Vet. Res., 31 (4), 597-617.

Mahmoud, A.Z. (1979): Electron microscopic study of morphology and morphogenesis of bovine leukemia virus in tissue culture from lymph and blood of cattle with leucosis, p.h.D., Moscow, Vet. Acad. Sci.

Menshkova, Z.N. and Kriukon, V.A. (1978): Morphological characterstic of culture leucocytes from cattle affected with leucosis. Cpornic Noachek Trdoov, 100, 99-101.

Topp, C.R. and Carlson, H.C. (1971): Studies on avian hetrophils. 1- Cell seperation. Avian Dis., 71, 364-368.

Tullis, J.L. (1953): Preservation of leucocytes. Blood, 8, 563-575.

Wildy, P. and Ridley, M. (1958): Seperation of human leucocytes from blood. Nature, 182, 1801-1803.

Table (1): Showing the mean values and their stander deviation

	Total		Diff. count					
			N		L		E	
	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S
Whole blood	3.72	1.0685	1.76	0.6979	1.52	0.3376	0.44	0.4758
After one hour	10.56	2.6359	6.72	1.3739	3.77	0.9487	0.67	0.4045
After 18 hour	16.74	4.2254	9.93	2.0089	6.02	2.3100	0.79	0.4846
After 48 hour	12.39	1.9128	5.35	2.0397	6.13	2.2749	0.97	0.5068
After 72 hour	8.22	1.1443	2.36	0.8546	5.14	0.7977	0.72	0.2315
After 96 hour	7.01	1.0885	0.77	0.3377	5.56	2.3290	0.68	0.3773

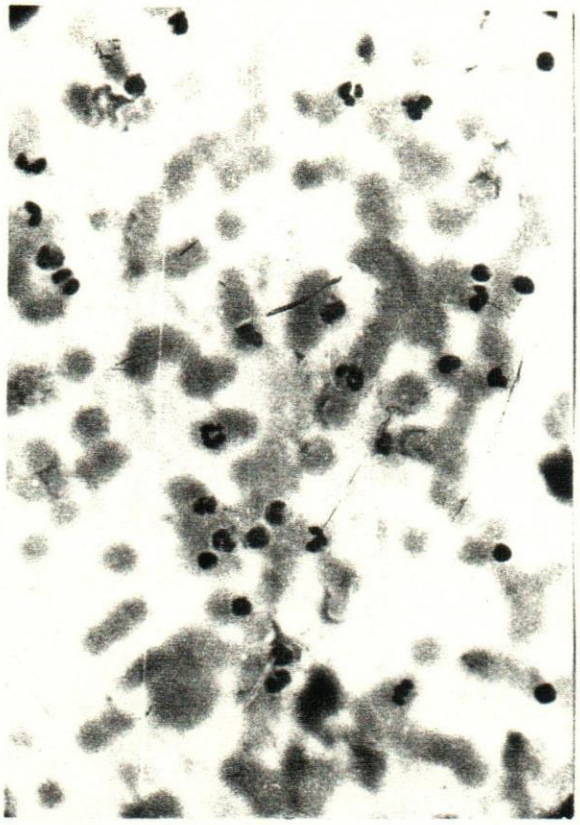

Fig. (1)

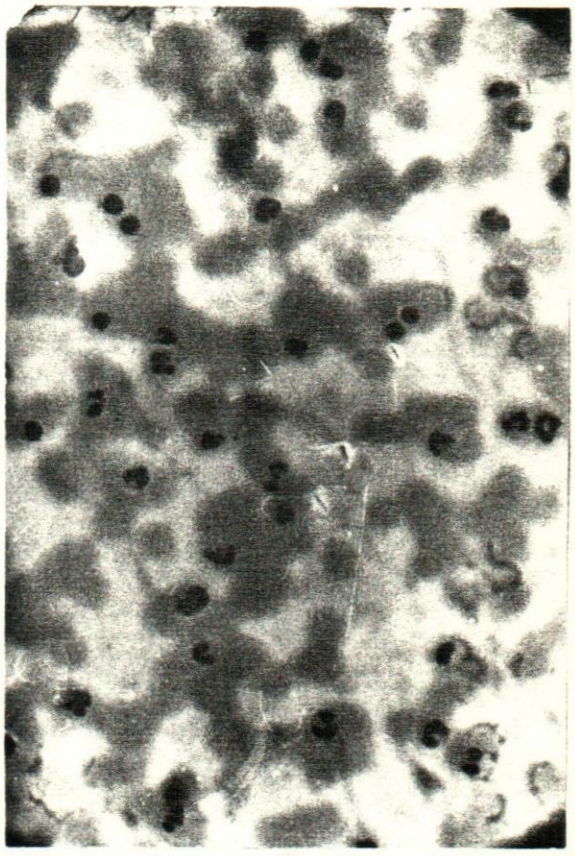

Fig. (2)

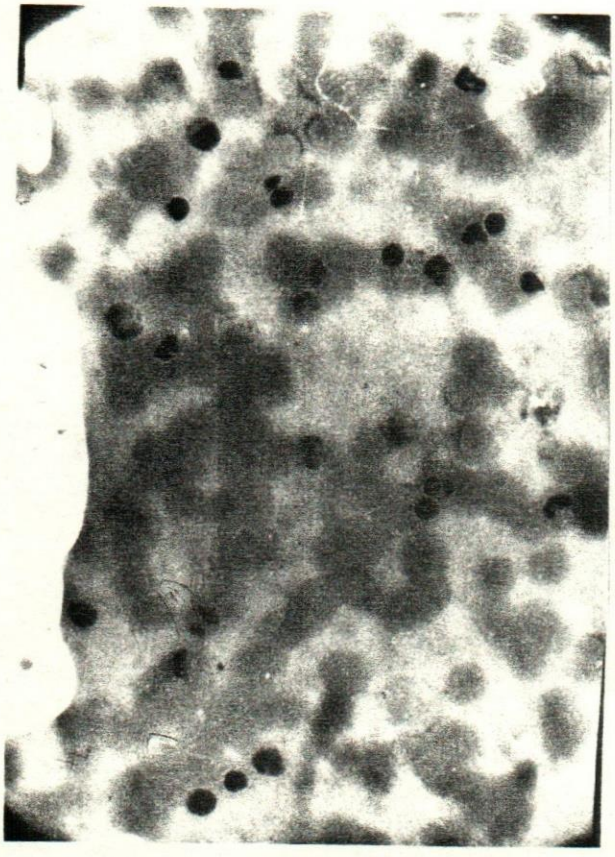

Fig. (3)

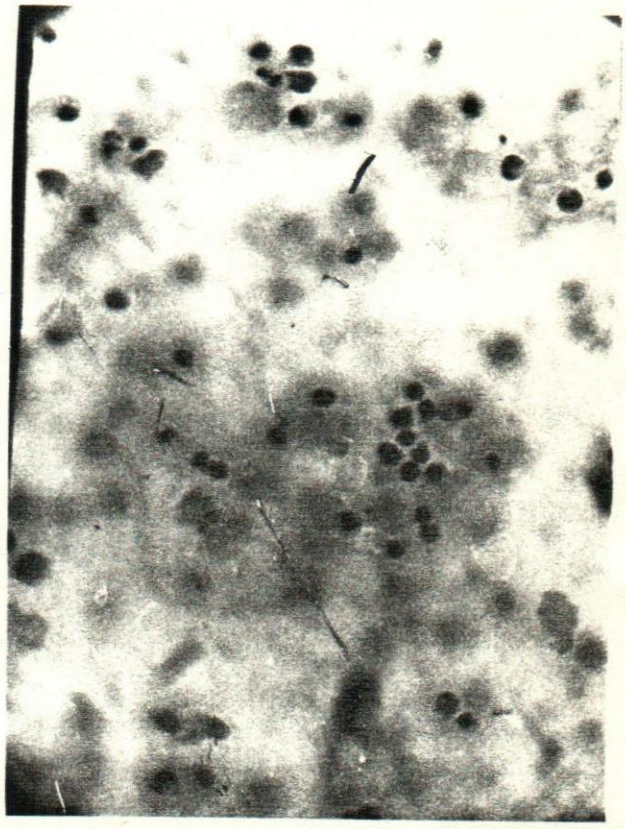

Fig. (4)

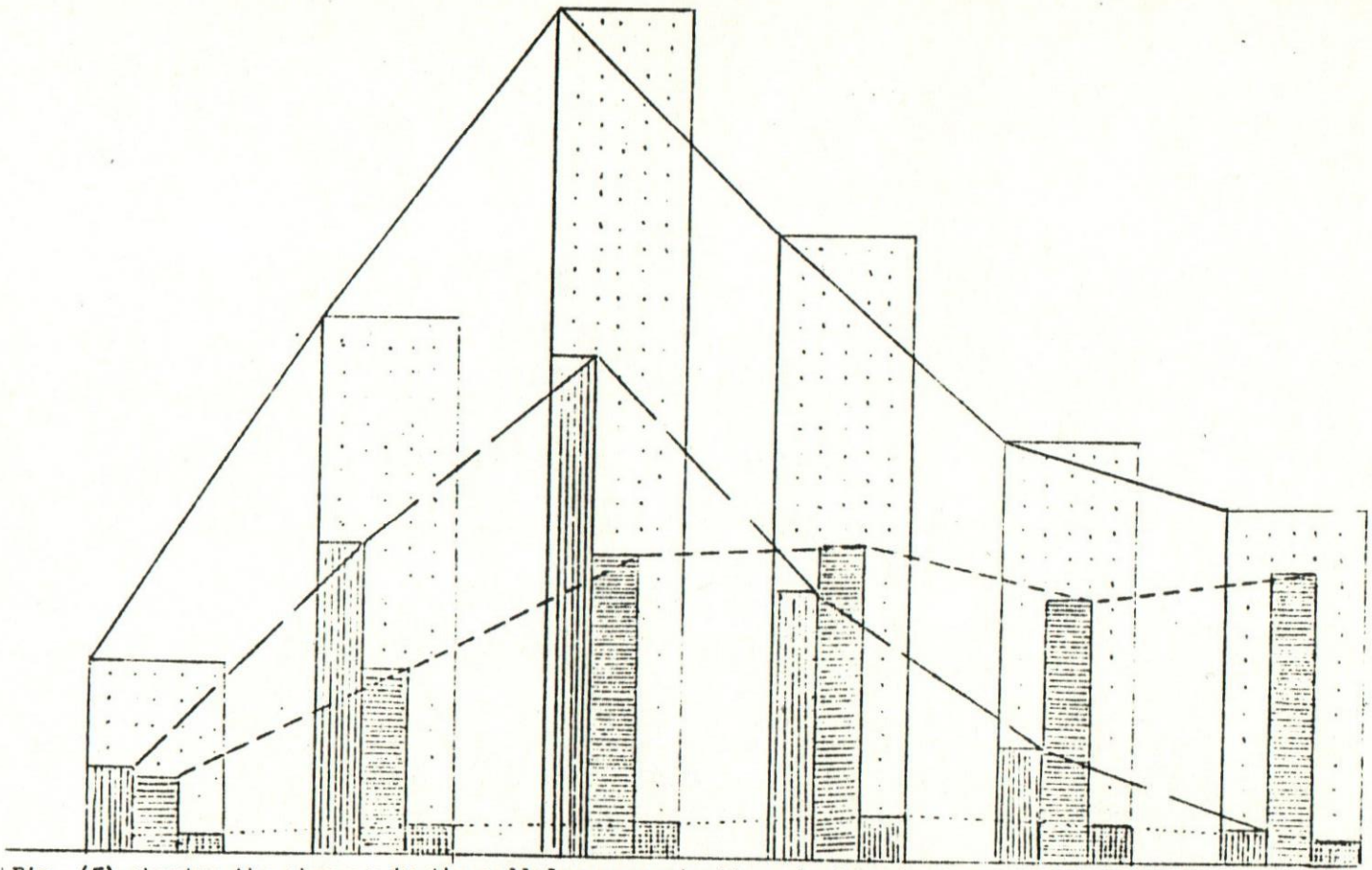

Fig. (5) showing the changes in the cellular concentrations during the different periods of preservation
Total number of leucocytes Neutrophilis concentration lymphocytie concentration
Esionophilic concentration.