قسم: الباثولوجي
كلية: الطب البيطري - جامعة أسيوط
رئيس القسم: أ. د / محمد ابراهيم الشرى.

فصل وحفظ كرات الدم البيض...
من دم الكلاب

محمود عبد الظاهر، محمد خيري ، عبد اللطيف بحومي

أجريت طريقة سهلة، سريعة، ورخيصة لفصل وتركيز وحفظ كرات الدم البيضاء من دم الكلاب. أخذت العينات من عدد عشرة كلاب وأجريت الطرقية وأخذت عينات من الدم الكلي وكذا بعد ساعتين، ثماني، ثماني وأربعون، أثنان وسبعون وستة وتسعون ساعة من الفصل.

فحصت العينات ميكروسكوبيا ودلت مورفولوجيا الخلايا وكذلك عدد هـ...
وأجريت دراسة إحصائية.

أسفرت النتائج أنه يمكن الحصول على أعلى تركيز من كرات الدم البيضاء بعد تسعين ساعة من الفصل، أما أعلى تركيز من الخلايا الليفية يمكن الحصول عليه بعد 72 ساعة من الفصل.
SEPARATION OF LEUCOCYTES FROM PERIPHERAL BLOOD OF DOGS
(With 1 Table & 5 Figs.)

By
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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 refrigeration, however, nearly pure, Maximum lymphocytic concentration could be obtained 72 hour after preservation.

INTRODUCTION

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

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

This study was initiated to investigate and establish an 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 hypotony was restored by adding 50 ml. of 4.5% sodium chloride to each.

The samples were preserved in the refrigerator 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 analyzed.

RESULTS

I. Micromorphological findings.

A. Whole blood smears:

The red blood cells were extremely 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 erythrocytes have undergone haemolysis, although remanents 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 erythrocytes 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 erythrocytes 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 overlapped the neutrophilic count in all squares.

F. Ninety-six hours after preservation:

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

Concerning the eosinophils, they appeared in small proportion 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.

SEPARATION OF LEUCOCYTES

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 theuffy coat layer technique for histopathological and ultrastructural studies, (BILKERNAN, et al. 1968; CUMONEN and AGACEN, 1968; DUNNE, et al. (1970), but leucocytes in this technique appeared to be admixed with amounts of primose; red blood corpuscles 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 sedimentation of leucocytes in refrigerator as well as the low speed centrifugation.

MENSHKOVA and KRIUKAN (1978), MAHMOUD (1979), stated that bovine neutrophils in short term tissue culture, showed degenerative changes after 24 hours and were completely 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 species 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


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

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Diff. count</th>
<th>N</th>
<th>L</th>
<th>E</th>
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<tr>
<td></td>
<td>$\bar{X}$</td>
<td>$S$</td>
<td>$\bar{X}$</td>
<td>$S$</td>
<td>$\bar{X}$</td>
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<td>Whole blood</td>
<td>3.72</td>
<td>1.0685</td>
<td>1.76</td>
<td>0.6979</td>
<td>1.52</td>
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<td>After one hour</td>
<td>10.56</td>
<td>2.6359</td>
<td>6.72</td>
<td>1.3739</td>
<td>3.77</td>
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<td>After 18 hour</td>
<td>16.74</td>
<td>4.2254</td>
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<td>After 48 hour</td>
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<td>1.9128</td>
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<td>After 72 hour</td>
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