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Cytochemical Characteristics of Blood and Bone Marrow Cells in Dog

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Abstract

The cytochemical characteristics of the hematopoietic cells in blood and bone marrow from 3 clinically healthy dogs were examined using a battery of cytochemical stains. Alkaline phosphotase activity was demonstrated in eosinophilic series and occassionally in progranulocytes. A variety of cells exhibited acid phosphatase activity, but tartrate-resistant acid phosphatase activity was seen only in eosinophilic series. Peroxidase activity was observed in myeloblasts to segmented cells of granulocytic series and in monocytes. α-naphthyl acetate esterase activity was found in monocytes and occassionally in lymphocytes. Naphthyl-AS-D-chloroacetate esterase marked neutrophilic series from myeloblasts to segmented cells. β-Glucuronidase activity was detected in a variety of cells except the cells of erythrocytic series. Periodic acid Schiff stain-positive granules were demonstrated in the neutrophilic and eosinophilic series from myelocytes to segmented cells and in monocytes and occassionally in lymphocytes. Sudan black B stain-positive granules marked granulocytic series from myeloblasts to segmented cells and monocytes.

Introduction

The correct identification of the leukemic cells is a prerequisite for development of more specific therapeutic regimens and for the characterization of various leukemias. The neoplastic cell type in the majority of leukemia in dogs can be classified with conventional Romanowski type stains. However, when primitive cells or abnormal cells predominate, morphologic differences are indistinct and the exact classification is difficult. In these cases, cytochemistry makes possible delineation of specific cellular features important in differentiating acute lymphocytic leukemia and acute myelogenous leukemia and it is known to be superior to morphologic subclassification of the myelogenous leukemia in human[3].

The cytochemical characteristics of hematopoietic cells in man are well documented, and cytochemical stains are used extensively in human medicine to identify blasts that appear poorly differentiated with Romanowski type stains. The cytochemical characteristics have been used not only for the classification of the cell types in leukemia and preleukemia

but also to monitor the therapeutic effects of antineoplastic drugs in human being.

Cytochemistry has been used increasingly in diagnosis of leukemias in dogs during the last 10 years[5,7,10,23], but comparatively little is reported about cytochemical characteristics of canine blood cells and bone marrow cells. Besides, various techniques have been employed for each cytochemical marker in human, and they make confusion because the results differ frequently depending on the technique used when they are applied for canie blood cells and bone marrow cells.

In the preliminary experiment, several techniques for each cytochemical marker were tried and selected one technique for each cytochemical marker which gives the best result for canine blood cells and bone marrow cells. In the present experiment, cytochemical characteristics of the canine blood cells and bone marrow cells were investigated by the selected methods. As the discussion for the comparison of the methods was not intended, this paper deals with only the results obtained by the selected methods for the discussion.

Materials and Methods

Circulating blood and bone marrow specimens were collected from three clinically healthy dogs without any anticoagulant. The bone marrow specimens were aspirated from the iliac crest of the dogs anesthesized with xylazine HCl.

Table 1. Summary of Selected Cytochemical Techniques

Cytochemical marker	Fixative	Substrate or Principal stain	Coupling dye	Reaction product	Reference
ALP	formalin-methanol	naphthol AS-MX phosphate	fast blue BB	blue	Nanba *et al.*[16]
ACP	citrate-buffered acetone	naphthol AS-BS	fast red ITR	red	Shibata & Sakaki[21]
Tartrate-resistant ACP	L-tartrate is added to naphthol AS-BS				
PO	formalin-ethanol	alpha-naphthol	brilliant cresyl blue	blue	Nagano *et al.*[15]
NAE	formalin-acetone	alpha-naphthyl acetate	hexazotized pararosaniline	red	Knoweles *et al.*[11]
CAE	formalin-acetone	naphthol AS-D chloroacetate	fast blue BB	blue	Saitoh *et al.*[19]
β-Gluc	formalin-acetone	naphthol AS-BI beta-glucuronide	fast red ITR	red	Kurokawa *et al.*[12]
PAS	absolute methanol	Schiff's reagent		magenta	Bauer-Sic[2]
Sudan black B	formalin vapor	sudan black B		black	Seehan & Storey[20]

Smears of blood and bone marrow were air-dried and fixed within 30 minutes after collection. The preparations were stained for alkaline phosphatase(ALP), acid phosphatase (ACP), peroxidase(PO), α-naphthyl acetate esterase(NAE), naphthol-AS-D-chloroacetate esterase(CAE), β-glucuronidase(β-Gluc), and with periodic acid Schiff(PAS), and sudan black B(SBB), within 1 hour after collection of the specimen.

The selected techniques are summarized in Table 1.

The positive reactions were evaluated by estimating the number of the positive granules distributed in the cytoplasm according the following criteria as described by Tsujimoto, *et al*[22].

negative(−) : with no positive granules
slightly positive(±) : with a small number of positive granules sparsely distributed
moderately positive(+) : with a moderate number of positive granules obviously recognized
markedly positive(++) : with abundant positive granules filling the cytoplasm

Results

The cytochemical characteristics of normal canine blood and bone marrow cells are shown in Table 2.

Positive ALP staining was seen in the cells

Table 2. Cytochemical Properties of Canine Blood Cells and Bone Marrow Cells

Blood or Bone marrow cell	ALP	ACP / Tartrate-resistant ACP	PO	NAE	CAE	β-Gluc	PAS	SBB
Myeloblast	−	−/−	±	−	occ +	occ ±	−	+
Progranulocyte	occ +	++/−	++	−	++	±	−	++
Neutrophilic								
myelocyte	−	+/−	+	−	++	+	+	++
metamyelocyte	−	+/−	+	−	++	+	+	++
band	−	±/−	+	−	++	occ +	+	++
segmented	−	±/−	+	−	++	−	±	++
Eosinophilic								
myelocyte	+	+/+	++	−	−	+	±	++
metamyelocyte	+	+/+	++	−	−	+	±	++
band	+	+/+	++	−	−	+	±	++
segmented	+	+/+	++	−	−	−	±	++
Lymphocyte	−	occ ±/−	−	occ ±	−	occ ±	occ ±	−
Monocyte	−	±/−	±	+	−	±	±	±
Rubriblast	−	±/−	−	−	−	−	−	−
Prorubricyte	−	±/−	−	−	−	−	−	−
Rubricyte	−	±/−	−	−	−	−	−	−
Metarubricyte	−	±/−	−	−	−	−	−	−

occ : occassional − : negative ± : slightly positive
+ : moderately positive ++ : markedly positive

of eosinophilic series(Fig. 1) and also in some but not all of progranulocytes(Fig. 2). The ALP activity of eosinophilic series was detected in the cytoplasm but not in the specific eosinophilic granules. Other cells were ALP-negative.

ACP activity was demonstrated in a variety of blood cells and bone marrow cells. Positive ACP staining was seen in progranulocytes, and from myelocytes to segmented cells in every granulocytic series. In eosinophilic series, ACP activity was detected in the specific eosinophilic granules(Fig. 3). Other granulocytes had from a few to many positive granules which stained more intensely than the granules of eosinophilic series. Positive ACP staining was also seen in erythrocytic series, from rubriblasts to metarubricytes, in monocytes and occassionally in lymphocytes.

Only the eosinophilic series showed ACP activity when the smears were incubated in the presence of tartrate(Fig. 5).

PO activity was recognized in myeloblasts, progranulocytes, and from myelocytes to segmented cell of neutrophilic series and eosinophilic series. Myeloblasts showed weakly positive fine granules, and progranulocytes(Fig. 6) exhibitied strong positive granules in the cytoplasm. Eosinophilic series revealed strongest PO activity(Fig. 7). A small number of dust like fine and weakly positive granules were noticed in the cytoplasm of monocytes(Fig. 8). However, lymphocytes and erythrocytic series were negative.

NAE-positive staining was seen in monocytes and some but not all lymphocytes. The NAE-positive granules in the cytoplasm of monocytes were diffusely distributed while those in positive lymphocytes revealed focal distribution. The color of the positive granules were more distinct in the positive lymphocytes than in monocytes.

CAE-positive staining was seen in myeloblasts(Fig. 10), progranulocytes and in the neutrophilic series from myelocytes to segmented cells(Fig. 11). Other cells were negative.

β-Gluc was demonstrated in a wide variety of cells. Myeloblasts occassionally showed weak β-Gluc activity(Fig. 12). Cells from progranulocytes(Fig. 13) to band cells of neutrophilic and eosinophilic series, and monocytes had β-Gluc-positive cytoplasmic granules. The positive granules were occassionally observed in lymphocytes. Cells of erythrocytic series did not show β-Gluc activity.

PAS-positive materials were demonstrated in neutrophilic and eosinophilic series from myelocytes to segmented cells, and neutrophilic series(Fig. 14) revealed stronger reaction than eosinophilic series. In eosinophilic series, PAS-positive materials were demonstrated in the cytoplasm and not in the specific eosinophilic granules(Fig. 15). Myeloblasts, progranulocytes, erythrocytic series and basophilic series did not exhibit PAS-positive reaction. Lymphocytes occassionally had a few but distinct PAS-positive cytoplasmic granules. Other cells were PAS-negative.

Myeloblasts, progranulocytes, cells of neutrophilic series(Fig. 16), and eosinophilic series showed strong reaction to SBB staining. In eosinophilic series, cytoplasm revealed strong positive reaction and the specific eosinophilic granules revealed moderate reacion to SBB staining.

Discussion

In human, neutrophils show ALP activity in the cytoplasm and it is suggested that it can facilitate differentiation of chronic myelogenous leukemia from leukemoid reaction or neutrophilic leukocytosis associated with non-malignant causes. ALP activity is markedly

decreased in the former, while it is normal or increased in the latter class of patients[17,21]. In the present experiment, neutrophils did not show ALP activity and only eosinophilic series and some progranulocytes revealed ALP activity. It has been mentioned that the cells of myeloblastic and myelomonocytic leukemia in dogs showed ALP-activity, and therefore, ALP could be used as a granulocytic marker in dog [10,23]. However, Facklam and Kociba[5] demonstrated that 38% of the dogs with lymphocytic leukemia had ALP-positive cells and that ALP should not be used singly as a granulocytic marker in dog.

As ACP activity was found in a variety of cells and it is, therefore, doubtful as a marker for differentiation of blood cells and bone marrow cells in dog. In human, on the other hand, tartrate-resistant ACP activity was demonstrated in hairy cell leukemia(reticuloendotheliosis), and hence applied to identify the hairy cell leukemia[21,25].

Granulocytic series and monocytes were PO positive. This observation was consistent with previous reports in dogs[5,8] and in man[4]. Negative staining of lymphocytic series was utilized for differentiation of acute lymphocytic leukemia from acute myelogenous leukemia in man. If the blast is PO-negative, it may be defined as non-lymphocytic, but if it is PO-negative, it can not be defined as non-myelogenous[14]. It has been reported that the blasts of granulocytic or myelomonocytic leukemia revealed PO-negative, and these findings imply that the PO stain is not sensitive enough to identify leukemic myeloid blasts in dogs[5,10].

NAE activity was demonstrated in monocytes and it was in accord with the earlier report of Jain[8]. Jain, and co-workers[10] classified the undifferentiated leukemic cells into myelomonocytic leukemic cells using NAE as monocytic marker. NAE activity was also demonstrated in some but not all lymphocytes. Wulff[24] demonstrated that NAE is a T-cell marker in dogs. Other proposed human T-cell marker such as rosette formation with human or guinea pig erythrocytes has been demonstrated to be unreliable in dogs[1]. Hasegawa *et al*[7]. could classify the leukemic cells from a dog with thymus-type malignant lymphoma as originated from T-cell system by the NAE-positive staining. Otherwise they might be impossible to differentiate in this patient as the cells revealed neither E-rosette formation nor surface immunoglobulin. Hasegawa *et al.*[7] also classified the leukemic cells from a dog with multicentric malignant lymphoma as originated from B-cell system by the NAE-negative staining. The NAE-positive lymphocyte and monocyte can be classified by the characteristics that lymphocyte has a small number of focal positive granules while monocyte has diffuse particle-like fine positive granules.

In this experiment, CAE activity was demonstrated in myeloblasts, progranulocytes, and neutrophilic series. Similarly Osbaldiston and Sullivan[18] could demonstrate CAE activity in various cell lines of dog by differing the pH of reaction mixture. So, when CAE is used as a neutrophilic marker, one should select a proper method and pH of the reaction mixture. Likewise, Facklam and Kociba[5] demonstrated CAE activity in undifferentiated cells of 4 dogs with myelomonocytic leukemia and suggested CAE as an efficient neutrophil marker in dogs. However, Jain *et al.*[10], found that CAE activity decreased in some dogs with myelomonocytic leukemia.

β-Gluc activity was seen in a variety of cells, and the similar results were reported in man by Lorbacher *et al.*[13]. It is commonly believed that β-Gluc activity decreases in chronic lymphocytic leukemia in man[12]. Little information is available on the β-Gluc activity in

animal blood cells and bone marrow cells. β-Gluc activity in normal feline blood cells is described by TsuJimoto et al.[22], and positive β-Gluc activity in contrast to negative NAE activity and negative PO activity in the cells of large granular lymphoma in cat is described by Goitsuka *et al.*[6]. The importance of the cytochemical demonstration of β-Gluc activity in blood cells for clinical hematology still remains to be elucidated in veterinary medicine.

PAS-positive staining was seen in myelocytes to segmented cells of neutrophilic and eosinophilic series, and in monocytes and in occassional lymphocytes. The results were consistent with Facklam and Kociba[5]. In human studies, some of erythroid precursor cells of all cell lines showed no positive staining by PAS(cited from Jain[9]). As the early precursor cells of all cell lines showed no positive staining by PAS in the present experiment, it suggests that PAS would hardly serve as a marker for any cell lines in leukemia of dogs.

Positive staining with SBB went hand in hand with PO-activity, but some myeloblasts were SBB positive without PO-activity. It is well agreed with the findings of Facklam and Kociba[5] that PO activity was negative in some myelomonocytic leukemia while the SBB stain was positive, thereby indicating that during granulopoiesis sudanophil manifests prior to PO activity.

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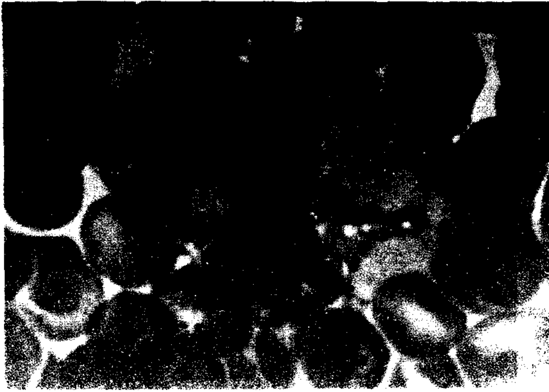
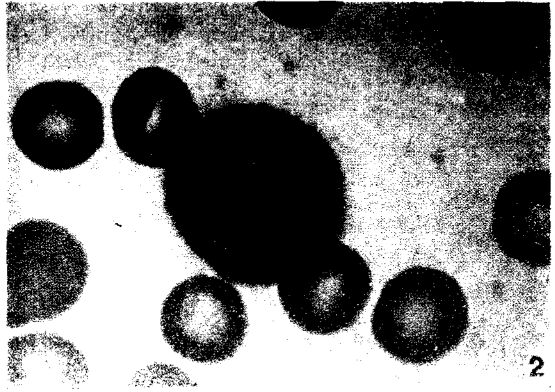
Legends for Figures

Fig. 1. Two alkaline phosphatase-positive eosinophils are illustrated.
Fig. 2. An alkaline phosphatase-positive progranulocyte is illustrated.
Fig. 3. An acid phosphatase-positive eosinophilic myelocyte is illustrated.
Fig. 4. An acid phosphatase-positive neutrophilic myelocyte is illustrated.
Fig. 5. A tartrate-resistant acid phosphatase-positive eosinophilic metamyelocyte is illustrated.
Fig. 6. A peroxidase-positive progranulocyte is illustrated.
Fig. 7. A peroxidase-positive eosinophilic myelocyte is illustrated.
Fig. 8. A peroxidase-positive monocyte(arrow) is illustrated.
Fig. 9. A peroxidase-positive neutrophilic metamyelocyte is illustrated.
Fig. 10. A naphthyl-AS-D-chloroacetate esterase-positive myeloblast is illustrated.
Fig. 11. A naphthyl-AS-D-chloroacetate esterase-positive segmented neutrophil is illustrated.
Fig. 12. A β-glucuronidase-positive myeloblast(arrow) is illustrated.
Fig. 13. A β-glucuronidase-positive progranulocyte(arrow) is illustrated.
Fig. 14. A PAS-positive segmented neutrophil is illustrated.
Fig. 15. A PAS-positive segmented eosinophil(arrow) is illustrated.
Fig. 16. Sudan black B-positive neutrophilic metamyelocyte, band, and segmenter(from left to right) are illustrated.

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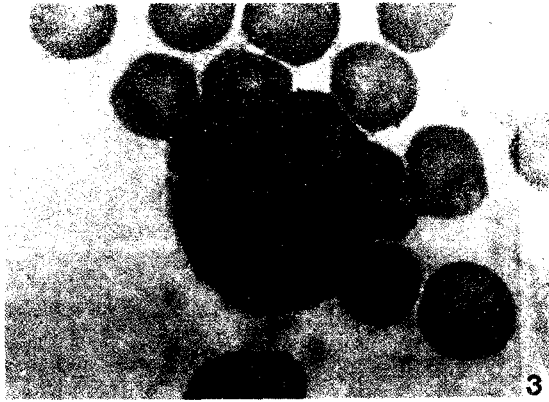
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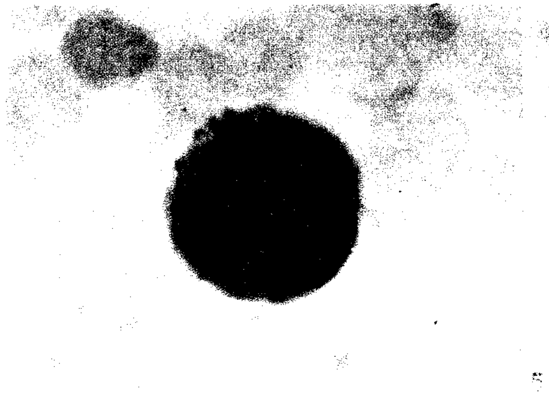

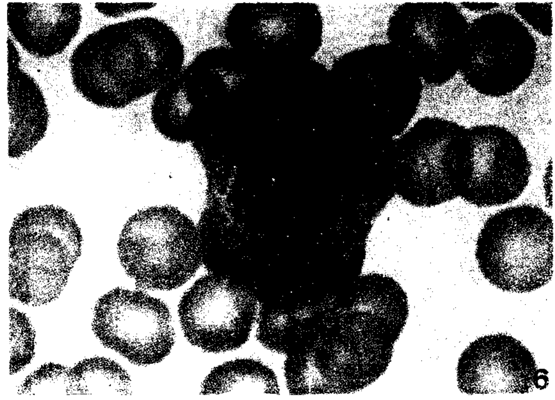
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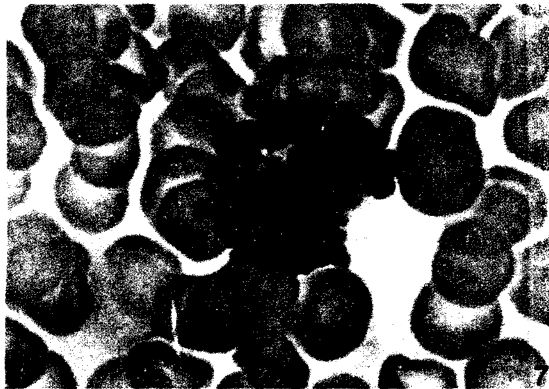
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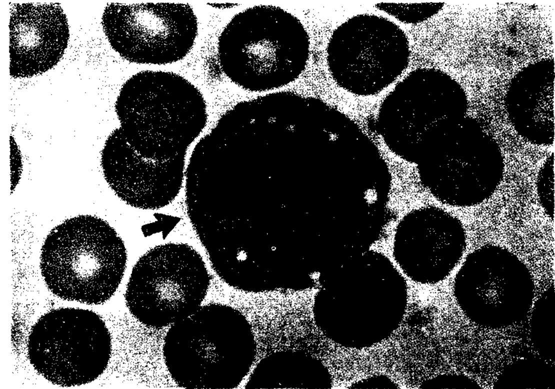

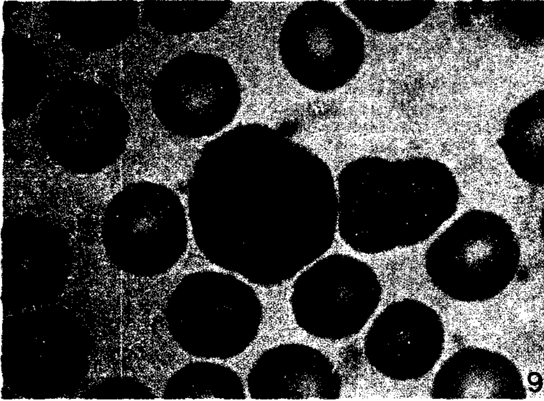
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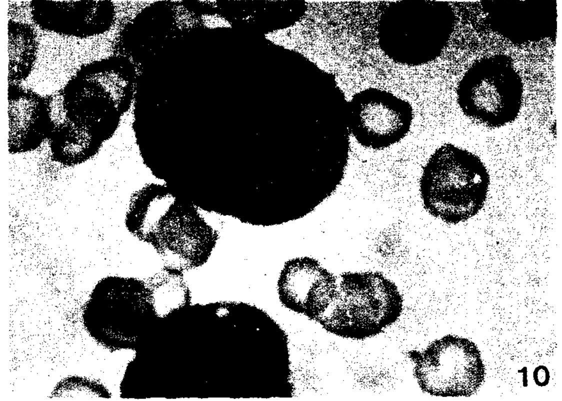
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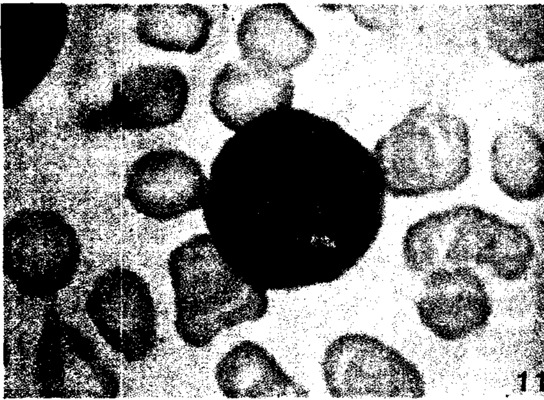
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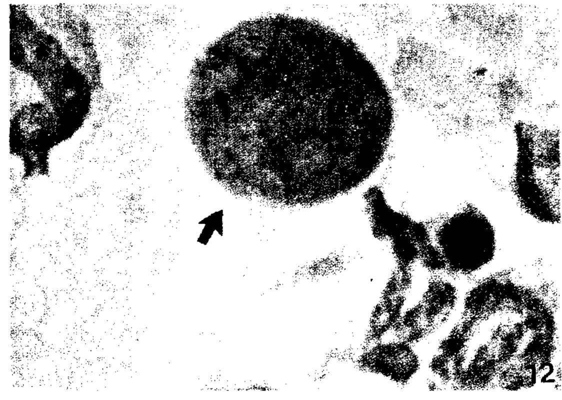
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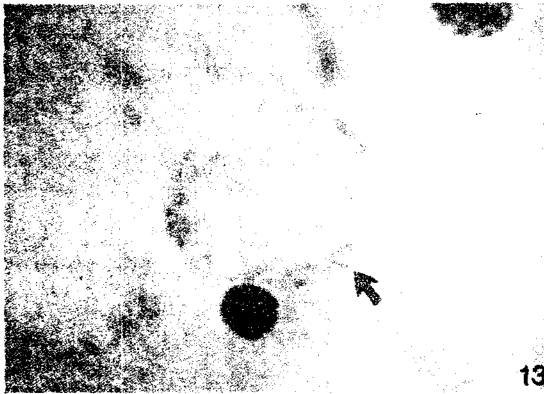
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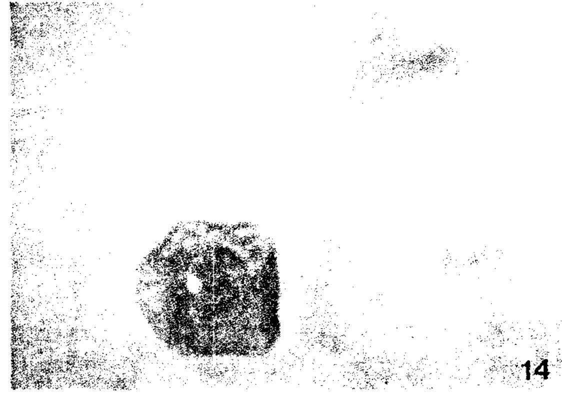
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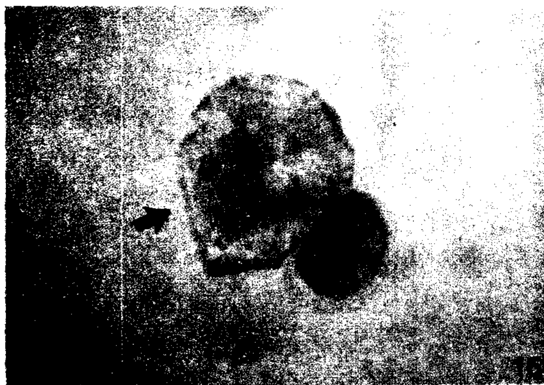

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개의 혈액 및 골수세포의 세포화학적 특성

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초 록

건강한 개의 혈액세포와 골수세포의 세포화학적 특성을 조사하였다. 이 실험에서 혈액과 골수 시료에 항응고제를 일체사용하지 않았으며 시료채취후 즉시 도말표본을 작성하여 30분이내에 반응을 실시하였다. 이 실험의 결과는 아래와 같다.

1. alkaline phosphatase의 활성은 호산구계통과 간혹 전골수구에서 양성반응을 나타내었다.
2. acid phosphatase의 활성은 대부분의 계통의 세포에서 양성반응을 나타내지만 tartrate로 억제하면 호산구계만 양성반응을 나타내었다.
3. peroxidase활성은 골수구계통의 모든 세포에서 양성반응을 나타내며 단구에서는 미약한 양성의 미세과립을 나타내었다.
4. naphthyl-AS-D-chloroacetate esterase활성은 호중구계 세포에서만 양성을 나타낸다.
5. α-naphthyl acetate esterase활성은 단구와 일부의 임파구에서 양성을 나타낸다.
6. Sudan black B 염색은 골수구계 세포와 단구계 세포에서 양성을 나타내었다.
7. β-glucuronidase활성은 적혈구계를 제외한 모든 세포에서 양성반응을 나타내었다.