Alkaline Phosphatase Activity in Leukocytes of Dogs and Cats*

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During recent studies on the distribution of alkaline phosphatase (ALP) activity in leukocytes of certain animal species, mature neutrophils of clinically healthy dogs and cats were found to be devoid of ALP [10]. Similarly, mature as well as immature neutrophils of dogs and cats with a leukemoid reaction and shift to the left were negative for ALP [9].

It has been shown that the leukocytic ALP of man and the rabbit is quite labile, affected by anticoagulants, varies with staining time and other technical variations, differentially affected by specific enzyme activators, and influenced by certain hormones [7,12,17,20,22]. Therefore, the present investigation was undertaken to determine whether the lack of ALP in mature neutrophils of the dog and the cat is an inherent species characteristic or is a matter of technical consideration.

Materials and Methods

Samples of blood were obtained from 61 dogs and 31 cats; all animals were clinically normal. Jugular blood was collected from all dogs and some cats using disodium ethylene diamine tetracetate (EDTA, 2 mg/ml of blood) as anticoagulant, unless otherwise indicated. Capillary blood from a marginal ear vein was obtained from many cats without any added anticoagulant and used immediately. Marrow samples were collected using heparinized syringes and smears were prepared immediately after collection. Hematologic determinations were performed using standard methods.

Air-dried coverslip blood and marrow smears were fixed in cold (0 to 4 °C) formaline-methanol 1:10 and stained for ALP according to the azo-dye technic of Kaplow [11]. Preliminary studies on the use of a fixative indicated that fixation with cold formaline-methanol was better than that with absolute methanol or acetone, and unfixed smears had altered cell patterns. Most of the smears were routinely placed in incubation mixture for 10 minutes and for 1 hour. The smears were mounted in glycerine jelly just before examination. The smears were examined the same day, or kept unmounted and examined within 1 to 3 days since ALP-activity faded in mounted smears. Control smears included smears stained similarly after they were immersed in boiling water for 1 minute to inactivate the enzyme or smears stained in incubation medium without the substrate.

The influence of various technical factors on leukocytic alkaline phosphatase activity was determined in several experiments. The factors included anticoagulants, length of the time of incubation in the substrate solution, temperature of incubation, pH of the buffer used for preparing the incubation mixture, and addition of some activators to the incubation mixture. Usually one variable was introduced at a time as indicated below. Blood samples from 2 to 4 animals of each species were used for each determination. So that the influence of different variables could be evaluated both for neutrophils and eosinophils, animals having high normal eosinophil counts were selected. The ALP-activity of eosinophils was

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graded from 0 to +4, as was done for neutrophils [11]. Usually a total of 50 eosinophils was graded on a smear, because of their comparatively small number in the blood; ALP-activity scores were calculated accordingly. When eosinophils were few, the whole smear was scanned.

**Time of making smear and length of incubation:** Smears were prepared within 10 minutes of sample collection and after blood was stored at 4°C for 1, 4 and 24 hours. Duplicate smears for each period were fixed, washed, and stored at 0°C to stain all together. They were incubated in the incubation solution at room temperature for 10, 30 and 60 minutes.

**Temperature of incubation:** Smears were prepared within 15 minutes of sampling. Duplicate smears were incubated in the incubation solution at 4, 24 (room temperature), and 37°C for 10, 30 and 60 minutes.

**pIq of the buffer:** Duplicate smears, prepared within 15 minutes of sample collection, were incubated in the incubation solution prepared with 0.05 M propandiol buffer adjusted to pH 7, 8, 9, 9.75 or 10. Incubation was done at room temperature for 10, 30 and 60 minutes.

**Anticoagulants:** Blood samples were collected using EDTA (2 mg/ml of blood), heparin sodium (10 units/ml of blood), or sodium citrate (3.8 per cent solution; 0.1 ml/5 ml of blood) as the anticoagulant. In addition, smears were prepared directly from venous blood without any added anticoagulant. Duplicate smears were incubated in the incubation solution at room temperature for 10, 30 and 60 minutes and 4 hours.

**Activators:** Duplicate smears, prepared within 15 minutes of sample collection, were placed in the incubation solution containing a specific activator. A small amount of the activator solution was added to the incubation solution to obtain the desired final molar concentration. The metal salts and amino acids used and their final molar concentrations (in parentheses) were as follows: Zn (10^4 to 10^-6), Mg (10^-4), Ca (10^-4), Mn (10^-3), glycine (10^-2 to 10^-3), alanine (10^-4 to 10^-5), histidine (10^-4 to 10^-5). Incubation was done at room temperature for 10 and 60 minutes.

**Alteration of Membrane Permiability:** The permeability of leukocyte membranes for staining reagents was increased by several means. Saponin was used in two ways; (a) before staining, fixed and unfixed smears were placed in saponin solution (0.25 ml of saponin + 9.75 ml of 0.85% NaCl solution) for 1, 5, 15 and 30 minutes; and (b) different amounts (0.5 to 1.0 ml) of 2% saponin solution (prepared in 0.85% NaCl solution) were added to the incubation solution before incubation. The smears were incubated for 10, 30, 60 and 180 minutes at room temperature.

In addition, fixed smears were placed in 0.5% NaCl and 1% NaCl solutions for 10 and 30 minutes and in 0.1 M citrate buffer of pH 3, 5 and 7 for 30 minutes before staining. The smears were then washed and incubated in the incubation mixture at room temperature for 60 minutes.

**Hormone administration:** The influence of ACTH (10 or 20 units), prednisolone (5 to 20 units), and progesterone (50 to 100 mg) on leukocyte ALP-activity was determined by injecting the hormone in a given dog or cat and collecting blood samples at 0, 6, 24, 48, 72 and 96 hours postinoculation. Smears were incubated at room temperature for 10 and 60 minutes and eosinophils on the whole smear were graded. For comparison, the average ALP-activity per eosinophil was calculated by dividing the activity score with the number of eosinophils graded.

**Other cytochemical methods:** The ALP-activity of leukocyte in some smears was determined also by the methods of Gomori [6], Kaplow [12], and Rulenberg et al. [18].

**Results**

**Blood Neutrophils:** Neutrophils of all the dogs and cats examined were negative for alkaline phosphatase activity (Fig. 1A). Variations of the staining technic used did not produce positive results. Neutrophils were devoid of ALP-