HÆMATOLOGY

Cytochemical Demonstration of Aminopeptidase Activity in Blood Platelets

A NUMBER of enzymes have been identified biochemically in platelets[1] and several of these have been localized cytochemically[1,2] as well. Aminopeptidase activity also can be demonstrated in platelets by an adaptation of Burstone's histochemical method[3,4].

Blood smears of healthy adult persons were briefly dried at room temperature and then fixed in cold acetone at 4° C for 1 h. Following this they were incubated for 22 h at room temperature in an incubating medium composed of 4 mg of alanyl-β-naphthylamide dissolved in 5 ml. of distilled water, 5 ml. of 0·1 M Sörensen's phosphate buffer at *p*H 6·8, 1 mg of magnesium sulphate and 5 mg of fast garnet *GBC*. An alternate, otherwise similar incubation medium contained 4 mg of 'Leucine' β-naphthylamide instead of alanyl beta-naphthylamide, and 1 mg of potassium cyanide instead of magnesium sulphate. After incubation in either of the foregoing solutions, the coverslips were rinsed for 10 min in tap water, briefly fixed in 10 per cent neutral formalin and mounted in glycerogel.

Microscopic examination of these preparations revealed fine granular orange red to rusty brown deposits in all platelets, an indication of considerable aminopeptidase activity (Fig. 1). A more intense reaction was seen with alanyl-β-naphthylamide, while incubation in medium from which the substrate was omitted yielded no evidence of enzymatic activity. Leukocytes also displayed aminopeptidase activity as described by Ackerman[5].

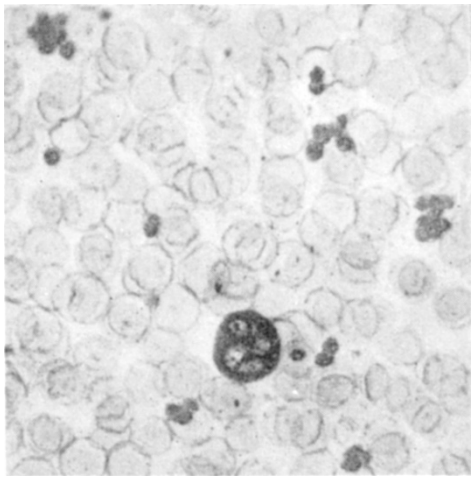

Fig. 1. Showing considerable aminopeptidase activity in human blood platelets (incubated in alanyl-β-naphthylamide). A neutrophilic leukocyte also reveals strong enzyme activity. (× 700)

The significance of the presence of aminopeptidases in platelets is uncertain, although as noted earlier their occurrence has been known for some time[6,7]. Recently Kocholaty[8], for example, has characterized several di- and tri-peptidases in normal human platelets. A more intense reaction was found with alanyl β-naphthylamide, but it is unlikely that this represents a difference in specific aminopeptidase-level in the platelets. From reports on other tissues[9,10] it would appear that more than one aminopeptidase may participate in histochemical reactions using chromogenic aminoacyl-naphthylamides, even though certain peptides may be preferentially hydrolysed. On the other hand, an increased peptidase activity has been reported in platelets during menstruation and in some hæmorrhagic disorders[11], while enzyme-levels decreased during the administration of dicoumarol[12]. These data tend to link aminopeptidase activity in platelets with blood clotting phenomena, but the true physiological role of these enzymes in platelets remains unknown.

This investigation was supported by a grant (*NB*–04155–02) from the National Institute of Neurological Diseases and Blindness, National Institutes of Health of the United States Public Health Service, Bethesda, Maryland.

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Elution Procedure for the Demonstration of Methæmoglobin in Red Cells of Human Blood Smears

THE peroxidatic activity of the red blood pigment can be inhibited by cyanide in the case of methæmoglobin (Hb^{III}), but not in the case of oxyhæmoglobin (Hb^{II}) (ref. 1). It seemed promising to use this difference for a differential staining procedure of Hb^{II}- and Hb^{III}-containing red cells by first adding cyanide to the heparinized or citrated blood in question and by staining smears prepared from it by a peroxidase reaction.

The results were relatively poor. Much better results were obtained, however, with the following elution procedure:

(1) To heparinized or citrated blood 1/50 vol. of a 0·4 M solution of potassium cyanide is added. Thin blood smears are prepared.

(2) The air-dried smears are immersed into the following mixture at room temperature: 80 ml. ethanol (96 per cent); 16 ml. 0·2 M citric acid; 5 ml. hydrogen peroxide (30 per cent b.w.). The smears are agitated up and down for 1 min and then allowed to stand for 2 min.

(3) Washing first in methanol, then in water, 30 sec each.

(4) Staining for 2 min in Ehrlich's hæmatoxylin and, after rinsing with tap water, for 2 min in 0·2 per cent erythrosin (Merck). Rinsing and drying.

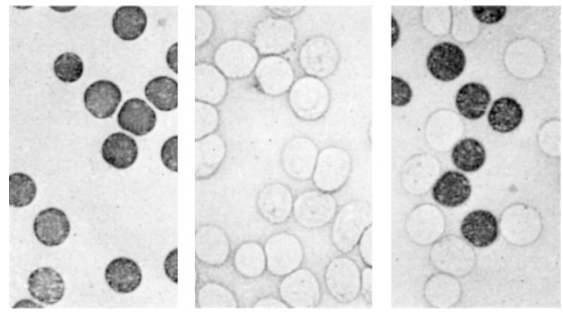

Fig. 1. Elution and staining of blood smears for the demonstration of methæmoglobin. Left, normal human adult blood; middle, same blood after preliminary conversion of the blood pigment to methæmoglobin by nitrite; right, mixture of normal and methæmoglobinæmic blood