PCMB inhibits RIA. This is particularly interesting since platelet aggregation induced by bovine factor VIII, which is thought to be similar to RIA, is not inhibited by another sulfhydryl inhibitor, PCMBS.

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To the Editor:

5'-Nucleotidase is an ectoenzyme of many mammalian cells, i.e., the active site(s) of the enzyme are located on the external surface of the plasma membrane. The high activity of this enzyme and its specific localization to the plasma membrane has led to its use as a marker for this cell component in fractionation studies of polymorphonuclear leukocytes (PMN) from guinea pigs and rabbits. We found little to no activity of this enzyme in human PMN or in membrane preparations from these cells, and thus, we were unable to use this convenient marker to identify the plasma membrane fraction from cell homogenates.

Human PMN were isolated from venous blood using a combination of dextran sedimentation, Ficoll-Hypaque gradient (Winthrop Laboratories, New York) and hypotonic lysis. The final leukocyte suspensions were free of erythrocytes and platelets and contained 98–100% PMN. These cells were >95% viable and exhibited normal bactericidal activity and postphagocytic oxidative metabolism. Fractions that were presumed to be plasma membranes by phase microscopy were prepared from homogenates of these cells by the method of Oliver et al., using a discontinuous sucrose gradient. Suspensions of guinea pig leukocytes containing greater than 90% PMN were obtained from peritoneal exudates collected 16-18 hr after the injection of shellfish glycogen.

The 5'-nucleotidase activity was determined by the method of DePierre and Karnovsky. Briefly, the PMN preparations were incubated at 37°C for 30 min in Krebs Ringer phosphate buffer (pH = 7.4) containing 1 μCi [32P] in 1 mM adenosine monophosphate as substrate for the 5'-nucleotidase and 1 mM p-nitrophenyl phosphate as substrate for the nonspecific phosphatase. The reaction mixtures were plunged into ice and a suspension of 20% acid-washed Norit (w/v) in 10% trichloroacetic acid was added. A basic principle of this method is that the intact substrate is absorbed to the Norit (activated charcoal) and is removed by filtration. The [32P] that is released by hydrolysis is not absorbed, and the radioactivity in an aliquot of the filtrate, as determined by liquid scintillation counting, is a measure of enzyme activity.

The results of a representative study are recorded in Table 1. The enzyme activity of human PMN, when expressed as either cpm of [32P] released or as nanomoles of substrate metabolized, was only slightly greater than that from the buffer and much less than that from guinea pig PMN which served as positive controls. The mechanism for the slight increase in activity following homogenization of the human PMN is unknown, but the following speculations can be made. (1) The increase was an artifact of homogenization and was not significant. (2) The enzyme in human PMN is not an ectoenzyme, and an intracellular source was released by homogenization. (3) The increase was due to the activation of nonspecific phosphatases by homogenization. The latter seems unlikely because of the presence of p-nitrophenyl phosphate. The lack of increase in activity following homogenization of guinea pig PMN supports the earlier observations that this is an ectoenzyme in these cells, and that additional enzyme was not made available from intracellular sources. The decrease in activity, noted in Table 1, that was not observed by DePierre and
Karnovsky, was probably the result of technical differences in homogenization. Little activity was demonstrated using 50 μg of membrane protein and there was no increase when the potential source of the enzyme was doubled.

Little to no 5'nucleotidase was demonstrated in human PMN. The enzyme was either absent or was inactive under the experimental conditions and could not serve as a suitable marker to identify plasma membrane fractions from these cells.

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Erratum: In the article “Selective Damage to Erythroblasts by 55Fe by Reinke et al. (Blood, Vol. 45, No. 6, June 1975, pp. 801–810), the next to the last sentence in the article, on p. 809, should read as follows: “The second category, typified by hypoxia and hemorrhage, induces secondary perturbation in the stem cell pool uncomplicated by stem cell destruction but suffers the disadvantage of limited control of “dosage” and mediator.” Also, please note that the origin of the paper should include the Mount Sinai School of Medicine of the City University of New York in addition to Brookhaven National Laboratory.
Letter: Activity of 5’nucleotidase in polymorphonuclear leukocytes

RG Strauss and SE Burrows