IDENTIFICATION OF α-NAPHTHYL BUTYRATE ESTERASE AS A PLASMA MEMBRANE ECTOENZYME OF MONOCYTES AND AS A DISCRETE INTRACELLULAR MEMBRANE-BOUNDED ORGANELLE IN LYMPHOCYTES*

By MAREK J. BOZDECH‡ and DOROTHY F. BAINTON

From the Departments of Pathology and Medicine, and the Cancer Research Institute, University of California School of Medicine, San Francisco, California 94143

A rust red, diffuse reaction for α-naphthyl butyrate esterase (ANBE),[1] that is inhibited by sodium fluoride (NaF), distinguishes the monocyte from other leukocytes in blood smears (1). A similar reaction that is resistant to NaF inhibition can be found within lymphocytes in small discrete punctate densities. This latter distribution of enzyme reaction correlates strongly with T cell domains in lymph node preparations (2, 3) and sheep erythrocyte (SRBC)-rosetting capability (4–9), and has led some investigators to suggest discrete esterase positivity in lymphocytes as a substitute for SRBC rosettes in identifying T lymphocytes (6, 7) or even possibly a T cell subset (10). This stain has frequently been called the nonspecific esterase (1–21).

The origin of this technique can be traced to Gomori, who observed esterase activity in myeloid and mast cells during studies in 1953 that used naphthol chloroacyl esters and diazonium salts (11). The first suggestion that monocytes might have a distinctive esterase was made by Braunstein in 1959 with the use of α-naphthyl acetate (ANA) as the substrate (12). In the same year, Davis and Ornstein introduced the use of hexazotized pararosaniline as the dye-coupling agent (13), which has become a mainstay of the current technique. Fischer and Schmalzl showed that NaF inhibited esterase reactivity with nonhalogenated naphthol esters in monocytes alone (18). Subsequently, Ansley et al. found that the butyrate side chain on α-naphthyl produced greater staining of monocytes and was more stable than the acetate (19). They combined all these findings in studies of an automated differential white blood cell counter, in which they suggested that monocytes could be best demonstrated with the use of short-chain α-naphthyl butyrate (ANB) ester as the substrate, hexazotized pararosaniline as the coupling agent, and a pH near 6.0 (20). It remained for Li et al. to relate cell specifications to enzyme profiles in neutrophils, monocytes, and mast cells by systematically combining clinical studies with zymograms of the enzymes (1). In their studies using polyacrylamide disc electrophoresis, ANB was hydrolyzed optimally at pH 6.0 and 6.3 by band 4 esterase isoenzyme, which was inhibited by

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‡ Supported by a National Institutes of Health Fellowship (CA-06043) from the National Cancer Institute and by Training Grant HL-07100. Present address: Section of Hematology, Department of Medicine, University of Wisconsin, Madison, Wis. 53792.

[1] *Abbreviations used in this paper:* ANA, α-naphthyl acetate; ANB, α-naphthyl butyrate; ANBE, α-naphthyl butyrate esterase, pH 6.3; DFP, diisopropyl fluorophosphate; SRBC, sheep erythrocyte(s).

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NaF, and was found primarily in monocytes and to a lesser extent in platelets and megakaryocytes. The NaF-resistant esterase in lymphocytes was found in band 2. These isozymes have recently been more extensively characterized utilizing isoelectric focusing (21).

The subcellular structures with which the ANBE is associated have remained obscure. Our studies were undertaken to determine whether the histochemical technique for demonstrating this esterase could be modified for use on the cytochemical, fine structural level. We approached this goal by systematically analyzing the techniques used in the collection, separation, fixation, incubation, and subsequent processing and embedding of human blood leukocytes for light and electron microscopy. We were successful and have determined for the first time the fine structural localization of ANBE and α-naphthyl acetate esterase in human blood monocytes and lymphocytes.[2]

Materials and Methods

Leukocytes. Normal human blood was obtained by finger puncture and smeared on glass cover slips or collected in heparinized (10 U/ml) polyethylene syringes by venipuncture. Suspensions of leukocytes were obtained either by (*a*) dextran sedimentation of red cells, (*b*) Ficoll-Hypaque centrifugation, or (*c*) buffy coat preparation using Kaplow tubes. The cells were washed twice in heparinized Hanks' balanced salt solution at 22°C. In some experiments, the cells were processed in the incubation medium directly after washing, without previous fixation.

Fixation. The fixation procedures (24–26) were first tested on smears shown in Table I. Because none was satisfactory for fine structural analysis, we systematically explored fixation with glutaraldehyde in combination with various other fixatives; we also varied the concentration, the pH, the buffer system, and the time of fixation. The fixatives, listed in Table I, were used in parallel experiments on cell suspensions processed for electron microscopy. We found that fine structural morphology and enzymatic preservation were best in cells fixed for 10 min at 4°C in 0.25% glutaraldehyde and 1% sucrose in 0.1 M sodium cacodylate buffer (pH 6.3). After fixation, the cells were washed three times at 4°C in 0.1 M sodium cacodylate containing 7% sucrose and could be stored overnight in the refrigerator.

Incubation. The fixed cells were incubated with substrate at 22°C or 37°C in a Dubnoff metabolic shaker. The substrate was 10 mg of ANB or ANA (both from Sigma Chemical Co., St. Louis, Mo.) dissolved in 0.5 ml of ethylene glycol monomethyl ether. When ANB was used, the dissolved substrate was added to 9.5 ml of 0.15 M phosphate buffer (pH 6.3). Then 0.05 ml of freshly prepared hexazotized pararosaniline (TAAB Laboratories, Emmer Green, Reading, England) was added. The pararosaniline was prepared by mixing equal volumes (1.0 ml) of 4% pararosaniline in 2 N HCl and 4% sodium nitrite newly made in a small, Parafilm-covered Ehrlenmeyer flask for 60 s. The cloudy mixture was filtered with no. 1 Whatman paper (Whatman Chemicals, Div. W & R Balston, Maidstone, Kent, England) because the osmolarity of the mixture was 1,070 mosmol by freezing point depression. The time of incubation ranged from 20 to 90 min. When ANA was used, the dissolved substrate was added to 8.9 ml of 0.15 M phosphate buffer to which 0.6 ml of hexazotized pararosanilin was then added. The final pH for both incubation media was 6.3 (1).

In some experiments, cells were washed with Michaelis buffer after the incubation for esterase and then incubated for peroxidase in a mixture of H_2O_2 and 3,3′-diaminobenzidine (27) at pH 7.6 at 22°C for 1 h.

Subsequent Processing. Blood smears to be examined by light microscopy were washed three times in distilled water, air-dried, and counterstained with 2% methyl green.

Cell suspensions to be examined by electron microscopy were washed three times in Michaelis buffer containing 7% sucrose and then postfixed in buffered 1% OsO_4 in distilled H_2O (pH adjusted to 7.6 as suggested by Smith and Van Frank [28]) for 90 min at 22°C. Dehydration

[2] Preliminary observations have been previously published in abstract form (22, 23).

TABLE I

Effect of Fixatives on ANB/ANA Reactivity of Monocytes and Lymphocytes

Fixative	pH	Substrate	Monocytes		Lymphocytes	
			Light*	EM*	Light	EM
Unfixed		ANB	++++	++++	–	–
Formalin/acetone	6.6	ANA	++++	ND	++++	ND
		ANB	++++	ND	++++	ND
Formalin, 4%	7.3	ANA	++++	++++	–	–
		ANB	++++	++++	–	–
Formalin, 2%/glutal,‡ 0.5%	6.3	ANB	+++	++	–	–
Formalin, 1%/periodate/lysine	6.2	ANB	++++	++++	–	–
Glutal,‡ 1.5%	7.4	ANA	+	–	+	–
		ANB	+	–	+	–
Glutal, 1.0%	6.3	ANB	++	+	++	–
Glutal, 0.5%	7.3	ANB	++	+	–	–
Glutal, 0.5%	6.3	ANA	++++	++	+++	+
		ANB	++++	++	++	+
Glutal, 0.25%	6.3	ANA	++++	+++	++++	+++
		ANB	++++	+++	++++	+++

* Refers to method of examination; light or electron microscopy (EM); ND, not done.
‡ Glutaraldehyde.

was carried out in ethyl alcohol, and specimens were embedded in Epon 812 or Spurr's medium with or without propylene oxide. Some of the thin sections were stained on grid with alkaline lead citrate to enhance contrast, and uranyl acetate was occasionally used as well. Sections were examined with a Siemens 101 electron microscope (Siemens Corp., Medical Industrial Groups, Iselin, N.J.) at 60 and 80 kV.

Controls and Other Variations. To test for inhibition of esterase reactivity by NaF, fixed cells were incubated first in 0.15 M phosphate buffer (pH 6.3) with 0.04 M NaF for 45 min at 22°C, and then in the incubation medium with 0.04 M NaF. In some experiments, the procedure was varied by boiling cell suspensions for 15 min after fixation or by omitting substrate or pararosaniline or both. In addition, cell suspensions frozen in liquid nitrogen and thawed to 37°C after fixation, but before incubation, were examined to determine whether the substrate penetrated the cells. Cell suspensions in which glycerol (final concentration 16%) and Triton X-100 (0.1%) had been added to the fixative were examined for the same reason.

Results

Blood Smears. With ANB or ANA as the substrate, the reaction in monocytes on blood smears was rust red, dense, and diffuse. It was equally strong in unfixed cells and cells fixed with formalin (Table I). Fixation with 1.5% glutaraldehyde resulted in markedly diminished reaction product, but the amount of reaction product increased as the concentration of glutaraldehyde was reduced (Table I). Decreasing the pH of the fixative to 6.3 and increasing the temperature of the incubation mixture from 22 to 37°C enhanced the reaction in monocytes fixed with glutaraldehyde. Inhibition of monocytic reactivity by NaF was confirmed.

Between one and three small (1 μm), discrete, darker rust red staining bodies were found in variable numbers (10–40%) of lymphocytes on blood smears as previously observed by others (1, 5, 8, 20). The reactivity in lymphocytes, like that in monocytes, varied with the type of fixation used. It was strongest in minimally fixed (0.25% glutaraldehyde) cells and diminished as the concentration of glutaraldehyde was increased (Table I). A comparison of the effects of formalin, pH, acetone, and the

concentration of glutaraldehyde, with both ANB and ANA as the substrate, confirmed these observations (Table I).

Ultrastructural Localization of Reaction Product in Monocytes. The reaction product was first observed in formalin-fixed monocytes, with ANB as the substrate. It had the appearance of a thick, flocculent, electron-dense precipitate covering the surface of the plasma membrane. The fine structure of the cells was, not surprisingly, poorly preserved (Fig. 1). Because our studies with light microscopy had suggested that the amount of reaction product varied inversely with the concentration of glutaraldehyde, we tried various concentrations of glutaraldehyde in an attempt to achieve a good compromise between density of reaction product and preservation of cellular structure and detail. Fixation in 0.25% glutaraldehyde (pH 6.3) at 4°C for 10 min provided the best compromise (Fig. 2). That the stained cells were monocytes was supported by their size (8–10 μm), nuclear shape, nuclear-cytoplasmic ratio, and cytoplasmic contents. Reaction product was found over the entire external surface of the plasma membrane, but none was observed within cells.

To determine whether the substrate or the coupling agent or both penetrated the cells, we performed experiments in which cells were frozen and thawed after fixation and others in which glycerol was added to the fixative. In neither case did monocytes show a different localization of reaction product. The addition of Triton X-100 to the fixative, the omission of the substrate or the coupling agent or both from the incubation medium, preincubation and the addition of NaF to the incubation medium, or boiling after fixation all abrogated reaction product in monocytes.

Because monocytes (in contrast with lymphocytes) contain peroxidase-positive granules, some of the cells were incubated first for esterase and then for peroxidase. Peroxidase-positive granules were present in varying numbers in monocytes showing esterase reaction product on the plasma membrane (Fig. 3). However, a small percentage (5%) of the monocytes that contained such granules showed no esterase reaction product. Conversely, some monocytes with strong esterase reactivity contained only a few positive granules (see upper monocyte in Fig. 3), and several contained none. (The absence of peroxidase-positive granules in a small percentage of monocytes examined on thin sections was previously reported by Nichols and Bainton [29].) We concluded that incubation for esterase and then for peroxidase allowed better discrimination between the two types of mononuclear cells (true monocytes and lymphocytes) than either method alone.

Ultrastructural Localization of Reaction Product in Lymphocytes. In preparations fixed for a short time with a low concentration of glutaraldehyde (0.25%), some lymphocytes showed intracellular structures (Fig. 4) corresponding in size, location, number, and density with the reactive bodies observed by light microscopy (Fig. 4*a*). These organelles were round or oval and from 0.3 to 1.2 μm in size. Each was limited by a single unit membrane (Fig. 4*b*). Their contents were homogeneous and dense, and stained strongly with ANB (Figs. 4*a*, *b*, and *c*) or ANA (Fig. 4*d*). The reactivity was not inhibited by NaF, in contrast with that in monocytes. The organelles were found—usually singly, but sometimes in groups of two or three–near the Golgi region, often adjacent to multivesicular bodies (Fig. 4*c*). Occasionally, the reaction product was seen only as a reactive rim surrounding a clear central area (not illustrated).

Although the use of low concentrations of glutaraldehyde permitted the consistent detection of reactive intracellular organelles, the diffusion of reaction product in the

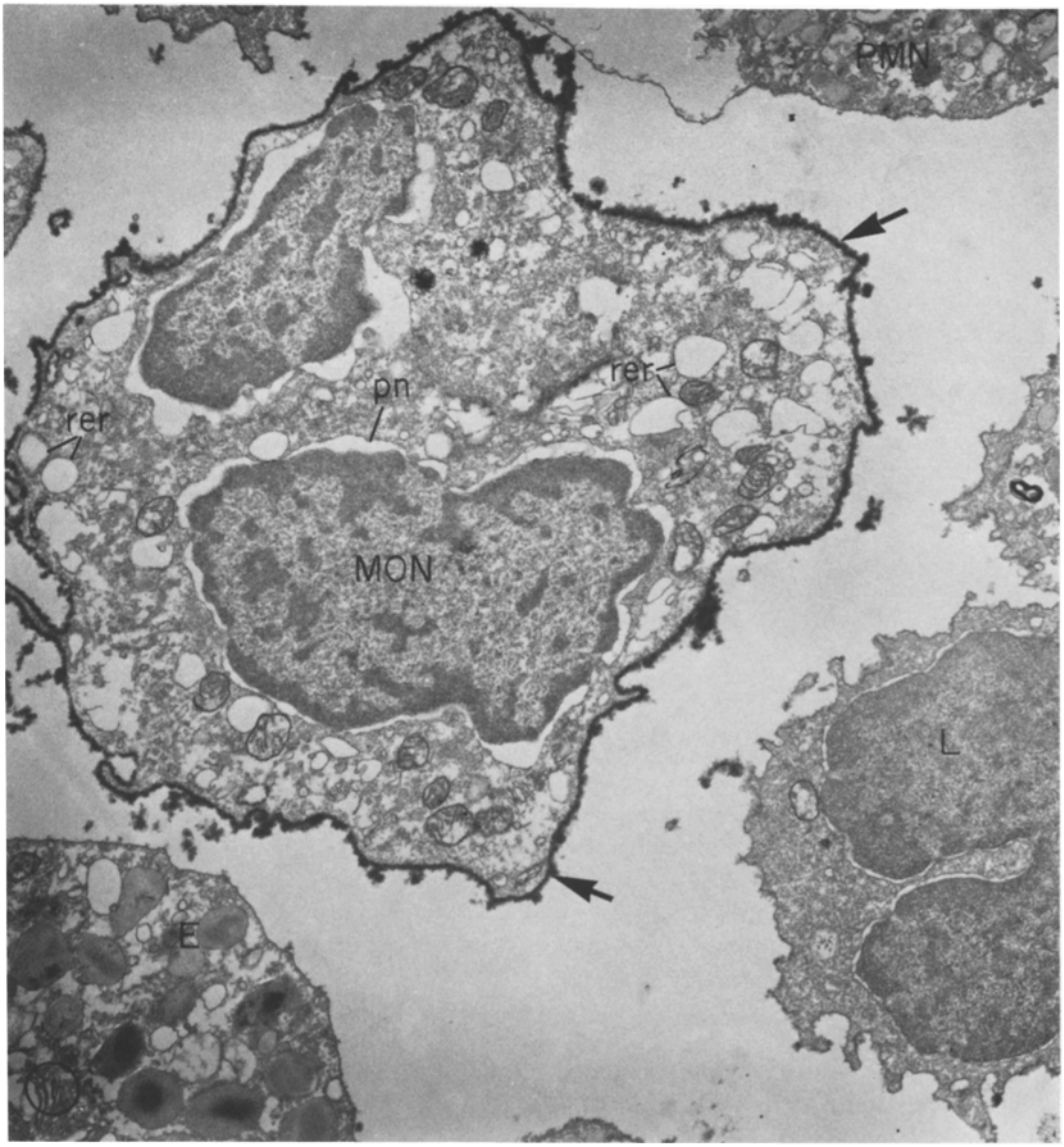

FIG. 1. Blood leukocytes fixed with 4% buffered formalin and incubated with ANB and hexazotized pararosaniline (pH 6.3). Monocyte (MON) shows a dense extracellular reaction product (arrows), but the morphologic preservation is poor. The rough endoplasmic reticulum (rer) and perinuclear cisterna (pn) are markedly dilated, and granules are difficult to identify. Note the absence of reaction product from the surfaces of an eosinophil (E), neutrophil (PMN), and lymphocyte (L). Postfixed with 1.5% glutaraldehyde and 1% OsO_4, embedded in Epon, and stained on grid with alkaline lead citrate for 30 min. × 14,000.

cytoplasm surrounding some reactive organelles (Fig. 4*d*) was a distinct disadvantage of this procedure. In addition, reaction product was sometimes present in the segments of plasma membrane adjacent to areas of cytoplasmic diffusion. Because neither the cytoplasmic nor the plasma-membrane reactivity was inhibited by NaF, we interpret

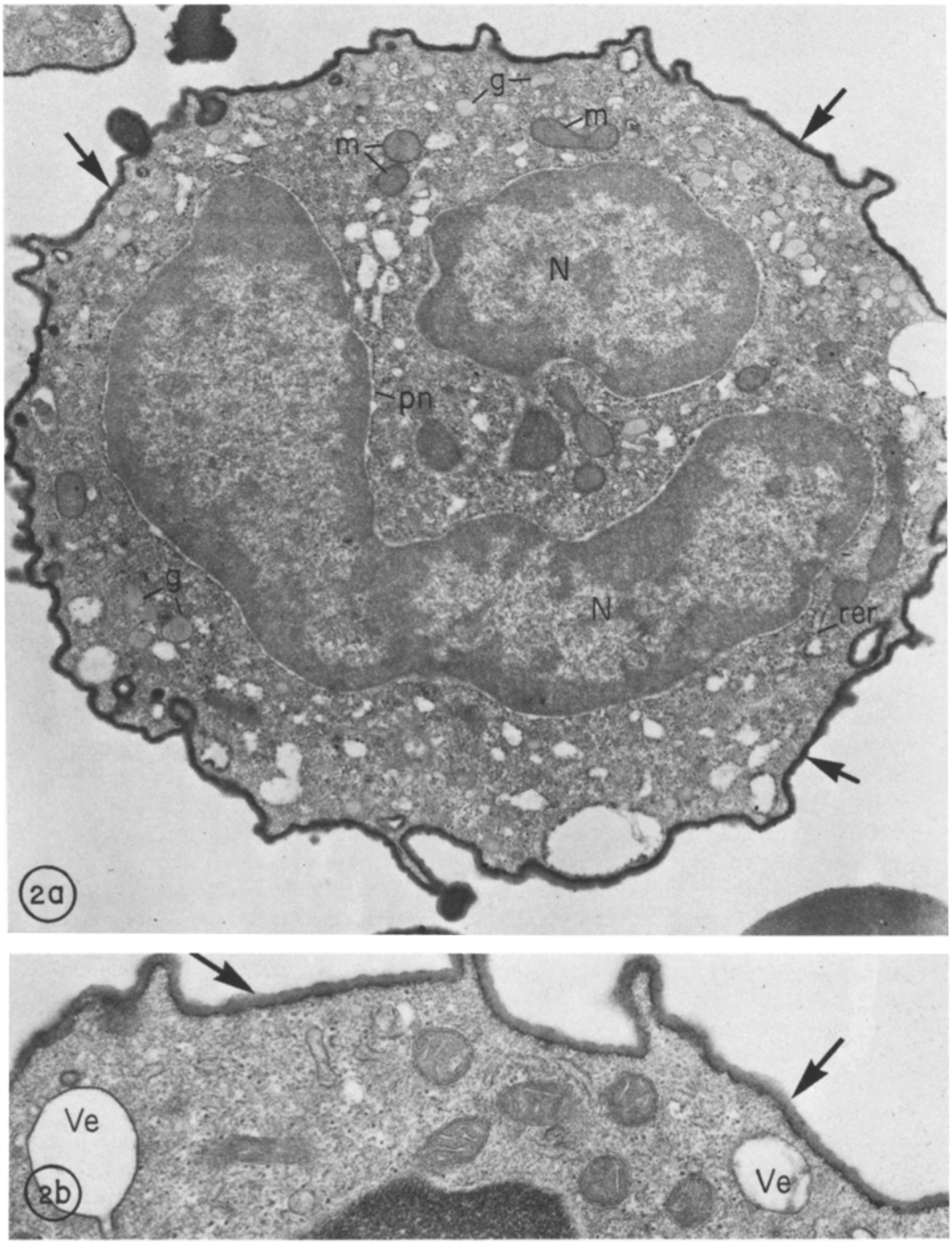

FIG. 2. (*a*) Blood monocyte fixed with 0.25% glutaraldehyde (pH 6.3) and incubated as in Fig. 1, but with ANA as the substrate. The extracellular reaction product (arrows) is similar to that observed with formalin fixation (Fig. 1), but cytoplasmic preservation is markedly improved. Note the well-preserved mitochondria (m) and small granules (g), and the lack of dilation in the perinuclear cisterna (pn) and small segments of rough endoplasmic reticulum (rer). Postfixed with 1% OsO_4, embedded in Epon, and stained on grid with lead citrate for 30 min. × 18,000. (*b*) A higher magnification view of a blood monocyte better illustrates the localization of reaction product on the external surface of the plasma membrane (arrows). Note that the large clear endocytic vesicles (Ve) are not reactive. Tissue prepared as in (*a*), but with ANB as substrate. × 30,000.

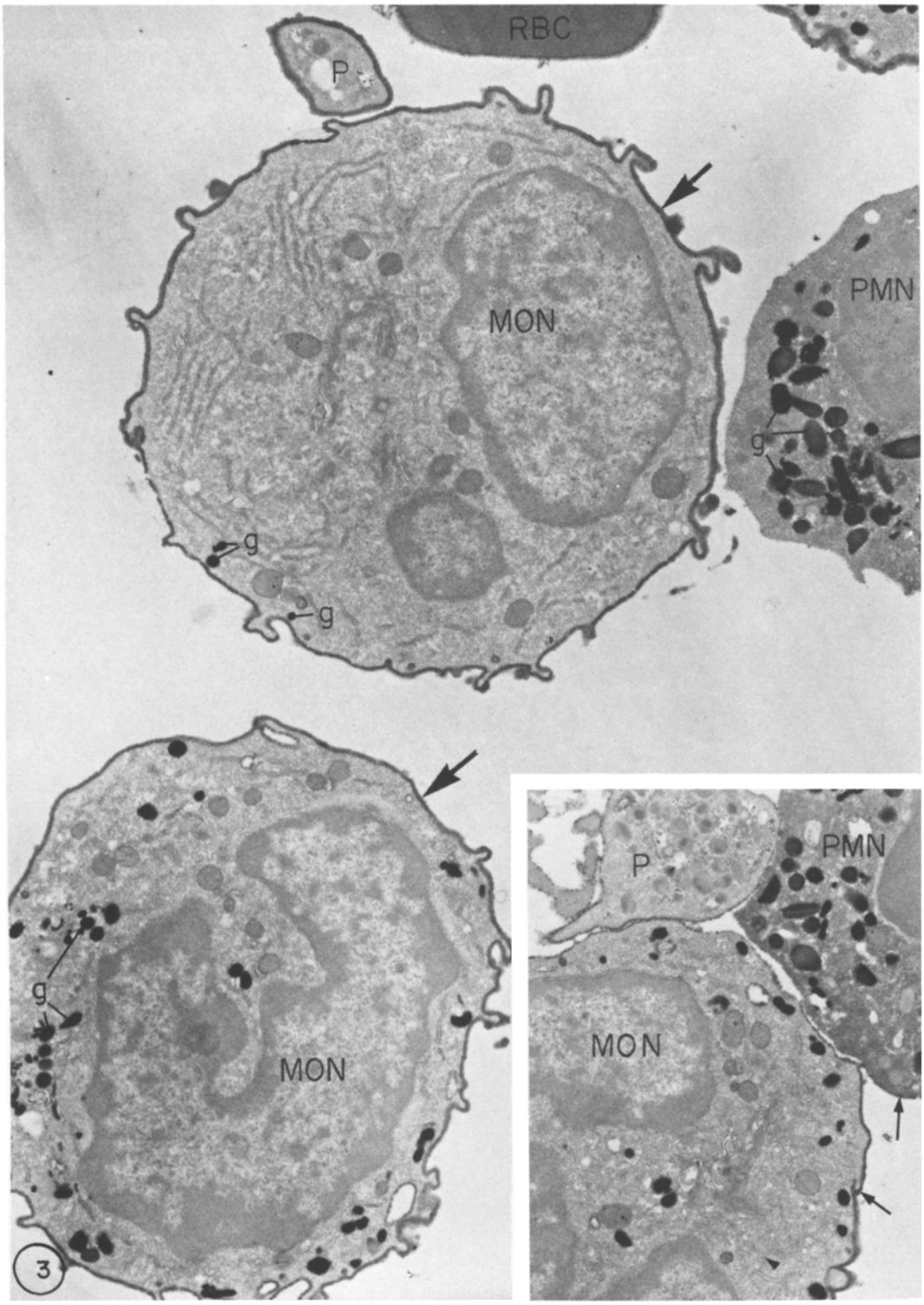

FIG. 3. Cells fixed with 0.25% glutaraldehyde (pH 6.3) and incubated first for esterase with ANB and then for peroxidase. The two monocyte (MON) plasma membranes show a strong reaction product for esterase (arrows), but the neutrophil (PMN) plasma membrane shows none. Intracellular peroxidase-positive granules (g) are larger and more numerous in PMN than in MON. In inset, note that a platelet (P) and a neutrophil (PMN) have reaction product (arrows) on the surface adjacent to a monocyte (MON), and this appears to diminish with increasing distance from the surface of the monocyte. Tissue was postfixed in alkalinized OsO_4, and embedded in Epon without propylene oxide. × 12,500; inset × 12,000.

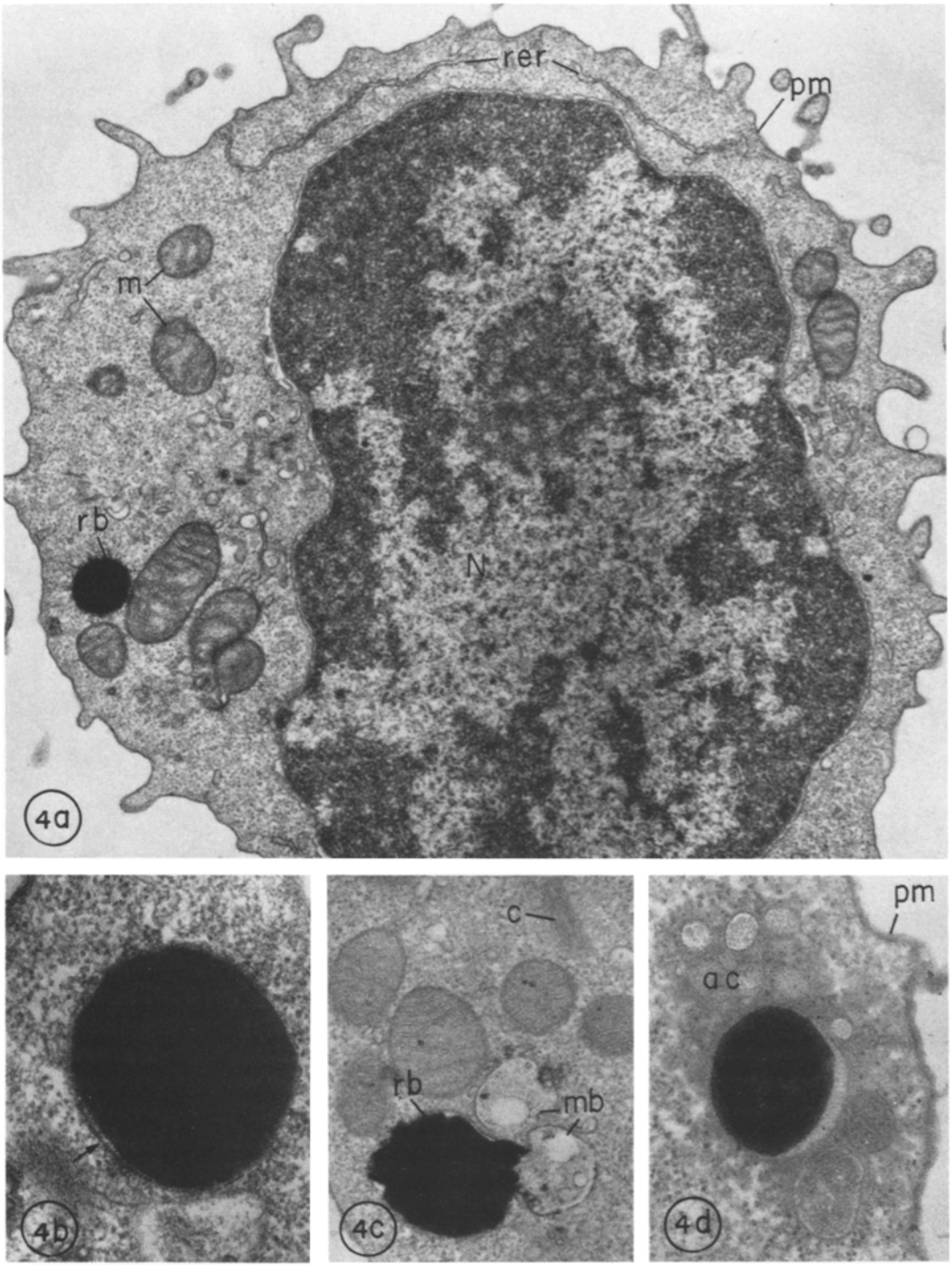

FIG. 4. (*a*) Blood lymphocytes illustrating the localization of esterase. In contrast with the monocytic reaction product, the lymphocytic reaction product is present exclusively within the cell, in a dense organelle corresponding in size and location with the reactive body (rb) seen by light microscopy. Note that at higher magnification (*b*), this organelle is homogeneously dense and is surrounded by a single unit membrane (arrow). We believe that the organelle corresponds with the Gall body (Discussion). It is usually seen near the centrioles (*c*) and the Golgi region, and may be located near multivesicular bodies (mb) (*c*). With the weaker glutaraldehyde fixatives, the reaction product may stain adjacent areas of the cytoplasm (ac) and plasma membrane (pm) (*d*). The reactivity at these sites is not inhibited by the addition of NaF. Nucleus (N), mitochondria (m), and rough endoplasmic reticulum (rer). Tissue fixed and processed as in Fig. 2 except 4*a* and *b* were washed in uranyl acetate after postosmification to enhance membrane staining. (*a*) × 20,000; (*b*) × 45,000; (*c*) × 26,000; (*d*) × 16,000.

the plasma-membrane reactivity as a diffusion artifact emanating from the reactive organelle.

As in monocytes, reaction product was not seen if the cells were boiled after fixation. Nor was it seen in preparations incubated without substrate or coupling agent or both. The matrix of the membrane-bounded body was considerably less dense in these preparations than in those which had been incubated in complete medium.

Specificity of Reaction Product. With the improved morphology and enzyme survival provided by low concentrations of glutaraldehyde fixative, reaction product was noted on a few platelets and erythrocytes, and in rare instances on polymorphonuclear leukocytes, particularly those in close proximity to monocytes (Fig. 3 and inset). The reaction product on these nonmonocytes was usually on the surface adjacent to a monocyte, seldom encircled the cell, and appeared to diminish in density with increasing distance from the surface of the nearby monocyte. A similar phenomenon was observed on the surface of some lymphocytes, as mentioned above, near intracytoplasmic reactive bodies whose contents appeared to have diffused into the cytoplasm and toward the plasma membrane (Fig. 4*d*).

The fine structural localization of reaction product in eosinophils and basophils also deserves comment. When fixed in 0.25% glutaraldehyde, occasional eosinophils contained one or two nonmembrane-bounded lipid droplets that were strongly reactive for ANBE, and occasional basophils showed reaction product on the plasma membrane.

Factors Influencing Intensity of Reaction Product. The specific cytochemical staining we observed was the same whether ANB or ANA was used as substrate and whether the cells had been collected and separated by dextran sedimentation, by Ficoll-Hypaque centrifugation, or by preparation of buffy coat. The staining was dependent, however, on variables of pH, temperature, duration of incubation, and cell processing, as well as the variables of fixation already noted. Incubation without prior fixation resulted in strong reaction on the plasma membrane of monocytes, but no intracellular staining in lymphocytes or any other type of cell. Fixation at pH 6.3 rather than 7.4 seemed to increase the reaction in monocytes with ANB as the substrate (ANA was not tested). Incubation at 37°C resulted in an increased reaction in monocytes, but at the cost of an increased reaction in nonmonocytes, whereas incubation at 22°C resulted in a slightly decreased reaction in monocytes, but also in a decreased reaction in nonmonocytes. Increasing the duration of incubation beyond 60 min did not enhance monocytic reactivity, but decreasing the duration to 20 min diminished it (not tested on punctate densities). Adjustment of osmic acid fixative to pH 7.6 appeared to heighten the contrast of the osmiophilic reaction product (28) in monocytes. Reaction product was preserved to the same extent with embedding in Epon and embedding in Spurr's resins. Because processing the cells in propylene oxide diminished the contrast between esterase reaction product and cytoplasm, we no longer use this procedure. Increased contrast could be obtained by using 60 kV during microscopy.

Discussion

Our results have demonstrated the localization of esterase activity on the entire outer surface of the plasma membrane of monocytes and within certain membrane-bounded cytoplasmic organelles in a subpopulation comprising 10–40% of lymphocytes. The circumferential distribution of the reaction product in monocytes explains

its diffuse appearance under the light microscope. A similar distribution has been observed in human alveolar macrophages by Jaubert et al. (30). The reactive intracellular bodies in lymphocytes correspond with the punctate densities or focal reaction product seen by light microscopy and seem to correspond with organelles called Gall bodies (see below). Our technique provides the first means of identifying these reactive bodies at the ultrastructural level.

The plasma membrane esterase observed on monocytes appears to be an ectoenzyme. Ectoenzymes are localized on the plasma membrane and have active sites that face the external medium rather than the cytoplasm (31). The major drawback of using cytochemical techniques to demonstrate ectoenzymes has been that it is unclear whether substrate penetrates cells or organelles (32). Proof that a substrate can penetrate cells is desirable in a study of an ectoenzyme on whole cells because it is then possible to assert that the absence of reaction product from the cytoplasm implies a true absence of enzyme (33). The unequivocal reaction product we observed within lymphocyte organelles shows that the substrates we used do penetrate cells. Consequently, we believe that the lack of reaction product within monocytes signifies the absence of any substantial amount of active intracellular esterase, whereas the presence of diffuse reaction product on the external surface of the plasma membrane probably means that the active site is externally directed. It must be recognized, however, that the reaction product may diffuse and not indicate the true location of the enzyme.

Little is known about the properties of the monocytic esterase. Diisopropyl fluorophosphate (DFP) and other organophosphates inhibit its activity (14, 34), thus defining it as a serine esterase (35). Such an ectoenzyme would be ideally located for interaction with the external milieu. Ectoserine esterase of neutrophils, for example, induces complement-mediated chemotaxis (36). Recently, esterase activity in human monocytes has been demonstrated to have a close correlation with chemotaxis (37). In addition, a serine esterase thought to be externally located on the guinea pig macrophages has been shown to counteract migration inhibition factor (38). Inhibitors of serine esterases have also recently been shown to enhance lymphokine-induced microbicidal activity in macrophages (39). In view of the monocyte's role in inflammation, it is interesting that Zucker-Franklin et al., using a technique similar to ours, has shown that an elastase-like protease, which is inhibited by DFP and which degrades the amyloid precursor protein serum amyloid A, appears by light microscopy to be present on the surface of the monocyte (40). Kitagawa et al. (41) have also recently presented evidence that monocytic serine proteases, possibly located at the cell surface, may be involved in superoxide production. Perhaps the development of this ultrastructural technique will aid in future investigations of the function of this esterase.

The reactive bodies that we observed in lymphocytes, which are resistant to inhibition by NaF, have been widely described in normal and abnormal lymphocytes (1–10, 12, 19–21). Workers in the laboratories of Knowles et al. (7) and Pangalis et al. (6) have suggested that these reactive bodies might serve as a substitute for the SRBC rosette in identifying the T cell subpopulation of lymphocytes. Grossi et al. (10) have further suggested that these bodies specifically mark the Tμ or helper T cells, which form the largest subpopulation of T cells. The membrane-bounded organelles in which we observed reaction product at the ultrastructural level are morphologically identical with the Gall bodies first described in 1936 (42). In 1952, Hempelmann and

Knowlton found these bodies to be birefringent, soluble in fat solvents, and stainable with lipid stains and basic dyes, and concluded that they were probably composed of phospholipids (43). Bessis published the first fine structural demonstration of the Gall body, which he noted was membrane-bounded and "perfectly round and composed of a gray center with a dark corona that contains a great deal of lipid" (44).

Two technical problems that need further comment are the esterase negativity of the plasma membrane of some cells that otherwise resembled monocytes and the esterase positivity of the plasma membrane of some nonmonocytes. Others have reported that from 2 (45) to 10% (46) of monocytes are negative for acid esterase by light microscopy. In preparations fixed with formalin alone, we found very few negative monocytes. In those fixed with 1.5% glutaraldehyde, we found very few positive monocytes. We attribute this discrepancy to the sensitivity of monocytic esterase to glutaraldehyde fixation, which has been noted by others (20). (A similar sensitivity has been noted in lymphocytic ectoenzymes such as 5′-nucleotidase [47].) When lower concentrations of glutaraldehyde at acid pH were used, more than 95% of the monocytes were reactive. The high degree of reactivity was especially evident in preparations incubated for acid esterase and then for myeloperoxidase. When this technique was used, it was exceedingly uncommon to find a monocyte not positive for one or the other enzyme. Because peroxidase alone may not be a reliable marker for macrophages in some of the later stages of development (26, 48–50), this combined technique may be useful in identifying mononuclear phagocytes in tissues.

The reaction product on the surfaces of nonmonocytes was most prominent when weaker concentrations of glutaraldehyde were used to improve preservation of enzymatic activity in monocytes (Fig. 3). Most of the reactive nonmonocytes were adjacent to monocytes, and the reaction product was usually present only on the surface proximal to the monocyte. Because lower concentrations of fixative stabilize cellular structures less effectively than higher ones, we assume that a relative deficiency in anchoring the enzyme to cellular structures may be responsible for these observations. The only exceptions to this pattern were the occasionally reactive platelets, which seemed to have circumferential reaction product similar to that seen in monocytes and which may indeed be additional cells with esterase ectoenzyme (1).

A third technical problem—the cytoplasmic diffusion and localized extracellular accumulation of reaction product adjacent to the strongly reactive intracellular bodies in lymphocytes (Fig. 4*d*)—was clearly an artifact. Also, because this lymphocytic reaction product is resistant to inhibition by NaF, it is easily distinguished from the monocytic ectoenzyme reaction product.

The presence of extracellular reaction product on some basophils cannot be readily explained. In addition, eosinophils contained occasional lipid droplets (51) that stained densely when processed for acid esterase. We are further investigating the reactivity of these cells.

Finally, although the function of esterase on monocytes is unclear, the ability to demonstrate its presence should allow investigation of its time of appearance, sites of synthesis, and mode of delivery to the plasma membrane when precursor cells in bone marrow are studied. In addition, observation of its fate and possible redistribution during later stages of development of the monocyte (i.e., in the tissue macrophage) or during a functional event such as endocytosis will now be possible. We feel that the

combination of the ANBE and myeloperoxidase techniques may be especially useful in this regard.

Summary

A reaction for an esterase, with a nonhalogenated, short-chain naphthyl ester (α-naphthyl butyrate or α-naphthyl acetate) as the substrate, has been used to identify mononuclear phagocytes by light microscopy. By analyzing techniques used in the collection, separation, fixation, processing, and embedding of human blood leukocytes for electron microscopy, we adapted the light microscopic method for use in determining the fine structural localization of this reaction. In monocytes, the reaction product covered the external surface of the plasma membrane. This distribution indicated that monocytic esterase is an ectoenzyme. The addition of NaF completely inhibited the monocytic reaction. In lymphocytes, the reaction product was localized in membrane-bounded intracellular organelles, similar to those previously shown to contain phospholipid and called Gall bodies. These organelles correspond with the punctate densities or focal reaction product observed by light microscopy. Other investigators believe that this distribution of enzyme in lymphocytes marks a subset of T cells, the Tμ. The lymphocytic reaction was not inhibited by NaF.

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