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Acid α-naphthyl acetate esterase: presence of activity in bovine and human T and B lymphocytes

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Summary. Non-specific acid α-naphthyl acetate esterase (ANAE) activity was demonstrated in a majority of bovine peripheral blood lymphocytes, confirming and extending the observations on murine and human lymphocytes made by previous workers. Simultaneous study of both ANAE activity and spontaneous erythrocyte (E) or erythrocyte-antibody-complement (EAC) rosetting capability of the same bovine lymphocytes showed directly that, while 64·2 ± 4·9 (SEM) % of bovine lymphocytes capable of forming E rosettes were ANAE positive, 38·3 ± 0·8% of those forming EAC rosettes were also ANAE positive. Similar studies of human peripheral blood lymphocytes showed also that, while 80·6 ± 2·2% of the lymphocytes capable of forming E rosettes were ANAE positive, 44·1 ± 2·6% of EAC forming lymphocytes were ANAE positive. Thus the presence of ANAE activity in a majority of T lymphocytes and a significant proportion of B lymphocytes of both human and bovine peripheral blood is indicated. Human and bovine lymphocytes from phytohaemagglutinin (PHA)-stimulated cultures demonstrated greatly enhanced intensity of ANAE activity.

INTRODUCTION

Non-specific acid esterase stain has been widely used for identifying monocyte-macrophages from other leucocytes in mixed mononuclear cell suspensions (Yam, Li & Crosby, 1971; Koski, Poplack & Blaese, 1976; Tucker, Pierre & Jordan, 1977; Kass, 1977). The recent discovery that non-specific acid α-naphthyl acetate esterase (ANAE) is also present in lymphocytes but with an unusual distribution within the cell of T lymphocytes of mice and humans has raised a great hope for using the histochemical method as a practical alternative to spontaneous erythrocyte rosette (E rosette) assay as a T lymphocyte marker (Mueller, Brun del Re, Buerki, Keller, Hess & Cottier, 1975; Mueller, Keller, Buerki, Hess & Cottier, 1976; Sher, Fripp & Wedee, 1977; Knowles, Hoffman, Ferrarini & Kunkel, 1978). This conclusion was made on the finding of a close connection between the percentage of lymphocytes containing a marked ANAE activity and the percentage of E-rosetting lymphocytes.

As reported here, however, simultaneous studies of ANAE activity in the same lymphocytes forming erythrocyte (E) and erythrocyte-antibody-complement (EAC) rosettes indicated that, while a majority of bovine and human T lymphocytes were ANAE positive, a significant proportion of B lymphocytes were also ANAE positive.

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MATERIALS AND METHODS

Cell preparation

Bovine mixed mononuclear cell suspension. Venous blood was collected into heparinized tubes (preserva-

tive-free sodium heparin, Fellows Medical Manufacturing Co., Inc., Oak Park, Michigan) from normal cows at the University of Connecticut Dairy Farm.

Ten millilitre aliquots of whole blood were centrifuged for 15 min at 400 *g* and the buffy coat diluted in phosphate-buffered saline (PBS) to 4 ml and layered over 3 ml Ficoll–Paque (Pharmacia Fine Chemicals, Inc., Piscataway, New Jersey). This was centrifuged for 40 min at 400 *g* and the cells in the interface diluted to 3×10^6 cells per ml in medium containing 10% heat-inactivated foetal calf serum (FCS), penicillin (100 units/ml), streptomycin (100 μg/ml), and fresh glutamine (2 mM). The mononuclear cells so prepared consisted of 45–87% lymphocytes with 12–32% monocytes as verified from Giemsa stained cytocentrifuged cell smears.

Human mixed mononuclear cell suspension. Mononuclear cells from adult individuals were separated by overlaying 4 ml buffy coat cell suspension onto 3 ml Ficoll–Paque and processed similarly.

Lymphocyte enrichment by iron powder treatment

For phagocytic cell depletion, the method of Sanderson, Clark & Taylor (1975) was used. Carbonyl iron (40 mg; No. 44890, Tridom Chemical, Inc., Hauppauge, New York) was suspended in distilled water, sonicated, washed, autoclaved, and resuspended in RPMI 1640–HEPES medium supplemented with 10% heat-inactivated FCS at a concentration of 20 mg/ml. After incubation at 37° for at least 1 h, it was centrifuged, immediately prior to use, at 1000 r.p.m. for 5 min and the supernatant decanted.

The mononuclear cell suspension (3×10^6/ml) in medium was incubated with 4 mg/ml of the treated iron powder for 60–90 min at 37° while mixing on a rocker platform (Bellco Glass, Inc., Vineland, New Jersey). The non-phagocytic cells were decanted, with the help of a strong magnet, into tubes which contained a teflon-coated magnetic bar, and allowed to stand for 5 min at room temperature. They were then decanted into conical tubes, centrifuged, and resuspended in fresh medium at a concentration of 1×10^7 cells/ml.

The mononuclear cells so prepared consisted of 92–99% lymphocytes and less than 5% monocytes in both bovine and human cell preparations, with virtually 100% viability by trypan blue exclusion test.

Tissue culture medium

RPMI 1640–HEPES medium supplemented with 10% heat-inactivated FCS, 100 units penicillin/ml, 100 μg streptomycin/ml, and 2 mM glutamine was used for lymphocyte cultures.

For rosette assays, the same medium supplemented with the FCS which had been pre-absorbed with sheep red blood cells (SRBC) was used.

Lymphocyte culture

Standard techniques, as described (Angus & Yang, 1978a), with minor modifications, were briefly as follows. Lymphocytes (1×10^6) were cultured in 1 ml of the above mentioned RPMI 1640–HEPES medium. Phytohaemagglutinin-purified (PHA-P, Difco Laboratories, Detroit, Michigan) was added at a final concentration of 1:1500 (11·33 μg/ml) and 1:100 (170 μg/ml) to one half (six to eight tubes) of bovine and human lymphocyte cultures, respectively. Incubation was for 72 h at 37° in a humidified atmosphere with 5% CO_2. Eighteen hours before harvest, each tube received 1 μCi [^{3}H]-thymidine (20 Ci/mmol, New England Nuclear, Boston, Massachusetts) in 0·1 ml medium. The cultures were harvested according to the method of Hall & Gordon (1976). Briefly, the tubes were centrifuged at 400 *g* for 10 min, decanted, and 1 ml cold (5%) trichloracetic acid (TCA) added to the pellet. After vortexing, they were centrifuged and the pellet washed twice with 1·5 ml methanol. 0·2 ml NCS tissue solubilizer (Amersham/Searle, Arlington Heights, Illinois) were added to the pellet and the tubes held for 30 min in a 56° water bath.

2·5 ml of toluene scintillation fluid were added to each tube and decanted into scintillation vials, and rinsed three more times with 2·5 ml scintillation fluid which were added to the vial. The samples were counted in a Packard Tricarb liquid scintillation spectrometer (Packard Instrument Co., Downer's Grove, Illinois) and the results expressed as the mean difference between counts per min for the mitogen-stimulated cultures and unstimulated control cultures (Δ c.p.m.).

The other half of the cultures, both with and without PHA-P, were harvested for 72 h for morphological and histochemical studies after cytocentrifugation at 1500 r.p.m. for 10 min in a Shandon cytocentrifuge (Shandon Southern Instruments, Inc., Sewickley, Pennsylvania).

Erythrocyte rosette (E rosette) assay

Bovine E rosettes. A modified method of Wardley (1977) was used. Briefly, SRBCs, which had been collected in Alsever's solution and stored at 4° for less

than 2 weeks, were washed three times in Hanks's balanced salt solution (HBSS) and resuspended in 200 volumes of 6% dextran saline (6% w/v dextran T-70 in 9% NaCl, autoclaved). Equal volumes (0·25 ml) of the lymphocyte suspension (3×10^6/ml) and the SRBC suspensions were mixed and incubated for 10 min at 37°, centrifuged at 200 *g* for 5 min, further incubated overnight at 4°, and counted by the method of Sandilands, Gray, Cooney & Browning (1975) described previously (Angus & Yang, 1978b). Lymphocytes with more than three SRBCs attached were considered as E rosetting cells.

Human E rosettes. The method described by Kaplan, Woodson & Clark (1976) was employed for preparing E rosettes using 2-5-aminoethylisothiouronium bromide-treated sheep red blood cells (AET-SRBC), and the method of Sandilands *et al.* (1975) was used for enumeration.

Erythrocyte-antibody-complement rosette (EAC rosette) assay

The method of Kenyon & Piper (1977) was employed. Briefly, 2 ml of sub-agglutinating dilution of anti-SRBC-haemolysin in HBSS was added to 0·01 ml of thrice washed, packed SRBCs in HBSS. After sensitization at room temperature for 15 min, 2 ml of 1:40 dilution of horse complement (non-lytic) was added, and the mixture incubated for 1 h at 37°. After centrifugation, the EAC reagent was resuspended in 2 ml of tissue culture medium. For EAC rosette assay, 0·25 ml of the EAC suspension was added to equal volumes of lymphocyte suspension (3×10^6/ml), centrifuged gently, incubated for 30 min at 37°, and counted by the method of Sandilands *et al.* (1975).

Cell smears

Smears of cells and E and EAC rosettes were prepared by centrifugation of less than 0·5 ml of cell suspension onto glass slides for 10 min at 1500 r.p.m. in a Shandon cytocentrifuge. They were fixed and stained by Giemsa or non-specific esterase stain.

Non-specific esterase stain. The method of Koski *et al.* (1976) was used in this study. The only modification made was the substitution of α-naphthyl acetate for α-naphthyl butyrate. In preliminary tests in our laboratory, no significant differences were found between these substrates when used for staining peripheral blood lymphocytes.

Fixative. Na_2HPO_4, 20 mg; KH_2PO_4, 100 mg; distilled water, 30 ml; acetone, 45 ml; and formaldehyde (30%), 25 ml; pH 6·6; and kept refrigerated.

Pararosaniline. Pararosaniline hydrochloride (Sigma Chemicals, St Louis, Missouri, No. P-3750), 1 g; 2 N hydrochloric acid (warm), 25 ml stored at 4°.

Methylgreen counterstain (0·5%). Methylgreen (Fisher, Medford, Massachusetts, No. 76110), 500 mg; distilled water, 100 ml; stored at 4°; and filtered before use.

M/15 Sorensen's phosphate buffer (pH 6·3). Na_2HPO_4, 2·128 g; KH_2PO_4, 6·984 g; distilled water, 1000 ml.

α-Naphthyl acetate solution. α-Naphthyl acetate (Sigma, No. N8505), 1 g; ethylene glycol monomethyl ether, 50 ml; mixed in glass bottle using glass pipettes; store at −20°, protected from light.

Sodium nitrite solution (4%). Sodium nitrite, 100 mg in 2·5 ml distilled water; prepared fresh for each use.

The slides were fixed in cold fixative for 30 s and rinsed through three changes of distilled water. They were then incubated (within 24 h) with a mixture of hexazotized pararosaniline and α-naphthyl acetate in Coplin jars in a 37° water bath for 45 min. The mixture was prepared by adding, in sequence, M/15 Sorensen's buffer, 44·50 ml, hexazotized pararosaniline, 0·25 ml, and α-naphthyl acetate solution, 3 ml. (Hexazotized pararosaniline was prepared by combining equal volumes of freshly filtered pararosaniline solution [pararosaniline hydrochloride] and 4% sodium nitrite, and allowed to stand 1 min before use.)

RESULTS

ANAE activity in bovine T and B lymphocytes

Acid α-naphthyl acetate esterase (ANAE) activity was demonstrated in a majority of bovine peripheral blood lymphocytes and monocytes but not in polymorphonuclear leucocytes. Although individual cells showed differences in the pattern and intensity of staining, such as pale pink cytoplasm or diffuse minute red granules present in part of the cytoplasm (−), a coarsely granular or solid red rim of the cytoplasm (+), and a bright red (++) to extremely vivid red (+++) of the entire cytoplasm, no distinctive stain-

Table 1. Comparison of percentages of bovine lymphocytes forming E rosettes or EAC rosettes and expressing acid α-naphthyl acetate esterase (ANAE) activity

Cow No.	Percentage positive* E rosettes	EAC rosettes†	ANAE	Total E and EAC rosettes
1	44·8	32·3	66·0	77·1
2	49·6	27·0	60·8	76·6
3	37·3	33·4	65·8	70·7

* Percentage of 500 cells counted.

† Percentage of bovine peripheral blood lymphocytes with surface membrane Igs (SmIg) was 27·6 ± 4·2%. Over 91% of EAC-rosetting lymphocytes were also SmIg-positive.

ing pattern, as reported by Knowles *et al.* (1978) for monocytes and lymphocytes was observed (Fig. 1 a–d).

The lymphocyte-rich preparations purified further by iron treatment showed 62·3 ± 1·6% (SEM) of ANAE-positive cells among cell populations with over 95% lymphocytes. As shown in Table 1, comparison of percentages of lymphocytes forming E (37 ~ 50%) and EAC (27 ~ 33%) rosettes and expressing ANAE activity (61 ~ 66%) suggests that both E and EAC rosetting lymphocytes are ANAE positive.

Simultaneous study of both ANAE activity and E- or EAC-rosetting capability of the same bovine lymphocytes, indicates directly that 64·2 ± 4·9% (56 ~ 73%) of the lymphocytes capable of forming E rosettes, and 38·3 ± 0·8% (37 ~ 40%) of the lymphocytes capable of forming EAC rosettes are ANAE positive (Table 2). It has been reported previously in humans, bovines, and rodents that most E-rosette forming cells are T lymphocytes and a majority of B lymphocytes, but not of T lymphocytes, possess C receptors (Bianco, Patrick & Nussenzweig, 1970; Dukor, Bianco & Nussenzweig, 1971; Kenyon &

Table 2. Simultaneous demonstration of both acid α-naphthyl acetate esterase (ANAE) activity and E-rosetting or EAC-rosetting capability in bovine lymphocytes

Cow No.	ANAE activity	E-rosette assay: Lymphocytes E-rosetting	E-rosette assay: Lymphocytes Non-E rosetting	EAC-rosette assay† Lymphocytes EAC-rosetting‡	EAC-rosette assay† Lymphocytes Non-EAC rosetting
1	+	58/103* (56·3%)	220/297 (74·0%)	26/66 (39·4%)	94/126 (74·6%)
	−	45/103 (43·7%)	77/297 (25·9%)	40/66 (60·6%)	32/126 (25·4%)
2	+	65/103 (63·1%)	112/197 (56·9%)	29.79 (36·7%)	210/321 (65·4%)
	−	38/103 (36·9%)	85/197 (43·1%)	50/79 (63·3%)	111/321 (34·6%)
3	+	68/93 (73·1%)	137/207 (66·2%)	32/82 (38·9%)	158/218 (62·5%)
	−	25/93 (26·9%)	70/207 (33·8%)	50/82 (61·0%)	60/218 (27·5%)

* Number of ANAE positive cells/number of lymphocytes.

† Majority (> 91%) of EAC-rosetting lymphocytes corresponded to surface membrane Ig (SmIg) positive cells as determined by anti-Ig immunofluorescence assay.

‡ Simultaneous demonstration of both ANAE activity and SmIg as determined by protein A-rosette formation on lymphocytes reacted with rabbit IgG anti-bovine IgsG, M, and A showed that 41% of SmIg-positive lymphocytes were ANAE positive and 69% of SmIg negative lymphocytes were ANAE positive.

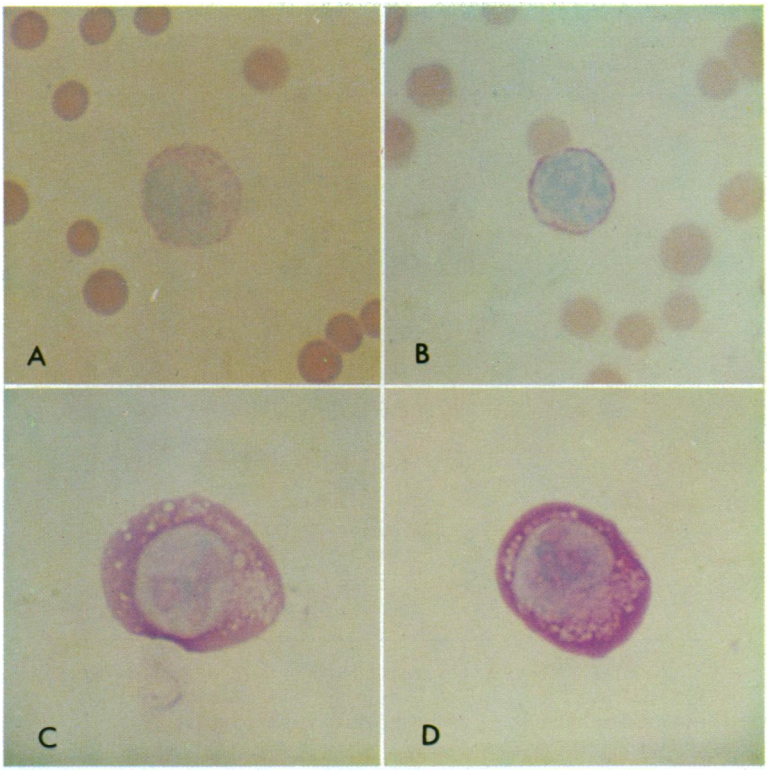

Figure 1. Cytocentrifuge smear of Ficoll–Paque separated peripheral blood mononuclear cells showing (A) − ANAE activity (human); (B) + ANAE activity (human); (C) ++ ANAE activity (bovine); and (D) +++ ANAE activity (bovine) (magnification × 1000).

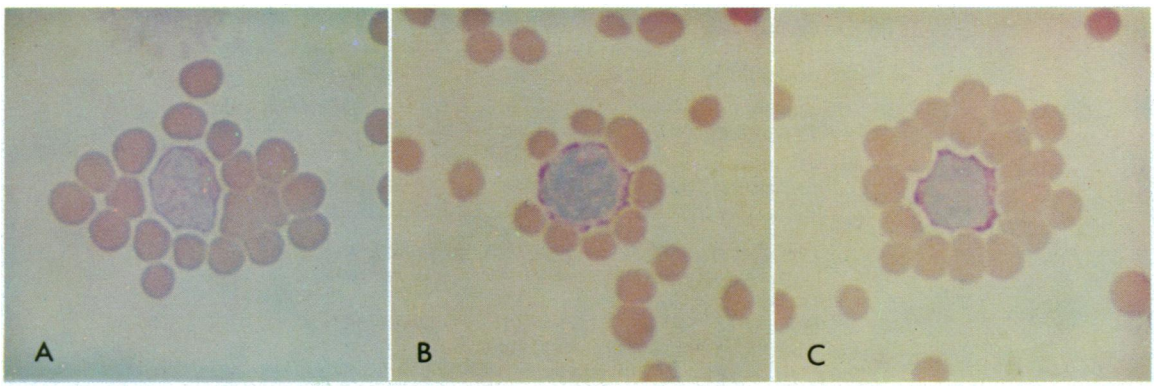

Figure 2. Cytocentrifuge smear of (A) ANAE (−) E rosettte, and simultaneous demonstration of ANAE activity in (B) E and (C) EAC rosette-forming human lymphocytes (magnification × 1000).

Table 3. Simultaneous demonstration of both acid α-naphthyl acetate esterase (ANAE) activity and E-rosetting or EAC-rosetting capability in human lymphocytes

		E-rosette assay		EAC-rosette assay†	
		Lymphocytes			
Subject	ANAE activity	E-rosetting	Non-E rosetting	EAC-rosetting	Non-EAC rosetting
1	+	192/250* (76·8%)	67/121 (55·4%)	58/147 (39·4%)	278/353 (78·8%)
	−	58/250 (23·2%)	54/121 (44·6%)	89/147 (60·5%)	75/353 (21·2%)
2	+	290/360 (80·6%)	115/239 (48·1%)	71/146 (48·6%)	204/253 (80·6%)
	−	70/360 (19·4%)	124/239 (51·9%)	75/146 (51·4%)	49/253 (19·4%)
3	+	246/291 (84·5%)	66/109 (60·6%)	58/131 (44·3%)	229/269 (85·1%)
	−	45/291 (15·5%)	43/109 (39·4%)	73/131 (55·7%)	40/269 (14·9%)

* Number of ANAE positive cells/number of rosetting lymphocytes.
† Majority (> 86%) of EAC rosetting lymphocytes corresponded to surface membrane Ig positive lymphocytes as determined by anti-Ig immunofluorescence assay.

Table 4. Acid α-naphthyl acetate esterase (ANAE) activity in phytohaemagglutinin (PHA)-stimulated and unstimulated bovine lymphocytes*

	ANAE activity†			
Lymphocytes	−	+	++	+++
Pre-incubation	37·9 ± 1·8	62·2 ± 1·8	—	—
Unstimulated 3-day culture	30·6 ± 4·8	69·4 ± 4·8	0·1 ± 0·1	—
PHA-stimulated 3 day culture‡	22·5 ± 1·7	33·4 ± 2·6	30·4 ± 0·9	13·8 ± 2·4

* The cell preparation consisted of over 95% lymphocytes.
† Percentage of 400 cells counted; mean ± SE of triplicate cultures from four cows.
‡ Majority (83 ~ 91%) of cells in PHA-stimulated cultures consisted of lymphoblasts. Parallel cultures from three cows pulse-labelled with [^{3}H]-thymidine showed Δc.p.m. (c.p.m. of PHA-stimulated cultures − c.p.m. of unstimulated cultures) ± SEM of 319,566 ± 41,999; 137,990 ± 4,678; and 159,278 ± 14,330.

Table 5. Acid α-naphthyl acetate esterase (ANAE) activity in phytohaemagglutinin (PHA)-stimulated and unstimulated human lymphocytes*

	ANAE activity†			
Lymphocytes	−	+	+++	++++
Pre-incubation	27·2±2·0	72·9±2·1	—	—
Unstimulated 3 day culture	26·8±4·8	72·4±4·7	0·5±0·1	0·3±0·1
PHA-stimulated 3 day culture	28·9±4·8	34·0±2·0	26·1±1·7	11·0±2·3

* The cell preparation consisted of over 93% lymphocytes.

† Percentage of 400 cells counted; mean±SE of triplicate cultures from three adults (one female; two males).

‡ Cells in PHA-stimulated cultures consisted of 80~91% lymphoblasts. Parallel cultures pulse labeled with [^{3}H]-thymidine showed Δc.p.m. (c.p.m. of PHA-stimulated cultures −c.p.m. of unstimulated cultures) ±SEM of 97,680±8,797; 115,479±7,323; and 97,746±1,148.

Piper, 1977; Grewel, Rouse & Babiuk, 1978; Stobo, 1978), thus the present finding indicates that while a majority of T lymphocytes are ANAE positive, a significantly high proportion of B lymphocytes are also ANAE positive. Additional studies by simultaneous ANAE staining of the lymphocytes with surface membrane Igs (SmIg) as determined by rabbit IgG anti-bovine Igs–protein A rosetting, indicated also that the EAC rosetting cells indeed represented B lymphocytes, *viz.*, similar percentage of SmIg-positive cells were ANAE positive (Table 2).

ANAE activity in human T and B lymphocytes

ANAE activity was demonstrated in a majority of human peripheral blood monocytes and lymphocytes but not in polymorphonuclear leucocytes. The staining characteristics were the same as those described above for bovine cells and a distinctive staining pattern characteristic of lymphocytes which could be readily distinguished from monocyte staining was demonstrated (Knowles *et al.*, 1978).

As shown in Table 3, while 80·6±2·2 (SEM) % (77~85%) of lymphocytes capable of forming E rosettes were ANAE positive, 44·1±2·6% (39~49%) of lymphocytes forming EAC rosettes were ANAE positive. In other words, 15~23% of E-rosetting lymphocytes were ANAE negative (Fig 2 a–c).

To exclude the possibility that EAC-rosetting lymphocytes were 'contaminated' with T lymphocytes, a lymphocyte preparation was depleted of T lymphocytes by centrifugation of E-rosetted cells over Ficoll–Paque and the EAC rosette test was performed on those lymphocytes remaining at the interface. In this T-depleted preparation, 85% of the lymphocytes formed EAC rosettes, and 48·1% of those forming EAC rosettes were ANAE possitive.

ANAE activity in PHA-stimulated and unstimulated bovine lymphocytes

As shown in Table 4, bovine lymphocytes from PHA-stimulated cultures showed enhanced ANAE activity as compared to those from unstimulated or pre-incubation preparations. The majority of cells in PHA-stimulated cultures with blastic transformation showed strong ANAE activity (vivid red staining) in greatly enlarged cytoplasm.

ANAE activity in PHA-stimulated and unstimulated human lymphocytes

As shown in Table 5, PHA-stimulated human lymphocytes also showed an increase in ANAE activity as compared to those from unstimulated cultures, although the increase was not as pronounced as that in bovine lymphocyte cultures.

DISCUSSION

Identification of monocyte-macrophages in mixed mononuclear cell suspensions frequently presents problems for investigators. The non-specific esterase staining method developed originally by Yam *et al.* (1971) and subsequently improved by Koski *et al.* (1976) has been used successfully in identifying these cells in humans and mice. In bovines the methods were anticipated to be of even greater importance because the Ficoll–Hypaque density gradient separation method generally yields greatly increased percentages of monocytes in mononuclear cell preparations, and because bovine monocytes are difficult to differentiate morphologically from the high number of large lymphocytes normally present in bovine peripheral blood.

In an attempt to employ the method of Koski *et al.* (1976) to identify monocytes from other leucocyte populations in bovine peripheral blood, we noticed that a large number of lymphocytes also showed nonspecific esterase activity. This confirms the recent reports of the presence of ANAE activity in human and murine lymphocytes (Muëller *et al.*, 1976; Knowles *et al.*, 1978; Horwitz, Allison, Ward & Knight, 1977). These reports indicated further a distinctive staining characteristic in T lymphocytes suggesting that the histochemical method might be of great use as a routine, practical alternative to E-rosette assays in identifying T lymphocytes.

Simultaneous demonstration of ANAE activity and E- or EAC-rosetting capability of the same lymphocytes, as reported in this paper, indicates that while a majority of human and bovine T lymphocytes are ANAE positive, a significant proportion of B lymphocytes are also ANAE positive (Tables 2 and 3). In our study, the procedures prescribed by Koski *et al.* (1976) were strictly followed, with the additional use of α-naphthyl acetate as suggested by Yam *et al.* (1971) and Li, Lam & Yam (1973). It is possible that slight differences in the methods employed by us and the previous workers might be responsible for the differences observed. The conclusions made by the previous workers, however, were generally based on comparison of percentages of lymphocytes forming E rosettes and expressing ANAE activity (Mueller *et al.*, 1975, 1976; Higgy, Burns & Hayhoe, 1977; Sher *et al.*, 1977; Knowles *et al.*, 1978) rather than on direct demonstration of ANAE activity in the same lymphocytes forming either E or EAC rosettes as we showed in this experiment. Indeed, Sundström, Nilsson, Ranki & Häyry (1978) showed recently the presence of ANAE activity not only in T-cell derived acute leukaemia lines but also in histiocytic lymphoma and myeloma lines and some of the Burkitt and lymphocytic lymphoma and non-neoplastic lymphoblastoid cell lines with B-cell surface markers. They concluded further that 'the ANAE marker is no longer T-specific when malignant lymphoid cells are considered and that the usefulness of this marker in routine diagnostic work therefore is limited'.

A discussion made in a recent report by Ranki (1978) on simultaneous demonstration of ANAE activity in $53 \pm 12\%$ of Fc receptor positive, $25 \pm 17\%$ of E-rosette depleted, and 10% of surface Ig-carrying human lymphocytes supports our findings on the presence of ANAE activity in B lymphocytes, although Ranki (1978) inclined to conclude that ANAE activity is predominantly confined to mature resting lymphocytes and largely lacking from B lymphocytes. Obrist, Albrecht & Nagel (1978) noted also that $25{\cdot}5 \pm 8{\cdot}8\%$ of non-E rosette-forming lymphocytes were ANAE positive. A previous report by Li *et al.* (1973) on the presence of moderate ANAE activity in plasma cells substantiates further the presence of ANAE activity also in the cells of B lymphocyte lineage. The role of esterases in cellular metabolism is not understood. The dramatic increase in intensity of ANAE activity we have seen in PHA-stimulated lymphocytes could reflect some functional capability of an activated cell (Ranki, Tötterman & Häyry, 1976).

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