engaged in different ways in their regulation and the genetic defects in TE-CFUs could be separated from the genetic defects in CFU-S[8].
Therefore, according to these results, in the murine hemopoietic system 2 separate classes of tripotent hemopoietic cells operate; CFU-S and TE-CFUs.

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2 C.V. Robinson, Proc. Soc. exp. Biol. Med. *124*, 118 (1967).
3 S.S. Boggs, P.A. Chervenick and D.R. Boggs, Blood *40*, 375 (1972).
4 C.J. Gregory, E.A. McCulloch and J.E. Till, J. Cell Physiol. *86*, 1 (1975).
5 W. Wiktor-Jedrzejczak, A. Ahmed, S.J. Sharkis, A. McKee and K.W. Sell, J. Cell Physiol. *99*, 31 (1979).
6 P.J. Smith, C.W. Jackson, L.W. Dois, C.C. Edwards and M.A. Whidden, Blood *56*, 52 (1980).
7 P.J. Smith, C.W. Jackson, M.A. Whidden and C.C. Edwards, Blood *56*, 58 (1980).
8 W. Wiktor-Jedrzejczak, C. Szczylik, P. Gornas, S.J. Sharkis and A. Ahmed, Cell Tissue Kinet. *14*, 211 (1981).
9 G. Silini, S. Pons and L.V. Pozzi, Br.J. Haemat. *14*, 485 (1968).
10 S.J. Sharkis, W. Wiktor-Jedrzejczak, A. Ahmed, G.W. Santos, A. McKee and K.W. Sell, Blood *52*, 802 (1978).
11 L.O. Jacobson, K.K. Marks, E.O. Gaston and E. Goldwasser, Blood *14*, 635 (1959).

Nonspecific esterase activity in monkey thymus lymphocytes; study of distribution in lymphocyte subpopulation

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Summary. A subpopulation of monkey thymus lymphocytes was investigated by direct nonspecific esterase staining of E-rosette forming lymphocytes. Thymus lymphocytes had high nonspecific esterase activity and E-rosette formation, but the level of their in vitro lectin responses was very low.

Nonspecific acid α-naphthyl acetate esterase (ANAE) activity, rosette formation with heterogeneic erythrocytes and responsiveness to some mitogens have been evaluated as markers for peripheral T-lymphocytes in laboratory rodents and in man. Whether the ANAE marker, E-receptor and lectin response would also be predominantly expressed by thymus lymphocytes was of considerable interest. Previously, we reported that human thymus lymphocytes had low blastogenic activity to lectin, although they had a high degree of E-rosette formation[1]. It has been reported that percentages of ANAE positive cells in the thymus from human[2,3] and mouse[4] were lower than those in peripheral blood. The present study was undertaken therefore to investigate the relationship among nonspecific esterase activity, cell surface receptor for sheep erythrocytes and in vitro lectin responses in thymus lymphocytes and blood lymphocytes from monkeys.

Materials and methods. Fresh thymuses were obtained from 30 young adult Japanese monkeys *(Macaca fuscata)*. A suspension of thymus lymphocytes was prepared by mincing the tissue into fragments on a 60 stainless steel mesh in chilled Hanks balanced salts solution. Blood lymphocytes were also prepared from heparinized venous blood from the animals from which the thymuses were obtained, using the Ficoll-Isopaque (Lymphoprep, Nyeggard & Co. As., Oslo) density gradient method for human lymphocytes, as previously described[5].
Spontaneous rosette formation between lymphocytes and sheep erythrocytes was assayed by the method reported in detail elsewhere[1]. Briefly, equal volumes (0.1 ml) of lymphocyte suspension (5×10^6/ml) and sheep erythrocytes (2×10^8/ml) were mixed in short test tubes. These mixtures were incubated at 37 °C for 10 min, then centrifuged at 700 rpm for 5 min. After 16 h incubation at 4 °C, the pellets

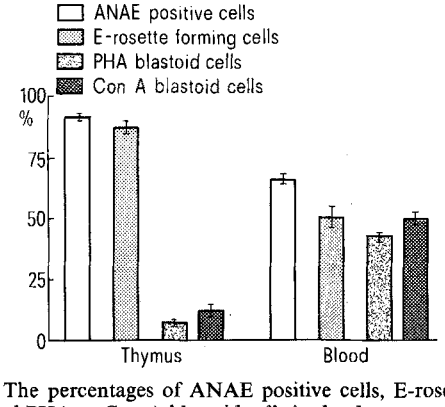

Fig. 1. The percentages of ANAE positive cells, E-rosette forming cells and PHA or Con A blastoid cells in the thymus and peripheral blood from monkey.

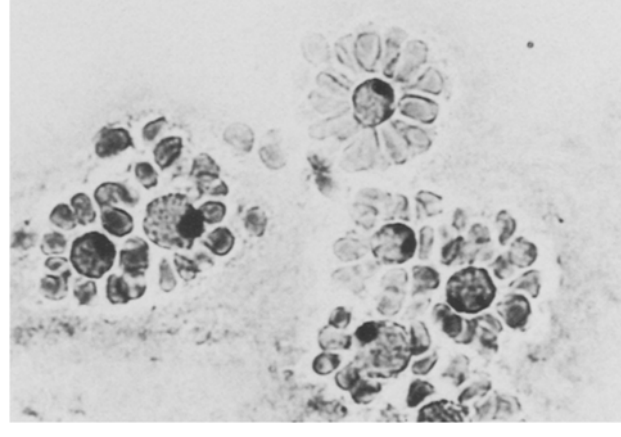

Fig. 2. ANAE positive and E-rosette forming lymphocytes from monkey thymus, × 700.

were gently resuspended and the samples examined by differential interference microscopy at 400× magnification. Cells binding more than 3 erythrocytes were scored positive.

ANAE activity of lymphocytes was detected according to the method of Knowles et al.[2]. The lymphocytes, subjected or not to the E-rosette test, were smeared on glass microscopic slides and then immediately fixed in buffered formalin acetone (pH 6.6) at 4 °C for 10 min. After washing, the slides were kept moist at all times and never allowed to dry. The demonstration of esterase activity was performed using α-naphthyl acetate as substrate and hexazotized pararosaniline as coupler. The fixed cell smears were also incubated with an irreversible inhibitor, diethyl-p-nitrophenyl phosphate (E 600, Sigma) at 37 °C for 1 h before ANAE staining by the method of Ranki[4]. The slides were incubated for 21 h at room temperature, washed with distilled water and then counterstained with 0.5% toluidine blue for 30 min.

Lymphocytes ($1 \sim 5 \times 10^6$/ml) from thymus or peripheral blood were suspended in RPMI-1640 culture medium containing 20% fetal calf serum (Gibco). The cells were incubated with PHA-P (10 μg/ml, Difco) or Con A (10 μg/ml, Miles-Yeda) in short test tubes (14 × 100 mm) at 37 °C for 72 h in a humidified atmosphere with 5% CO_2. The percentages of PHA or Con A blastoid cells were determined according to the morphological method previously described. A microculture method using plastic test plates (0.2 ml, Nunch) containing 2×10^5 cells in the medium was also performed. 16 h before harvest, each well (0.2 ml) received 0.2 μCi ^{3}H-TdR (45 Ci/mmole, The Radiochemical Centre, Amersham, England). The cells were harvested on glass filter paper (Whatman, CF/A). Radioactivity was measured in a Beckman liquid scintillation counter.

Results and discussion. ANAE activity was detected in lymphocytes and monocytes but not in polymorphonuclear leucocytes. The majority of ANAE positive lymphocytes displayed a prominent, red-brown, dot-like staining pattern adjacent to the cell membrane whereas monocytes contained diffuse, cytoplasmic red-brown reaction products. This ANAE reaction was completely inhibited by preincubation of the cell preparation in a 10 mM concentration of E 600. The percentages of ANAE positive lymphocytes in the thymus were much higher than those in the peripheral blood (fig. 1) although the pattern and intensity of ANAE staining of both lymphocytes were the same. In preliminary experiments we also observed high ANAE activity in the thymus lymphocytes from mouse, rat and guinea-pig. These results differ from those reported in human and mouse by some authors[2-4]. We obtained a close correlation of percentages of ANAE positive cells and E-rosette forming cells in the thymus from 10 monkeys: 91.7 ± 1.3% of thymus lymphocytes were ANAE positive and 87.6 ± 19% formed E-rosettes (fig. 1).

In the present studies ANAE activity in lymphocytes forming E-rosettes was demonstrated directly. The intense, dot-like reaction product in thymus lymphocytes forming E-rosettes was localized at the periphery of the cells (fig. 2). These results suggest that the E-rosette forming cell population appears in most instances to be entirely contained within the ANAE positive cell population. The role of esterases in cellular metabolism is not fully understood. However, the distinctive staining characteristic of thymus lymphocytes and T-lymphocytes suggests that the histochemical method may be of great use for identification of T-lymphocytes. In lymphocyte cultures the percentages of PHA or Con A blastoid cells in the thymus were markedly lower than those in peripheral blood (fig. 1). The number of cells carrying out DNA synthesis as estimated by ^{3}H-TdR incorporation into thymus lymphocytes in PHA or Con A cultures was also lower than that of blood lymphocytes obtained from the same donor in 3 cases (PHA; thymus 7477 cpm, blood 19,410 cpm, Con A; thymus 11,109 cpm, blood 28,381 cpm). This is in agreement with the result obtained in human thymus[1]. It is concluded that there is no definite correlation between the ANAE activity or E-rosette formation capability of thymus lymphocytes and their in vitro lectin responses.

1 S. Kato, K. Yamaguchi, K. Awaya and M. Nishioka, Acta haem. Jap. *41*, 45 (1978).
2 D.M. Knowles, H.T. Hoffman, M. Fovarini and H.G. Kunkel, Cell. Immun. *35*, 112 (1978).
3 R. Obrist, R. Albercht and G.A. Nagel, Experientia *34*, 660 (1978).
4 A. Ranki, Clin. Immun. Immunopath. *10*, 47 (1978).
5 S. Kato, Bull. Yamaguchi med. Sch. *24*, 1 (1977).

Precocene treatment of the female tsetse fly *Glossina morsitans morsitans* sterilises her female offspring

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Summary. When adult female *Glossina morsitans morsitans* were treated with either precocene I or precocene II, females of the F_1 generation, emerging from pupae of the 2nd, 3rd and 4th reproductive cycles failed to develop oocytes.

Tsetse flies (*Glossina* spp.) reproduce by adenotropic viviparity. To achieve this end, the female tsetse fly produces a single egg, in the right ovary, which ovulates on day 9 of adult life in the case of *G. morsitans*[2]. The fertilized egg undergoes embryogenesis and development is completed within the mother, the developing larva being nourished by secretions of the modified accessory glands. During this time, the 2nd egg develops in the left ovary. Following larviposition, the 3rd instar larva soon pupates, the pupal stage lasting about 30 days, after which adult emergence occurs. 1-2 h after the 1st larviposition[2], the 2nd egg ovulates and the cycle repeats itself. The corpus allatum is known to be involved in regulating the reproductive cycle of female tsetse flies[3,4].

Precocenes[5] are known to affect corpus allatum activity[6,7] and oocyte development in insects[5,8]. All reports on the effects of precocenes on insects focus attention on their action on the treated (parental) generation. The only exception is the work on aphids[9], where the effect of precocene II was carried over to the F_1 generation to produce alate