ics with hepatitis and/or cirrhosis, the liver dysfunction may be responsible for the presence of SIF in the peripheral circulation rather than the alcoholic state per se.

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Alkaline phosphatase in human lymphocyte subpopulations

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Summary. The levels of membrane alkaline phosphatase have been measured on different lymphocyte fractions from human peripheral blood separated on bovine serum albumin discontinuous gradients. The peak in enzyme activity was observed in a non-T-, non-B-cell fraction, rich in 'null' lymphocytes; the lowest values were found in the fraction with the highest proportion of T-cells.

Alkaline phosphatase (E.C. 3.1.3.1.) has been demonstrated on the surface of normal and leukaemic lymphocytes[1-3]. No correlation has been established so far between enzyme activity and any particular lymphocyte subpopulation. Ruuskanen et al.[1] suggested that it is present in immature T-cells, based on their findings in guinea-pig thymocytes. High values of alkaline phosphatase were recently demonstrated in the mononuclear cells of human cord blood[3], which contains a low proportion of T-lymphocytes and a high proportion of cells negative for T- and B-cell markers ('null')[4,5].

In the present study we have investigated the levels of enzyme activity in the different lymphocyte fractions from human blood separated by bovine serum albumin (BSA) discontinuous gradient.

Materials and methods. Blood samples collected in CPD (citrate-phosphate-dextrose) were obtained from 4 normal adult donors, washed once and diluted 1:1 with sodium chloride. The mononuclear cell population was separated with 'Lymphoprep' (Nyegaard & Co., AS., Oslo, Norway), washed twice with TC 199 medium, resuspended in a small volume (3 ml) and then layered on a BSA discontinuous gradient as modified from Dicke et al.[6]. Briefly, decreasing concentrations of BSA solutions (from 35% to 11%, in 4% decrements) were prepared and 3 ml of each fraction was carefully laid on top of the fraction with the next highest concentration of BSA. The interface between 11% and 15% BSA was considered as fraction 1, and so on, up to fraction 6, between 31% and 35% BSA. The gradients with the lymphocytes (between 19×10^7 and 50×10^7) were centrifuged at 10 °C for 30 min at $900\times g$. Cells from each interface were collected and washed 3 times with TC 199 medium. On each fraction and on a sample of cells before BSA separation the following tests were performed:

1. T-lymphocyte percent was assessed by the capacity for forming rosettes with papain-treated sheep RBC (E-rosettes)[7] (blood lymphocytes of normal adults 62% ± 8 SD).
2. Surface immunoglobulins were assessed by direct immunofluorescence with a polyvalent $F(ab)_2$ antiserum to human Ig; samples were incubated at 37 °C in serum-free medium for 1–2 h before adding the antiserum (blood lymphocytes of normal adults 14.1 ± 4.8 SD).

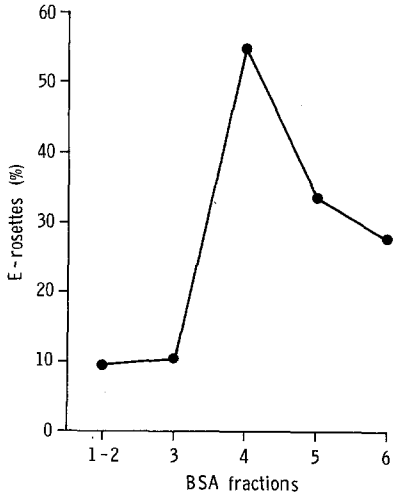

Fig. 1. Normal human blood separated on a BSA discontinuous gradient: distribution of T-lymphocytes as shown by percentage of E-rosette forming cells in each fraction.

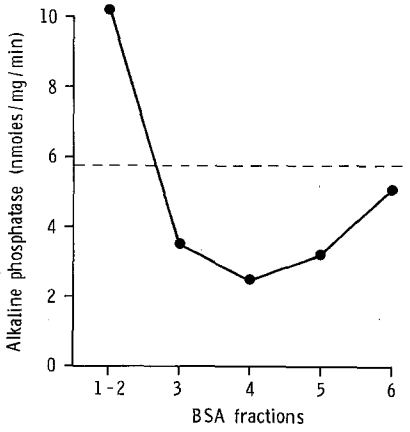

Fig. 2. Normal human blood separated on a BSA discontinuous gradient: levels of alkaline phosphatase activity (the broken line shows the mean value for unseparated normal blood lymphocytes). Results are expressed as nmoles of activity per mg of protein per min.

3. Alkaline phosphatase activity was evaluated by a biochemical assay with sodium p-nitrophenol as substrate. The assay is described in detail elsewhere[3]. Cytocentrifuge slides for morphological examination were prepared from each fraction.

Results. The polymorphonuclear contamination in all the BSA fractions was always less than 4% and the proportion of monocytes ranged between 1% and 20%. As the cell recovery in fractions 1 and 2 was always very low, between 0.4% and 1% of the initial number of cells, the cells recovered from these 2 fractions were pooled.

Figure 1 shows the percent of E-rosettes in the mononuclear cell population of each fraction. In the lower density fractions (1–3) the proportion of T-cells was always low; in 2 of the 4 samples it was < 5%, in 1 sample < 10% and in the last one 23.5%. The peak of T-cells was observed in fraction 4; the values decreased in fractions 5 and 6, but were still higher than in fractions 1–3. The results from fractions 3, 4, 5 and 6 were similar in the samples from the 4 subjects tested. The percent of B-lymphocytes appeared to be uniformally distributed throughout the different fractions (ranging between 9% and 20%).

Figure 2 shows the alkaline phosphatase activity in each cell fraction. In all the experiments the highest activity (mean 10.1±1.6 SEM) was observed in the pooled samples from fractions 1 and 2 where the values were twice those observed in normal unseparated lymphocytes (broken line in figure 2). The lowest enzyme values (mean 2.4±1.4 SEM) were found in fraction 4. The levels of activity increased slightly in the higher density fractions.

Discussion. The observation[3] that high alkaline phosphatase values are found in the mononuclear cells of human cord blood, which contains few T-lymphocytes compared with adult blood and many non-T-, non-B-cells, suggested that the enzyme activity might be associated with the latter cells. The high values in 'common', non-B-, non-T- acute lymphoblastic leukaemia[3] may support this view. The findings by Ruuskanen et al.[1] also suggested that alkaline phosphatase activity may be a feature of immature T-cells and that maturation may be associated with a loss in the enzyme content. The present studies demonstrate that the peak of alkaline phosphatase activity, well above values for unseparated lymphocytes, was observed in fractions 1–2 where the proportion of T-lymphocytes was lowest and the proportion of non-T-, non-B-cells highest. Others have shown that null-cells with a high rate of spontaneous DNA synthesis are found in the low density fractions[8].

The possibility that the high enzyme levels in fractions 1–2 may be related to the residual subpopulation of T-cells is unlikely because their percentage was always very low in these fractions. The lack of a relationship between T-lymphocytes and alkaline phosphatase activity is further shown by the low values observed in fraction 4, where the highest proportion of T-cells was found.

The uniformly low proportion of polymorphonuclear cells throughout all fractions rules out the possibility that neutrophil alkaline phosphatase contributed to the observed values. In experiments with cord blood removal of most of the monocytes and neutrophils from the mononuclear cell layer and incubation with the detergent Triton X-100 did not result in significant changes in alkaline phosphatase activity[3].

The recent suggestion[2] that alkaline phosphatase may be associated with a subtype of B-cell lymphocyte cannot be completely excluded from this study because the only B-cell marker performed was SmIg with a polyvalent reagent, which does not enable to distinguish between various B-cell populations. However, it seems unlikely that B-cells can be responsible for the enzyme activity because they were distributed in low proportion (< 20%) throughout all fractions.

Our results indicate that high surface-membrane alkaline phosphatase activity is associated with non-T-, non-B-cells, but these experiments do not permit further characterization.

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Mise en évidence de l'induction de la coagulation plasmatique par les hémocytes chez *Locusta migratoria*

Role of hemocytes in hemolymph coagulation in *Locusta migratoria*

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Summary. When performed at low temperature (4 °C), centrifugation of hemolymph from *Locusta migratoria* separates a plasma which does not exhibit any coagulation when it is preserved from the hemocyte pellet. Blood cells added to plasma, induce a rapid and normal coagulation (precipitation of coagulogen).

La majorité des hématologistes d'insectes admettent que la coagulation plasmatique est induite par les hémocytes. Cette conclusion repose uniquement sur l'observation des transformations de certains hémocytes au moment de la coagulation de l'hémolymphe (travaux de microscopie optique[1,2] et de microscopie électronique[3,5]). C'est pourquoi Gupta et Sutherland[6] ont pu émettre l'hypothèse que ces transformations des hémocytes pouvaient être l'effet et non pas la cause de la coagulation, hypothèse reprise par Crossley[7].

L'objet du présent travail est d'apporter une preuve expérimentale formelle du rôle des hémocytes dans l'induction de la coagulation plasmatique chez l'orthoptère *Locusta migratoria.*

Chez cet insecte, nous avons montré que la coagulation de l'hémolymphe se traduit par la précipitation d'un complexe