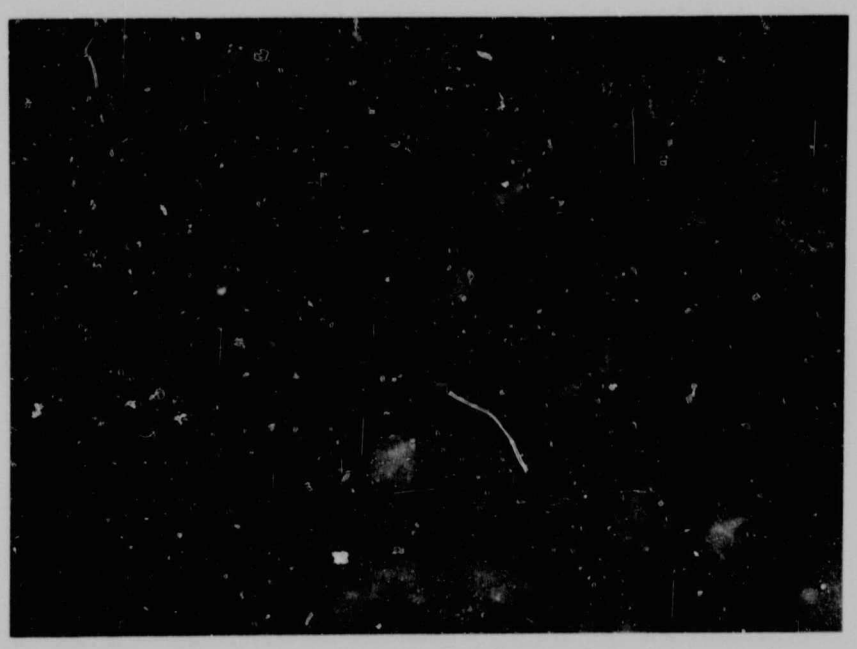

Figure 14 Anti-T cell antiserum activity on CLL cell suspension, x 600.

rosettes to follicular areas (Figure 3), together with both EAC and EA adherence to macrophages in the medullary and subcapsular sinuses. Native erythrocytes were lysed in this frozen section process, so intact red cells in the figure are adhering sheep cells. The naphthalene black technique of Allison et al., (1976) failed to give adequate differentiation of black red cells against grey tissues, but dark ground illumination showed good contrast between attached cells and tissue.

As discussed in section 12.5, non-specific esterase activity could not be taken as a specific marker for T cells.

Sections were also treated with commercial antisera against human immunoglobulins to detect surface bound immunoglobulin. This reagent detected surface immunoglobulin on the membranes of lymphocytes localised in the follicular centres of the lymph node cortex (Figure 15). Both this reagent and a non-immune rabbit serum conjugate gave weak background staining superimposed with intense non-specific fluorescence of macrophages and elastin. These are common problems in immunofluorescence being almost impossible to eradicate, so they become particularly obtrusive when studying tissues rich in such elements. Figures 21 and 22 show incidentally, the marked non-specific fluorescence of elastin-rich sub-dermal connective tissue.

The results of applying anti-immunocyte conjugates to tissues are shown in the following figures (16-22). Anti-thymocyte reagent was capable of detecting T cells by intense staining of lymphocytes throughout the thymus (Figure 16), and paracortical areas of the lymph node,

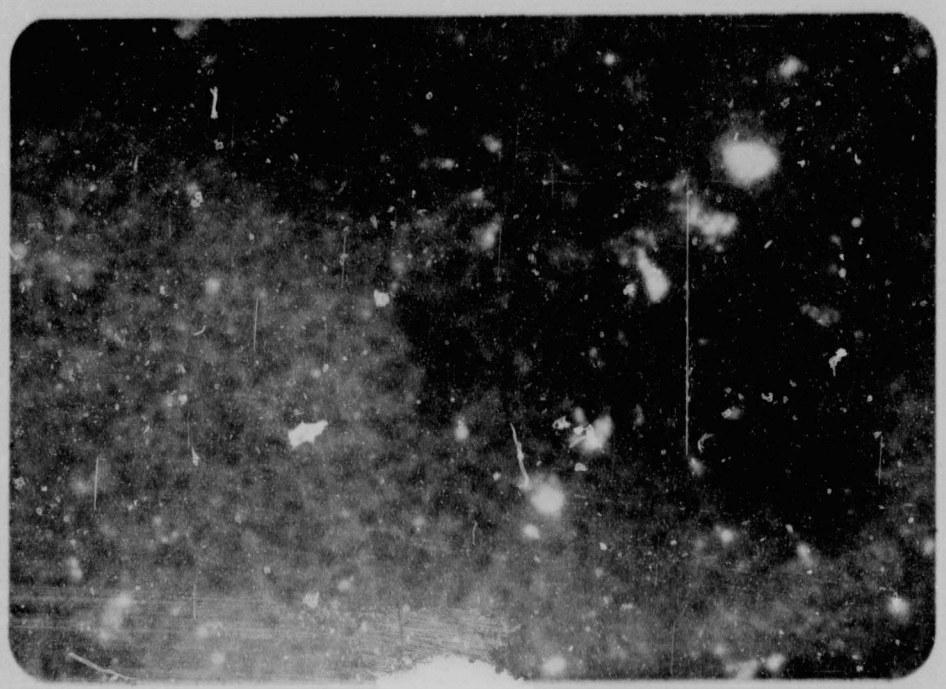

Figure 15 Immunoglobul[illegible]ted by fluorescent antisera, localised in the c[illegible]tre of a human lymph node follicle. The bright granules are macrophages, x 350.

(Figure 17). There was weaker staining of the medullary cords but cortical follicles, although not completely unstained, showed very weak fluorescence of cell membranes. The anti-CLL conjugate however showed weak staining of all cells of the thymus and lymph node with little differentiation. (Figure 18 and 19). There was some intensification of staining at the centres of follicles but this tended to correlate in distribution and appearance with specific fluorescence with anti-human globulin reagent.

A pathological lymph node with benign hyperplasia was studied by similar techniques. Three separate attempts with different EAC preparations failed to describe any significant localisation of B cells in the cortex. The stained paraffin section showed loss of follicular architecture and the anti-thymocyte conjugate gave diffuse staining of cell membranes throughout the cortex. Finally figures 20, 21 and 22 illustrate the application of the anti-lymphocyte reagents to lymphocytes in other tissues. The stained section shows round cell infiltration in sub-dermal connective tissues. The anti-CLL reagent shows no fluorescence in these areas, but the significance of this finding is inconclusive in the light of the foregoing observations. The anti-thymocyte conjugate gave weak, but specific membrane fluorescence of the infiltrating cells, with confirmation of their T cell identity afforded by their inability to cause EAC or EA indicator systems to adhere to their surfaces.

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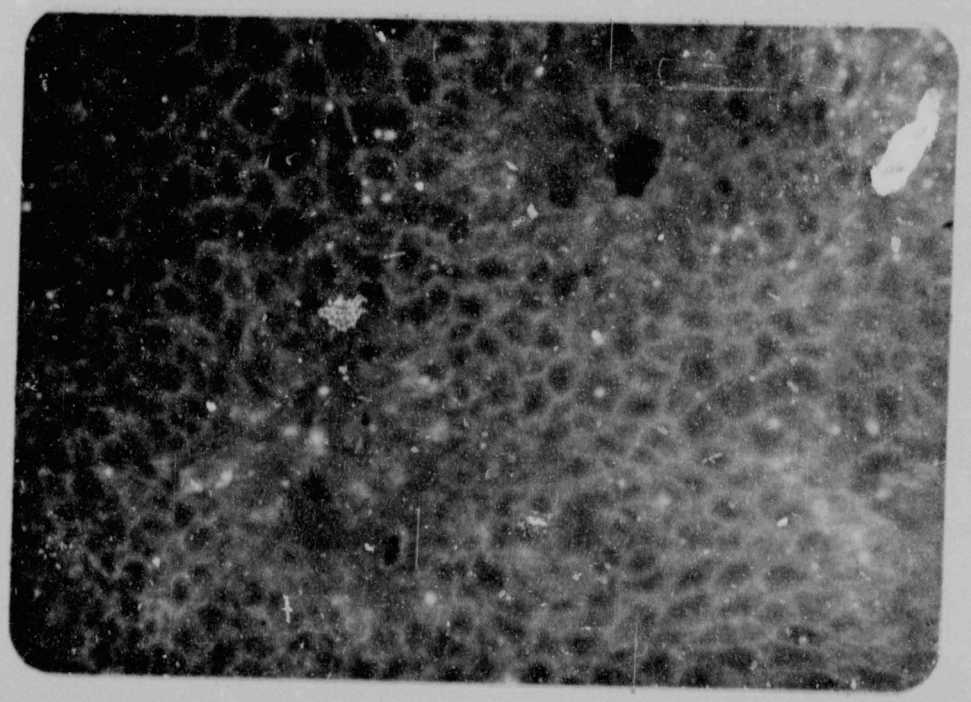

Figure 16 Anti-thymocyte reagent applied to a frozen section of human fetal thymus. Membranes of all cells are stained, x 700.

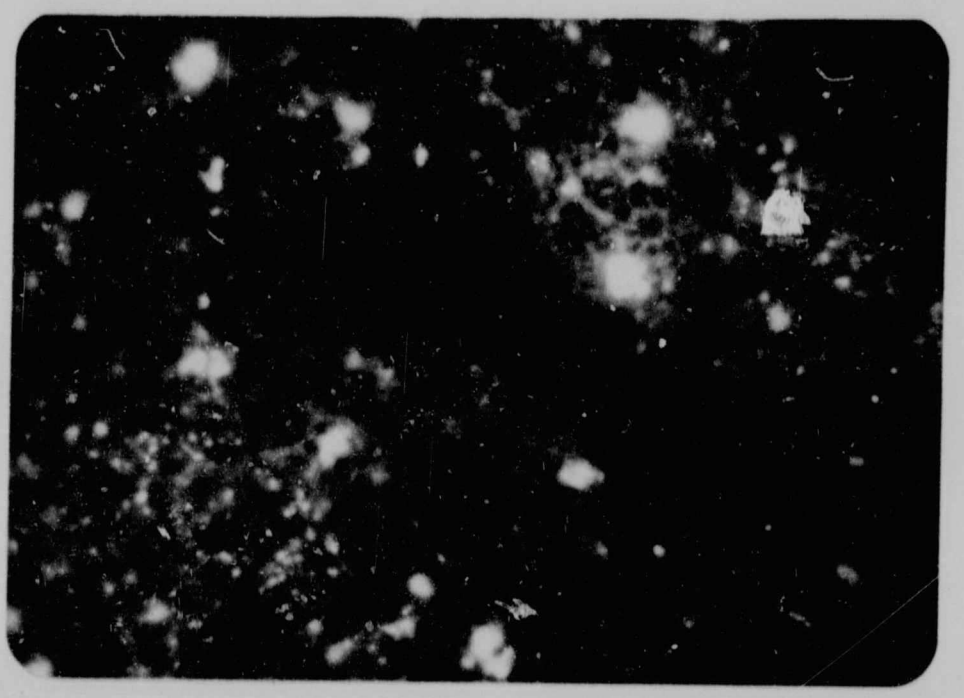

Figure 17 Anti-thymocyte reagent applied to human lymph node. Perivascular T cells are stained, x 700.

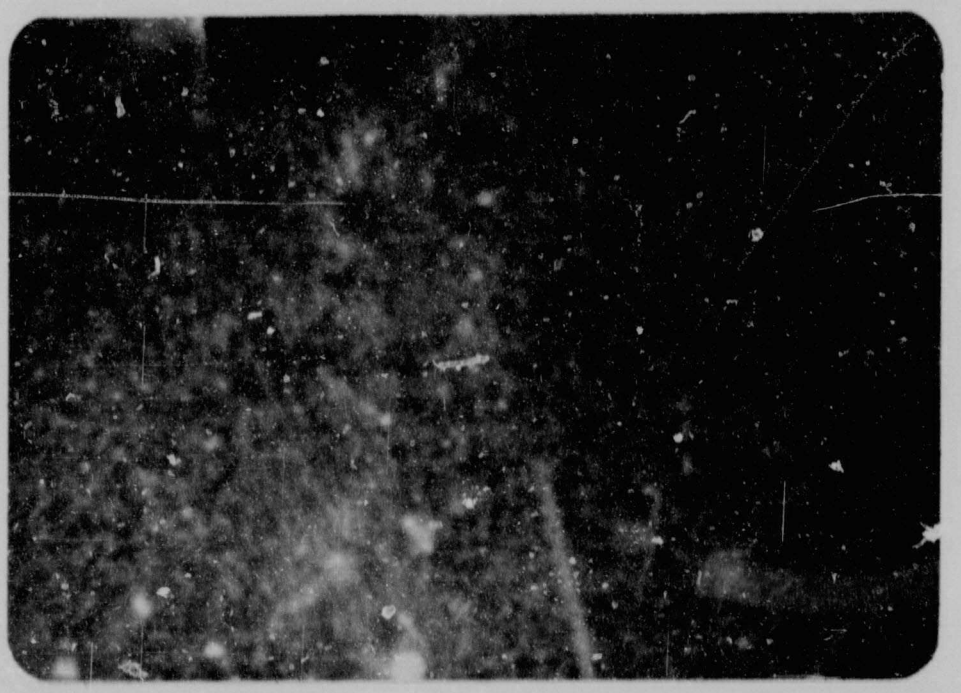

Figure 18 Anti-CLL cell reagent applied to human fetal thymus, x 700.

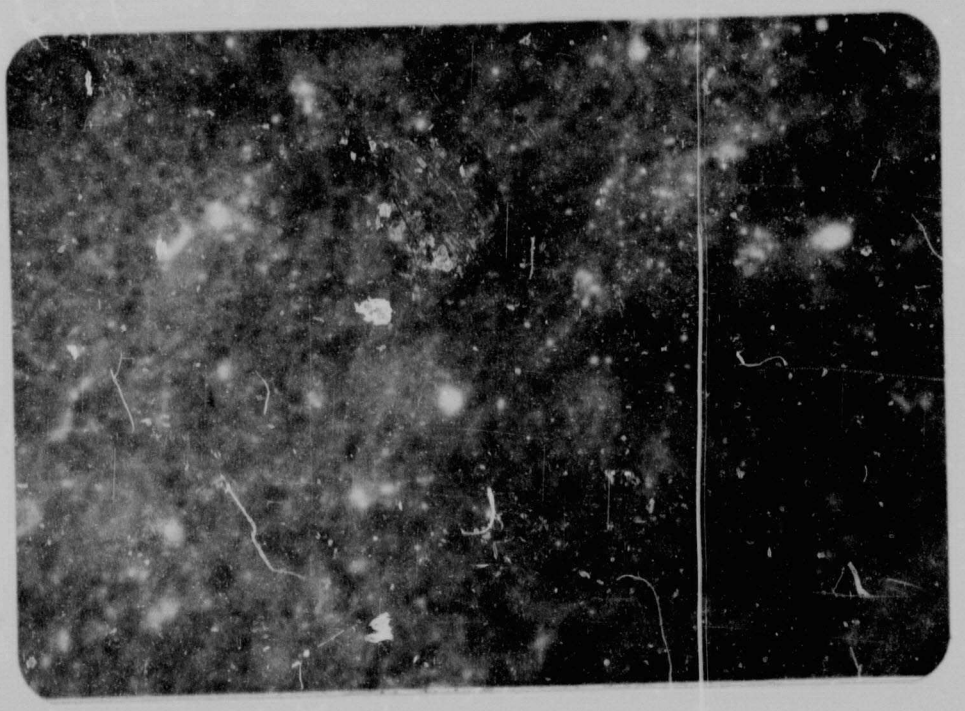

Figure 19 Anti- CLL cell reagent applied to human lymph node, x 700

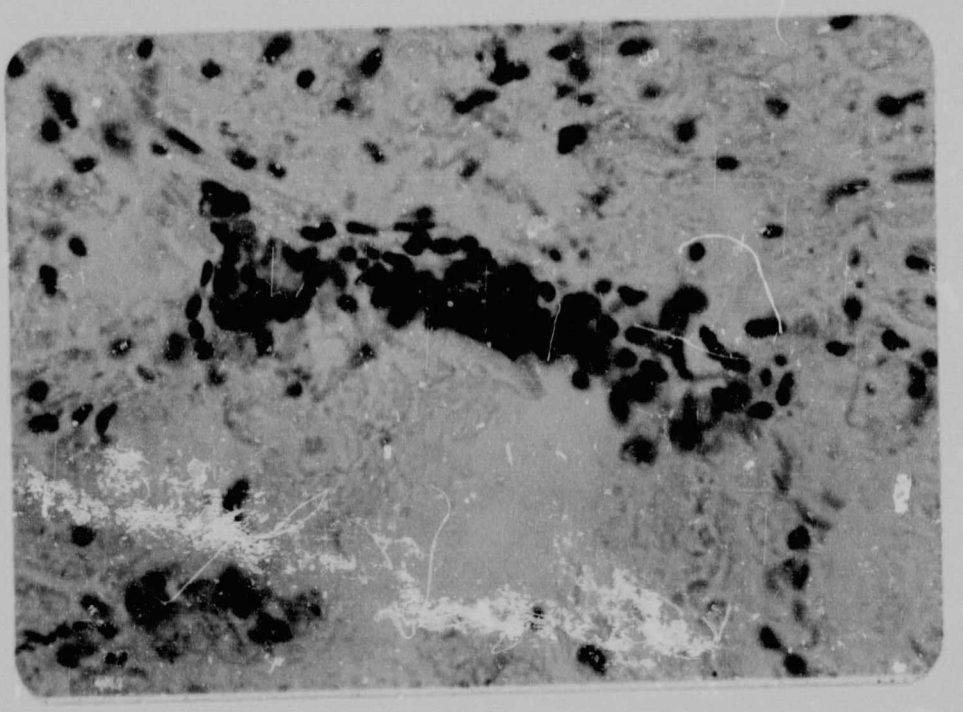

Figure 20 Frozen section of human skin showing round cell infiltration in sub-dermal connective tissue, H & E x 600.

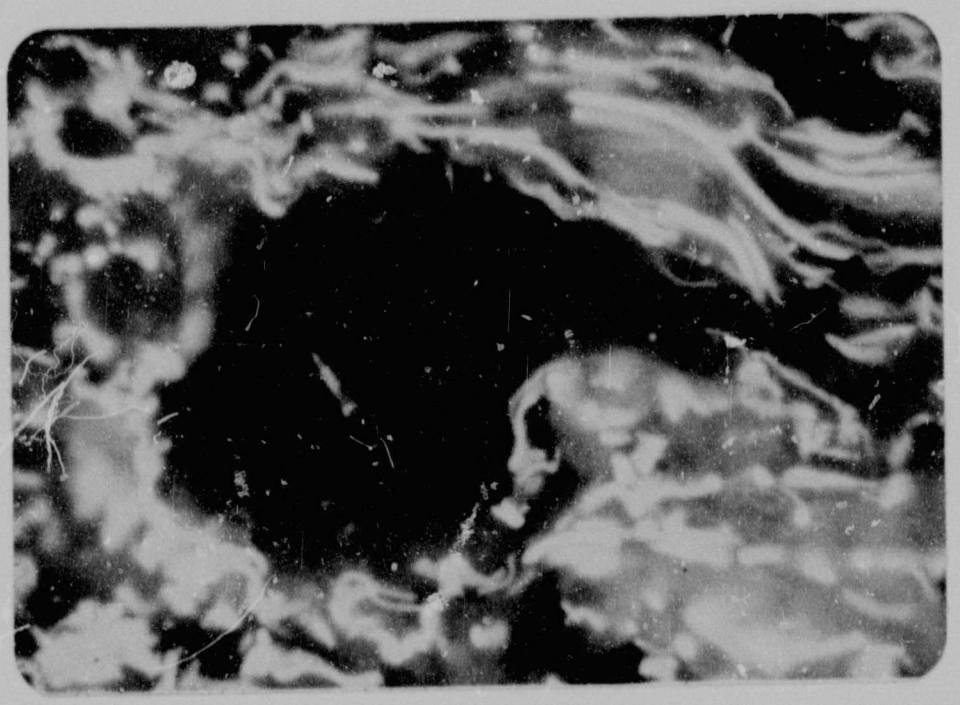

Figure 21 Adjacent section to figure 20, stained with anti-thymocyte reagent. T cell membranes are weakly fluorescent, x 700.

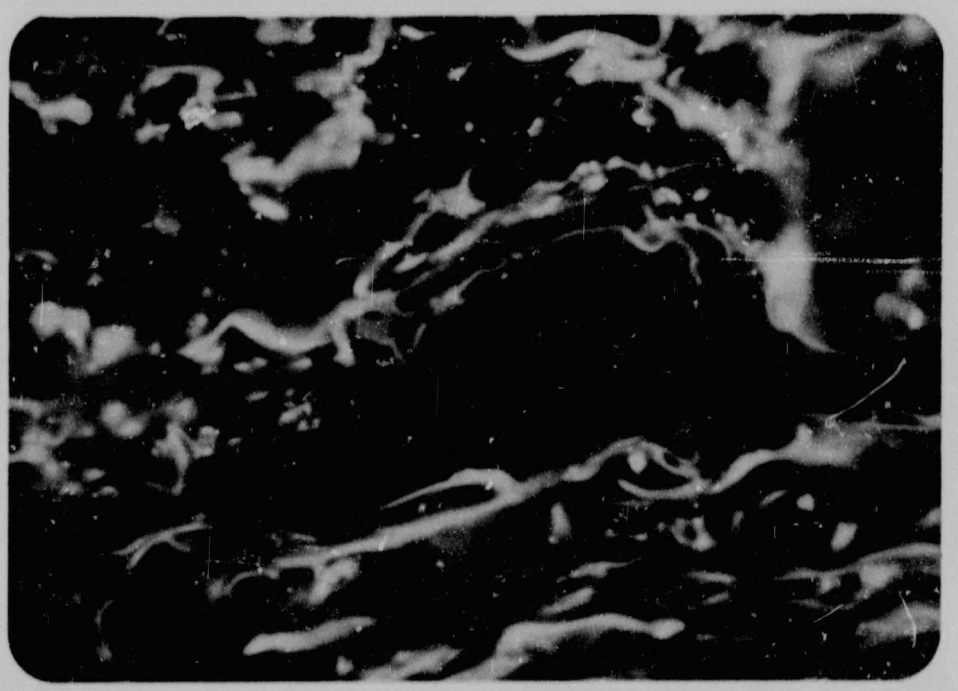

Figure 22 Adjacent section to figure 20, stained with anti-CLL cell reagent. No specific fluorescence is seen, x 700.

23 CONCLUSIONS.

The results of this work confirm the few reports that lymphocytes may be differentiated by fluorescent techniques using anti-immunocyte preparations as immunodiagnostic reagents. A useful anti-thymocyte serum was produced that fulfils all the criteria for specific identification of T cells in tissue sections or suspensions, although the intensity was too weak to detect individual cells. As the thymocyte preparations used in this work proved to be of exceptional purity, extended immunisation schedules, over two or three months, would enhance antibody titre dramatically without the danger of spurious antigens impairing specificity. This project will continue in order to produce sufficient volumes of reagent suitable for routine diagnostic use. The four sera described showed cytotoxic function and lymphocyte agglutination, in addition to weak immunofluorescence, so they would be useful for a variety of diagnostic, investigative or preparatory procedures. The obvious application that comes to mind is the separation of T cells from B cells in peripheral blood lymphocyte suspensions by sedimentation and separation of agglutinated T cells. Cytotoxicity, proved to be of a weak, antibody-dependent, complement-independent nature, without the potent lytic properties of complement-dependence. The reagent would have only limited application in cytotoxic studies.

The attempt at preparing anti-B cell reagent was not successful. The sera showed little evidence of specific cytotoxicity for B cells and the immunofluorescent reagents failed to differentiate B and T cells successfully. The T

cell staining was not removed by repeated thymocyte absorptions, being of low titre and probably non-specific. Dilution of the reagent beyond 1:20 also negated any B cell staining, this being localised at the centre of lymph node follicles - a similar distribution to the staining obtained with anti-human globulin reagent (Gajl-Peczalska et al. 1969). The most likely explanation lies in the finding during this work that lymphocytes from patients with chronic lymphatic leuaemia do not behave as peripheral blood B cells in conventional tests for marker systems. The low density of complement receptor sites on the surface of leukaemic cells is now the subject of a separate investigation by staff of the Department of Haematology. Greaves and Brown, (1973) report successful anti-B cell reagents prepared by the procedures described above, but do not comment on any anomalous observations on the leukaemic lymphocytes used as immunising antigen. The evidence would indicate that membrane bound immunoglobulin of injected lymphocytes in this study stimulated the formation of anti-immunoglobulin antibodies at the expense of normal surface antigens. Loss of such antibody after absorption with insolubilised serum proteins would support this hypothesis. Goldschneider and McGregor (1973) produced successful anti-rat B cell reagents from rabbits immunised with peripheral blood lymphocytes. They were made specific by extensive absorptions with T cells, bone marrow and macrophages. On the evidence from this work with human lymphocytes it would seem that such extensive absorptions would seriously impair specific antibody effectiveness. In the profusion of reports on the preparation of anti-immunocyte reagents, there have been many antisera produced that were specific for either human

or animal T cells. Anti-human B cell antisera have not featured significantly in the reports, with only one group (Williams et al., 1973) admitting to the difficulties that were encountered. Fortunately there are well documented marker systems available for B cell identification in tissues, without recourse to immunofluorescence. As this is not so for T cells, the production of an anti-T cell serum contributes a valuable addition to the armamentarium of the cellular immunologist.

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