otherwise undifferentiated cases of AML; this permits their separation from undifferentiated leukaemia (UL) which includes childhood lymphoblastic leukaemia. A positive acid phosphatase reaction in UL has been associated with the presence of T lymphocyte markers in the leukaemic cells (Catovsky *et al.*, 1974).

3. Products released by leukaemic cells

Consistently elevated serum and urine lysozyme (muramidase) concentrations are found in AML with predominantly monocytic differentiation (Perillie and Finch, 1973). This enzyme can also be demonstrated in single cells by a cytochemical test (Catovsky and Galton, 1973). Serum levels of vitamin B₁₂ binding protein (Transcobalamins I and III) seem to parallel granulocytic differentiation in the bone marrow.

4. Immunological markers

These have been dealt with in more detail by previous speakers. Their main application in AL is in the study of the morphologically and cytochemically undifferentiated cell types. The majority of childhood UL cases lack recognizable markers of B and T lymphoid cell differentiation ("null" blasts.) About 20% have been shown to have T-cell markers (Catovsky, *et al.*, 1974; Borella and Sen, 1974; Brown *et al.*, 1974), some of these cases present as a malignant lymphoma. B-cell markers may be found in cases of UL with morphological features resembling Burkitt's lymphoma cells and in adult cases of poorly differentiated lymphoma with blood and bone marrow involvement. Some of the findings in the latter cases show differences from the B-cell markers which are often found in chronic lymphocytic leukaemia.

5. Electron microscopy (E/M)

- (a) Transmission electron microscopy allows a more detailed study of the cell structure, degree of nuclear maturation, presence of cytoplasmic granules, etc. In AL it is of value when used in combination with
- (b) Cytochemical techniques at E/M level: Myeloperoxidase is a specific marker of the early "azurophilic" granules which appear during myeloid differen-

tiation. This enzyme may sometimes be demonstrated in a few cytoplasmic granules and/or in membranous structures of the cell in cases where the same reaction appears negative by light microscopy. Acid phosphatase with a special localization in the structure of the Golgi apparatus has been found in T-lymphoblastic leukaemia (Catovsky *et al.*, 1975).

- (c) Scanning electron microscopy (SEM): Differences in the surface structure of B and T lymphocytes were reported by Polliack *et al.* (1973). Few studies have been reported in AL. We have not observed differences in the surface appearances of T and "null" blast cells in cases of childhood UL (Catovsky *et al.*, 1975).

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EXPRESSION OF SURFACE ANTIGENS IN RELATION TO THE MITOTIC CELL CYCLE

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"... for many years we and others have compared the special biochemical properties of the cancer cell with what we called the corresponding normal cell. The great bulk of these studies utilized the corresponding normal *adult* cell, and we now begin to see how misleading this comparison can be. It is likely that the real comparison ought to be between the cancer cell and a *normal cell growing rapidly*" (Haddow, 1967).

This opinion is enforced by a failure to find *real* phenotypic differences between normal and transformed cells. Innumerable biological properties, originally considered peculiar to tumour cells, have subsequently been shown to be a feature of dividing cells. These include changes in electro-negative charge (Purdom, Ambrose and Klein, 1958; Ben-Or, Eisenberg and Doljanski, 1960), membrane permeability (Cunningham and

Pardee, 1969; Holley, 1972), lectin agglutinability (Burger and Goldberg, 1967; Ozanne and Sambrook, 1971), cell surface components (Kijimoto and Hakamori, 1971; Hynes and Bye, 1974) and more recently, the leukaemia-associated nuclear antigen (Klein *et al.*, 1974).

In this talk, the variable expression of surface antigens with cell growth were illustrated and evidence presented for the occurrence of "division membrane antigens" in man. These antigens can only be detected at the cell surface during cell division and may therefore contribute to antigenic differences between tumour cells (dividing) and normal adult cells (resting).

Division membrane antigens specific for human lymphoid cells were first recognized using heteroantisera raised in rabbits against human thymocytes or Burkitt lymphoma cells (Thomas and Phillips, 1973). After absorptions with normal adult tissues, sera were specific for thymocytes and T lymphoblasts or B lymphoblasts and Ig-positive lymphoid cell lines. Recently, it was shown that cold agglutinin, anti-i sera recognize a determinant unique to dividing human cells, which has been designated the i^1 antigen (Thomas, 1975). This antigen is present on the surface membrane of various cell types including lymphoblasts, fibroblasts, erythroblasts, and thymocytes and absent from normal adult tissues. Absorption studies have shown that the i^1 determinant is distinct from the i^1 antigens of erythrocytes.

To determine the temporal expression of division antigens during the life cycle, cultured lymphoblasts have been fractionated according to size, and therefore age, by velocity sedimentation in a zonal rotor. Rotor fractions were analyzed for the i^1 antigen or blast-specific antigen and cells were assigned to a position in the cell cycle according to size and ability to incorporate ^3H -thymidine into DNA. A majority of cell fractions from the rotor corresponding to the G_1 (or G_0) interval were negative for both specificities, whilst there was an enrichment of antigen-positive cells at the S and G_2 interval. This indicates that surface markers exist which recognize cells "in cycle".

The above antigens are present on tumour cells, embryonic cells, and normal dividing cells; oncofoetal antigens have been demonstrated on tumour and embryonic cells, but as yet no attempt has been made to establish whether they are re-expressed on normal

dividing cells. Are division antigens oncofoetal antigens? There still remains a need for "comparison . . . between the cancer cell and a normal cell growing equally rapidly".

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ROLE OF TARGET CELLS IN DETERMINING LEUKAEMIC CHARACTERISTICS

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The bone marrow cell population may be described as a "three tier" structure of interrelated cell populations: (a) the pluripotent stem cells, (b) the "committed" precursor cells, and (c) the maturing "end line" cells. The inter-linking is in effect provided by the two differentiation steps which create populations (b) and (c) respectively, the rate of differentiation, in part at least, being controlled by the appropriate population sizes.

Both the "committed" precursor and the maturing "end line" populations are transit types of cell populations in the sense that while they possess proliferative capacity, this is limited to a varying number (4–10) of cell cycles. This enables a highly elastic amplification—depending on demand—in the transit populations, but only up to the limit of their proliferation capacity.

Both these transit populations undergo "age changes" *i.e.* maturation during their amplification transit and their rate of maturation—which thus limits their proliferation capacity—can be altered by physiological controlling factors.