Until some direct evidence, either from *in vitro* experiments or from studies of mutant strains of *B. subtilis* carrying conditional lethal mutations in the structural gene specifying the 70,000-dalton polypeptide, is obtained the role of this polypeptide in the specificity of transcription during sporulation must remain uncertain.

LEUKAEMIC CELLS

Mutagenic Polymerase

from our Cell Physiology Correspondent

REPORTS about the physiology of various tumours and more particularly about the relationship between the host and the malignant cells are now legion, but although knowledge of the molecular biology of malignancy is fundamental to its control, information on this point is sadly lacking. It is true that many workers are investigating reverse transcriptase, but there are innumerable other facets of the molecular workings of these cells which also warrant study.

Springgate and Loeb have started on a new track, for they are now trying to determine whether DNA synthesis in malignant cells is less accurate than that of normal cells. The idea seems to be something of a shot in the dark, but in their recent report (*Proc. US Nat. Acad. Sci.*, **70**, 245 ; 1973) they show that the cells from four patients with acute lymphatic leukaemia have a DNA polymerase which is considerably less exact and makes a far greater number of mistakes when copying a template than does the DNA polymerase from normal human lymphocytes.

After removal of indigenous DNA the accuracy of synthesis was assessed by determining the proportion of radioactively labelled dGTP or dCTP incorporated during replication of a synthetic poly(dA-dT) · poly(dA-dT) template. The enzymes from leukaemic cells apparently lack the fidelity of those from normal lymphocytes, for the proportion of cytidine residues in DNA synthesized by polymerases from cells of patients with acute lymphatic leukaemia was some ten times higher than that made by DNA polymerases from normal lymphocytic polymerases the mic polymerases the level of errors lay between 1 in 250 and 1 in 850, with normal lymphocyte polymerases the error frequency was of the order of 1 in 2,000 to 1 in 30,000.

Control experiments indicate that the dCTP really is incorporated into the polynucleotide chain and is not merely attached to it non-specifically. In identical conditions the *Escherichia coli* enzyme was found to have an error frequency somewhat lower than 1 in 100,000 and that of T4 phage was in the region of 1 in 10,000 ; these values agree very closely with those found by other authors. Other simple experiments also suggest that the observation is not an artefact, for the addition of cold, unlabelled dCTP to the nucleotide pool reduced the proportion of label incorporated into the freshly synthesized polymer and the requirements of the assay system for Mg^{2+}, template, nucleotides and enzyme are exactly the same for incorporation of both correct or incorrect bases. If the correct bases, dATP and dTTP, are labelled with ^{32}P and the incorrect base, dCTP, with ^{3}H, then the ratio between ^{3}H and ^{32}P remains the same, irrespective of both the amount of enzyme protein added to the reaction mixture and also to the time over which the reaction is measured.

Springgate and Loeb believe these experiments indicate that the incorrect dCTP is physically incorporated into the replicating polynucleotide ; much stronger support for this contention comes from two further experiments. In the first experiment, caesium chloride density gradients showed the product of the polymerase reactions to band in the position expected for a poly(dA-dT)· poly(dA-dT) and, as would also be expected if the dCTP were an integral part of the molecule, ^{3}H-dCTP was found in exactly the same band. Again, if the freshly synthesized polynucleotide was labelled with ^{32}P-dTTP and ^{3}H-dCTP and then sequentially hydrolysed with snake venom phosphodiesterase the release of the two bases closely paralleled one another. These results indicate that the cytosine residues are incorporated into the DNA and that they are evenly distributed.

Apparently it was not possible to assess the extent of inaccuracy using a poly(dG) poly(dC) template because the level of infidelity was too low to allow for accurate measurement. Results with the poly(dA-dT) · poly(dA-dT) template strongly suggest, however, that these acute lymphatic leukaemic cells

Adherent Cells and B Cell Immunity or Tolerance

FOLLOWING the realization that many immune responses cannot be thought of in terms of the interaction of antigen with a single species of lymphocyte, cellular immunology has entered a more realistic but more complicated phase of analysis. Broadly there are three kinds of cell known to be involved—T lymphocytes from the thymus, B lymphocytes from the bone marrow (or bursal equivalent) and adherent cells which also derive from the bone marrow but which are probably macrophage-like cells rather than lymphocytes.

All three sorts of cell are thought to have specific and non-specific parts to play in an immune response. The favourite notion at present is that specifically-activated T cells synthesize both non-specific and specific factors as a result of contact with specific antigen. The specific factor, which is thought to be an immunoglobulin referred to as IgT, becomes bound to the surface of a macrophage where in turn it binds antigen. The antigen-IgT complex can stimulate B cells to produce the specific antibody. It is possible that the macrophage stimulated by antigen-antibody complexes and the activated B cell in their turn secrete non-specific and perhaps specific (in the case of B cells) regulators of the cellular consortium. In next Wednesday's *Nature New Biology* (March 21), Feldmann illustrates how the presence of an adherent cell can determine whether B cells are induced to synthesize a specific antibody by activated T cells or become specifically tolerant.

All Feldmann's results relate to experiments performed *in vitro* and carry the limitation that the antibodies produced are IgM only. The crucial part of the study is that activated thymocytes in contact with DNP (dinitrophenyl) conjugated to the activating antigen (fowl gamma globulin) liberate into the supernatant a factor which can induce spleen cells previously primed *in vivo* to DNP flagellin and in the presence of DNP-POL (POL is polymerized flagellin) to produce anti-DNP antibody as measured by the Jerne technique. If adherent cells are removed from the spleen cell population before incubation with the supernatant, then little anti-DNP antibody but anti-POL antibody is produced. Feldmann's interpretation of this experiment is that IgT-DNP complexes can specifically immunize primed B cells when presented to the B cells by an adherent cell population, but can also specifically tolerize if they come in direct contact with the B cells.

Feldmann feels that these experiments have an *in vivo* parallel in those situations in which large numbers of activated T cells can suppress immunity. He distinguishes three phases in an immune response ; first, an exponential phase in which the amount of IgT is less than the number of receptors available on macrophages and therefore in the absence of unbound IgT-antigen complexes immunity will develop ; second, a plateau phase during which complexes and receptors are present in roughly equal numbers and in the absence of free complexes only immunity can result ; and third, a tolerance phase in which free IgT complexes are present and these effectively tolerize B cells.