other residual DNA polymerase activity, DNA polymerase II, which can be isolated free of membrane and template. The DNA-membrane complex obtained from these cells continues for short periods to synthesize DNA *in vitro* (whether this synthesis is brought about by DNA polymerase II is an open question). The DNA made *in vitro* sediments with the bulk template parental DNA until the protein component of the complex is removed. After the removal of the protein the DNA made *in vitro* sediments as fragments ranging in sedimentation coefficient from 10S to 25S. At least some of these fragments, the 10S fraction in particular, seem to contain molecules in which parental and DNA sequences made *in vitro* are covalently linked while the remainder of the fragments seem to comprise parental DNA hydrogen bonded to DNA made *in vitro*. Moreover, kinetic studies indicate that the covalent linkage of parental and *in vitro* DNA chains is transient.

Strätling and Knippers believe that these observations indicate that parental DNA is nicked by endonucleases to produce free 3′ hydroxy termini which not only allow free rotation of the DNA strand but also serve as a primer for DNA synthesis. They further suggest that the parental/progeny molecules so produced are cut by endonucleases to separate parental from newly made DNA and then the two sorts of DNA fragments must be relinked to form continuous parental and progeny strands.

Strätling and Knipper's data, as they comment, are consistent with Haskell and Davern's model which envisages that DNA replication involves the introduction of nicks ahead of the replication fork, with the nicked pieces serving as primers for DNA synthesis. The recent experiments of Werner (*Nature*, **230**, 570; 1971) also indicate that progeny and parental DNA chains are transiently linked by covalent bonds, although Werner interprets his findings differently. In short, it seems that during *E. coli* DNA synthesis parental and progeny sequences are linked but how and why remains to be unequivocally explained.

HAEMOGLOBIN

Models for All Tastes

from our Molecular Biology Correspondent

As to the choice of a kinetic scheme for haemoglobin oxygenation, most workers in the field will still prefer, like H. L. Mencken's politician, to sit on the fence while keeping both ears to the ground. Although the structural differences between the oxygenated and deoxygenated states have been defined in minute detail by Perutz, who was able to infer from them many features of the mechanism of oxygenation, this has been insufficient to quell the wrangle about which of the several feasible mechanistic models best fits the equilibrium and kinetic data.

MacQuarrie and Gibson (*J. Biol. Chem.*, **246**, 5832; 1971) have gathered some interesting evidence from a comparison of carbon monoxide binding rates and release of the cofactor, 2,3-diphosphoglycerate, which binds in a ratio of one molecule per haemoglobin tetramer to the deoxygenated form only. More exactly, they have used a fluorescent analogue of diphosphoglycerate, a hydroxypyrenetrisulphonate, which competes with the cofactor for the single site, and has a qualitatively identical effect on the oxygen uptake curve. The binding of this form is easily measured, for when it is bound its fluorescence is quenched by the haem groups.

When carbon monoxide is introduced into a solution of haemoglobin containing some of the cofactor, the kinetics of binding of ligand and release of cofactor can be simultaneously measured by fast spectroscopy. It is at once apparent that the two processes follow different rates. This leads immediately to the conclusion that intermediate oxygenation states occur in significant concentration. MacQuarrie and Gibson then go on to interpret their results in terms of alternative ligand binding mechanisms, assuming only that there are not more than two affinity states in respect of the cofactor. They fit the rate data first in terms of an Adair model, with four sequential binding reactions, in which the liganded conformational state reacts rapidly with carbon monoxide and does not bind the cofactor. The cofactor, in this scheme, is released after the attachment of the third haem ligand. A least-squares fit of both the carbon monoxide and cofactor binding curves shows that this formation is capable of satisfying the observations within the error of the experiment. If, on the contrary, one assumes that the cofactor is released after binding the second ligand, the fit is much worse (though one

The Lymphocyte Trap Sprung

THE jargon of the cellular immunologist contains words such as population, microenvironment, migratory, which would be perfectly proper on the lips of an ecologist. This perhaps provides a clue to some of the present trends in cellular immunology because to an ever increasing extent lymphocytes are being studied in relation to their environment. It is quite feasible to think of lymphocytes as organisms of which the behavioural, morphological and functional characteristics can be arranged in relation to their environment in the intact vertebrate. Such a concept stresses that the lymphocyte in its most interesting form is abundantly alive. The old days of the strictly morphological approach based exclusively on the perusal of long dead slices of tissue are happily past.

In *Nature New Biology* next Wednesday (December 22), Zatz and Lance present a communication entitled "Lymphocyte Trapping in Tolerant Mice". This study, with its undertones of polecats and the frozen North, concerns the phenomenon of retention of migratory lymphocytes in lymphoid organs which are involved in responding to an antigenic stimulus. The method is simple. Lymphocytes are labelled with ^{51}Cr *in vitro* by incubation in an appropriate solution of "hot" sodium chromate. Subsequently, the labelled cells are injected intravenously into mice at various times after antigenic stimuli. It is a reasonable approximation to say that for a day or so the amount of radioactivity found in various lymphoid organs of the body will be an accurate indicator of the presence of the labelled cells. The lymphoid organs are always harvested 24 h after the injection of labelled cells.

Lance and his associates have previously demonstrated that in lymph nodes which drain the site of application of a foreign skin homograft more of the injected cells were found than in comparable nodes which drain the site of a skin autograft. The maximum differential between the two kinds of node was observed at about the tenth day after application of the skin homograft, before the graft itself is rejected. The functional significance of the phenomenon cannot at the moment be specified but in their most recent experiments Zatz and Lance find that in parental mice rendered tolerant by neonatal injection of F_1 spleen cells trapping does not operate in the lymph nodes draining the site of an applied (and tolerated) skin homograft.

In other studies it has been shown that some degree of engorgement of lymph nodes can follow the introduction into afferent lymphatics of some of the products of stimulated lymphocytes (Kelly, R. H., *Clin. Exp. Immunol.*, in the press, 1972). It is tempting to suppose that some such mechanism is operating (or failing) in the lymph nodes of the mice of Zatz and Lance. It will be interesting to learn when and with what intensity the trap operates in animals which have been injected with either immune or normal cells in order to break the tolerant state.