latent period of the effect suggests that the path is unlikely to be important in that aspect of cerebellar function which currently attracts most attention—the control of evolving movements. A role as a long-term regulator of cerebellar cortical responsiveness seems more plausible.

Interest in the result is enhanced when it is viewed against a more general background. Histochemical studies by Olson and Fuxe (*Brain Res.*, **28**, 165; 1971) have shown that the axons of LC neurones terminate at destinations as diverse as the cerebral cortex, the hypothalamus and the lateral and medial geniculate bodies, not to mention the pathway terminating in the cerebellar cortex. The pathway to the cerebral cortex has been investigated in a general way by observing the behavioural changes caused by pharmacological agents believed to stimulate or to depress it. These experiments suggest that high LC activity leads to behavioural arousal and an alerting of the cerebral cortex and this activity is signalled by desynchronization of the electroencephalograph. Reduced activity in the nucleus, or outright destruction of it, leads to the synchronous electroencephalograph which is characteristic of light sleep or waking repose. The new results apparently show that the LC axons to the cerebellum are inhibitory and the odds are therefore high that the other LC projections are also inhibitory, in which case the path to the cerebral cortex clearly does not cause arousal directly by exciting cortical neurones. It may prove necessary to regard the projection as functioning by turning down some cerebral cortical element the unchecked activity of which leads to electroencephalographic synchronization and behavioural repose.

HAEMOGLOBIN

Hammering the Sickie

from our Molecular Biology Correspondent

THIS week, dear reader, a tale with a moral, about molecular remedies for molecular disease. Sickie-cell haemoglobin is in all respects a normal functional molecule, except for the property, which the single substitution in the β -chain confers on it, of associating in the deoxygenated state to large tactoidal aggregates. When this happens in the cell, the consequences are exceedingly disagreeable. A simple problem, one

might suppose, and an open challenge to protein chemists, to prove that theirs is a useful art, such as the hard-faced men by the National Institutes of Health should appreciate in tangible form. Better not to dwell on the more risible details, but to say only that until now they have failed prodigiously.

A new approach by a group of clinicians to the treatment of the condition was recently given wide publicity: having apparently encountered allusions to hydrophobic interactions between proteins, and the effect of urea on such forces, they proceeded to inject their patients with urea solutions. Protein chemists, knowing about the urea concentrations that might be effective in such situations, and guessing at the difficulties which might be involved in filling a patient with 8M urea, might have been permitted a pitying sneer. Astonishingly, however, this primitive ruse seemed to work. It is now apparent that this was in fact a consequence of the gradual formation in urea solutions—as every protein chemist also knows—of cyanate ions, which react with protein amino-groups.

The urea (which would not work anyway if too freshly made up) should therefore clearly be replaced by suitable doses of sodium cyanate. Kraus and Kraus (*Biochem. Biophys. Res. Commun.*, **44**, 1381; 1971) have now improved on this strategy by introducing carbamyl phosphate, a physiological substance, which is evidently capable of entering the red cell, with beneficial effects on the sickling tendency. They suggest that because phosphates in general compete with the natural cofactor, 2,3-diphosphoglycerate, for its site on the haemoglobin molecule, the carbamyl phosphate functions as an affinity reagent, entering the putative diphosphoglycerate site at the N-termini of the β -chain, and carbamylating the α -amino-groups (which indeed would be the most reactive at physiological pH). Indeed Caldwell, Nagel and Jaffé (*ibid.*, 1504) have shown that not only cyanate ions, but also carbon dioxide, which is known to bind non-covalently at the same site, will chase off diphosphoglycerate from normal human haemoglobin. One may also reflect that the loss by carbamylated haemoglobin of the capacity to bind the cofactor will lead to a relative increase in oxygen affinity (because the diphosphoglycerate binds only to the deoxygenated form). Thus the red cells will be less readily deoxygenated, and if they contain sickie-cell haemoglobin, the sickling will be inhibited. It is interesting to note that this provides a new retrospective rationale for the attempts once made to mitigate sickie-cell anaemia by administering carbonic anhydrase inhibitors. In addition to the decrease in pH that the accretion of

carbon dioxide will engender, thereby bringing into play the Bohr effect to increase the oxygen affinity of the haemoglobin, inhibition by carbon dioxide of diphosphoglycerate binding would work in the same sense.

The ramifications of diphosphoglycerate binding are evidently endless. Lian, Roth and Harkness (*ibid.*, 151) now report that in intact red cells, the shift in the oxygen uptake curve on addition of the cofactor is substantially increased when the cells contain sickie-cell haemoglobin. It has long been known that the oxygen affinity of such cells is lower than those containing only normal haemoglobin, and Lian *et al.* also now find that this difference is still present, though much diminished, when the cofactor is absent. These findings must be set against the well-established identity of both the oxygen and diphosphoglycerate affinity of normal and sickie-cell haemoglobins in dilute solution, out of the cell. The situation is complex, and Lian *et al.* indulge in some contorted efforts at explanation. The formation, however, of aggregates in sickie-cell haemoglobin only at high concentrations must stabilize the deoxygenated state, and it is therefore reasonable to suppose that this will decrease the oxygen affinity, and increase the diphosphoglycerate binding in the cell which in turn will enhance the change in oxygen affinity.

The aggregation reaction in sickie cells is complicated by possibilities of participation of other forms of haemoglobin that are commonly present, such as normal adult and foetal haemoglobin. The true complexity of the situation has now been revealed by Bookchin and Nagel (*J. Mol. Biol.*, **60**, 263; 1971), who use the device of introducing cyanmethaemoglobins, which have the oxyhaemoglobin conformation, but do not bind oxygen, into mixtures. Sickie-cell haemoglobin forms gels only in the deoxygenated state, but the normal adult pigment will co-aggregate with it both in the deoxy- and the cyanmet- (that is, oxy-) conformations. Foetal haemoglobin by contrast will enter the aggregates in the cyanmet- but not in the deoxy- state. In another abnormal variant (Harlem C), capable of aggregating when deoxygenated, the interactions with other forms and other conformations are quite different. The results are by no means easy to explain in detail, but the authors are able, starting from the notion, originally advanced by Pauling, that the aggregates are helical structures, to make some inferences about the existence of separate secondary interaction sites across successive turns of a tight helix of low pitch; these are envisaged as distinct from the primary association in which the sickie-cell haemoglobin molecule is regarded as bivalent.