scale) the amount of rapid haemoglobin produced varies with the extent of saturation with carbon monoxide. At low carbon monoxide saturation no rapid haemoglobin is formed. This is difficult to account for on any scheme governed by the reaction of dimers. Gibson and Parkhurst suggest instead that the change from the low to high reactivity conformation occurs after three ligands have combined with the haemoglobin tetramer—a scheme which can be fitted (with a sprinkling of adjustable parameters) to the observed data.

In support of this, Gibson and Parkhurst refer to results of flash photolysis experiments on hybrids, in which the two α-chains bear nitric oxide ligands and the β-chains are in the deoxy-state. The nitric oxide is not subject to photolysis, and it is therefore possible to follow the carbon monoxide equilibrium of the β-chains alone. It is argued that if the functional unit is the dimer, only one carbon monoxide molecule is bound, and there must be only a single rate process. In practice it turns out that the rate of recombination with carbon monoxide after flash photolysis is again a function of the extent of dissociation. Because aggregation–disaggregation of the protein is presumed to be slow on this time scale, it is deduced that tetramers rather than dimers must be the operational species. Just how these results relate to the dimer mechanism which has been demonstrated under different circumstances is not clear. Carboxy and nitric oxide-haemoglobins, of course, are not oxyhaemoglobin, and one might well wish to reserve one's judgment on the bearing of these interesting results on oxygen binding under physiological conditions.

Another interesting finding on the conformational behaviour of haemoglobin during oxygenation comes from the electron spin label experiments of McConnell, Ogawa and Horwitz (*Nature*, **220**, 787; 1968). The label is attached to cys-93 of the β-chains, and the electron paramagnetic resonance spectrum responds to changes in the magnetic environment of the label. There is a large difference between the EPR spectra of deoxy and oxyhaemoglobins, and it has now been found that the spectra over a range of oxygen saturation do not show an isosbestic point. There are thus more than two components in the system, and it is inferred that states of intermediate oxygenation possess minor conformational differences in the region of the spin label, compared with pure oxy and deoxyhaemoglobins. It follows that the local conformation near β-93 is to some extent influenced by the state of oxygenation of the α-chains. This was borne out by oxygenation of species in which either the α or the β-haem iron atoms were in the ferric and therefore non-functional state: oxygenation of the β-chains (α-haems ferric) led to the usual large change in the spectrum of the label, whereas oxygenation of the α-haems, with the β-haems ferric, led to a small, but also definite, change. The proximity of the label to a critical α–β-chain contact is consistent with the observations of Perutz and his group of changes in this region on oxygenation.

A further contribution by Enoki *et al.* (*J. Mol. Biol.*, **37**, 345; 1968) on the isolation and properties of γ-chains (the foetal counterpart of the β-chains) shows that this species, like the adult β-chains, forms tetramers, and reaffirms that such homotetramers show no haem–haem interactions, and no Bohr effect.

MEDICINE

Daily Enzyme Variations

from our Medical Biochemistry Correspondent

THE traditional "three times a day after meals" dosage schedule is more an aid to the patient's memory than a rational system of drug therapy. Recent work with mice has shown that the response to some drugs including ouabain, pentobarbital, lidocaine and chlordiazepoxide may depend on the time of day the drug was given.

Radzialowski and Bousquet (*J. Pharmacol. Exp. Therapeut.*, **163**, 229; 1968) have now shown that there are very large daily variations in the activities of enzymes metabolizing drugs in the livers of rats and mice. They measured the demethylation of the drugs aminopyrine and *p*-nitroanisole, the oxidation of hexobarbital and reduction of 4-dimethyl aminoazobenzene (4-DAB) in the microsomes and 9,000 *g* supernatant from homogenates of rat and mouse liver. Normal rats showed a diurnal rhythm in all the enzyme activities with maximum activity at 0200 hours and a minimum at 1400 hours: the maxima and minima were exactly 12 hours different from the maximum and minimum values for plasma corticosterone in the rats. Removal of the adrenals reduced the enzyme activities for the first three drugs to below the minimum found in the normal animals and the activity remained constant during the day, but adrenalectomy did not affect the diurnal rhythm in the metabolism of 4-DAB. The daily rhythm could also be abolished by maintaining the plasma corticosterone levels, but the rhythm was not affected by fasting. The variation in enzyme activity is probably due to synthesis of new enzyme, for the increase in activity was found chiefly in the microsomal fraction which is synthesizing new protein.

Because the maximum activity in normal rats was often almost double the minimum activity, the rate of metabolism of some drugs, and therefore their effectiveness, must differ considerably at different times of day. Does the same thing happen in man? Indirect evidence from studies of amino-acid variations suggests that it does. Earlier this year, Wurtman *et al.* (*New Engl. J. Med.*, **279**, 171; 1968) showed that the plasma concentrations of tyrosine, tryptophane and phenylalanine in normal humans varied during the day with a minimum value at 0200 and maximum in the morning, with possibly a secondary peak in concentration in the evening. The samples showing the highest and lowest concentrations of tyrosine were analysed for 16 other amino-acids, and all the amino-acids showed some diurnal variation in plasma concentration. The percentage variation was very large for amino-acids whose concentration was low, and less spectacular for those normally present in large quantities. The diurnal rhythm was not affected by large differences in the quantity of protein ingested, though there was some evidence that more frequent ingestion of protein reduced the amplitude of the variation. The authors suggested that the variation was therefore due to differences in the rate of utilization of the amino-acids, similar to the well established variations in tyrosine and tryptophane-metabolizing enzymes, which are known to be affected by the adrenocortical hormones. Thus in man, enzyme synthesis probably shows a diurnal variation, and the effectiveness of a drug may depend on the time of administration.