mid research. Perhaps the discovery of new proteins could be confined to rigorously controlled (P4) conditions, or a two-year moratorium on gels could be held. At least that would let us catch up with the literature. □

Metabolic studies using ^{31}P NMR

from G. E. Chapman

THE state of proton NMR spectroscopy of biological macromolecules is somewhat akin to that of X-ray crystallography before methods of solving the phase problem had been devised. There is an embarrassment of detail, with as yet no reliable methods of interpreting it in terms of molecular conformation, though it is relatively simple to work backwards from a known structure to the observed spectrum. It is not surprising therefore that much effort is now being directed towards the NMR study of those heavier nuclei where there is less detail in the spectra, and interpretation is easier.

The increased use of ^{31}P NMR in biological research in the past year or two owes much to magnet development. In the continuing search for greater sensitivity, the NMR spectrometer designer has usually opted for higher magnetic field strengths, stretching superconducting magnet technology to the limit. However, there are indications that increasing magnetic field strengths are not achieving increases in sensitivity for the heavier nuclei when they are incorporated in macromolecules. Hence one alternative development has been that of magnets capable of maintaining very homogenous fields over large sample volumes. This is particularly advantageous where the sensitivity is limited by the concentration of the sample rather than by the amount of material available. The following recent work has been made possible by these developments.

The monitoring of phosphate metabolite levels in living cells and tissues by NMR, being non-destructive, is aesthetically more pleasing than the conventional freeze-clamp techniques that have been used in the past by biochemists. However, it could be argued that both methods give the same results, and that a great many freeze-clamp experiments can be done for the price of buying and maintaining a modern advanced NMR spectrometer. This question probably cannot be resolved at present. A hybrid of the two methods has recently been illustrated, where the phosphate metabolites from the extract of a quick-frozen heart were analysed by ^{31}P NMR (Garlick *et al. Biochem. biophys. Res. Commun.* **74,** 1256; 1977).

One type of measurement by ^{31}P NMR in living cells however is giving results which have not been obtained reliably and accurately by other methods. This is the measurement of intracellular *p*H. It is achieved by observation of the inorganic phosphate chemical shift, which changes with *p*H in the region of neutrality due to its second acidic dissociation, and is capable of considerable accuracy (better than ±0.02 *p*H units near *p*H 7). The intracellular *p*H of living *Escherichia coli* suspensions has been measured in a variety of metabolic conditions (Navon *et al. Proc. natn. Acad. Sci. U.S.A.* **74,** 888; 1977). It was shown that the intracellular *p*H was consistently higher than that of the external medium during respiration, but if respiration ceased, then the *p*H values rapidly equalised. This supports the hypothesis that an outward directed proton pump mechanism operates during respiration. Experiments on chromaffin granules (the catecholamine-storing vesicles of the adrenal medulla) by ^{31}P NMR also used chemical shifts to detect *p*H changes within the vesicles (Casey *et al. Biochemistry* **16,** 972; 1977) though in this case the resonance monitored was the γ phosphate of ATP rather than inorganic phosphate.

Although *in vivo* experiments are inherently more spectacular than *in vitro* ones, from the view point of the biologist, the latter type of study can be better controlled. Investigations of the important metabolic regulatory enzyme system glycogen phosphorylase, is a classic example of the importance of *in vitro* studies of metabolic processes. There are at least five phosphate compounds associated with the enzyme as substrate, cofactors and allosteric effectors. It has been investigated by ^{31}P NMR in the past, but the high molecular weight has meant the use of very high protein concentrations to achieve adequate molar concentrations of the bound phosphates, which in turn has given rise to considerable viscous broadening of the resonances. This has recently been overcome by the use of large sample volumes, and in the latest paper (Feldmann & Hull *Proc. natn. Acad. Sci. U.S.A.* **74,** 856; 1977) the role of pyridoxal phosphate in the enzyme complex has been investigated. In such an NMR experiment, one would expect some of the resonances to overlap, and the authors overcome this by the ingenious device of using thiophosphate analogues, where these were known to be active in the enzyme system. Thiophosphates have chemical shifts about 40 p.p.m. downfield from those of the corresponding phosphate compounds. Hence the resonances of all the phosphates could be resolved. In this way, much was learnt about the interaction of the molecular species involved.

The above exemplifies, I believe, how good communication and teamwork between the biochemists and physical technique specialists can advance the application of a technique to biological problems. Here the interdisciplinary approach is paying handsome dividends. □

G. E. Chapman is a member of staff at the Biophysics Laboratories, Portsmouth Polytechnic

The nucleotide-binding fold revisited

from C. C. F. Blake

SOME time ago in these columns (*Nature* **250,** 284; 1974) I discussed the significance of a particular protein fold that had been noticed by Rossman to constitute part or all of the structures of four NAD-dependent dehydrogenases and a number of other enzymes, some but not all of which also bind nucleotides. In lactate-, alcohol-, and glyceraldehyde-3-phosphate dehydrogenase, the fold corresponds to a separately organised NAD-binding domain, that in each molecule is linked to a second domain whose tertiary structure is specific to the particular enzyme. On the basis of this structural pattern Rossman and his colleagues (*Nature* **250,** 194; 1974) proposed that the NAD-binding domains had evolved by divergence from a common ancestor, and had been incorporated into the dehydrogenases by gene fusion with another protein fragment, different in each enzyme, that became responsible for that enzyme's particular catalytic and substrate binding properties. Potentially this proposal provides a profound insight into the evolution of those groups of enzymes that catalyse the same type of reaction, often using common cofactors, but working on quite different substrates. The key to the proposal is the assumption that an extensive structural similarity, unsupported by sequence homology, represents the relic of a very distant gene duplication. However, so little is known about the factors controlling protein

C. C. F. Blake is a lecturer in Molecular Biophysics in the University of Oxford.