Lithium therapy, combined with regular assays of serum lithium, is now restored to clinical practice.

Dr Marks claimed that, contrary to various reports, there is no reliable assay for consumed LSD or cannabis in blood, urine or breath. Detecting morphine and amphetamine is simpler: thin-layer chromatography of urine extracts often suffices. But Dr Marks warned that even here results can be confusing, chiefly because of the tendency of hard drug users to dose themselves with anything they can lay their hands on. He displayed some of the resulting chromatograms, with spots in kaleidoscopic profusion.

Dr J. R. Hobbs of the Royal Postgraduate Medical School gave a fascinating account of the diagnostic use of serum proteins. Many diseases relate to characteristic patterns of immunoglobulin deficiency, and there is evidence that an increase in α-foetoprotein heralds the onset of primary carcinoma of the liver, at any rate in Bantu.

The new MRC recommended assays for steroids were discussed by Professor V. H. T. James of St Mary's Hospital Medical School. He went on to describe some sophisticated assays, not as yet taken up by the MRC, which depend on the displacement of label, by the test sample, from steroid binding proteins loaded with labelled steroid. Professor James ended by describing the growing evidence for a link between some parameters of steroid output and responsiveness to surgery for breast cancer.

Finally, Dr A. C. Pollard of the Charing Cross Hospital Medical School gave some dire warnings for those clinical biochemists contemplating the purchase of computers for their laboratories. Such luxuries are very low on the Ministry of Health's list of priorities, perhaps rightly, and there is a great shortage of the programming talent required to make full use of complex installations. Technicians who at present spend half their day on some routine assay, and half the day working out results, may not feel a computer is a boon if its effect is simply to make them spend all of each day on the routine assay. Dr Pollard advised his audience to make do with a small self-programmed computer unless their needs were very special.

MOLECULAR BIOLOGY

Active and Inactive Sites

from our Molecular Biology Correspondent

RECENT high resolution crystallographic studies of several enzymes have awakened in enzymologists the hope of divining the mechanism of action by a sufficiently intense scrutiny of the geometry of the active site. Many hairs have been enthusiastically split, and some concrete ideas appear also to have taken shape. Moreover, experiments are now being designed to test some of these, along the lines, for example, of introducing small selected changes in the local geometry.

A good example is the approach used by Bender and by Koshland of converting the vital serine hydroxyl group in serine proteases into a thiol. Some studies of subtilisin, modified in this manner, are reported by Neet, Nanci and Koshland (*J. Biol. Chem.*, **243**, 6392; 1968). The thiol-subtilisin appears from its physical properties to retain essentially unchanged the gross conformation of the native enzyme. Towards proteins, and esters, such as the common substrate, *N*-acetyl-tyrosine ethyl ester, it is inactive. Binding measurements, however, revealed that the capacity of the protein to bind the ester was almost unimpaired. The inactivation was clearly therefore associated with the failure of a catalytic step. Now there is good evidence that the action of the serine proteases involves acylation of the serine by the substrate, the ester bond of the latter being hydrolysed. The acylated enzyme, which can be isolated as an intermediate, is then deacylated, to yield the carboxylic acid product. Evidently it is the acylation step which fails in the thiol enzyme, for there is no build-up of acylated protein. Neet *et al.* therefore tried as substrate the much more reactive ester, *p*-nitrophenyl acetate, which is known to be a very powerful acylating agent. Towards this substrate the enzyme showed appreciable activity. Moreover, the deacylation step, though much slower than in the native enzyme, proceeded satisfactorily, showing again that it is the acylation reaction that is inhibited by thiolation.

In mechanistic terms, it is clear from reactions of simple compounds that thiols are in general better nucleophiles than alcohols; moreover, the steric effect of substituting sulphur for oxygen is very small, amounting only to a difference of 0·4 Å between the covalent radii. It is extraordinary therefore that the effect on the activity is so drastic. Neet *et al.* conclude that the minute change in geometry within the active site is alone sufficient to destroy the activity, and they offer this argument as a riposte to criticisms of Koshland's induced-fit hypothesis, arising from the vanishingly small steric changes induced (as shown crystallographically) in ribonuclease and lysozyme, for example, by the substrate. If a shift of 0·4 Å measures the hairsbreadth between the active and inactive states, they argue, then a change of similar order on entry of the substrate will be enough to produce the same effect in the native enzyme.

The active site of two subtilisin-type enzymes has been further defined by Markland, Shaw and Smith (*Proc. US Nat. Acad. Sci.*, **61**, 1440; 1968). It was surmised from activity data that an unionized histidine residue forms part of the active site. The alkylating agent and substrate analogue, carbobenzoxyphenylalanine bromoethyl ketone, inactivates the enzyme specifically. The reaction was carried out with labelled reagent, and after enzymatic and chemical fragmentation, the label was located in his-64, which is thus part of the active site. Neet *et al.* note that the active serine is residue 221, which is near the other end of the chain, and point out the similarity to chymotrypsin and trypsin, where the corresponding residues are his-57, ser-195, and his-46, ser-184, respectively. A structural and evolutionary relation between all these proteases may be cautiously inferred.

Another example of modified specificity within this family comes from Coletti-Previero *et al.* (*J. Mol. Biol.*, **39**, 493; 1969), who find that when one or more tryptophan residues are in a modified (formylated) state in trypsin, the proteolytic activity is annihilated, but the esterase activity survives almost undiminished. Moreover, the addition of non-aqueous solvents to native trypsin has precisely the same effect. Only one active site is present in the enzyme, and so it is reasonable to suppose that the differential inactivation is a consequence of the effective diminution of its hydrophobic character relative to the solution.

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