after transport periods of up to eight hours. This was clearly not the result of a failure of tritium to enter the sieve tubes; exudate from stylets situated at the point of application of the tracer showed that large amounts of tritium had moved laterally into the phloem tissues. Any longitudinal movement of tritium that could be detected was shown to be the result of diffusion.

If a mass flow mechanism of the type envisaged by Münch were operating in the phloem, then water should be at least as mobile as the solutes carried. Peel and his colleagues think it unlikely that lateral exchange of radioactivity between the tritiated water and water in the surrounding cells could account for the laggardly movement of water in the sieve tubes. The overall conclusion must be that sugars and phosphates move in the phloem by a mechanism other than mass flow, which agrees with the earlier suggestions of Biddulph and Cory (*Plant Physiol.*, **32**, 608; 1957) and Choi and Aronoff (*Plant Physiol.*, **41**, 1119; 1966). Furthermore, it seems that all the water in the exudate from stylets must come through the walls of the tapped sieve element, suggesting that sieve elements are much more osmotically permeable than had been thought.

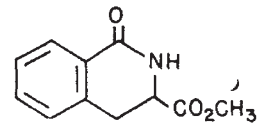
ENZYMES

Rigid Substrates map Active Site

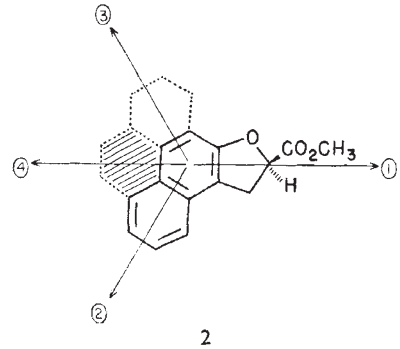
THERE is still much to know about the active site of chymotrypsin in spite of the 2 Å resolution X-ray structure published two years ago. In the latest issue of the *Journal of Biological Chemistry* (**244**, 4164; 1969), Y. Hayashi and W. B. Lawson report mapping of the aromatic binding site in relation to the active site. *In vivo*, chymotrypsin is a protease which cleaves the peptide bond between the C-terminal of an aromatic amino-acid and the N-terminal of the adjacent amino-acid. Chymotrypsin also hydrolyses ester links at the C-terminal of aromatic amino-acids, and much work has been done on this reaction as well. Hayashi and Lawson have prepared several rigid ester substrates and have measured their rates of hydrolysis. The rates are very dissimilar and Hayashi and Lawson suggest that the shape which is hydrolysed most easily should be a rough molecular plaster-cast of the active site.

Hayashi and Lawson's rigid substrates have two special features: the asymmetric α-carbon atom is held in a ring and there is a naphthalene nucleus. A rigid ring substrate of this type is already known for chymotrypsin; it is methyl dihydroisocarbostyril-3-carboxylate (1). This is composed of C-methyl D-phenylalaninate with the α-amino group condensed in an amide ring onto an additional *ortho* carboxylate residue. Chymotrypsin substrates can also be designed out of naphthalene structures. These substrates are analogous to the typical model substrate of chymotrypsin, N-acetyl-L-tryptophanate, because naphthalene is isosteric with the aromatic nucleus in tryptophan.

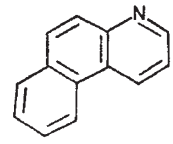
There is enormous variation in the rates of hydrolysis of these rigid structures, and, in the diagram (2), the structural features necessary for this to be optimum are indicated by arrows. Arrow 1 indicates that the D-configuration is desirable, which means that, as it is drawn, the carboxyl group should stick up, out of the plane of the paper. Arrow 2 indicates where an aromatic ring is desirable, arrow 3, where it is undesirable, and arrow 4, where it is very undesirable. A hydrogen bond acceptor may be localized in direction 4, where it could interact with the *para*-OH of tyrosine and the ring NH of tryptophan. The compound shown by the full lines is by far the most rapidly hydrolysed. Hayashi and Lawson conclude that the aromatic binding site of chymotrypsin is planar, elongated and L-shaped.



1



2



3

This shape immediately accounts for the powerful inhibitory action of 5,6-benzquinoline (3). It also suggests the configuration taken up by the aromatic L-amino-acid in the normal protein substrate of chymotrypsin, before it is hydrolysed.

BLOOD COAGULATION

Fibrinolysis in Pregnancy

from our Medical Biochemistry Correspondent

WHILE we are naturally concerned about the effects of external agents on the body, we often know all too little of the response of enzymes in "normal" physiological conditions. The importance of the complex factors involved in blood coagulation and fibrinolysis, for example, is only now beginning to be recognized. Fibrinolysis is the digestion of excess fibrin by the proteolytic enzyme, plasmin, which removes thrombi that might otherwise block blood vessels. Bonnar, McNicol and Douglas (*Brit. Med. J.*, **3**, 387; 1969) have now shown that during pregnancy there is a decrease in the ability of the blood to lyse fibrin, even though the concentration of the plasmin precursor, plasminogen, increases considerably.

Plasminogen reacts with plasminogen activators present in the blood or released from tissues to become the active proteolytic enzyme, plasmin. Bonnar *et al.* measured the concentrations of plasminogen and fibrinogen in the blood throughout pregnancy, during labour and one and six weeks after delivery in ten normal pregnant women. On each blood sample they