in the inhibitor molecule was found to have a profound effect on the inhibition constant of the acid. Saturated dicarboxylic acids, with the exception of oxalate, were less inhibitory as the molecular weight increased. A *cis* effect was observed in the inhibition by unsaturated organic acids; compounds with two *cis*-carboxyl groups were much more inhibitory than the corresponding *trans*-isomers. This position effect was investigated quantitatively by measurement of the distances between carboxyl groups in space-filling models of the inhibitors. When these distances were plotted against the inhibition constant of the inhibitor, the degree of inhibition was observed to increase linearly with the increasing proximity of the carboxyl groups.

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Inhibition of Blood Platelet Function by Cytochalasins: Effects on Thrombosthenin and on Glucose Metabolism

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The cytochalasins are mould metabolites that inhibit cytokinesis and cell movement (Carter, 1967). Cytochalasin B can also inhibit platelet aggregation (White, 1971) and clot retraction (Shepro *et al.*, 1970; White, 1971). An attempt has been made to correlate the effects of cytochalasins A and B on platelet function with biochemical changes.

Citrated pig platelet-rich plasma at 37°C was used. Platelet aggregation was initiated with ADP (10 µM) and clotting with bovine thrombin (10 N.I.H. units/ml) in both cases 5 min after addition of cytochalasin. Cytochalasin A inhibited aggregation completely at 20 µg/ml (42 µM), but cytochalasin B only partially at 100 µg/ml (209 µM). Clot retraction was inhibited completely by 4 µg/ml of cytochalasin A and by 20 µg/ml of cytochalasin B. Cytochalasin A (but not cytochalasin B) reacted with added cysteine, which blocked its effects.

Thrombosthenin (platelet actomyosin) was extracted from pig platelets (Grette, 1962; Haslam & Mustard, 1969). The effects of 0.1 mM-ATP on the turbidity of stirred suspensions of thrombosthenin in 60 mM-KCl at pH 7.0 were recorded. In the presence of 4 mM-MgCl₂, ATP cleared the suspension. This effect was inhibited by cytochalasin A (5–50 µg/ml) but not by cytochalasin B (100 µg/ml). With 15 mM-

MgCl₂, ATP caused a rapid superprecipitation which was similarly inhibited by cytochalasin A but not by cytochalasin B. Thrombosthenin was also isolated from platelets that had been incubated with cytochalasin A (4 or 20 µg/ml) for 5 min and then washed. Only with 20 µg/ml were the clearing and superprecipitation phenomena markedly inhibited. Thus only the inhibition of aggregation by cytochalasin A was associated with marked changes in thrombosthenin.

Washed rabbit platelets depleted of endogenous substrates rapidly form ¹⁴CO₂ from [6-¹⁴C]glucose (McElroy *et al.*, 1971). In this system cytochalasin A at 4 µg/ml and at 20 µg/ml inhibited ¹⁴CO₂ formation by about 30 and 70% respectively, whereas these concentrations of cytochalasin B inhibited by 91 and 98%. Thus the relative effectiveness of cytochalasins A and B was reversed in these experiments.

The biological activities of cytochalasin B have been attributed to disruption of contractile microfilaments (Schroeder, 1970; Wessells *et al.*, 1971). The effect of cytochalasin B on glucose metabolism and its lack of effect on thrombosthenin suggest that this conclusion may be premature.

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Amino Acid Sequences in Penicillo-pepsin: Evidence for Homology with Porcine Pepsin and Chymosin

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Penicillo-pepsin is an extracellular acid protease secreted by *Penicillium janthinellum*. Its purification and properties have been reviewed (Šodek & Hofmann, 1971). Earlier it was shown that the amino acid sequence around the active-site aspartic acid residue is almost identical with that of porcine pepsin (Šodek & Hofmann, 1970). In further sequence studies peptides have now been obtained from tryptic, chymotryptic and thermolytic digests by separation on

Sephadex G-75, G-50 and G-25F columns, and further purification has been achieved by standard electrophoretic and chromatographic procedures. The tryptic digests with diphenylcarbamoyl chloride-treated trypsin gave four of the five lysine residues in unique peptides and a considerable number of peptides originating from chymotryptic cleavages. The *N*-terminal region and overlapping sequences around some of the lysine residues were obtained from a subtilisin digest of the maleylated protein by the diagonal electrophoresis method. The sequences of the purified peptides were determined by the dansyl-Edman method (Gray, 1968). Almost all of the 310 residues of the protein have been accounted for in 30 unique peptides. These were compared with the partial sequences available for porcine pepsin (see Dayhoff, 1969) and for chymosin (B. Foltmann, personal communication). The comparison shows three areas with similarity in addition to the active-site peptide. These are: 17 residues at the *N*-terminal of the active enzymes, Ala-Ala-Ser-Gly-Val-Ala-Thr-Asn-Thr-Pro-Thr-Ala-Asn-Asx-Glx-Glx-Tyr-; the sequence -Asp-Phe-Gly-Phe-Ile-Asp-Ser-Ser-Lys-Tyr-; and 27 residues at the *C*-terminal end of the molecule, -Ser-Ile-Gly-Asp-Ile-Phe-Leu-Lys-Ser-Gln-Tyr-Val-Val-Phe-Asx-Ser-Asx-Gly-Pro-Glx-Leu-Gly-Phe-Ala-Pro-Gln-Ala. These findings provide good evidence for the homology between penicillopepsin and the mammalian acid proteases.

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Evidence for an Acyl Intermediate in Pepsin-Catalysed Reactions

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During a study of the action on the *S*-sulpho-B-chain of insulin by penicillo-pepsin (Mains *et al.*, 1971), the formation of Leu-Leu was observed. This sequence does not occur in insulin and thus is probably the product of a transpeptidation reaction. A study with a number of peptides showed that penicillo-pepsin and porcine pepsin catalyse transpeptidations that differ from those discovered some years ago by Neumann *et al.* (1959) involving an 'amino'-enzyme intermediate. We have observed the

formation of Phe-Phe from Phe-Tyr-Thr-Pro-Lys-Ala (peptide B-4), Leu-Leu from Leu-Tyr-Leu, and Met-Met from Met-Leu-Gly. We postulate that with these substrates an acyl transfer of the *N*-terminal amino acid via an 'acyl'-enzyme to a second substrate molecule occurs. The following evidence supports this.

(a) The major products of the action of both pepsins on Leu-Tyr-Leu after prolonged incubation (24h, pH 3.6, 35°C; [Leu-Tyr-Leu]=6.4mM; [enzyme]=6μM) were Tyr-Leu, Leu-Leu, leucine, Leu-Tyr and tyrosine in order of decreasing amounts. Almost twice as much Leu-Leu as free leucine was formed.

(b) Free amino acids cannot act as acceptors. When Leu-Tyr-Leu and [¹⁴C]leucine (1:10, molar ratio) were incubated with porcine or penicillo-pepsin, no radioactivity was incorporated into Leu-Leu.

(c) When peptide B-4 and Leu-Tyr-Leu (2:1, molar ratio) were incubated together with penicillo-pepsin, the main products were Tyr-Leu, Leu-Phe, Phe-Leu, Leu-Leu, Phe-Phe and Tyr-Thr-Pro-Lys-Ala. Thus either substrate can act as acceptor for the *N*-terminal of the other.

(d) Although penicillo-pepsin did not act on Leu-Tyr, this dipeptide was an acceptor for leucine from Leu-Tyr-Leu. When the two peptides were incubated together, the yield of Leu-Leu doubled and that of tyrosine increased many-fold over a control from which Leu-Tyr was absent.

(e) Trace amounts of Leu-Leu-Tyr-Leu (from Leu-Tyr-Leu) and Met-Met-Leu-Gly (from Met-Leu-Gly) have been isolated. This suggests that the reaction proceeds by way of an intermediate tetrapeptide.

The experiments reported provide evidence for the 'acyl'-enzyme postulated on the basis of ¹⁸O exchange reactions (Sharon *et al.*, 1962), and support the mechanism proposed by Bender & Kezdy (1965) for pepsin-catalysed hydrolysis reactions involving both 'acyl'- and 'amino'-enzyme intermediates. The two types of transpeptidation reactions (acyl transfer and amino transfer) are presumably special cases of the hydrolytic reaction. Some of the implications on the mechanisms discussed by Knowles (1970) are being considered.

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