centre sequence as thrombin. If, on the other hand, it turns out to be present in stoicheiometric proportion to thrombin it could be part of the covalent structure of prothrombin.

It is clear (Magnusson, 1965*d*) that thrombin formed in both citrate activation and thromboplastin activation contains the *N*-terminal sequence Ile-Val-Glu-Gly-. Recent evidence for the amino acid sequence (S. Magnusson & B. S. Hartley, unpublished work) of the B-chain of bovine thrombin isolated from thromboplastin activation mixtures (Magnusson, 1965*c*) shows sufficient sequence homology with the pancreatic serine proteinases for one to feel confident that the activation mechanism is very similar to that of chymotrypsinogen (Sigler, Blow, Matthews & Henderson, 1968), the proteolytic cleavage of the peptide bond to produce the *N*-terminal isoleucine being the critical step that produces active thrombin. Since the amino acid composition of prothrombin is very similar to that of thrombin and the molecular weight nearly twice that of thrombin, we have tried to obtain evidence that prothrombin of molecular weight 68000 is a dimer. Reduction and carboxymethylation, even if followed by maleylation, failed to produce material of smaller molecular weight as evidenced by gel filtration (S. Magnusson & B. S. Hartley, unpublished work). Unless one invokes some kind of covalent bond other than a disulphide bond to hold two polypeptide chains together, the best hypothesis at the moment seems to be that prothrombin consists of only one polypeptide chain with the B-chain of thrombin (*C*-terminal serine) constituting the *C*-terminal part and the A-chain constituting part of the polypeptide chain between the *N*-terminal alanyl sequence and the B-chain. This leaves about 250 amino acid residues and about 75% of the carbohydrate of prothrombin to be accounted for as 'non-thrombin' or 'pro' material. This, incidentally, is sufficient polypeptide material to include a second serine proteinase. The key questions at the moment are whether the second proteinase is in fact part of the covalent structure of prothrombin or, as has been suggested by others (Seegers, 1969), prothrombin is an aggregate of two different proteins held together by non-covalent forces. The second important question is whether the second proteinase can be activated by some other means than proteolysis, which could be the common denominator of all the different systems that are known to trigger off prothrombin activation, such as concentrated salt solutions, phospholipids and basic polyamino acids.

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Prochymosin and Chymosin (Prorennin and Rennin)

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The milk-clotting enzyme from the fourth stomach of the calf has for many years been called rennin in the English-speaking countries. Confusion very often occurs, however, between rennin (EC 3.4.4.3) from the calf stomach and renin (EC 3.4.4.15) from the kidneys. The milk-clotting enzyme was called chymosin 50 years before the term rennin was introduced (Deschamp, 1840; Lea & Dickinson, 1890), and to avoid further misunderstanding I return to the old nomenclature. The chemistry of the enzyme has been reviewed (Foltmann, 1966).

Like other acidic proteases from the gastric juice, chymosin is secreted as an inactive precursor, prochymosin. The precursor is stable at weakly alkaline pH whereas the active enzyme is rapidly denatured at pH values above 7. Below pH 5 prochymosin is converted into chymosin by a limited proteolysis during which a peptide segment is cleaved from the *N*-terminus.

If the increase of milk-clotting activity is plotted against time, the course of the activation at pH 4·5–5 appears as more or less sigmoid curves, indicating that the activation is at least partly autocatalytic. Experiments carried out at lower pH show a different course of reaction. The lower the pH the higher is the initial rate of reaction and the sigmoid shape of the curve disappears.

Chromatographically purified preparations of prochymosin are activated very rapidly at pH 2 and no specific activating agent has been found. To explain this, the hypothesis has been advanced that the zymogen is stabilized in its inactive form by electrostatic interactions. At low pH the carboxylic groups become uncharged and the prochymosin may rearrange into an active conformation. Having undergone such a change in conformation the zymogen molecules undergo irreversible activation by splitting off the activation peptide.

By this limited proteolysis the molecular weight is decreased from approx. 36000 for the zymogen to approx. 31000 for the active enzyme. Simultaneously the isoelectric point is shifted from approx. pH 5·0 to pH 4·6.

Analysis of the primary structure of chymosin is in progress. The sequences of 21 residues from the *N*-terminus and 28 residues from the *C*-terminus are known. Three disulphide bridges are present, two of these forming relatively short loops. 45 residues around the disulphide bridges have been located. Analyses of methionine-containing fragments have shown that four methionine residues are clustered in a sequence of 38 residues. From these investigations it is possible to account for 132 amino acid residues in chymosin. The *C*-terminal sequence and the sequence around the two short disulphide loops show a pronounced homology with hog pepsin (Foltmann, 1969). The *N*-terminal sequence has no resemblance to any known sequence from pepsin.

The proteolytic activity of chymosin has optimum pH about pH 3·5. The proteolytic specificity is somewhat similar to that of pepsin.

The well-known milk-clotting activity of chymosin is due to a limited proteolysis of the κ-fraction of the casein. The κ-casein consists of a hydrophobic and a hydrophilic part (Hill & Wake, 1969). In milk this protein stabilizes the casein micelles against aggregation. During milk-clotting, a Phe-Met bond is hydrolysed, the hydrophilic moiety of the κ-casein is liberated and aggregation occurs. The Phe-Met bond seems to be particularly exposed in casein, since the same bond is not hydrolysed by chymosin in a tryptic digest of κ-casein (Jollès, Alais & Jollès, 1968).

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Kallikreins

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The central property of the kallikreins (EC 3.4.4.21) is the hydrolysis of certain serum proteins, the kininogens, whereby pharmacologically highly active hypotensive peptides, the kinins, are liberated. Kininogenase activity, however, is also shown by certain other proteolytic enzymes, and this makes a precise definition of which enzymes should be called kallikreins very difficult, particularly as kallikreins from different sources show very different properties with regard to unspecific proteolysis, activity against synthetic substrates or sensitivity against inhibitors. Most of these studies, however, have been conducted with materials of doubtful purity, and this might in part explain the diversified results. It has been proposed that the name of kallikrein should be restricted to those kininogenases so named by their discoverers, and this proposal will be adhered to here. This limitation, however, is in contrast with the view of the Commission on Enzymes, which gave to kallikrein a system number of its own. The final clarification will need much more experimentation with pure enzymes.

Serum kallikrein from the pig was isolated by Habermann & Klett (1966). It had a molecular weight of 97000, liberated bradykinin from kininogen, and hydrolysed α-*N*-benzoyl-L-arginine ethyl ester, α-*N*-toluene-*p*-sulphonyl-L-arginine methyl ester, α-*N*-benzoyl-DL-arginine *p*-nitroanilide and casein. The isolation of bovine serum kallikreinogen (prekallikrein) was reported by Nagasawa, Takahashi, Koida, Suzuki & Schoenmakers (1968). The proenzyme was activated by Hageman factor or trypsin to an enzyme hydrolysing α-*N*-toluene-*p*-sulphonyl-L-arginine methyl ester but not casein. Rabbit serum kallikrein is inhibited by L-7-amino-1-chloro-3-toluene-*p*-sulphonamidoheptan-2-one (Paskhina *et al.* 1968). Guinea-pig serum kallikrein was shown to hydrolyse, among others, *N*-acylated lysine esters with *N*-acetylated amides of serine and tyrosine, but not threonine, as alcoholic components (Holman, Lowe, Morley & Smithers, 1968).

Simplified purification procedures for porcine submandibular-gland and urinary kallikreins are being worked out by the group of Fritz at our institute, but not much work has been done on the properties of these preparations as yet. Four different isoenzymes, having a molecular weight of about 25000 and able to hydrolyse α-*N*-benzoyl-L-arginine ethyl ester, α-*N*-benzoyl-DL-arginine *p*-nitroanilide and α-*N*-benzoyl-L-arginine β-naphthylamide, were isolated from submandibular glands of rats by Ekfors, Riekinnen, Malmiharju &