Gut, 1968, **9**, 134-138

Pepsinogens and pepsins[1]

Although it has long been known that the cow produces more than one gastric proteinase, it has been assumed until lately that non-ruminant mammals had only a single enzyme, pepsin. Until recently, therefore, no problem of nomenclature existed since pepsin was obviously the proteinase of gastric juice. The demonstration of a number of distinct enzymes and possible isoenzymes in the gastric juice of many mammals[1,2] has made the matter of nomenclature more difficult. The problem may eventually be solved by the identification of the peptide-bond specificity of the individual enzymes and the use of a nomenclature based on this. Until such time, a pepsin may be defined as 'a proteinase which is active at low *p*H, coagulates milk protein, and is inactivated under neutral or slightly alkaline conditions; it is secreted into the gastric juice and exists in the gastric mucosa in an inactive zymogen form which is alkali stable and from which the enzyme is formed in the presence of acid'. Under this definition, the rennins could be regarded as types of pepsin; indeed, the presently recognized rennins and pepsins have so many similarities that this is not unreasonable.[3,4,5] An excellent recent review of prorennins and rennins is that of Foltmann.[5]

There are technical problems in the demonstration of heterogeneity in proteinases from any tissue or secretion. The activation of zymogen to enzyme involves the cleavage of a number of peptide bonds in the zymogen molecule. Auto-digestion of the enzyme can also occur, and it is thus possible that a single zymogen may give rise to a number of partial hydrolysis products with enzymatic activity which may be separable by chromatographic or other techniques and mistakenly interpreted as indicating the presence of several enzymes. Thus, the mere demonstration of heterogeneity in the proteinases of a tissue or secretion does not inevitably imply the presence of more than one distinct zymogen. Much better evidence of the presence of more than one enzyme is the demonstration of several zymogens. This requires that care be taken to ensure that partial hydrolysis of zymogen does not occur during analysis or extraction. The presence of distinct enzymes is confirmed if it can be shown that the distinguishable zymogens, when activated, give enzymes with different properties.

Since activation of pepsinogen takes place in the presence of acid and is catalyzed by pepsin which is unstable in alkali, it is important that the initial extraction be done with weak alkali which prevents activation of the zymogen and destroys any traces of preformed enzyme. Hydrolysis of pepsinogen preparations to the extent of activation may be detected by testing them for enzymatic activity: however, since the usual assays for proteolytic activity require acid conditions which at once convert zymogen to enzyme, they cannot be used for this. The only suitable method is the measurement of milk coagulating activity which can be done at a *p*H at which activation of the zymogen, if it occurs at all, is very slow. The demonstration of milk clotting activity in a zymogen preparation indicates that it has been converted partially or completely to enzyme.

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Most of the present confusion about the human pepsinogens and pepsins stems from the fact that three groups of investigators have studied the human pepsinogens by three different techniques, the results of which have not been correlated.

Richmond, Tang, Wolf, Trucco, and Caputto[6] were the first to show the presence of more than one protease in human gastric juice. They chromatographed gastric juice on Amberlite IRC 50, a cation exchange resin, and demonstrated two distinct peaks of proteolytic activity, one with an optimum for hydrolysis of haemoglobin of *p*H 2, and the other with an optimum of *p*H 3·2. The first they termed 'pepsin' and the second 'gastricsin'. Gastricsin was later isolated and crystallized[7] and shown to be different from the pepsin preparation. In subsequent studies, an extract of human gastric mucosa was chromatographed on a column of DEAE-cellulose which was eluted with an increasing gradient of phosphate. Two zymogen fractions were obtained, one of which was called IA and the other IB. On activation of zymogen IA, both gastricsin and 'pepsin' were obtained.[8] It was concluded that the gastric mucosa contained a zymogen which on activation gave gastricsin and 'pepsin'.

Seijffers, Segal, and Miller[9] chromatographed extracts of human gastric mucosa on a column of DEAE-cellulose which was eluted with a carefully controlled chloride gradient. They found three zymogen fractions which they termed pepsinogens I, II, and III in the order of their appearance from the column. On rechromatography, the individual zymogen fractions showed the same behaviour as on the first occasion. Pepsinogen I on activation gave a single pepsin, pepsin I, and pepsinogen III a single pepsin, pepsin III. Activation of pepsinogen II gave two pepsins termed IIA and IIB suggesting that the pepsinogen II was really a mixture of two zymogens. On activation pepsinogen I gave only pepsin I which was identical in its chromatographic behaviour to gastricsin. Pepsin I could not be produced by activation of any of the pepsinogen fractions other than pepsinogen I. Human urine was found to contain only pepsinogens II and III, and on activation these never gave rise to gastricsin.[10] Mucosa from the antrum of the human stomach was found to contain only pepsinogen I.

Kushner, Rapp, and Burtin[11] studied an extract of human gastric mucosa by agar-gel electrophoresis and demonstrated four distinct bands of enzymatic activity which they named I, II, III, and IV in order of decreasing electrophoretic mobility. On activation with acid and subsequent neutralization bands I, II, and III were destroyed, but band IV persisted, suggesting that band IV represented a cathepsin and that only bands I, II, and III contained pepsinogens.

We are in the process of correlating the results of these three methods of investigation. Any of the statements which follow on the identity of the various fractions obtained from the different procedures must be regarded as tentative since they are based on a small number of observations which require confirmation.

It appears that the correlation between the findings of the electrophoretic method of Kushner[11] and the DEAE-cellulose chromatography method of Seijffers is quite close. As would be expected, the pepsinogens with the greatest electrophoretic mobility are the most acidic and are eluted last from the DEAE column. It thus appears that band IV of Kushner corresponds to a minute trace of enzymatic

activity eluted very early from the DEAE column which is probably a cathepsin. Band III of Kushner appears to correspond to pepsinogen I, and band II of Kushner seems to contain pepsinogens II and III which are not completely separated by electrophoresis. The fastest running band, band I of Kushner, seems to correspond to a late-appearing peak of zymogen obtained from some DEAE columns which has tentatively been labelled pepsinogen IV. Gastric mucosal extract which we prepared and chromatographed after the method of Tang and Tang demonstrated two peaks of zymogen activity apparently corresponding to the zymogens IA and IB obtained by them. Subsequent examination of this 'zymogen IA' by DEAE-cellulose chromatography by a modification of the method of Seijffers *et al.* revealed[12] that it contained three bands of zymogen activity corresponding to pepsinogens I, II, and III of Seijffers, Segal, and Miller. Electrophoresis of this 'zymogen IA' by a method similar to that of Kushner, Rapp, and Brutin also indicated that it was heterogeneous.[12] It seems that it will soon be possible to correlate the results of these methods. The present evidence indicates the existence in human gastric mucosa of a number of distinct zymogens each of which, on activation, gives a single enzyme. It is likely that the zymogen granules of the chief cells contains pepsinogens II and III although it is not yet known which cell produces pepsinogen I. A possibility is the mucous neck cell, but this is pure speculation.

In spite of highly sensitive methods for pepsin which may even be used to measure the pepsinogens from gastric biopsies,[13] the laborious nature of the chromatographic techniques and the particularly difficult features of the separation methods for the pepsins have inhibited the clinical application of these procedures. We cannot hope for much increase in our knowledge of the clinical significance of these enzymes until less tedious methods have been developed.

The first steps in this direction have already been taken. There is little doubt that pepsin I (gastricsin) hydrolyzes very different peptide bonds from those attacked by other pepsins. Chiang *et al.*[14] measured total pepsins by haemoglobin hydrolysis and their pepsin by hydrolysis of N-acetyl-L-phenylalanyl-L-diiodotyrosine (APDT). This substrate is little affected by gastricsin, and the difference in total peptic activity observed with haemoglobin and the peptic activity measured by APDT was used to give a measure of gastricsin. This method probably gives a good approximation to the gastricsin activity. However, there is some evidence that others of the chromatographically distinguishable pepsins are less active in hydrolyzing APDT than pepsin III: undetected variations in these other enzymes may therefore possibly represent a small source of error. An alternative approach to the measurement of pepsin I is to destroy the other pepsins in gastric juice by preincubation since it has been found that the differences in stability at *p*H 7·25 and 25° allow the separate measurement of gastricsin.[15] This method is simple and may have some applicability. It seems very likely that specific peptide substrates for gastricsin will soon be produced, but at the present time it is by no means clear if the peptide bond specificities of the other chromatographically detectable pepsins are sufficiently different to allow the development of specific substrates. The APDT method of Chiang *et al.*[14] has already been used to demonstrate that histamine stimulates the output of gastricsin in gastric juice in man, and we have obtained evidence that histalog stimulates all three chromatographically distinct

pepsins.[16] No other studies of the secretion of the individual pepsins in man are available at present.

Only pepsinogens II and III are found in the urine,[10] but to date we do not have reliable information on the enzymes present in the serum. Both the serum and urinary pepsinogen levels are raised in some patients with superficial gastritis and duodenal ulcers.[17] The cause of the elevation of serum enzymes is obscure. A similar rise in serum pepsinogen has been shown in rabbits immunized against hog pepsinogen.[18] The animals produced antibodies which reacted with their own pepsinogens, and the rise in pepsinogen level may have been due to the development of antigen-antibody complexes although this was not proven conclusively. Antibodies against human pepsinogens have not been identified in adult patients with pernicious anaemia although antibodies to the intrinsic factor and to other components of the parietal cell[19,20,21] are common.

The role of pepsin in the production of peptic ulcer has been almost entirely neglected. Many, however, believe that a peptic ulcer cannot develop in the absence of pepsin and acid,[22] and the recent studies on the prevention of peptic ulcers in animals by pepsin inhibitors do appear to implicate the pepsins in the ulceration process.

Levey and Sheinfeld[23] found that the administration of chondroitin sulphate to Shay rats[24] markedly reduced the incidence of peptic ulceration in these animals. Other sulphated polysaccharides have shown similar affects in guinea-pigs and rats.[25,26] The mechanism of the protective effect shown by these compounds is unknown but is generally believed to be related to their antipeptic activity.

One sulphated amylopectin (SN 263, 'depepsen') has been made available for clinical trial. It shows considerable pepsin inhibitory activity while being a relatively poor anticoagulant.[27] On oral administration it significantly reduces gastric ulceration in Shay rats and appears to inhibit the gastric ulceration produced by △-1-cortisol in rats and histamine in guinea-pigs. Clinical trials of depepsen in peptic ulceration have already been reported;[28] symptomatically it appeared more effective than a placebo but less than some anticholinergic drugs. Depepsen combined with anticholinergic drugs was shown to have a significant effect in preventing recurrences of duodenal ulcers.[29] In view of the marked effects in animals, further trials in man would seem worthwhile.

Alkalis are among the most potent destroyers of pepsin, and their action is largely irreversible. It has been clear for a long time that oral alkalis may neutralize acid for only short periods, whereas symptomatic relief may last for hours. It is possible that the effect may result from destruction of pepsin rather than neutralization of acid.

Further investigation of the gastric proteinases may define their role in peptic ulceration. The significance of the demonstrated differences in peptide bond specificity of gastricsin and the other enzymes is still obscure. Could any peptide malabsorption develop in the absence of one or more gastric enzyme? Even now some believe that pepsin deficiency is responsible for the B_{12} malabsorption of pernicious anaemia. Are some pepsinogens antigenic? If so, do they contribute to the development of gastritis? Are there different patterns of pepsin secretion in different disease states? These are just a few of the many questions remaining to be answered.

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