Biochem. J. (1970) **118**, 587–594
Printed in Great Britain

The Pepsins from Human Gastric Mucosal Extracts

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(*Received* 16 *January* 1970)

1. The pepsins and pepsinogens of the gastric mucosal extracts of two normal subjects, of seven patients with gastric adenocarcinoma and of two patients with duodenal ulcer have been investigated by agar-gel electrophoresis and by ion-exchange chromatography. 2. Of the eight zones of proteolytic activity that have previously been reported in normal human gastric juice, seven can be detected in activated fundic mucosal extracts. Of these seven, four can be attributed to discrete pepsins, numbered 1, 3a, 3 and 5. 3. Zone 7 results from the activity of one or more enzymes that are alkali-stable and are best referred to as gastric proteinases rather than as pepsins. Zone 7 is much more evident in mucosal extracts than in gastric juice. 4. Zones 4 and 6 may result respectively from the activity of a pepsin–inhibitor complex and of an unactivated zymogen. 5. It was not possible, by the chromatographic methods employed, to separate satisfactorily the individual pepsins from activated extracts or their precursors from unactivated extracts, so that the ascribing of a pepsin to a specific zymogen must be considered tentative. Even so, pepsin 3 appears to arise from at least two major precursors, if not from three, whereas pepsins 1 and 5 each arise from a single major precursor. 6. Pyloric mucosal extracts contain principally zone 5 but also zones 6 and 7. These zones in general behave similarly to the corresponding zones of fundic extracts, but pyloric pepsin 5 migrates slightly faster on agar-gel electrophoresis than does fundic pepsin 5 and is a different enzyme. Zones 1 to 4 are absent.

The observation that normal human gastric juice may exhibit as many as eight zones of proteolytic activity after electrophoresis at pH 5.0 on agar gel (Etherington & Taylor, 1967; 1969*a*) prompts an investigation into the origin of the responsible enzymes in the gastric mucosa. This is particularly needed because earlier work does not enable firm conclusions to be drawn about the number of human mucosal pepsins and pepsinogens.

Thus Merten, Schramm, Grassmann & Hannig (1952) showed that gastric mucosal extracts contained two proteolytically active components that could be separated by moving-boundary electrophoresis. Taylor (1959*b*), by means of proteolytic pH–activity curves, also identified two enzymes, from the fundic and pyloric glands of both human and pig mucosa, that differed in their biochemical action upon several protein substrates. Seijffers, Segal & Miller (1963*a*) subsequently confirmed, by ion-exchange chromatography, that human pyloric mucosal extracts contained a pepsinogen that had similar chromatographic properties to one of three pepsinogens which they demonstrated at the same time in fundic mucosal extracts. On activation the three pepsinogens yielded four pepsins (Seijffers, Segal & Miller, 1963*b*). Kushner, Rapp & Burtin (1964), using a similar technique, separated four pepsinogens that on activation yielded five pepsins. Hanley, Boyer & Naughton (1966), using starch-gel electrophoresis, demonstrated two principal pepsinogens. Tang & Tang (1963) provided contrasting evidence by obtaining the bulk of the mucosal pepsinogens in a single chromatographic peak. Their material was homogeneous on ultracentrifugation and starch-gel electrophoresis, but acid-activation yielded two pepsins, which they thus claimed were derived from a single zymogen. In the pig the picture is less uncertain for Ryle and his colleagues (Ryle & Hamilton, 1966) have described individual pepsinogens B, C and D which are the respective precursors of pepsins B, C and D.

Additional reasons for investigating the gastric mucosal pepsins arise from the earlier observations on agar-gel electrophoresis of human gastric juice (Etherington & Taylor, 1969*a*) that of the eight proteolytic zones, one, numbered 4, may represent a complex of a pepsin with an inhibiting peptide, like those described by Seijffers, Miller & Segal

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(1964); that another, numbered 6, may represent an unactivated pepsinogen, and that a third, numbered 1, cannot be ascribed to any of the pepsins and pepsinogens of Seijffers *et al.* (1963*a*,*b*), because the material was eluted from DEAE-cellulose only by raising the salt concentration above 0.21M, to a range not employed by the latter group.

MATERIALS AND METHODS

Mucosal extracts. Stomachs were obtained at operation from patients with gastric adenocarcinoma or duodenal ulcer. Two stomachs were also obtained at necropsy, within 2h of death, from subjects without gastric disease, and whose gastro-intestinal tract appeared normal macroscopically. Each specimen was placed immediately in a beaker inserted into a thermos flask containing ice and was transferred to the cold-room (2°C). There it was rinsed in cold iso-osmotic NaCl (0.9g/100ml) to remove adhering mucus and debris, and divided into the fundic and pyloric portions (Taylor, 1959*b*); the diseased portion was carefully cut away. The mucosa was then separated from the overlying muscle, chopped into small pieces and macerated in 2% (w/v) NaCl solution, essentially as described by Taylor (1959*a*). The debris, collected by centrifugation, was macerated further in fresh NaCl solution and recentrifuged. The supernatants, after passage through a glass-wool filter to remove the surface lipid film, were combined.

The extracts contained pepsinogens that were activated by acidification to pH2.0 followed by incubation at 37°C for 10min. For some experiments with fresh extracts, this incubation period at pH2.0 was extended to 30min for one portion of the extract, and a second portion of activated extract was carefully adjusted to pH3.5 with 1.0M-and 0.1M-NaOH and then again incubated at 37°C for 20min. Extracts, after activation, were stored at pH3.5–4.0 at −15°C.

Agar-gel electrophoresis. Zymograms of the pepsins in activated mucosal extracts were obtained after electrophoresis at pH5.0 in agar gel (Etherington & Taylor, 1969*a*). For electrophoresis at pH8.2, 0.025M-sodium barbitone–HCl buffer was used for the gels, with 0.05M buffer in the electrophoresis tank (Lapresle & Webb, 1960, 1962).

The results are expressed in a semi-quantitative fashion; the amount of extract added to each gel was carefully adjusted to avoid overloading on the one hand and to ensure, on the other hand, that the principal enzyme in the extraction gave a zone of intensity graded ++ or +++, so that enzymes present in very small concentrations did not go undetected.

Ion-exchange chromatography. Acidified mucosal extracts were fractionated on columns (2.5cm×20cm) of Amberlite CG-50 (Rohm and Haas Co., Philadelphia, Pa., U.S.A.) as described previously (Etherington & Taylor, 1969*a*). The extracts (50–150ml) were concentrated against Carbowax (20M grade) in 1.9cm Visking tubing, dialysed against two changes of the starting buffer, 0.2M-sodium citrate, pH2.9, and then centrifuged at 1500*g*. The supernatant, (10–15ml) was then applied to the column and fractions (11ml) were collected at a flow rate of approximately 100ml/h.

Chromatography on DEAE-cellulose (Whatman, grade DE-52) was a modification of the method of Seijffers *et al.* (1963*a*). The microgranular exchanger was prepared for use and packed into columns (2.5cm×20cm) as recommended by the manufacturers. Mucosal extracts (50–100ml) (unactivated) were dialysed directly against three changes of 0.1M-sodium acetate buffer, pH5.3, followed by centrifugation at 10500*g* for 10min. The supernatant was applied to the column and unadsorbed protein was washed through with 100ml of pH5.3 buffer.

We found, confirming the observations of Seijffers *et al.* (1963*a*) and Kushner *et al.* (1964), that the pepsins are eluted from DEAE-cellulose as pepsinogens. The latter were eluted by using (from 0.10 to 0.16M) a linear gradient of NaCl in the same buffer. A total volume of 1800ml was used in the gradient, and this was followed by a second gradient to 0.8M-NaCl with a total volume of 900ml. Fractions (13.5ml) were collected at a flow rate of approximately 50ml/h.

Proteolytic activity. Proteolytic activity was determined as previously described (Etherington & Taylor, 1969*a*). The zymogens were not activated before being added to the digestion mixture, which at pH1.9 gave an almost instantaneous conversion of the pepsinogens into pepsins (Seijffers *et al.* 1963*a*). The protein content of the fractions was followed from the extinction at 280nm. When eluate fractions were analysed by agar-gel electrophoresis, samples from the DEAE-cellulose columns were activated by acidifying with 1.0M-HCl to pH2.0 and incubating at 37°C for 10min. Eluate fractions from the Amberlite chromatograms contained only active pepsins and this step was omitted.

RESULTS

Agar-gel electrophoresis of fundic mucosal extracts. The uninvaded gastric mucosa of patients suffering from gastric carcinoma can be regarded as normal mucosa, both on histological grounds and from a consideration of pH–activity curves (Taylor, 1960). The gastric mucosa of patients with duodenal ulcer cannot be regarded as normal (Taylor, 1959*c*). Because of the difficulty of obtaining sufficient normal material, mucosa from two patients with duodenal ulcer was used at certain appropriate points in this investigation; these are specifically mentioned in the text.

The zymograms of the activated fundic mucosa from one normal subject and of the activated uninvaded fundic mucosa of two patients with gastric carcinoma are shown in Fig. 1, together with a diagram indicating and numbering the individual zones of activity, as observed in normal human gastric juice (Etherington & Taylor, 1969*a*). The activity of each zone for the mucosa of five subjects is shown semi-quantitatively in Table 1. It is clear that the proteolytic zones of the activated mucosal extracts are located in the same positions on the zymograms as are the zones of normal gastric juice.

As with normal gastric juice, the principal zones are 3 and 5, with 3 being the most abundant. Zone 1 was weakly present in only two extracts and zone 4 in one. Zone 2 was not certainly identified in any of the extracts. Zone 3a was identified in three of the five extracts. Activity at zones 6 and 7 seemed more pronounced than in normal gastric juice. Zone 7 occurred in all mucosal extracts and zone 6 in all but one, whereas they occur in only 20% and 4% respectively of normal gastric juices.

Agar-gel electrophoresis of pyloric mucosal extracts (*Fig.* 2). Activated extracts of pyloric mucosa gave zymograms that show principal activity at zone 5. Zones 6 and 7 were also present, but zones 1 and 2 were not detected. Zone 3 was absent or very much decreased in amount, when compared with fundic extracts and with gastric juice. The pepsin responsible for zone 5 migrated slightly faster than the pepsin 5 of the fundic extracts and in patients with duodenal ulcer, at least, the two enzymes are not identical (Fig. 2), for when the fundic and pyloric extracts are mixed together, the two enzymes do not migrate together as a single zone.

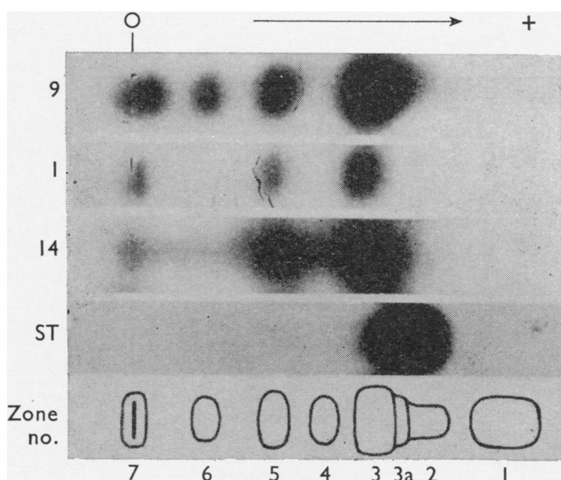

Fig. 1. Agar-gel electrophoresis at pH5.0 of acidified fundic mucosal extracts from a normal subject (9) and from the uninvaded portion of the stomachs of two patients with gastric adenocarcinoma (1,14). Crystalline swine pepsin (ST) was used as the standard marker and the numbering of the zones is shown diagrammatically. The gels were incubated at pH1.9. o, Origin; +, anode.

Preincubation at pH3.5. After preincubation at pH3.5 and 37°C for 20min all zones showed some decrease in activity, but this was particularly noticeable for zones 4, 6 and 7 of fundic extracts (Fig. 3) and for zones 6 and 7 in pyloric extracts. It should be noted that in Fig. 3 (30A) a zone 4 is shown by fundic extract of subject 30, but not by the same extract in Fig. 2 (30F). This has arisen because during the interval between carrying out the experiment of Fig. 3 and then that of Fig. 2, the mucosal extract was stored at pH3.5 at −15°C. Under these conditions zone 4 is labile (Etherington & Taylor, 1969*a*). The absence of zone 4 in the experiments of Fig. 2 has made it easier to demonstrate, in this subject, the separate fundic and pyloric zones 5.

Alkali stability. Acidified fundic and pyloric mucosal extracts were tested for alkali stability by careful adjustment to pH8.2 with 1.0M- and then with 0.1M-sodium hydroxide. At this pH, pepsins are characteristically destroyed. The extracts were then subjected to agar-gel electrophoresis at pH5.0 and pH8.2 (Fig. 4). At pH5.0 the activity at zone 7 persisted, whereas activity at the other zones was destroyed. Thus zone 7 behaved unlike pepsins generally but similarly to the 'pepsin 4' of Kushner *et al.* (1964). When the extracts were subjected to electrophoresis at pH8.2, the activity of zone 7 was located towards the anode as two closely running zones for both pyloric and fundic mucosa, instead of the one found at pH5.0.

Ion-exchange chromatography of activated extracts containing pepsins. Activated fundic and pyloric

Table 1. *Agar-gel electrophoresis at pH*5.0 *of activated human fundic mucosal extracts*

The frequency and intensity of the different proteolytic zones are given. It was not possible to make satisfactory semi-quantitative estimates for zone 3a.

Subject	Zones of activity							
	7	6	5	4	3	3a	2	1
Normal								
9	++	+	++		+++	Detected		±
Gastric carcinoma patients								
1	+		+		++	Not detected		
4	++	+	++		++	Not detected		±
14	+	±	++	+	++	Detected		
15	+	+	+		++	Detected		

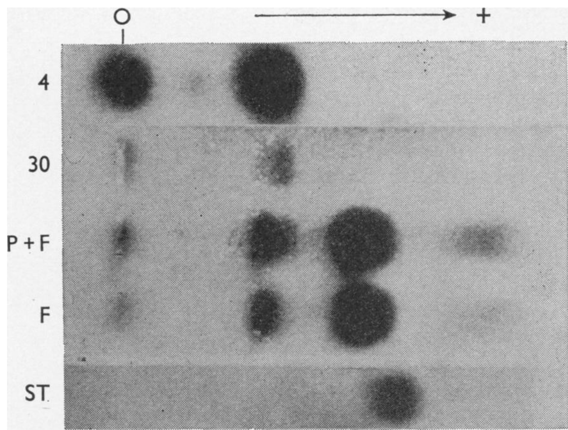

Fig. 2. Agar-gel electrophoresis at pH5.0 of two pyloric mucosal extracts, one from a patient with gastric adenocarcinoma (4) and one from a patient with duodenal ulcer (30). The electrophoretogram of the fundic mucosal extract of the latter subject is also shown (F), and that of the mixed pyloric and fundic extracts (P+F). ST is the standard marker of crystalline swine pepsin. Pyloric and fundic zones 5 migrate at different rates.

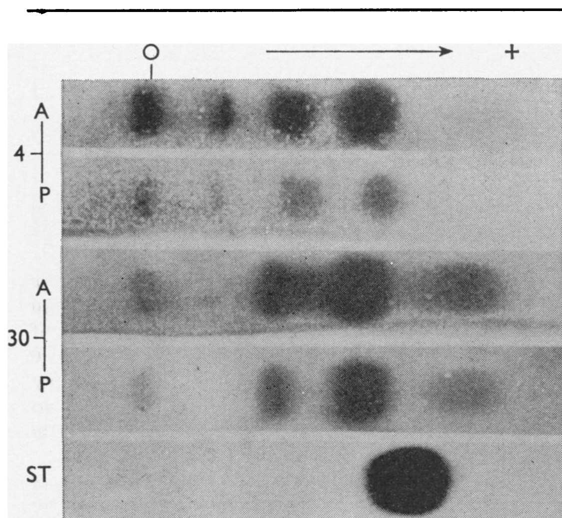

Fig. 3. Agar-gel electrophoresis at pH5.0 of two activated fundic mucosal extracts (4, 30), which had been either (P) preincubated at pH3.5 and 37°C for 20min or (A) adjusted directly to pH5.0. ST is the standard marker of crystalline swine pepsin. Zone 4 disappears after preincubation. Zones 6 and 7 also diminish in intensity. The same initial quantities of extract were applied to the gel in both the preincubated (P) and the directly adjusted (A) experiments.

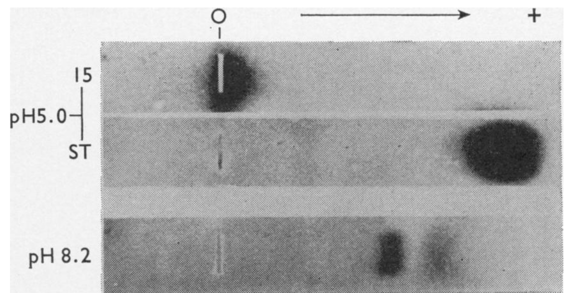

Fig. 4. Agar-gel electrophoresis at pH5.0 of an activated fundic mucosal extract (15) that had been adjusted to pH8.2. ST is the standard marker of crystalline swine pepsin. Peptic activity has been totally abolished, but activity at zone 7 persists. Agar-gel electrophoresis at pH8.2 shows this activity to migrate to the anode as two zones.

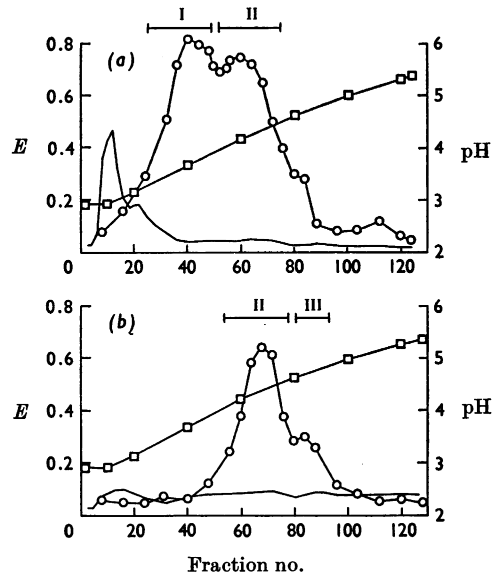

Fig. 5. Chromatography of activated (*a*) normal human fundic and (*b*) normal human pyloric mucosal extracts (subject 9) on columns (20cm×2.5cm) of Amberlite CG-50. Peptic activity was eluted by a proportionately linear gradient between 0.2M-sodium citrate buffers of pH2.9 and 6.0. The concentration of Na^+ was maintained at 0.36M by the addition of NaCl. Fractions (11ml) were collected. ——, E_{280} (total protein); ○, E_{700} (peptic activity); □, pH.

mucosal extracts were chromatographed on Amberlite CG-50 (Fig. 5). The enzymic activity of the fundic extracts was eluted as two peaks at pH3.7–3.9 and 4.2–4.4, as was found for gastric juice (Richmond, Tang, Wolf, Trucco & Caputto, 1958; Etherington & Taylor, 1969*a*). Activated extracts of pyloric mucosa did not exhibit the peak at pH3.7–3.9, but the peak at pH4.2–4.4 was always

Table 2. *Chromatography of activated fundic and pyloric mucosal extracts on Amberlite CG-50 and subsequent analysis of the effluent peaks by agar-gel electrophoresis at pH* 5.0

It was not possible to make satisfactory semi-quantitative estimates for zone 3a. (S) indicates a shoulder to the main peak.

Subject	Peak	pH for maximal elution and relative size	pH range of 'cuts'	Zones of activity							
				7	6	5	4	3	3a	2	1
Normal fundic mucosal extracts			2.9–3.3					++	Detected		+
9	I	3.7 ++	3.3–4.0					+++	Detected		
	II	4.2 ++	4.0–4.5			++		++			
	III	4.7 (S) +	4.5–4.8	±		+					
20	I	3.8 ++	3.2–4.0					+++			++
	II	4.4 (S) +	4.0–4.4			+++		+++			
			4.4–4.8			+		+			
Uninvaded fundic mucosal extracts											
1	I	3.9 +	3.3–4.0					+++	Detected		++
	II	4.3 ++	4.0–5.0			+++		+++	Detected		
12	I	3.8 +	3.3–4.1					+++			++
	II	4.3 ++	4.1–4.8	±		++		+++			
Normal pyloric mucosal extracts											
9	II	4.4 ++	4.0–4.6	+		+++		±			
	III	4.7 (S) +	4.6–4.9	++		+					
20	II	4.4 ++	4.2–4.5			+++					
	III	4.6 (S) +	4.5–4.7	±		+					
Uninvaded pyloric mucosal extracts											
22	II	4.3 ++	4.1–4.4			+++					
	II	4.7 ++	4.5–4.9	++		+					
33	II	4.4 ++	4.1–4.5			++					
	III	4.7 ++	4.5–5.0	+		+					

found. A shoulder or very small peak was located on the more alkaline side of the pH 4.2–4.4 peak in the fundic chromatograms, around pH 4.6, and a shoulder or distinct minor peak in the pyloric chromatograms was located also at pH 4.6–4.7.

Analysis of the effluent fractions by electrophoresis in agar gel at pH 5.0 (Table 2) showed that the distribution of activity in the fundic chromatograms was similar to that previously obtained in the chromatograms of gastric juice. The first peak exhibited activity mostly at zone 3, together with the faster zone 1, and with small amounts of zone 3a in two subjects. The second peak contained pepsin 5 along with pepsin 3. The shoulder of this second peak was found to contain the proteinase of zone 7. The major peak in the pyloric chromatograms contained the faster, 'pyloric', pepsin 5 and the minor peak exhibited activity at zone 7.

Ion-exchange chromatography of unactivated extracts containing pepsinogens. Chromatography of unactivated fundic mucosal extracts on microgranular DEAE-cellulose at pH 5.3 gave a total of five peaks with potential peptic activity. Fig. 6 shows that the first sodium chloride gradient (0.10–0.16 M) eluted four pepsinogens, and the fifth pepsinogen peak was eluted by the second gradient (0.16–0.80 M). A small amount of activity (peak P) was unadsorbed by the column, and emerged from the column before peak I. Eluate fractions were acidified to pH 2.0 and incubated at 37°C for 10 min before analysis by agar-gel electrophoresis. The zymograms are reproduced in Fig. 7. They show that peak I contained principally the zymogen of pepsin 5 and peak V the zymogen of pepsin 1. Peaks II, III and IV, on activation, each yielded principally a pepsin with the mobility of pepsin 3. The small amount of activity eluted at P was located at zone 7. This proteinase remained at the origin whether the sample was acidified or not before electrophoresis.

Extracts of pyloric mucosa yielded a single zymogen peak at a position corresponding to fundic peak I when chromatographed on DEAE-cellulose, as found by Seijffers *et al.* (1963*a*). Upon activation and subsequent agar-gel electrophoresis, the peptic activity was found to be due to pyloric pepsin 5,

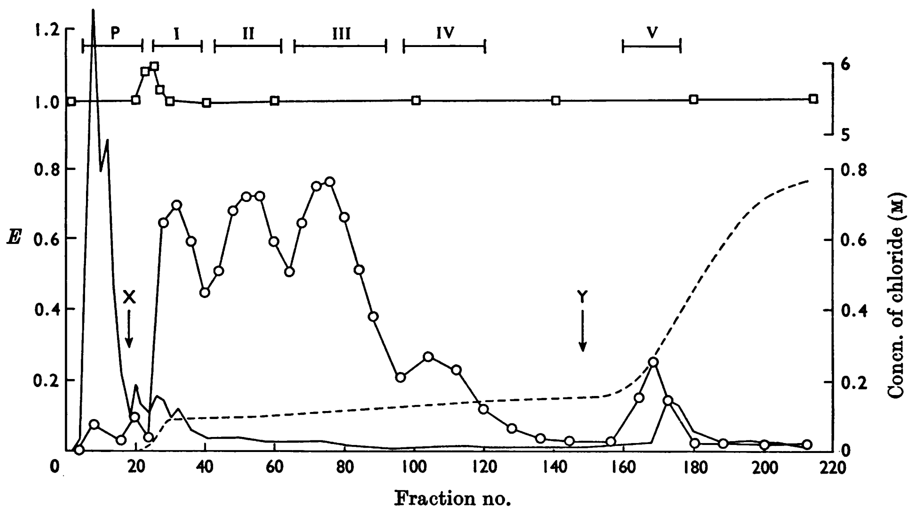

Fig. 6. Chromatography of the pepsinogens of a human fundic mucosal extract from a patient with duodenal ulcer (subject 43) on a column (20cm × 2.5cm) of DEAE-cellulose equilibrated with 0.1M-sodium acetate buffer, pH5.3. The pepsinogens were eluted by an increasing concentration of NaCl in the same buffer. X shows the commencement of a linear gradient from 0.10M- to 0.16M-NaCl. At Y a linear gradient was applied from 0.16M- to 0.8M-NaCl. Fractions (13.5ml) were collected. ——, E_{280} (total protein); ○, E_{700} (peptic activity); □, pH; -----, chloride concentration.

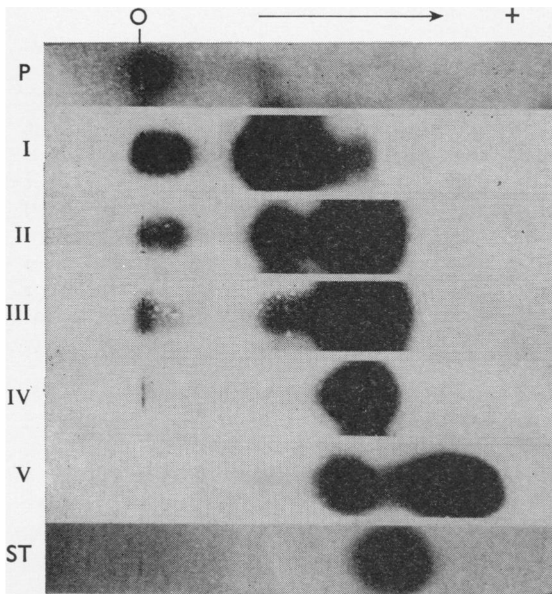

Fig. 7. Analysis of the activated pepsinogen peaks I–V eluted from the DEAE-cellulose column (see Fig. 6) by agar-gel electrophoresis at pH5.0. P is the small peak of activity not adsorbed by the column. ST is the standard marker of crystalline swine pepsin.

with very small amounts of activity at zones 6 and 7. A small amount of unadsorbed activity again preceded this pepsinogen peak, and showed, on agar-gel electrophoresis, activity at zone 7.

DISCUSSION

We are now in a position to answer some of the questions asked in the introduction. Taking the zones in numerical order, pepsin 1 occurred in only two out of five activated normal mucosal extracts that were analysed initially, and then only weakly. It occurred in the gastric juice of 48% of normal subjects (Etherington & Taylor, 1969*a*). The enzyme could be concentrated from the activated mucosa of two of the five subjects, and from two other normal subjects, in more readily demonstrable quantities by chromatography on Amberlite CG-50 (Table 2). Agar-gel electrophoresis of the fractions obtained from Amberlite CG-50 showed that pepsin 1 is eluted between pH2.9 and 4.1, but that separation from pepsin 3 cannot be achieved by this chromatographic technique. Larger quantities of pepsin 1 were found in activated mucosal extracts from patients with duodenal ulcer (e.g. Fig. 3). When such extracts (unactivated) were subjected to chromatography on DEAE-cellulose (Fig. 6) to separate the unactivated zymogens, the precursor

of pepsin 1 was located in a similar chromatographic position to the pepsin 1 of human gastric juice (Etherington & Taylor, 1969*a*). As was noted with gastric juice, a small amount of activity with the mobility of pepsin 3 was also located in this fifth chromatographic peak. Pepsin 1 thus occurs in the gastric juice of some normal subjects and is represented in fundic muocsa by a distinct precursor which can be called pepsinogen 1. It would seem to be the same enzyme as the pepsin 1 of Kushner *et al.* (1964), but has not been previously recognized by column chromatography. The increased amounts of this enzyme that were demonstrated in patients with duodenal ulcer may perhaps be associated with the known differences in gastric proteolytic pH–activity curves between normal subjects and such patients (Taylor, 1959*c*) and require further investigation.

Pepsin 2 (zone 2) occurs in the gastric juice of only 32% of normal subjects and was not certainly identified electrophoretically or chromatographically in any of the normal mucosal extracts. The absence of this enzyme in the chromatographic fractions may possibly be because of the relatively small number of extracts examined. The mucosal origin of pepsin 2 remains therefore uncertain.

Pepsin 3a is difficult to locate and identify because of its close association with pepsin 3 (Etherington & Taylor, 1969*a*). It occurred in at least three of the five activated normal mucosal extracts (Table 1) and is illustrated most typically in Fig. 1 subject 9. The enzyme was also identified in two of the four activated extracts of normal mucosa that were chromatographed on Amberlite (Table 2). It was eluted over a wide pH range of 2.9–5.0. It was not identified in the unactivated extracts that were chromatographed on DEAE-cellulose. No definite zymogen for pepsin 3a has yet therefore been identified, although it is undoubtedly of mucosal origin.

Pepsin 3 was the most abundant pepsin in all the fundic extracts that were examined, as it is in normal human gastric juice. The principal problem about pepsin 3 is its occurrence in virtually every chromatographic fraction from the Amberlite columns (Table 2) and in each of the zymogen peaks (after activation) from DEAE-cellulose columns (Figs. 6 and 7). The simplest explanation of the occurrence of more than a single pepsin in many of the electrophoretograms of the chromatographic fractions of human gastric mucosal extracts is that the different pepsins, and their zymogens, are each eluted from the columns over wide and overlapping ranges. If this is so, it must be open to doubt whether the chromatographic peaks separated by earlier workers really consist of single pure enzymes, or of single zymogens, as has been claimed. Others have noted the impurity of their chromatographic fractions and have explained the phenomenon by postulating that an enzyme may dissociate into two components (Tang & Tang, 1963; Kushner *et al.* 1964). This is indeed an alternative explanation of our own results, but we would need to postulate that peaks III and V, at least, if not I and II, dissociate in such a fashion. The present results do not establish either hypothesis firmly.

Zone 4 was demonstrated in one of the five, activated, normal gastric mucosal extracts, as compared with a frequency of 54% in normal human gastric juice. It was also demonstrated in the extract from a patient with duodenal ulcer. It was not demonstrated by either of the chromatographic techniques. These observations would be compatible with our earlier suggestion (Etherington & Taylor, 1969*a*) that this zone is caused by a complex of a pepsin with an inhibiting peptide that has itself been released during activation. Such a complex would normally be seen in gastric juice, and not in mucosal extracts, but might appear during activation *in vitro*, as in preparing the extracts for agar-gel electrophoresis. Seijffers *et al.* (1964) have shown that complexes of this type can be destroyed by incubation at 37°C and pH3.5, as was the zone 4 in one of our extracts (Fig. 3). This observation gives further support to the possibility that zone 4 represents such a complex.

Pepsin 5 was the second most abundant of the pepsins and occurred in all the fundic extracts examined. In both these respects it resembled the similar zone in normal gastric juice. On Amberlite columns it was eluted over the pH range 4.0–5.0, as was the pepsin 5 of gastric juice. On DEAE-cellulose, peak I was, upon activation, found to contain mainly pepsin 5. Neither chromatographic procedure yielded a pure preparation, and some pepsin 5 was located, after activation, in peaks II and III eluted from DEAE-cellulose.

Pyloric mucosal extracts differed from fundic mucosal extracts in exhibiting, when activated, principally pepsin 5 and no pepsins 1 or 4. Pepsin 3 was only occasionally present and its occurrence probably represents the accidental inclusions of some fundic mucosa during the cutting-away of the pyloric portion. The principal enzymes from the pyloric and fundic glands are thus different, an observation first made by Glaessner (1902) and demonstrated in terms of pH–activity curves by Taylor (1959*b*). Pyloric pepsin 5 was eluted from Amberlite over a similar pH range to fundic pepsin 5. On the other hand, the fundic and pyloric pepsins 5 could be distinguished electrophoretically (Fig. 3), and close examination of normal pyloric zymograms shows that zone 5 moves slightly faster than the fundic zone 5. The two pepsins 5 would appear therefore to be different enzymes. Normal gastric juice exhibits pepsin 5 in the fundic position

and there is no certain proof that the pyloric enzyme is actually secreted into human gastric juice although it is in the pig; Taylor, 1959*a*).

Zone 6 occurred in four of the five fundic extracts initially examined, and in one of the two pyloric extracts. The loss of activity on preincubation at pH 3.5 suggests that zone 6 might represent an enzyme–inhibitor complex, as postulated for zone 4, rather than residual zymogen, as was postulated for the similar zone 6 in normal gastric juice (Etherington & Taylor, 1969*a*). The frequency of occurrence of the zone in mucosal extracts is, however, much greater than in normal gastric juice (4%). This is the reverse of the findings with zone 4 and is best explained by the 'zymogen' hypothesis for zone 6. It may be that when zone 6 is found in gastric juice, it arises from desquamated mucosal cells, rather than as a true secretion.

The activity that remained at the origin, zone 7, was alkali-stable (Fig. 4) and was thus similar to the 'pepsin IV' of Kushner *et al.* (1964). However, whereas these workers found that this enzyme showed a slightly increased mobility at pH 5.6 after acidification, we have not been able to detect any difference of mobility for zone 7 between the activated and unactivated extracts at pH 5.0. At pH 8.2 two components of zone 7 could be demonstrated to move towards the anode. Because this enzyme(s) is alkali-stable it is probably best called gastric proteinase, to distinguish it from the gastric pepsins. Kushner *et al.* (1964) found moreover that 'pepsin IV' was immunologically unrelated to the pepsins.

Ion-exchange chromatography on Amberlite CG-50 showed that the gastric proteinase of zone 7 was located in the terminal portion of the second of the two chromatographic peaks. The relatively greater abundance of the gastric proteinase in pyloric extracts was shown in the chromatograms as a separate peak adjacent to the peak containing pyloric pepsin 5. On DEAE-cellulose the gastric proteinase of zone 7 was found to be virtually unadsorbed, emerging as a small peak before the zymogen peak I. Seijffers *et al.* (1963*a*,*b*) also noted this unadsorbed activity, which they did not investigate further, but Kushner *et al.* (1964), who had equilibrated their DEAE-cellulose columns with a 0.01 M-phosphate buffer, pH 7.0, obtained the enzyme only after raising the ionic strength of the eluting buffer and just before the first zymogen peak. That this enzyme was weakly present in the gastric juice of only 20% of normal subjects (Etherington & Taylor, 1969*a*) but more markedly in all the mucosal extracts examined, requires explanation. Intestinal mucosal extracts and extracts of gastric adenocarcinomata contain an enzyme with similar biochemical, chromatographic and electrophoretic properties (Etherington & Taylor, 1969*b*) so that the enzyme is unlikely to be a complex or a zymogen. It may be that the enzyme is simply inactivated during the process of secretion in some patients, or that it arises in normal gastric juice only from desquamated mucosal cells.

Of the eight proteolytic zones of normal gastric juice, all but zone 2 have thus been positively identified in activated fundic mucosal extracts. Zone 4, a possible enzyme–inhibitor complex, may well be present in mucosal extracts only as an artifact of the activation procedure. Zone 6 may well represent unactivated zymogen. The enzymes resulting from the 'activation' of zones 4 and 6, if these hypotheses are correct, are not yet known however. Zone 7 represents a gastric proteinase with properties dissimilar to the pepsins. The existence of four pepsins 1, 3a, 3 and 5 in fundic extracts is firmly established. Pepsins 1 and 5 have each one major pepsinogen precursor, which can be identified by chromatography on DEAE-cellulose. Pepsin 3 has two or three; but pepsin 3 is released from all five precursor peaks, and pepsin 5 from three. Pepsin 3a does not appear to have a precursor that can be separated from those giving rise to pepsin 3.

We thank Mrs Philippa Parker and Miss Pamela Gale for skilled technical assistance and the surgeons of the Liverpool Royal Infirmary for samples of gastric mucosa.

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