Studies on Gastric Proteolysis

3. THE SECRETION OF DIFFERENT PEPSINS BY FUNDIC AND PYLORIC GLANDS OF THE STOMACH*

By W. H. TAYLOR
Department of Clinical Biochemistry, Radcliffe Infirmary, University of Oxford

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It has been shown (Desreux & Herriott, 1939; Northrop, Kunitz & Herriott, 1948; Hoch, 1950; Merten, Schramm, Grassmann & Hannig, 1952; Taylor, 1956, 1959*b*) that crystalline swine pepsin contains more than one proteolytically active component. Merten *et al.* (1952) and Taylor (1959*b*) have also found more than one proteolytic component in pooled human gastric juice. The latter described two electrophoretically homogeneous components of human gastric juice which moved to the anode at pH 2·5 in the Tiselius apparatus and thus resembled pepsin. Each component digested serum albumin with two pH maxima, one near pH 2 and the other near pH 3·5.

The origin of these active components of crystalline pepsin and gastric juice has not been satisfactorily settled. As Northrop *et al.* (1948) point out, crystalline pepsin from a single individual animal has never yet been isolated, so that different components may represent nothing more than pepsins from the different individuals who contributed to the original starting material. However, in the electrophoretic experiments mentioned above the gastric juice from which the two anodal-moving components were prepared was contributed by only two normal subjects; the pH maxima for digestion by the smaller component were at lower pH values than those of the larger, whereas the pH–activity curves of the original juice of each subject showed maxima close to those of the larger component. It seemed therefore that the smaller component was not the main pepsin of either of the individuals but a substance with its activity masked in the original juices. This evidence suggests that there might be in each individual at least two pepsins, each exerting proteolytic action with two maxima below pH 5, but with definite differences in the pH values at which the maxima occur.

A further clue to the origin of these components arose during the investigation of the proteolytic activity of extracts of gastric mucosa from different parts of the stomach (removed at operation) of patients with gastric or duodenal ulcer. Extracts of pyloric mucous membrane often gave lower pH maxima than extracts of fundic mucosa (Taylor, 1956) and appeared to have different proteolytic properties. The possibility immediately suggested itself that the different proteolytic components of normal human gastric juice and swine pepsin might be derived from fundic and pyloric parts of the stomach. An investigation of this hypothesis is now described.

* Part 2: Taylor (1959*a*).

EXPERIMENTAL

The methods and materials used have been described previously (Taylor, 1957, 1959*a*). Swine pyloric juice was kindly supplied by Professor H. W. Florey, F.R.S., and Dr N. G. Heatley from an animal with a pyloric pouch. This juice was secreted at neutral pH, brought to about pH 3 by adding 0·1 M-HCl and stored at 0°.

Pyloric extracts from human and swine stomachs were made from the portion of gastric mucosa which extends 1 in. cephalad from the pyloro-duodenal junction. The next inch of gastric mucosa and the cardiac mucosa were discarded. The remaining portion, consisting of fundus and body mucosa, and containing the fundic glands, was made into a fundic extract. Normal human gastric mucosa was obtained within 2 hr. of death and swine mucosa within 15 min. of death.

RESULTS

Human fundic and pyloric mucosal extracts. In Table 1 are shown the pH maxima of digestion of several protein substrates by extracts of the pyloric and fundic portions of the mucous membrane from five normal human stomachs. Fig. 1 shows representative pH–activity curves. It will be seen that all five fundic extracts gave maxima for the digestion of proteins within the ranges pH 1·8–2·4 and pH 3·4–3·9. Four pyloric extracts, on the other hand, gave pH maxima between pH 1·5 and 1·6 and 3·1 and 3·2. It would seem therefore that the pepsins of human fundic and pyloric mucosa differ from each other in the normal subject.

Swine fundic and pyloric mucosal extracts. Similar observations have been made upon fundic and pyloric mucosa from fresh swine stomachs (Table 1, Fig. 2). Fundic extracts gave maxima at pH 2·0–2·2 and 3·5–3·9, whereas pyloric extracts showed maxima at pH 1·7–1·9 and 3·2–3·4.

Table 1. pH *maxima for digestion of proteins by extracts in* 2% *(w/v) sodium chloride of fundic and pyloric mucosa from normal human and pig stomachs*

In this table and the figures, the amounts of liberated amino acid nitrogen are those in the whole digest, which usually consisted of 3 ml. of buffer, 1 ml. of enzyme extract or gastric juice and 1 ml. of plasma or 5% (w/v) fraction V, or 2 ml. of 2% (w/v) casein.

		pH 1·5–2·4		pH 3·1–4·0	
Extract	Substrate	Maximum pH	Amino acid N (mg.)	Maximum pH	Amino acid N (mg.)
Human					
Fundus 1	Plasma protein	2·1	0·62	3·4	0·67
Fundus 2	Plasma protein	1·8	0·71	3·5	0·80
	Bovine plasma albumin	2·2	0·63	3·5	0·74
	Casein	2·0	0·22	3·9	0·42
Fundus 3	Plasma protein	2·4	0·74	3·6	0·77
Fundus 4	Plasma protein	1·8	0·63	3·4	0·83
Fundus 5	Plasma protein	2·0	0·48	3·5	0·56
Pylorus 2	Plasma protein	1·6	0·64	3·2	0·64
Pylorus 3	Plasma protein	1·5	0·60	3·1	0·78
Pylorus 4	Plasma protein	1·6	0·52	3·2	0·76
Pylorus 5	Plasma protein	1·6	0·17	3·2	0·23
Pig					
Fundus 1	Bovine plasma albumin, fraction V	2·2	0·38	3·9	0·48
Fundus 2	Fraction V	2·0	0·36	3·5	0·49
	Plasma protein	2·2	0·38	3·6	0·53
Fundus 3	Plasma protein	2·1	0·42	3·7	0·51
Pylorus 1	Fraction V	1·9	0·24	3·4	0·28
Pylorus 2	Plasma protein	1·7	0·34	3·3	0·41
Pylorus 3	Plasma protein	1·7	0·21	3·2	0·30

Swine pyloric juice. Swine pyloric juice digested serum albumin with two maxima at pH 1·6 and 2·6 (Fig. 3), that is to say, at lower pH values than have been given by any other materials studied in the pig.

Crystalline swine pepsin. Fig. 4 shows the pH–activity curves which result from the digestion of crystalline bovine plasma albumin by pepsin A and by the component of crystalline pepsin remaining after separation of pepsin A. Whereas the maxima for pepsin A occur at pH 1·6 and 2·8, those for the second component occur at pH 2·0 and 3·2. The former maxima are very close to those observed with pig pyloric juice, and the difference between the pH maxima of the two components resembles that seen between the electrophoretic components of human gastric juice (Taylor, 1959*b*) and that seen between fundic and pyloric mucosal extracts.

DISCUSSION

The demonstration that pyloric mucosal extracts and pyloric juice exert proteolytic activity are of interest, for relatively little work has been carried out upon the pyloric secretion and none upon the normal human pylorus. Furthermore, the conclusions to be drawn from previous studies are in many ways conflicting.

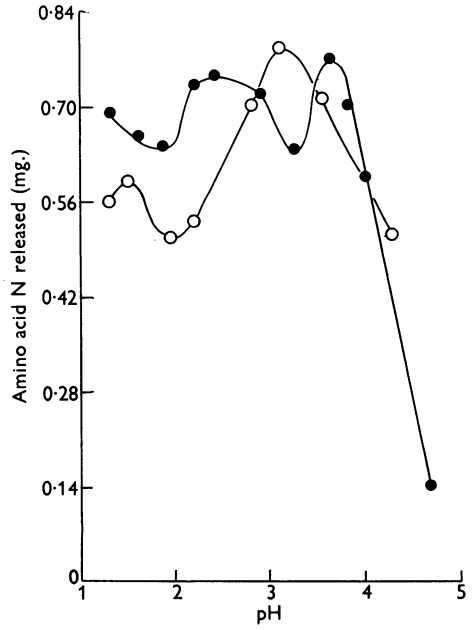

Fig. 1. pH–activity curves for the digestion of plasma protein by normal human fundic (●) and pyloric (○) mucosal extracts. Temp., 37°; time, 3 hr.

Wassman (1839) observed that acid extracts of pyloric mucosa digested protein, but more slowly than fundic extracts; after washing the pyloric mucosa three or four times with water he could no longer detect proteolytic activity. He concluded that only the fundic glands supplied pepsin, and that the activity of the pyloric mucosa was due to adherent fundic secretion. This view was supported by several authors (Wittich, 1873; Wolffhügel, 1873; Herrendorfer, 1875) but attacked by Ebstein & Grützner (1874), who found a digestive ferment constantly present in the pyloric mucosa of dogs and showed that it was contained in the deep glands, whereas the overlying mucous secretion was virtually pepsin-free. Glaessner (1902), in an important paper which summarizes previous work, concluded from observations in the dog, pig and rabbit that the pyloric mucosa contains a proteolytic ferment and that this exhibits differences from fundic pepsin. He named this enzyme pseudopepsin ('...und so führen diese Versuche zu der Folgerung, dass die peptische Wirkung der Pylorusmucosa nur durch die Anwesenheit von 'Pseudopepsin' zu erklären ist, und dass dieses aller Wahrscheinlichkeit nach das einsige peptische Ferment des Pylorus darstellt'). Glaessner produced no evidence that pseudopepsin could be secreted into gastric juice, but it is important to learn, in view of the observations described above, that the idea of separate fundic and pyloric pepsins was put forward as early as 1902. Glaessner also established that 'pseudopepsin' acts in an acid medium. Further confirmation of the proteolytic activity of pyloric mucosal extracts is given by the observations of Holter & Linderstrøm-Lang (1935), who found pepsin to be present most abundantly in swine pyloric mucosa, at a depth of 2 mm. from the surface. This is the depth at which most of the pyloric chief cells are to be found.

Pyloric juice itself has been collected from pyloric pouches of laboratory animals by a number of

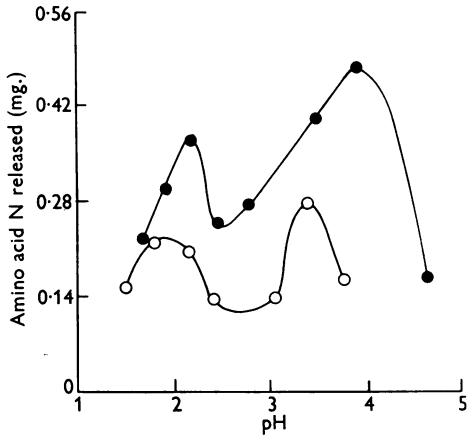

Fig. 2. pH–activity curves for the digestion of bovine plasma albumin fraction V by fundic (●) and pyloric (○) mucosal extracts of the pig. Temp., 37°; time, 3 hr.

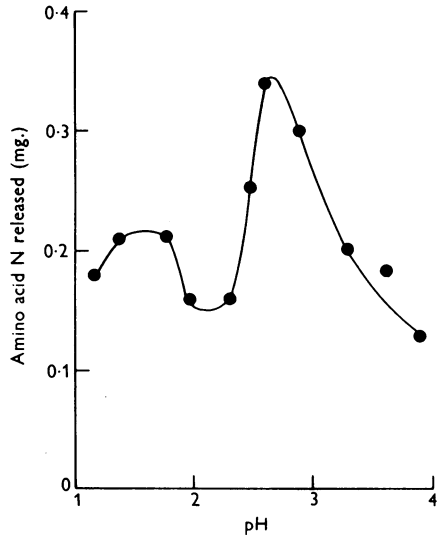

Fig. 3. pH–activity curve for the digestion of human serum albumin by swine pyloric juice. Temp., 37°; time, 3 hr.

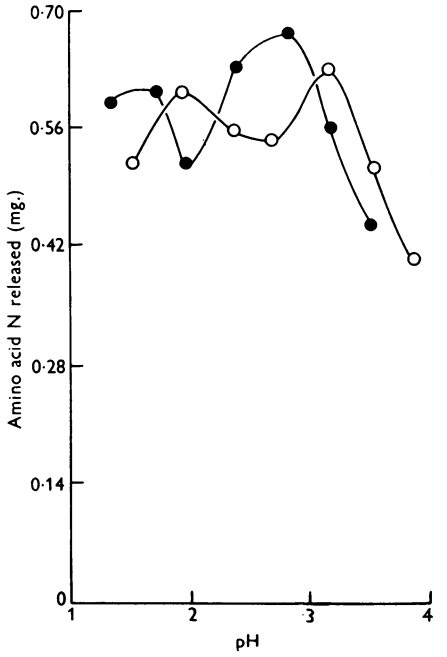

Fig. 4. pH–activity curves for the digestion of crystalline bovine plasma albumin by pepsin A (●) and by a second electrophoretically homogeneous component of crystalline swine pepsin (○). Temp., 37°; time, 3 hr.

investigators. All early experiments showed pepsin to be present in the secretion even though this was alkaline (Klemensiewicz, 1875; Heidenhain, 1878; Contejean, 1893; Akerman, 1894). Ivy (1919), in his early experiments, also found small quantities of pepsin to be present, but subsequently (Ivy & Oyama, 1921) modified this observation, concluding that pepsin was not present in the pyloric secretion of dogs. Takata (1923) repeated Ivy's work in the dog but concluded that an enzyme 'of the peptic character' was present in pyloric juice. More recently Jennings & Florey (1940) have found that the pyloric secretion in the cat is alkaline and has a trace of proteolytic activity at pH 2·0 but none at pH 6·8 and 8·0.

Although experimental results, even in the same animal, differ inexplicably, the balance of evidence favours the presence of proteolytic activity in pyloric mucosa and in pyloric juice. The evidence additionally presented here from the pig and man provide further support for this view, and make its alternative less plausible.

Not only do pyloric juice and pyloric extracts possess proteolytic activity, but it is now shown that they digest proteins with two pH maxima below pH 4, just as do human gastric juice, swine pepsin and human and swine fundic extracts. The pH values of the maxima for human and swine pyloric extracts and for swine pyloric juice are, however, consistently lower than those for the corresponding maxima of fundic extracts. It would seem therefore that the pepsin (or pepsins) of the fundic and pyloric glands are biochemically different in both species.

Evidence is also presented that crystalline pepsin and human gastric juice each contain two principal components, one of which has, in both species, pH maxima resembling those of fundic pepsin, and the other, again in both species, maxima resembling those of pyloric pepsin. One possible explanation of these facts might be that the respective components of human gastric juice and crystalline swine pepsin are derived from fundic and pyloric glands. Unfortunately no proof is possible in man that human pyloric juice has proteolytic activity with maxima at pH values close to those of human pyloric extracts, for pure human pyloric juice cannot be obtained. It has, however, been found (Taylor, 1956) that in pernicious anaemia, a small number of patients have appreciable proteolytic activity in their gastric juice and that in each one the pH maxima for digestion of proteins *in vitro* falls within the ranges pH 1·8–2·0 and 2·6–3·2. These ranges resemble those of pyloric mucosal extracts rather than of fundic extracts or of normal human gastric juice. It is known, moreover, that in pernicious anaemia the pyloric mucosa is sometimes not degenerated (Magnus & Ungley, 1938). If this were the case in these patients, the gastric juice secreted on histamine stimulation would be principally derived from the pyloric mucosa and would be expected to exert proteolytic action upon plasma protein with the pH maxima that have in fact been found.

Observations made by Linderstrøm-Lang, Holter & Ohlsen (1935) are in keeping with the possibility that more than one type of pepsin is present in gastric mucosa. They investigated the proteolytic activity of sections of swine fundic and pyloric mucosa which were cut at right-angles to the surface at progressive depths towards the submucosa. From the surface inwards they recognized epithelial cells, neck chief cells and chief cells as containing proteolytic activity. They observed that 'only the pepsin in the deeper layers of fundus can be extracted by 30% glycerine, while the pepsin of all the regions of tissue investigated (pylorus and fundus) is extracted quantitatively by edestin and hydrochloric acid'. It might be therefore that there are two types of chief cell, for example, those of the neck and those of the body of the gland, each yielding a slightly different type of pepsin. If one type were predominantly present in the fundus and body of the stomach and the other in the pylorus the results that have been obtained with fundic and pyloric mucosa would be explained. Such a hypothesis would be in keeping with the observation that in both human gastric juice and swine pepsin, the two components appear to be more nearly equal in amount than the respective sizes of the mucosal areas of fundus and pylorus. In this connexion it is interesting to find that Harvey (1906) in a histological study in the dog writes '. . . it appears that the pyloric glands correspond to the neck region of the fundus gland without the parietal cells'.

Finally it might now be possible to explain the observation made previously (Taylor, 1959*a*) that samples of gastric juice obtained at different times from the same subject show different pH maxima when digesting the same sample of substrate. If these samples contained differing amounts of the two pepsin components, variable pH maxima might be expected. The existence of two pepsins may perhaps also provide a basis for explaining why gastric enzymes other than 'pepsin', active at pH 2·0, have been described (Brücke, 1875; Sundberg, 1885; Kraut & Tria, 1937).

SUMMARY

1. Extracts of human and swine pyloric mucous membrane digest proteins with two pH maxima below pH 5. These maxima occur at lower pH values than the corresponding maxima for fundic extracts.

2. Swine pyloric juice also exhibited two proteolytic pH maxima, at pH values 1·6 and 2·6, which are lower than those of swine or human fundic extracts or of human gastric juice.

3. Crystalline swine pepsin can be separated into two components, one of which digested proteins with pH maxima close to those found with swine fundic mucosa and the other with maxima close to those found with pyloric mucosa.

4. It is concluded that in man and the pig there are two main pepsins, one of which predominates in the pyloric mucosa and the other in the mucosa of the fundus and body of the stomach.

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Formation Constants for the Complexes of Adenosine Di- or Tri-phosphate with Magnesium or Calcium Ions

By K. BURTON
Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Oxford

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The formation of complexes between adenosine phosphates and bivalent metal ions has been quantitatively studied by the effects of the metals on the acid–base titration curves (Smith & Alberty, 1956*a*; Martell & Schwarzenbach, 1956) and by the use of ion-exchange resins (DiStefano & Neuman, 1953; Nanninga, 1957; Walaas, 1957, 1958). These methods have been extensively used to study many chelation complexes but there is poor agreement between the several values that have been reported for the formation constants of the complexes of the adenosine phosphates. Further study of these complexes therefore seemed to be desirable and this paper describes measurements by an independent method in which the spectral changes of 8-hydroxyquinoline are used to determine the amount of free bivalent metal ion present in solutions containing adenosine di- or tri-phosphate and magnesium or calcium ions. For most of the measurements, tributylethylammonium bromide was added to obtain a convenient ionic strength (usually 0·11). The concentration of sodium or potassium ions was usually kept low because these ions form weak complexes with the nucleotides (Melchior, 1954; Smith & Alberty, 1956*b*).

MATERIALS AND METHODS

Nucleotides. The sodium salts of adenosine triphosphate (ATP; Schwarz Laboratories Inc., Mount Vernon, N.Y., U.S.A.) and adenosine diphosphate (ADP; Sigma Chemical Co., St Louis, Mo., U.S.A.) were dissolved in water and