GASTRIC PROTEOLYSIS IN DISEASE

1. THE PROTEOLYTIC ACTIVITY OF GASTRIC JUICE FROM PATIENTS WITH PERNICIOUS ANAEMIA

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Fenwick (1870) was the first to discover that patients with pernicious anaemia have a deficiency of gastric pepsin, and this observation has been amply confirmed (Stockton, 1904; Levine and Ladd, 1921; Teschendorf, 1927; Faber, 1927; Faber and Holst, 1928; Johansen, 1929; Pollard and Bloomfield, 1930; Castle, Heath, and Strauss, 1931; Davies, 1931; Wilkinson, 1932; Helmer, Fouts, and Zervas, 1932; Hartfall, 1933; Maltby, 1934; Griffiths, 1934; Mullins and Flood, 1935; Ihre, 1938; Witebsky, Klendshoj, and Vaughan, 1942; Janowitz and Hollander, 1951; Aitken, Spray, and Walters, 1954). Pepsin secretion is usually either absent or slight, whether the gastric juice is obtained in the resting state or after stimulation with histamine or insulin. Low values are still obtained when the stomach is washed with dilute HCl during the collection of juice in order to prevent the inactivation of pepsin at the neutral pH of gastric secretion in pernicious anaemia (Ihre, 1938). Occasionally patients have been observed who, despite achlorhydria, secrete appreciable though still sub-normal amounts of pepsin (Faber, 1927; Davies, 1931; Hartfall, 1933; Griffiths, 1934; Jones and Wilkinson, 1938; Aitken, Spray, and Walters, 1954; Taylor, 1956).

The proteolytic activity of the gastric juice in pernicious anaemia has also been investigated at neutral pH. In contrast to the mild proteolysis observed with normal gastric juice, no activity or decreased activity has been reported by Griffiths (1934), Helmer, Fouts, and Zervas (1932), Lasch (1937), and Taylor, Castle, Heinle, and Adams (1938). Slight activity, which does not significantly differ from that of normal subjects, has been observed by Maltby (1934), Emerson and Helmer (1936), Jones, Grieve, and Wilkinson (1938), and Gessler, Dexter, Adams, and Taylor (1940).

In all this work there has been an underlying assumption that the differences which exist between normal gastric juice and that from

patients with pernicious anaemia result from a simple quantitative deficiency of normal proteolytic activity. Usually determinations of proteolytic activity have only been carried out at pH 2.0 or pH 7.4. Only one pH activity curve for proteolysis by gastric juice from a patient with pernicious anaemia has been reported (Jones and Wilkinson, 1938). This showed a single maximum at pH 2.2, but no determinations were made between pH 3 and 4 where a second peak occurs in normal subjects (Taylor, 1959a). The possibility that the gastric juice in pernicious anaemia may contain different enzymes from normal gastric juice or differing proportions of normal enzymes does not seem to have been considered. An investigation of this possibility is now described.

Experimental

Determinations of proteolytic activity were carried out by formol titration using the technique already described (Taylor, 1957, 1959a). Samples of gastric juice from patients with pernicious anaemia were usually secreted at pH 6 to 8, brought quickly to a pH of about 4 with 0.1 M HCl, and stored at 0 to 4° C. until used.

Pernicious anaemia was diagnosed clinically by the presence of a macrocytic hyperchromic anaemia, a megaloblastic bone marrow, and a histamine-fast achlorhydria in the absence of clinical and laboratory evidence of steatorrhoea, and was confirmed by a positive response to treatment with vitamin B₁₂. In several patients the presence of subacute combined degeneration of the cord, the findings of a gastric biopsy, and the inability to absorb more than 40% of an oral dose of (⁶⁰Co)-vitamin B₁₂ have increased the certainty of the diagnosis.

Results

Action on Protein Substrates below pH 5.— Twenty-six samples of gastric juice from 23

TABLE I
PROTEINASE ACTIVITY BELOW pH 5 OF GASTRIC JUICE FROM PATIENTS WITH PERNICIOUS ANAEMIA*

Patient	Substrate	pH 1.5 to 2.4		pH 2.5 to 3.2		pH 3.3 to 4.0		pH 4.1 to 5.0	
		Maximum pH	Amino-acid N (mg.)	Maximum pH	Amino-acid N (mg.)	Maximum pH	Amino-acid N (mg.)	Maximum pH	Amino-acid N (mg.)
1. Histamine juice ..	Plasma protein	2.4	0.09	—	—	3.7	0.07	Inactive	
2. " " " "	" "	Inactive		Inactive		Inactive		"	
3. " " " "	" "	"		"		"		"	
4. " " " "	" "	"		"		"		"	
5. " " " "	Egg albumen	"		"		"		"	
6. " " " "	Plasma protein	2.0	0.35	3.0	0.32	Declining activity		Declining activity	
7. " " " "	Egg albumen	1.6	0.20	Declining activity		Inactive		Inactive	
8. " " " "	Plasma protein	1.8	0.10	2.6	0.08	"		"	
9. " " " "	" "	1.9	0.06	3.2	0.07	Declining activity		"	
10. " " " "	" "	2.0	0.08	3.2	0.11	"		"	
11. Insulin " " "	" "	Declining activity		2.7	0.29	"		"	
12. Histamine " " "	" "	"		3.2	0.08	"		"	
13. " " " "	" "	"		2.8	0.11	"		"	
14. " " " "	Egg albumen	2.0	0.04	Declining activity		Inactive		"	
15. " " " "	Plasma protein	Inactive		"		3.7	0.13	Declining activity	
16. " " " "	" "	"		"		3.6	0.09	"	
17. " " " "	" "	"		"		3.5	0.06	"	
18. " " " "	" "	" †		"		3.7	0.10	"	
19. " " " "	" "	"		"		3.5	0.07	Inactive	
20. " " " "	Serum albumin	Declining activity		2.7	0.23	Declining activity		"	
21. " " " "	" "	2.3	0.18	Declining activity		Inactive		"	
22. " " " "	Plasma protein	Inactive		"		Declining activity		4.4	0.09
23. " " " "	" "	"		"		"		4.2	0.07
	" "	" †		"		"		4.3	0.09
	" "	Declining activity†		"		"		4.6	0.10
	" "	Inactive		"		"		4.1	0.05
	" "	2.4	0.07	"		"		4.2	0.05
	Serum albumin	Declining activity		2.6	0.12	3.8	0.10	Declining activity	

* In the tables and figures the amounts of amino-acid nitrogen released are those in the whole digest, which usually consisted of 3 ml. of buffer, 0.5 to 2 ml. of gastric juice, and 1 ml. of plasma or 5% (w/v) albumin. 37° C., 3 hr.
† No determinations below pH 2.0.

patients were incubated with plasma protein, human serum albumin, or egg albumen at pH 1.5 to 5.0 for 3 hr. at 37° C. (Table I, Figs. 1 and 2). Only one patient (No. 1) exhibited the two normal

maxima, falling within the pH ranges 1.7 to 2.4 and 3.3 to 4.0, which have been established for normal gastric juice under similar experimental conditions (Taylor, 1956, 1959a). The remaining

FIG. 1.—The pH activity curves showing two maxima for the digestion of plasma protein by the gastric juice of two patients with pernicious anaemia (incubated at 37° C. for 3 hr.).

FIG. 2.—The pH activity curves showing single maxima for the digestion of plasma protein by the gastric juice of four patients with pernicious anaemia (incubated at 37° C. for 3 hr.).

patients fall into four main groups. Group I consists of three patients (Nos. 2, 3, and 4) in whom there was no demonstrable proteolytic activity of the gastric juice. Group II consists of four patients (Nos. 5 to 8) who exhibited two *pH* maxima but in whom the upper maximum occurred at *pH* 2.6 to 3.2. Group III comprises eight patients (Nos. 9 to 16) whose gastric proteolytic *pH* activity curves exhibited only a single maximum between *pH* 2.0 and 3.7. Group IV consists of five patients (Nos. 17 to 21) who exhibited only a single maximum at *pH* 4.1 to 4.6. In one patient from Group III and two from Group IV there was insufficient gastric juice to extend the *pH* activity curve below 2.0, so that the possibility of a second peak below this *pH* cannot be excluded. Apart from patients 5, 9, and 15, the amount of activity at the *pH* maxima was slight in comparison with that of normal subjects.

Action on Proteins above *pH* 5.—The histamine-stimulated gastric juices of 15 patients were incubated with plasma protein or human serum albumin at *pH* 5 to 8 (Table II). In all but one patient proteolytic activity was detected. *pH* maxima were exhibited at *pH* 6.8 to 7.1 in three patients, at *pH* 7.6 in eight patients, and at *pH* 7.9 in one. In this last patient the activity was high and the gastric juice also exhibited carboxypolypeptidase activity upon chloracetyl-L-tyrosine, indicating contamination with pancreatic juice.

Maxima sometimes occur with normal subjects at *pH* 6.8 to 7.2 (seven out of 14) and at *pH* 7.6 (three out of 14) (Taylor, 1959d) and it has been shown that the former is caused by an enzyme in

TABLE II
PROTEINASE ACTIVITY AT *pH* 5 TO 8 OF HISTAMINE GASTRIC JUICE FROM PATIENTS WITH PERNICIOUS ANAEMIA INCUBATED AT 37° C. FOR 3 HR.

Substrate Patient	Maximal <i>pH</i>	Amino-acid N Liberated (mg.)
<i>Plasma protein</i>		
2.	6.8	0.12
3.	7.6	0.09
7.	7.6	0.13
20.	7.6	Inactive
24.	7.6	0.15
25.	7.6	0.06
26. (Bile-stained)	6.9	0.20
27.	7.6	0.20
28.	7.6	0.29
29.	7.6	0.13
30.	7.6	0.16
	Active, no maximum	
<i>Serum albumin</i>		
24.	7.1	0.07
31.	Active, no maximum	
32.	"	"
33.	"	"
34. (Bile-stained)	7.9	0.67
35.	"	"

TABLE III
EFFECT UPON PROTEOLYTIC ACTIVITY OF NORMAL GASTRIC JUICE AT *pH* 2.5 OF ADDING TO DIGESTION MIXTURE GASTRIC JUICE FROM PATIENTS WITH PERNICIOUS ANAEMIA

Patient No. :	Formol Titration			
	12	18	21	4
4 ml. buffer + 1 ml. plasma + 1 ml. normal gastric juice + 2 ml. water. Incubated 37° C., 3 hr.	Mixed	11.93	12.09	12.35
1 ml. buffer + 1 ml. P.A. gastric juice. Incubated 37° C., 3 hr.				
5 ml. buffer + 1 ml. plasma + 1 ml. normal gastric juice + 1 ml. P.A. juice. Incubated 37° C., 3 hr. Then 2 ml. H ₂ O added				
	12.12	12.25	12.31	12.22

In a preliminary experiment 1 ml. of normal gastric juice incubated with 1 ml. plasma had produced an increased formol titration of 0.53 ml. N/10 NaOH. The values in the table are the means of duplicate estimations. Formol titrations expressed in ml. N/10 NaOH.

gastric juice, but that the latter probably results from contamination with a salivary enzyme. The observations in pernicious anaemia differ therefore from those of normal subjects only in the proportions of patients exhibiting these two maxima. They do not reveal the existence of abnormal *pH* activity curves.

Presence or Absence of an Inhibitor of Gastric Proteolysis.—A possible explanation of the occurrence of *pH* activity curves with single maxima in the region of *pH* 2.7 to 4.6 might be the presence in gastric juice in pernicious anaemia of a substance inhibiting peptic action at *pH* 1.5 to 2.5. This hypothesis has been tested at *pH* 2.5 by incubating normal human gastric juice with plasma protein alone and with gastric juice from each of four patients with pernicious anaemia. In each instance there was no decrease of formol titration in the tube containing pernicious anaemia juice and thus no evidence of inhibition of normal gastric proteolysis was obtained (Table III).

Proteolytic Activity of Normal Duodenal and Ileal Mucosal Extracts.—Biopsy and necropsy studies show that the gastric mucosa in pernicious anaemia occasionally contains patches of cells showing intestinal metaplasia. If these cells were to liberate a proteinase into the gastric juice, an unusual *pH* activity curve might appear. An attempt to evaluate this source of error was made by determining the proteinase activity of extracts of duodenal and ileal mucosa which had been removed at operation from patients with carcinoma of the stomach and ascending colon. The duodenal extracts exhibited two maxima (Taylor, 1959e) similar to those exhibited by

pyloric extracts (Taylor, 1959c) but quite different from the abnormal single maxima that occur in pernicious anaemia. Two ileal extracts, however, upon incubation with plasma protein, exhibited mild proteinase activity with a single maximum at pH 3.5 to 3.7. The possibility cannot therefore be excluded that abnormal curves with single maxima in this pH range result from secretion into the gastric juice of a proteinase derived from cells showing intestinal metaplasia, although proof of such a secretion, or of the identity of the ileal and the metaplastic cells, is lacking.

Discussion

The complete absence of proteolytic activity in the patients of Group I is not surprising in view of the existing evidence that peptic activity at pH 2 is frequently absent from the gastric juice of patients with pernicious anaemia. The unexpected feature is that the proportion of inactive juices was so low. However, had peptic activity been determined solely at pH 2, only 13 of 23 patients (Table I) would have been recorded as secreting pepsin in even minimal amounts. Estimations at pH 2.0 give, therefore, a false picture of the proteolytic power of the gastric juice in pernicious anaemia, for when activity was sought at pH 2.6 to 5.0 it was detected in as many as 20 out of 23 patients.

Those patients whose gastric juices were active below pH 5 exhibited a wide variety of pH activity curves. In order to interpret the abnormal curves, it is necessary to recollect certain features of the proteolytic activity of normal gastric juice. It has been established previously (Taylor, 1956, 1959a) that, under the same experimental conditions as those described in this paper, normal human gastric juice will digest plasma protein and human serum albumin below pH 5 with two maxima in the pH ranges 1.7 to 2.4 and 3.3 to 4.0. Egg albumen is digested with only one maximum at pH 1.5 to 1.8. It has also been shown (Taylor, 1959b) that the two maxima result from the presence in the pepsin molecule of two different types of active centre, and that at least two different pepsin molecules, each exerting two proteolytic maxima below pH 5, occur in normal gastric juice (Taylor, 1959c). One of these molecules preponderates in pyloric mucosa and the other in the mucosa of the body and fundus of the stomach. The "pyloric" pepsin exhibits maximal activity at pH values of 1.5 to 2.0 and 2.6 to 3.2, which are lower than those for normal gastric juice or for fundic mucosal extracts.

The patients of Group II thus exhibit pH activity curves which closely resemble those obtained with extracts of human pyloric mucosa. One hypothesis that would account for such curves is that the peptic activity in these four patients is derived from pyloric mucosal cells. This hypothesis is supported by the knowledge that in man (Fox and Castle, 1942) the fundus and body of the stomach contain intrinsic factor whereas the pyloric mucosa does not, and that in pernicious anaemia the fundus and body mucosa degenerates but the pyloric mucosa is unaffected (Magnus and Ungley, 1938). Were this the case in these patients, the gastric juice secreted after 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.

Three hypotheses may be put forward to explain the occurrence of abnormal pH activity curves with single maxima. The first of these, the presence of a substance inhibiting one of the two normal pH maxima, must, from the experimental evidence, be abandoned. The second, that cells showing intestinal metaplasia secrete an abnormal enzyme, could only account for the curves with maxima at pH 3.5 to 3.7, observed in three patients. The hypothesis would not account for the existence of pH activity curves with single maxima below 3.2 or above 4.0. The third hypothesis is that the gastric mucous membrane in pernicious anaemia may secrete abnormal gastric proteinases, i.e., enzymes which have a different chemical structure from the two normal pepsins. The curves of Group III would be explained if such abnormal enzymes consisted of fragments of normal pepsins in which only one active centre was present or if they were comprised of more or less normal pepsins in which one active centre was absent. The curves of Group IV would, however, only be explained by the production of a pathological enzyme with a different active centre from those normally present. This last explanation is the only one that would satisfy all the experimental observations of Groups III and IV.

The concept of the production by diseased tissues of abnormal and pathological enzymes will need to be confirmed by other evidence, such as the isolation and characterization of the enzymes themselves, before it can be regarded as firmly established. It should be noted, however, that the pathological features of pernicious anaemia are compatible with the possible elaboration and secretion of abnormal enzymes. Thus the gastric

mucous membrane is degenerated and produces enzymes in an environment that is deficient in vitamin B₁₂, in folic acid, and perhaps also in oxygen. The disordered protein synthesis which the elaboration of an abnormal enzyme implies might well arise under such conditions. Lastly, it may be pointed out that, although the existence of abnormal enzymes in disease is not well recognized, the elaboration of abnormal proteins is widely accepted, for example, the Bence Jones protein of multiple myelomatosis, the production of cryoglobulins in liver disease, or the presence in many diseases of abnormal haemoglobins in erythrocytes.

Summary

The proteolytic pH activity curves for gastric juice from patients with pernicious anaemia have been determined below pH 5 in 23 patients and between pH 5 and 8 in 15.

Below pH 5 only one patient exhibited a normal curve with two proteolytic maxima in the pH ranges 1.5 to 2.4 and 3.3 to 4.0.

Of the remaining patients, three secreted inactive juices, four secreted juices with activity resembling that of normal pyloric mucosal extracts, and 13 secreted juices exhibiting only single maxima. In five of these 13 patients the single maximum occurred above pH 4 and thus at a point where normal gastric proteolytic activity declines rapidly.

The abnormal pH activity curves are tentatively explained as follows:

(a) In those patients who show no activity, the fundic and pyloric glands are completely atrophic.

(b) In those who show the pyloric type of activity, the pyloric mucous membrane is preserved.

(c) In those who show abnormal maxima, abnormal enzymes are elaborated and secreted.

Determination of proteolytic action only at pH 2 gives an underestimate of the proteolytic power of the gastric juice in patients with pernicious anaemia.

The gastric juice of only one of 15 patients failed to show proteolytic activity at pH 5 to 8. Maxima occurred in three patients at pH 6.8 to 7.1 and in eight at pH 7.6. In normal subjects maxima occur in the same pH ranges, but that at pH 6.8 to 7.1 is the more common.

Some implications of the concept that abnormal enzymes may occur in human disease are considered.

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Addendum

Since this paper was written, Martin (1958) has drawn attention to the change of substrate pattern for serum pseudocholinesterase found in liver disease by Kekwick (1955) and has pointed out that one explanation of these results might be the release from the damaged liver cell of a "broken-backed" enzyme."

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