

# pH stability and activity curves of pepsin with special reference to their clinical importance

D. W. PIPER AND BARBARA H. FENTON

*From the Department of Medicine, the University of Sydney, and the Unit of Clinical Investigation, the Royal North Shore Hospital of Sydney, Sydney, Australia*

**EDITORIAL SYNOPSIS** A study of the effect of pH on the stability and activity of pepsin showed that 70% of maximal peptic activity was still present at pH 4.5 and that pepsin was irreversibly inactivated at pH 8. The clinical implications of these findings are discussed.

The concept of peptic ulceration and its treatment is based on the ulcerogenic action of acid and pepsin (Card, 1952). It appears that pepsin plays the predominant role in this action (Schiffirin, 1940; Schiffirin and Warren, 1942; Le Veen, 1947), the acid merely providing the correct pH for optimal peptic activity. Consequently, it is of some importance to know the effect of pH on the peptic activity of pepsin. One of these effects, the effect of pH on peptic activity, has been adequately studied (Christensen, 1955; Taylor, 1959a, b and c); the effect of pH on enzyme stability has received little attention apart from the observations of Langley in the last century (Langley, 1881a and b) and those of Northrop, Kunitz, and Herriott (1948). As both of these effects are of considerable importance in clinical gastroenterology, the present study was undertaken of the effect of pH on the stability and activity of pepsin.

## METHODS

**EFFECT OF pH ON THE STABILITY OF PEPSIN** Solutions of pepsin were incubated at 37°C. for 10 minutes at varying pH levels, then titrated to pH 2 and peptic activity estimated using the radioiodinated serum albumin method (Klotz and Duvall, 1957; Piper, 1960). The peptic activity in these reaction mixtures was compared, after corrections had been made for the dilution involved in the back titration, with the peptic activity of a standard solution that had not been incubated at any different pH. The solutions of pepsin used were Parke Davis reference pepsin (400 mg.%) and unpurified solutions of human pepsin obtained from the homogenate of human gastric fundic mucosa.

As the time the pepsin was at the high pH could be crucial, a solution of Parke Davis reference pepsin and of human pepsin was incubated at pH 7.2 for varying times (1, 2.5, 5, 10, 20, 40, and 60 min.) and the effect on peptic activity on restoration to pH 2 determined as above.

**EFFECT OF pH ON PEPTIC ACTIVITY** A pH activity curve for human pepsin was determined using radioiodinated serum albumin as substrate and the method previously described (Piper, 1960). The pepsin was that of gastric juice obtained from a patient suffering from a gastric ulcer. The pH of the reaction mixture was adjusted using varying proportions of buffers, 0.1 M glycine HCl or 0.2 M acetate HCl buffers, as described by Taylor (1959a).

## RESULTS

The result of the studies on the effect of pH on peptic activity and stability is shown in Figure 1. The pH stability curve in this figure represents that of pepsin in human fundic mucosa; Parke Davis reference pepsin gave an identical curve. These results show that pepsin is stable at pH ranges as high as pH 6. Above this pH, pepsin is rapidly irreversibly inactivated and elevation of pH of the reaction mixture to pH 8 results in complete inactivation of pepsin. The pH activity curve shows a maximum at pH 2 and almost no peptic activity at pH 5.5; 70% of the maximal peptic activity is still present at pH 4.5. The shape of the curve is consistent with two maxima being present, the major one at pH 2 and a second partially obscured one at pH 4.

Investigation of the time necessary for the inactivation of pepsin by pH as depicted in the pH stability curve shows that the reaction is almost instantaneous. Incubation at the higher pH at intervals of from one to 60 minutes produces a similar degree of denaturation of pepsin.

## DISCUSSION

The pH of the environment of an enzyme may affect the activity of the enzyme in several ways. First, each enzyme has its pH optimum, at which the

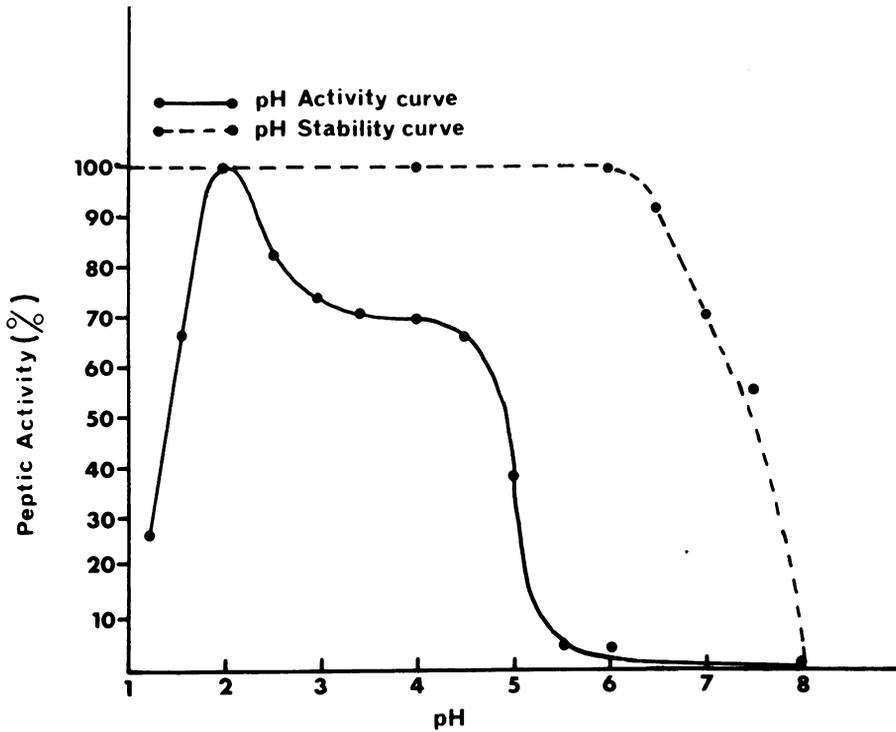


FIG. 1. The pH stability and pH activity of pepsin.

enzyme is most active, but the enzyme is stable within certain limits on each side of the optimum and if not at the pH where full activity is possible, adjustment of the pH of the reaction mixture to the optimum pH will restore maximal activity. Secondly, the environmental pH of the enzyme may influence its stability, and at extremes of acidity or alkalinity the enzyme may be denatured. Thirdly, the pH of the reaction mixture may cause dissociation of the substrate and so by its action on the substrate influence the character of the pH activity curve and the pH optimum. These factors have been discussed by Fruton and Simmonds (1958).

The pH activity curve has been fully defined using modern techniques and several substrates (Christensen, 1955; Taylor, 1959a and b; Piper, 1960). The variations found are considerable and are related to the method of measuring peptic activity and the origin of the pepsin. The pH stability curve has received little attention. The only satisfactory studies were those of Northrop *et al.* (1948), who, using swine pepsin, found pepsin inactivation and denaturation increased at pH levels above 6.8 and that the percentage of loss of activity was equal to the percentage of protein that had been denatured, and of Michaelis (1918), who found that the rate of inactivation of pepsin was proportionate to the fourth power of the hydroxyl ion concentration

between pH 6.0 and 8.0. The early observations of Langley (1881a and b) are consistent with our observations and those of Northrop *et al.* (1948), but were based on rabbit and sheep pepsin, and the pH produced by the addition of  $\text{Na}_2\text{CO}_3$  to the reaction mixture is not recorded.

If we study on a composite diagram the pH activity and stability curves, we find pepsin pH relationship may be divided into four parts:

- 1 pH 1.5 to pH 2.5, the range of maximal peptic activity: the actual pH optimum will vary slightly with the method used.
- 2 pH 2.5 to pH 5, a range where approximately 70% of maximal peptic activity persists.
- 3 pH 5 to pH 6.5 to 7.5, a range within which no peptic activity is possible but within which range pepsin is stable and restoration of the pH to pH 2 restores maximal peptic activity.
- 4 pH levels above 7.5 where pepsin is irreversibly inactivated.

The effect of pH on peptic activities has several applications in clinical medicine.

**IN THE TREATMENT OF PEPTIC ULCERATION** The effect of pH on pepsin perhaps attains its greatest practical application in antacid therapy. The latter forms the basis of the medical treatment of peptic ulcer and various antacids vary in the extent to which they may potentially raise the pH of gastric juice. This

varies from a maximum of  $pH$  4 attained by aluminium hydroxide gel to a maximum of  $pH$  8.5 that may be produced by sodium bicarbonate. Consequently some antacids may exert their effect merely by altering  $pH$  activity and others by altering both the  $pH$  activity and the  $pH$  stability. The high  $pH$  peaks that may be achieved by some antacids such as sodium bicarbonate therefore not only have the effect of inhibiting temporarily  $pH$  activity but also lead to the irreversible alkali denaturation of all pepsin present in the stomach at that time.

In the past the maintenance of the  $pH$  of the gastric contents above  $pH$  7 has been discouraged by the concept of acid rebound. Recent investigations have, however, shown that this phenomenon does not occur (Pereira-Lima and Hollander, 1959a and b; Gillespie, 1959). Probably too, in deference to this concept, some methods of antacid assay have stipulated that the  $pH$  of the reaction mixture must not be elevated above 5 by the antacid (*Brit. Pharm.*, 1963). The observations in this study show that elevation of the  $pH$  of the gastric contents above 8, even if transiently, not only inhibits peptic activity but leads to irreversible denaturation of pepsin. This is clearly an effect which antacid therapy should aim to achieve, assuming this effect can be attained without systemic effects.

The  $pH$  activity curve constructed in this study shows that the  $pH$  of the reaction mixture (or gastric juice) must be above  $pH$  5.5 if complete inactivation of pepsin is to be produced. This is higher than the  $pH$  level usually considered essential; Hollander in 1939 considered the crucial  $pH$  level to be  $pH$  4.5 which he termed the proteolytic neutralization point. Our findings of considerable peptic activity in the  $pH$  range of  $pH$  4-5 are consistent with the observations of Taylor (1959a, b and c) who found that in many preparations containing pepsin considerable peptic activity was still present in the  $pH$  range above 4.5.

**PEPTIC ACTIVITY IN ACHLORHYDRIC STATES** Pepsin is secreted as pepsinogen; the conversion of pepsinogen to pepsin begins slowly at  $pH$  6 and is almost instantaneous at  $pH$  2.0 (Ege, 1923; Ege and Menck-Thygesen, 1933). Accepting achlorhydria as defined by Card and Sircus (1958) as failure of the  $pH$  to drop below 6.0 after maximal histamine stimulation, pepsinogen would only be converted to pepsin to a slight degree in the achlorhydric state. The  $pH$  found in the stomach in achlorhydric patients in the basal state usually varies between  $pH$  6 and  $pH$  8.5 and consequently any pepsin formed would probably be irreversibly inactivated by normal intragastric  $pH$

fluctuations. This supports the clinical observation of the failure of peptic ulceration to occur in the achlorhydric patient (Kahn, 1937).

The work was supported by a grant from the Post Graduate Committee of the University of Sydney. The helpful advice of Professor C. R. B. Blackburn, Professor of Medicine, University of Sydney, is gratefully acknowledged.

#### REFERENCES

- British Pharmacopoeia* (1963). The Pharmaceutical Press, London, pp. 33.
- Card, W. I. (1952). Aetiology (of peptic ulcer). In *Modern Trends in Gastroenterology*, 1st series, edited by F. Avery Jones, pp. 380-388. Butterworth, London.
- and Sircus, W. (1958). Anacidity. In *Modern Trends in Gastroenterology*, 2nd series, edited by F. Avery Jones, pp. 177-192. Butterworth, London.
- Christensen, L. K. (1955). Concerning the  $pH$  optimum of peptic hydrolysis. *Arch. Biochem.*, **57**, 163-173.
- Ege, R. (1923). Eine Modifikation des Fuldscen Verfahrens zur Bestimmung von Pepsin. *Hoppe-Seylers Z. physiol. Chem.*, **127**, 125-136.
- and Menck-Thygesen, P. (1933). Über die Aktivierung des Propepsins. *Biochem. Z.*, **264**, 13-23.
- Fruton, J. S., and Simmons, S. (1958). *General Biochemistry*, 2nd ed., p. 272. Wiley, New York.
- Gillespie, I. E. (1959). Influence of antral  $pH$  on gastric acid secretion in man. *Gastroenterology*, **37**, 164-168.
- Hollander, F. (1939). What constitutes effective neutralization of gastric contents. *Amer. J. dig. Dis.*, **6**, 127.
- Kahn, J. R. (1937). Absence of peptic ulcer in pernicious anemia. *Amer. J. med. Sci.*, **194**, 463-466.
- Klotz, A. P., and Duvall, M. R. (1957). The laboratory determination of pepsin and gastric juice with radioactively iodinated albumin. *J. Lab. clin. Med.*, **50**, 753-757.
- Langley, J. N. (1881a). On the histology of the mammalian gastric glands, and the relation of pepsin to the granules of the chief-cells. *J. Physiol. (Lond.)*, **3**, 269-291.
- (1881b). On the destruction of ferments in the alimentary canal. *Ibid.*, **3**, 246-268.
- LeVeen, H. H. (1947). Chemical, physiological, and pathological observations on the role of pepsin and hydrochloric acid in the production of experimental ulcers. *Gastroenterology*, **8**, 648-661.
- Michaelis, L. (1918). Die Bestimmung und Bedeutung der Fermente im Magensaft. *Dtsch. med. Wschr.*, **44**, 685-689.
- Northrop, J. H., Kunitz, M., and Herriott, R. M. (1948). *Crystalline Enzymes*, 2nd ed., Columbia University Press, New York.
- Pereira-Lima, J., and Hollander, F. (1959a). Gastric acid rebound—a review. *Gastroenterology*, **37**, 145-153.
- , — (1959b). Basal secretion of gastric acid following administration of alkali (acid rebound). *Ibid.*, **37**, 154-157.
- Piper, D. W. (1960). The estimation of peptic activity in gastric juice using radioiodinated serum albumin as substrate. *Ibid.*, **38**, 616-621.
- Schiffman, M. J. (1940). Production of experimental jejunal ulcer. *Proc. Soc. exp. Biol. (N.Y.)*, **45**, 592-594.
- and Warren, A. A. (1942). Some factors concerned in the production of experimental ulceration of the G-I tract in cats. *Amer. J. dig. Dis.*, **9**, 205-209.
- Taylor, W. H. (1959a). Studies on gastric proteolysis. I. The proteolytic activity of human gastric juice and pig and calf gastric mucosal extracts below  $pH$  5. *Biochem. J.*, **71**, 73-83.
- (1959b). Gastric proteolysis in disease. II. The proteolytic activity of gastric juice and gastric mucosal extracts from patients with chronic gastric and duodenal ulcer. *J. clin. Path.*, **12**, 338-343.
- (1959c). Gastric proteolysis in disease. III. The proteolytic activity of gastric juice in chronic hypochromic anaemia and in idiopathic steatorrhoea. *Ibid.*, **12**, 473-476.