

# Measurement of Pepsin in Porcine Gastric Juice

JOAN FOURIE, R. S. ARNOT, J. CARTER, ROSEMARY HICKMAN,  
J. TERBLANCHE

## SUMMARY

The method for measurement of pepsin in human gastric juice as devised by Anson and modified by Berstad has been used for testing pepsin secretion from Heidenhain pouches in pigs. Twenty samples may be analysed at one time with accuracy. Variations in ambient temperature and humidity, and contamination of samples with bile, were found to interfere with the accuracy of the method. There was no change in activity after immediate freezing at  $-20^{\circ}\text{C}$  or after storage at  $-20^{\circ}\text{C}$  for up to 3 months. After repeated thawing and refreezing on 4 consecutive days, there was a fall in activity on the fourth day only.

*S. Afr. Med. J.*, **48**, 1873 (1974).

The pig is unique among large animals in its tendency to develop spontaneous gastric ulcers.<sup>1</sup> This fact has stimulated its use for experimental studies of gastric secretion which have been performed in our laboratory, using porcine Heidenhain pouches,<sup>2</sup> and in the course of these studies, pepsin was measured.

In one previous study of pepsin in pig gastric juice<sup>3</sup> the original method of Anson<sup>4</sup> was used. A modification of this method to study human gastric juice has recently been described by Berstad.<sup>5</sup> This article relates certain refinements of Berstad's method which resulted in reproducible values being obtained in porcine gastric juice. The effects of prolonged storage and repeated freezing and thawing are also described.

## METHOD

### Principle

Acidified human haemoglobin was used as substrate for pepsin activity. Trichloro-acetic acid-soluble products resulting from the proteolytic action of pepsin (including tyrosine and phenylalanine) were measured at 280 nm in comparison with the effect of a standard crystalline pepsin solution.

### Departments of Surgery and Chemical Pathology, University of Cape Town

JOAN FOURIE, B.S.C.

R. S. ARNOT, CH.M., F.R.C.S.

J. CARTER, M.B. CH.B.

ROSEMARY HICKMAN, M.D., CH.M.

J. TERBLANCHE, CH.M., F.C.S. (S.A.), F.R.C.S.

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Reprint requests to: Dr Rosemary Hickman, Department of Surgery, University of Cape Town Medical School, Observatory, Cape.

## Preparation of Reagents and Samples

**Haemoglobin:** One litre of citrated human blood was centrifuged at 3 000 rpm to remove plasma and leucocytes, and the red cells washed six times with 1 litre 0,9% saline. The final volume of red cells was measured and two volumes of distilled water added to lyse the cells. The pH was adjusted to  $5,8 \pm 0,1$  with 0,2N NaOH to precipitate the cell stroma. Complete separation was essential and the supernatant was collected after further centrifugation at 3 000 rpm for 30 minutes. The pH was readjusted to  $7,1 \pm 0,1$  and the solution was again centrifuged for the same period. Final haemoglobin concentration was measured by standard technique<sup>6</sup> and the clear solution was stored in aliquots of 25 ml at  $-20^{\circ}\text{C}$ . Immediately before use it was thawed and the haemoglobin concentration diluted with distilled water to 2,5 g/100 ml. This diluted substrate was stable at  $4^{\circ}\text{C}$  in the refrigerator for 1 - 2 days.

**Blank:** A pooled sample of gastric juice was used for the estimation of blank values.

**Standard:** Pepsin (3 × crystallised; Miles Laboratories) 0,015 g was dissolved in a little distilled water and made up to 50 ml with 0,01M HCl. This was diluted 1:10 with 0,01M HCl (pepsin concentration 3,0 mg/100 ml) and four standards were prepared for each test with 1,5 ml dilute standard plus 1,0 ml 0,01M HCl (pepsin concentration 1,8 mg/100 ml). The preparation was made just prior to use, since activity fell slowly after acidification (see Results).

**Other reagents** were: 0,3N HCl and 0,3N TCA (accuracy is important, as stressed by Berstad).<sup>5</sup>

## Gastric Juice Samples

### Test:

	Std (ml)	Blank (ml)	Test (ml)
Hb (2,5 g/100 ml)	2,0	2,0	2,0
0,3N HCl	0,5	0,5	0,5
Incubation at $25^{\circ}\text{C}$ for exactly 5 minutes			
Add 0,3N TCA	—	5,0	—
Diluted filtered gastric juice (1:50)	0,5	0,5	0,5
Incubation at $25^{\circ}\text{C}$ for exactly 10 minutes			
Add 0,3N TCA	5,0	—	5,0

Shake well and filter through Whatman No. 1 paper. Read against water at 280 nm using matched quartz cuvettes (1,0 cm).

**Calculation:**

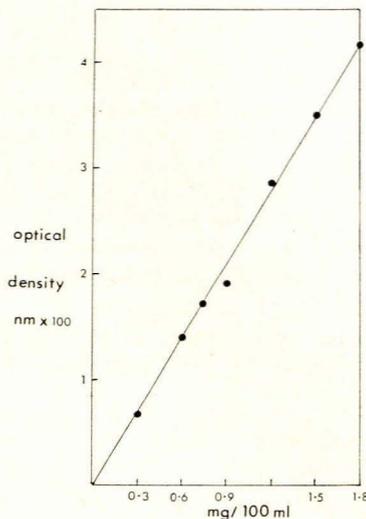
$$\frac{A_{\text{test}} - \text{mean } A_{\text{test blank}} \times \text{conc. of STD}}{\text{mean } A_{\text{STD}} - \text{mean } A_{\text{STD blank}}} \times 50 \text{ dilution factor}$$

and values expressed as mg pepsin/100 ml (A = absorbance).

**RESULTS**

**Assessment of Accuracy and Reproducibility of Test**

One hundred samples of gastric juice were randomly selected for duplicate analysis and no significant difference was found ( $P > 0.3$ ). The standard curve for pepsin is shown in Fig. 1. The range of values obtained in these studies of secretion from Heidenhain pouches under histamine stimulation was 10-100 mg/100 ml. With dilution, all readings could be made with reproducibility. Further details of the study of Heidenhain pouch secretion are reported elsewhere.<sup>7</sup>

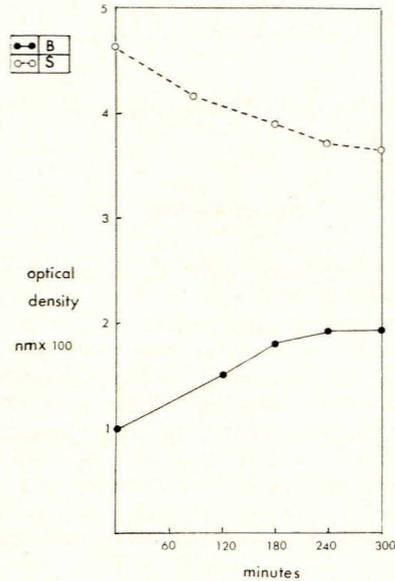


**Fig. 1. The standard curve for crystalline pepsin.**

Porcine gastric juice from the intact stomach was found to be contaminated with bile<sup>7</sup> and a comparison was made between samples collected from a gastric fistula and from the Heidenhain pouch. Minimal pepsin activity was detectable in samples contaminated with bile.

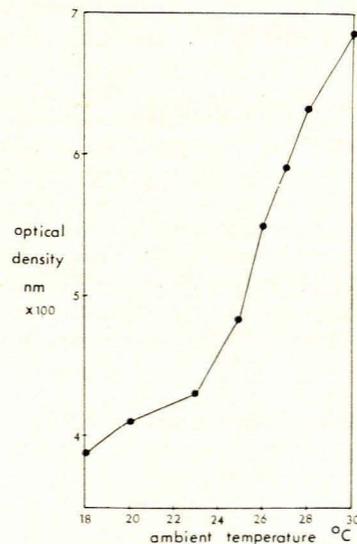
**Other Factors Influencing Accuracy**

**Acidification:** Whereas haemoglobin at room temperature was stable, on acidification there was a rise in blank values with time (Fig. 2), especially if incubated at 25°C



**Fig. 2. The effects of acidification upon blank (B) and standard (S) readings.**

or if bench temperatures exceeded this. This fact may not previously have been documented for climatic reasons, since previously published methods were reported from temperate countries. It is suggested that haemoglobin should be acidified within one hour of running the test. Readings of standards fell with time after acidification (Fig. 2). Sample values were not affected by short periods of storage after acidification, but Hunner *et al.*<sup>3</sup> and



**Fig. 3. The effect of ambient temperature upon readings of pepsin standard.**

Berstad<sup>8</sup> showed the effects of long storage on acidified samples.

**Temperature and humidity:** Berstad reported that major causes of inaccuracy were variations in temperature of the water bath or TCA concentrations. These observations, together with the fact that the rate of enzyme activity is measured over an arbitrary period and not carried to conclusion, militate for implicit accuracy in all timing.

Increased ambient temperature caused a rise in pepsin standard absorbance (Fig. 3) as did extreme humidity, and since pepsin is hygroscopic, it was stored in a dessicator and weighed just before use.

All samples, standards, reagents and the haemoglobin substrate were kept on ice and the laboratory temperature was controlled to 20°C. The acidified haemoglobin substrate was warmed to 25°C for 5 minutes just before use.

### Effects of Freezing and Thawing

**Initial freezing:** Most of the samples assayed in this study had been frozen within 6 hours of collection and were tested within 10 days. Two samples were studied for the effect of immediate freezing upon pepsin activity. Sample A (high pepsin value) was tested without freezing, immediately and after storage at 4°C for 6 hours after collection. Aliquots were stored at -20°C and were assayed at 3 and 7 days. Sample B (low pepsin value) was tested without freezing, immediately and after storage at 4°C for 6 hours after collection, and stored aliquots were assayed at 5 days and 1 month. There was no change in activity—in sample A, the mean values immediately, at 6 hours, and 3 and 5 days, ranged from 96,6 to 98,9 mg/100 ml (individual values ranged from 89 to 103 mg/100 ml). In sample B, mean values ranged between 43,0 and 45,3 mg/100 ml (individual sample range 39 - 49 mg/100 ml).

**Prolonged storage:** In 125 samples, pepsin values were measured after initial freezing, and again after storage at

-20°C for 3 months. There was no significant fall in activity ( $P > 0,4$ ).

**Repeated freezing and thawing:** The effect of repeated freezing and thawing for 4 consecutive days was assessed in 20 samples. For the first 3 days there was no alteration, but on day 4 a significant decrease in activity occurred ( $0,0125 < P < 0,025$ ). Thus a sample could be measured on two or three occasions without loss of activity. Berstad<sup>8</sup> noted a loss of activity with prolonged storage of human gastric juice and Hunner *et al.*<sup>3</sup> confirmed the observation in canine juice. In the latter study, samples were acidified before storage. In our report, gastric juice samples were stored undiluted.

### CONCLUSION

It is concluded that with care and recognition of certain influences pepsin may be accurately measured in porcine gastric juice. In addition, samples may be frozen initially, and stored for up to 3 months without loss of activity. Freezing and thawing may be performed on the sample up to three times without loss of activity, but thereafter, values fall.

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