Protein content of gastric juice

Part I A study of the validity of the biuret reaction in determining protein concentration after peptic digestion and acid hydrolysis of protein

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EDITORIAL SYNOPSIS Initially it is demonstrated that the biuret method is an accurate method of determining protein concentration when the protein has been subjected to varying degrees of acid hydrolysis and peptic digestion. Using continuous gastric suction, and the biuret method of measuring protein concentration, the protein content of gastric juice in a series of conditions was determined. Gastric cancer patients have a higher basal protein concentration than control subjects and duodenal ulcer patients and gastric ulcer patients have a higher protein concentration than duodenal ulcer patients. In stimulated gastric juice, duodenal ulcer patients have a lower protein concentration than gastric ulcer and gastric carcinoma patients and control subjects. The basal protein outputs were similar in the four groups studied.

There was no correlation between the protein and D.N.A. content of gastric juice. The protein lost into the stomach may vary between 1 and 16 g. per day.

Proteins in gastric juice are in most patients rapidly subjected to varying degrees of peptic digestion and acid hydrolysis. Because of this, methods of detecting and measuring protein in gastrointestinal secretions have been in the past isotopic, involving either labelled PVP or albumin (Gordon, 1959; Citrin, Sterling, and Halstead, 1957; Jeejeebhoy and Coghill, 1961). However, most methods of estimating protein concentration depend upon the biuret reaction, this reaction depending upon the violet colour with copper sulphate in alkaline solution given by all compounds with peptide bonds linked directly or through an intermediate carbon atom (White, Handler, Smith, and Stetton, 1954). This method would clearly be applicable to the estimation of protein in gastric juice, assuming that peptic digestion does not alter the apparent protein concentration as determined by this reaction. As this method had not previously been applied to the investigation of gastric juice protein and as evidence was not available that peptic digestion and acid hydrolysis of protein did not alter the colour reaction produced in the biuret reaction, the present study was undertaken of the effect of peptic digestion

and acid hydrolysis of protein on its apparent concentration as determined by the biuret reaction and the method of Gornall, Bardawill, and David (1949).

METHOD

The albumin used was human albumin prepared by the New South Wales Red Cross Blood Transfusion service, and the pepsin was Armour reference pepsin.

EFFECT OF PEPTIC DIGESTION OF ALBUMIN A solution of albumin (600-800 mg. %) at pH 1.5 was prepared and Armour reference pepsin added to a final concentration of 100 mg. %. An aliquot was immediately diluted 1 : 4 with 0.5 N NaOH and protein concentration estimated by the biuret reaction and the method of Gornall *et al.* (1949). The solution was incubated at 37°C. for 24 hours, and, at intervals of 30 minutes, one hour, two hours, four hours, and 24 hours, aliquots of the reaction mixture were taken, diluted 1 : 4 with 0.5 N NaOH as before, and the concentration of protein in terms of that in the original solution determined.

To demonstrate activity of the pepsin and digestion of the albumin, the digest after 24 hours' incubation was titrated to pH 7, dialysed, lyophilised and subjected to

paper electrophoresis according to the method of Piper, Stiel, and Builder (1962).

To confirm the results with human gastric juice, the protein concentration of a sample of gastric juice was determined immediately on collection, the gastric juice being neutralized immediately on collection. An aliquot of the same sample but not neutralized was incubated at 37°C. at its natural pH (pH 2) for six hours. The protein concentration was determined in the neutralized and acid-incubated specimens and compared after corrections had been made for the dilution factors introduced.

ACID HYDROLYSIS OF ALBUMIN Solutions of human albumin of concentration 600-800 mg. % were subjected to acid hydrolysis under the following conditions:

- 1 Albumin dissolved in 1N HCl at 37°C.
- 2 Albumin dissolved in 3N HCl at 37°C.
- 3 Albumin dissolved in 1N HCl at 80°C.
- 4 Albumin dissolved in 3N HCl at 80°C.

The protein concentration was determined initially with the albumin dissolved in distilled water and in the four reaction mixtures given above at intervals of 30 minutes, one hour, two hours, four hours, and 24 hours after the beginning of incubation. At those intervals

aliquots of the reaction mixture were removed, titrated to pH 7, and the protein concentration determined by the biuret reaction (Gornall *et al.*, 1949). The effect of the acid hydrolysis on the electrophoretic properties of the albumin was determined as described above.

RESULTS

The effects of peptic digestion and acid hydrolysis of albumin on its concentration as determined by the biuret reaction are given in Table I.

The effect of these procedures on the electrophoretic properties of albumin is shown in Figure 1. These results show:

Peptic digestion of albumin for six hours did not alter the biuret reaction; electrophoretically, this degree of digestion resulted in partial digestion of the albumin and the appearance of several other electrophoretic bands.

Acid hydrolysis in 1N HCl and in 3N HCl at 37°C. for 24 hours did not alter the biuret reaction; electrophoresis showed that the resultant band

TABLE I
EFFECT OF PEPTIC DIGESTION AND ACID HYDROLYSIS ON THE CONCENTRATION OF ALBUMIN AS DETERMINED BY THE BIURET REACTION

Peptic Digestion	Apparent Albumin Concentration after Varying Incubation Periods (mg. %)					
	Standard	30 Min.	One Hour	Two Hours	Four Hours	24 Hours
Peptic digestion	600	580	588	580	580	600
Acid Hydrolysis						
1N HCl at 37°C.	680	680	688	688	704	704
3N HCl at 37°C.	800	760	760	746	746	796
1N HCl at 80°C.	680	680	672	680	680	536
3N HCl at 80°C.	688	584	504	448	408	288

FIG. 1. Effect of peptic digestion and acid hydrolysis on the electrophoretic mobility of the specimens of albumin studied.

migrated more rapidly than unhydrolysed albumin when incubated at 37°C. in N HCl and when incubated at 37°C. in 3N HCl the migration of the main peak was unaltered but the resultant band was more diffuse.

Acid hydrolysis in 1N HCl at 80°C. did not alter the biuret reaction at intervals of up to four hours; at 24 hours there was a 21% reduction of the apparent albumin concentration. Electrophoresis of the hydrolysate showed a band that migrated more rapidly than unhydrolysed albumin.

Acid hydrolysis in 3N HCl at 80°C. resulted in a reduction of the apparent concentration of albumin throughout the period of incubation. Electrophoresis showed a band that remained at the application point.

The protein concentration as determined by the biuret method was not decreased by incubation at pH 2 in the sample of gastric juice.

DISCUSSION

The biuret reaction was first described in 1914 and the following year Autenreith and Mink (1915) utilized this reaction for the determination of protein concentrations. Since then many modifications have been suggested, the most commonly used being those of Gornall *et al.* (1949) and of Lowry, Rosebrough, Farr, and Randall (1951). It is given by all compounds with two amine or peptide bonds linked directly or through an intermediate carbon atom. Tripeptides and proteins possessing the structures below produce this reaction (White *et al.*, 1954).

Dipeptides and amino-acids, with the exception of histidine, serine, and threonine, do not give this reaction (White *et al.*, 1954; Ellman, 1962). With certain interesting exceptions, including glucosamine, the above amino-acids, and the slight reaction with glucose and starch, the reaction is characteristic of the peptide bond (Ellman, 1962). The colour deepens as the number of peptide bonds in a series of synthetic peptides is increased.

In the digestion of protein, pepsin acts at peptide linkages and shows a preference for peptide bonds involving aromatic amino-acid residues (Fruton and Simmonds, 1958); the action of crystalline swine pepsin on bovine serum albumin leads to the formation of fragments that contain, on the average,

seven amino-acid residues (Beloff and Anfinsen, 1948); when acting on horse globin, pepsin first breaks it into very large peptides and medium-sized peptides (about 12 residues); the larger peptides are later reduced to tri- and tetra-peptides (Desnuelle, Ravery, and Bonjour, 1950). Acid hydrolysis of protein may result in mixtures containing varying proportions of amino-acids and peptides, depending upon the state at which the hydrolysis is interrupted (White *et al.*, 1954). The pH to which the albumin was exposed in these experiments was more acid than that met in man because in gastric juice the pH is rarely below pH 1. The pepsin concentration and albumin concentrations used were comparable to those found in gastric juice (Piper, 1960).

The above results show that peptic digestion and acid hydrolysis at 37°C. of albumin for periods of up to 24 hours do not influence the colour reaction produced by the biuret reaction. Since pepsin attacks primarily the aromatic amino-acid residues of a protein molecule which comprise only a small portion of the albumin molecule, limited proteolysis only would result, the fragments being mostly polypeptides in nature; these polypeptides produce the same degree of colour reaction with the biuret reaction as does the protein from which they were derived.

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