The electrophoresis of human gastric juice

Part I In normal controls

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EDITORIAL SYNOPSIS The electrophoretic pattern of normal human gastric juice is described. The effect of autodigestion of gastric juice and of the peptic digestion of albumin is described. The fallacies involved in the study of gastric juice proteins where peptic digestion of the protein constituent has not been prevented are emphasized. In this study the gastric juice was neutralized within the stomach to prevent changes due to autodigestion.

The study of the human gastric secretion began with Reamer's discovery of the acid character of human gastric juice in 1752. His observations were confirmed in man by Stevens in 1777 and later Prout identified the acid present in the stomach as muriatic acid and Schwann demonstrated the presence of pepsin in gastric juice. Over the past two centuries since Reamer's observations the patterns of secretion of the stomach as regards electrolytes and pepsin to various stimuli have been fully studied. In the last decade many studies have centred around the protein portion of gastric juice and the interest in this fraction of gastric juice has been stimulated by the pioneer electrophoretic studies of Grossberg, Komarov, and Shay (1951), Glass, Stephanson, and Rich (1956), and Henning, Kinzlmeier, and Demling (1953), and by the recognition of a series of clinical syndromes characterized by increased loss of protein into the gastrointestinal tract (Citrin, Sterling, and Halsted, 1957; Schwartz and Jarnum, 1959; Schwartz and Thomsen, 1957; Gordon, 1959).

METHOD

ELECTROPHORETIC ANALYSIS OF GASTRIC JUICE All patients were in a fasting state and were instructed not to swallow saliva during the collection of gastric juice. Gastric juice was stimulated by histamine, using the technique of Kay. The gastric juice was neutralized intragastrically using varying dilutions of a 5% solution of sodium bicarbonate. The residue was aspirated and about 40 ml. of a 10% solution of the above solution of sodium bicarbonate was injected into the stomach and aspirated five to 10 minutes later. This was done on two or three occasions at 10-minute intervals to free the stomach of any residue. Thereafter 40 to 60 ml. of a

10 to 20% solution of the sodium bicarbonate solution was injected into the stomach and its pH checked each few minutes by aspiration and testing with multirange indicator paper, sufficient sodium bicarbonate solution being added to keep the pH above 7. At the end of each 10 minutes the gastric contents were partially aspirated, more sodium bicarbonate solution injected, and the above procedure repeated. The diluted and neutralized gastric fluid was collected into tubes surrounded by ice and any visible mucus was removed by filtering through gauze. The gastric juice was then dialysed through Visking dialysis tubing for 24 hours at 5°C. against five changes of distilled water. It was filtered through Whatman No. 1 filter paper and freeze dried. The residue was then dissolved in borate buffer at pH 9 and the following two procedures were carried out:

1 The buffer solution corresponding to approximately 1 mg. of the lyophilized gastric juice was applied to cellulose acetate electrophoresis paper and electrophoresis performed, using horizontal electrophoresis with borate buffer at pH 9 and of ionic strength 0.12 and current of 0.4 amp./cm. and 120 v. for five and a half hours. The strips were dried in the oven for half an hour, stained with Amido black 10B, dried between blotting paper, and scanned using a Spinco Analytrol.

2 The protein fractions were labelled with ^{131}I according to the method of Verschure (1959), and electrophoresis performed as above, except that Whatman No. 1 electrophoresis paper was used. The strips were dried in the oven and autoradiography performed using x-ray film.

In some patients phosphate buffer at pH 7 and of ionic strength 0.15 was used in order to compare the pattern when electrophoresed at different pH levels. To determine the effect of autodigestion, gastric juice of some patients was collected without being neutralized; this was compared with gastric juice neutralized with NaHCO_3 solution immediately after being collected, and with gastric juice neutralized intragastrically.

The patients studied comprised a group of 24 controls who were hospital in-patients free of clinical gastrointestinal disease, disorders of serum proteins, and evidence of heart disease.

To determine the effect of the above procedure on the serum proteins a specimen of serum was diluted with sodium bicarbonate solution, dialysed and freeze dried, and the electrophoretic pattern of serum so treated was compared with the pattern given by the same serum sample before being subjected to the above procedures.

PEPTIC DIGESTION OF ALBUMIN Human albumin supplied by the Red Cross Blood Transfusion Service was digested by Armour reference pepsin. A solution of albumin (2 g. %) was adjusted to pH 3 with 0.2 N HCl and 100 mg. of Armour pepsin was added. After incubation at 37°C. for five, 15, 30, and 60 minutes aliquots were removed and digestion terminated in the aliquots by addition of borate buffer pH 9 which raised the pH to 7. A part of each aliquot was dialysed in Visking dialysis tubing against five changes of distilled water at 5°C. for 24 hours. Electrophoresis of both parts of the aliquot, the dialysed and the undialysed, was performed, using the electrophoretic technique outlined above, except that Whatman No. 1 electrophoresis strips were used.

RESULTS

ELECTROPHORETIC PATTERN IN NORMAL HUMAN GASTRIC JUICE Using the technique outlined above involving paper electrophoresis, the normal pattern of gastric juice consists of three major bands that move towards the anode and a band that remains at the application site. In 13 of the 24 cases, a small band appeared that moved towards the cathode. In some cases two further bands that moved towards the anode appeared; one migrated slowly and the other more rapidly than the main band. The anodal bands were named from left to right 1 to 6, band 1 corresponding to the band that remained at the application site (Fig. 1); band 5 migrated at all pH levels at the same rate as human albumin and therefore was presumed to consist of human albumin. Expressing the distances migrated by each band in terms of the distance migrated by band 5, a band was considered band 1 if it remained at the application site, band 2 if it migrated up to 29% of this distance, band 3 if it migrated 30% to 68% of this distance, and band 4 if it migrated

FIG. 1. Electrophoretic pattern found in normal human gastric juice.

69% to 84% of this distance. Any band anterior to band 5 was termed band 6, and any band that migrated towards the cathode, band C. In the 24 patients studied bands 1, 3, and 5 were invariably present, band 4 was present on 23 occasions, band C on 13 occasions, and bands 2 and 6 on two and seven occasions respectively. Band 3 was frequently subdivided into two or three minor peaks.

After labelling the gastric juice proteins and autoradiography, there was one main band that moved towards the anode at the rate of human albumin (Fig. 2). In the gastric proteins of some patients two or three less prominent slowly moving bands were present; in those of two patients a small band anterior to the main anodally moving band appeared. The study using autoradiography did not appear to give information more comprehensive than that obtained by staining with Amido black after electrophoresis and this technique has since been abandoned. The unsatisfactory nature of this method is due to the fact that only one of the protein-staining bands takes up ^{131}I avidly and this obscures the other bands that stain well with Amido black yet do not readily bind iodine.

A comparison of the electrophoretic pattern of gastric juice neutralized intragastrically with a specimen from the same patient not neutralized intragastrically is shown in Fig. 3. It is seen that the band with the electrophoretic mobility of albumin is absent from the specimen not neutralized, and is replaced by three bands moving less rapidly towards the anode.

It was found that dilution with sodium bicarbonate solution, dialysis, and freeze drying did not

FIG. 3. Comparison of electrophoretic pattern of gastric juice neutralized intragastrically with that obtained when the juice is not neutralized and when the juice is neutralized immediately after collection.

alter the electrophoretic pattern of a specimen of serum.

PEPTIC DIGESTION OF ALBUMIN The progressive changes as the albumin is digested by pepsin are shown in Fig. 4 (a to e). It is noted that the first change is the separation from the albumin of one less rapidly moving component and this is accompanied by a slowing of the migration rate of the remainder of the albumin molecule. As the digestion proceeds, the main albumin band gradually disappears, as smaller, more slowly moving bands

FIG. 2. Comparison of electrophoretic pattern after electrophoresis and staining with Amido black with that obtained after labelling proteins with ^{131}I and identifying the proteins after electrophoresis by autoradiography.

FIG. 4. Effect of peptic digestion on human albumin, a to e. Fig. 4f shows the effect of dialysis on the albumin digest.

appear. It will be noted that no bands moving towards the cathode were observed, and though some of the bands moving less rapidly towards the anode were reduced by dialysis, especially in those samples where digestion was continued for 30 minutes or longer, the main pattern of the digestion of albumin was not altered by dialysis (Fig. 4f).

DISCUSSION

Electrophoresis of gastric juice was first attempted with canine gastric juice by Grossberg *et al.* in 1951 using the Tiselius method. The most detailed studies of the electrophoretic pattern of human gastric juice have been made by Glass and his colleagues (Glass *et al.*, 1956; Glass and Ishimori, 1961) in the U.S.A.

and by Henning and his colleagues (Henning *et al.*, 1953) in Germany. Glass *et al.* (1956) found that normal histamine-stimulated gastric juice showed on electrophoresis eight to 11 components, four to six of which were cathodal and four to five anodal. He showed the component migrating most rapidly towards the anode to be, by elution, pepsin. Henning usually found four bands on electrophoresis, the fast moving fraction corresponding to human albumin. A major advance was made when Gullberg and Olhagen (1959) studied the electrophoretic pattern of gastric juice, autodigestion of which had been prevented by intragastric neutralization with phosphate buffer. They found three main protein-staining components. The component moving most rapidly had the same mobility as crystalline pepsin,

the second corresponded with human albumin, while the third component had a high carbohydrate content and represented a mucoprotein complex.

The major problem in the interpretation of the findings in electrophoretic studies of gastric juice has been lack of knowledge of the extent to which the various constituents secreted by the stomach are altered by autodigestion, by acid or alkali denaturation, and by the various procedures involved in the preparation of the material for electrophoresis, such as dialysis and freeze drying. Assuming that two components of gastric juice are albumin and pepsin and that the juice is secreted and collected at a pH below 5, the most obvious complication is autodigestion. As has been shown in the present study, autodigestion results in the disappearance of the albumin peak, and when albumin is digested *in vitro* the digestion products produce three or four less rapidly moving peaks. It was found too that neutralization of gastric juice immediately after collection was not adequate to prevent autodigestion despite the fact that the juice was collected by continuous suction. It can be assumed too that intragastric neutralization involves neutralization of the acid secretion only after it reaches the gastric lumen, and consequently it is possible that some protein constituents of gastric juice may be altered by the action of pepsin while still within the lumen of the gastric glands. It was found that when albumin is digested by pepsin one of the first changes is a slowing of the migration rate of the main protein fraction; this is a possible source of error when attempts are made to identify the albumin fraction by its migration rate on electrophoresis. If it can be assumed that the proteins in gastric juice are similar to the serum proteins, it can be concluded as a result of the findings reported here that the pH and temperature changes involved in intragastric neutralization, dialysis, and freeze drying do not alter the electrophoretic motility of the protein. Also, we have found in the case of anacid patients, such as those suffering from pernicious anaemia, that the untreated gastric juice produces the same electrophoretic pattern as gastric juice which has been diluted with sodium bicarbonate as outlined in the technique.

There is confusion in the published work regarding the identity of the band moving most rapidly towards the anode. Glass *et al.* (1956) showed by elution that this band was pepsin but Henning *et al.* (1953) considered it to be albumin. This confusion has apparently arisen from several sources. First, human albumin and swine pepsin at least are not widely separated by electrophoresis at pH 9. Secondly, after intragastric neutralization, the pepsin should be in the form of its precursor, pepsinogen, the

migration rate of which would differ from that of pepsin because of its higher molecular weight and more alkaline isoelectric point; if the gastric juice is not neutralized intragastrically, pepsinogen would be converted to pepsin which would be subsequently inactivated irreversibly at the pH at which electrophoresis is performed. It appears from our studies that where electrophoresis is performed at two different pH levels the most rapidly moving anodal band always has a motility that approximates to that of human albumin. Gullberg and Olhagen (1959) demonstrated that this band was albumin using the Ouchterlony immunological technique. We have observed in some cases a small band migrating more rapidly than the main albumin band, as did Gullberg and Olhagen (1959) but we have been unable to demonstrate peptic activity in this band after elution. The evidence that this band is pepsin is based on the work of Glass *et al.* (1956); one would not have expected the demonstration of peptic activity after electrophoresis because the latter workers did not neutralize the gastric juice intragastrically, consequently pepsinogen would be converted to pepsin and the latter irreversibly inactivated at the pH of the electrophoresis buffer. Also, it is not reasonable to identify this band by its electrophoretic motility unless human pepsin is available for comparison. It has been shown that hog pepsin migrates more rapidly than human pepsin at pH 5.0 (Tang, Wolf, Caputto, and Trucco, 1959). Certainly a band is not usually present anterior to the main anodally moving band. If pepsin were present at this site one would expect it to show constantly on autoradiography in view of the avidity with which pepsin takes up iodine. It is possible that a small band anterior to the main albumin band found in our radiographic study of the samples from two patients was due to partial digestion of the albumin by pepsin; if the products of peptic digestion of albumin are labelled with iodine and demonstrated by autoradiography instead of being stained with Amido black as in the present study, it was observed that as the digestion proceeds bands appear that move more rapidly than the main albumin residue and are not revealed by protein stains.

We have found sodium bicarbonate more satisfactory in producing intragastric neutralization than phosphate buffer, as recommended by Gullberg and Olhagen (1959). The neutralizing power of the latter is slight and if gastric secretion is stimulated by histamine, large volumes of phosphate buffer are needed and, in hypersecretors, we have found it almost impossible to maintain the pH above 7 with phosphate buffer; however, with the use of varying concentrations of sodium bicarbonate and repeated

sampling of the gastric juice and the use of multi-range indicator paper, we have found it relatively easy to maintain the pH of the gastric juice above 7. We have found too that phosphate buffer at pH 7 to 9 gives results as good as or better than borate buffer, when used for electrophoresis. We have, however, continued to use borate buffer, as most previous workers have used this buffer following the method of Glass *et al.* (1956).

In the present study no bands moving towards the cathode were observed consistently either with gastric juice or as a result of the digestion of albumin with pepsin when stained with Amido black. This is at variance with the findings of Glass *et al.* (1956); the difference is probably due to the fact that we used horizontal, whereas Glass *et al.* (1956) used vertical, electrophoresis. The cathodal bands were probably carried towards the cathode by endosmosis and it is possible that a part of the large band that remained at the site of application of the albumin digest may have been carried towards the cathode under different electrophoretic conditions.

The intermediate products of peptic digestion of bovine albumin have recently been studied by Schlamowitz, Peterson, and Wissler (1961) by ultracentrifugal analysis and by fractionation with solutions of trichloroacetic acid and urea. Intermediates varying in molecular weight from about 69,000 to 5,000 were found and they were shown to disintegrate in a regular fashion during digestion, six major fractions appearing to result. The first fraction formed had a molecular weight similar to that of bovine albumin and was considered to result from rupture of the peptide bond(s) without major change in molecular weight; this fraction probably is identical with the major band formed at the commencement of the peptic digestion in the present study. They also found that this fraction disappeared if the period of digestion was prolonged.

Finally, our conclusion regarding the technique and future possibilities of electrophoresis of gastric juice are as follows:—

1 Using gastric juice neutralized intragastrically four major components are detected by electrophoresis on paper at pH 9. These findings are comparable with those of Gullberg and Olhagen (1959) who also studied gastric juice neutralized intra-

gastrically. There is little doubt that the major band moving towards the anode is albumin, and that the bands of intermediate mobility consist of mucopolysaccharides. The evidence does not permit one to state whether or not pepsin or pepsinogen contributes a band to the electrophoretic pattern of gastric juice.

2 Unless the gastric juice is neutralized intragastrically, the albumin band may not be present and be replaced by several bands which represent the products of peptic digestion of albumin.

3 Apart from its research interest, electrophoresis of gastric juice no doubt will be of clinical value in the diagnosis of conditions associated with protein loss into the stomach, *i.e.*, the protein-losing gastroenteropathies. In such studies, where the size of the albumin band is of diagnostic significance, the prevention of intragastric digestion is essential.

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