# ISOLATION OF GASTROFERRIN FROM HUMAN GASTRIC JUICE[1]

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Summary. A method is reported for the isolation of gastroferrin - the high molecular weight iron-binding fraction of human gastric juice. This method is based on the ability of the iron-gastroferrin complex to be removed from solution by coprecipitation with ferric hydroxide. When iron is removed from the iron-gastroferrin complex, the gastroferrin is shown to regain its iron chelating ability. Preliminary examination of the product so obtained showed it to be a glycoprotein of molecular weight 260,000. It consists of approximately 85% carbohydrate and 15% protein. Glucosamine, galactosamine, galactose, fucose, and sialic acid were the only carbohydrate residues detected. Serine, threonine, and proline predominated among the 17 amino acids observed; aromatic and sulfur containing amino acids were present in only small amounts.

Human gastric juice has the ability to chelate iron. This property resides predominantly in a high molecular weight fraction totally excluded from Sephadex G-200. The term gastroferrin has been applied to this fraction of gastric juice, and it is normally secreted in sufficient amount to bind much of the 15 mg of iron present in a typical daily diet. (Davis, Luke and Deller, 1967). Little direct chemical information is available on the nature of gastroferrin. It has been proposed on the basis of its high molecular weight, its iron-binding ability and its resistance to peptic and tryptic digestion that its physiological role might be the inhibition of mucosal iron absorption. Thus gastroferrin would form part of the control mechanism regulating the extent of gastro-intestinal iron absorption (Davis, Luke and Deller, 1966; Luke, Davis and Deller, 1967; Luke, Davis and Deller, 1968).

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The interaction of iron with gastric juice has been further studied by Morgan et al. (1969) who demonstrated that gastroferrin is able to hold iron in solution at pH 8 only within a limited iron concentration. At higher concentrations of the metal the iron-gastroferrin complex is coprecipitated with the ferric hydroxide. In this communication we report a method for the isolation of gastroferrin from gastric juice based on its coprecipitation with ferric hydroxide, and include some preliminary observations on the chemical and physical characteristics of this high molecular weight, iron-binding fraction of human gastric juice.

## Materials and Methods

Gastric juice: Samples of gastric juice, free as far as was possible from swallowed saliva and nasopharyngeal secretions, were collected after an overnight fast. The pooled samples were centrifuged at 18,000 g for 15 min. at 0° C and the supernatant fluid was stored at -20° C until required for use. The iron-chelating ability was determined by the method of Davis, Luke and Deller (1966) on a serial dilution of a portion of the pooled sample. A 10% v/v dilution of a typical sample will bind the iron present in an equal volume of 0.001 M $FeCl_3$.

Isolation of gastroferrin: To the pooled gastric juice was added twice the amount of iron (as solid $FeCl_3 \cdot 6H_2O$) necessary to saturate the iron-binding capacity of the sample. The mixture was stirred until the added ferric chloride dissolved and the pH was then raised to 8.0 by the slow addition of concentrated $NH_4OH$ with rapid stirring. The material was centrifuged for 15 min. at 18,000 g and the supernatant fluid was discarded. The precipitate was dissolved in the minimum volume of 10 N HCl, and a concentrated solution of ammonium nitrilotriacetate (NTA), sufficient to chelate the amount of iron used in the preparation was added slowly with stirring. After titration to pH 8.0 with $NH_4OH$ the preparation was dialyzed overnight against running water to remove the low molecular weight iron-NTA complex. The removal of iron from the high molecular weight components was completed by a further

treatment with NTA followed by dialysis. The non-dialyzable material was resolved by upward passage through a G-200 Sephadex column using 0.01M $NaHCO_3$ as eluting buffer. Gastroferrin is contained in the excluded fraction.

Analytical Methods: Protein was determined by the method of Lowry et al. (1951) using crystalline bovine albumin (Pentex Inc.) as a standard. Amino acid analysis was performed on the Beckman Automatic Amino Acid Analyzer after hydrolysis of the samples in 6 N HCl at 110° C for 24 and 36 hours. Total neutral hexose was determined by the anthrone method as described by Spiro (1966), using a 1:1 w/w galactose-mannose standard. Corrections were made for the contributions of fucose and sialic acid to the color developed by the anthrone reagent. Fucose was determined on unhydrolyzed samples by the Dische-Shettles (1948) method, using a 10 min. heating period. The thio-barbituric assay of Warren (1959) was used for the determination of sialic acid on samples which had been hydrolyzed with 0.1 N $H_2SO_4$ at 80° C for one hour. Hexosamines were determined after hydrolysis of the sample under nitrogen in 4 N HCl at 100° C for six hours. Ion exchange resin chromatography on Dowex 50-X4 ($H^+$) (200-400 mesh) coupled to a column of Dowex 1-X10-formate (200-400 mesh) was used to separate the amino from the neutral sugars (Spiro, 1966). The Nelson-Morgan method was employed to measure the separated hexosamines (Boas, 1953). Paper chromatographic resolution of amino and neutral sugars was carried out using 1-butanol:pyridine:0.1 N HCl (5:3:2 v/v) as developing solvent, and silver nitrate as stain (Neuberger and Marshall, 1966).

## Results and Discussion

The gastroferrin isolation procedure is shown diagrammatically in Figure 1 and the relative proportions of neutral hexose, protein and iron-binding substances found during a typical preparation are listed on Table 1. One liter of the pooled gastric juice starting material in the preparation cited was found to contain 880 mg neutral hexose, 4.0 g protein and to bind the iron in an equal volume of 0.001 M $FeCl_3$ when diluted to 12% v/v. The final product contained only 260 mg neutral hexose and 80 mg protein, but

contained 43% of the iron binding ability of the starting material.

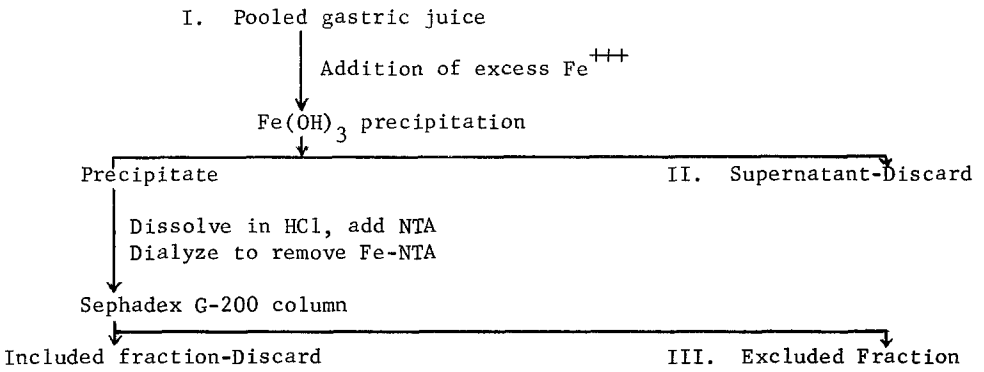


Figure 1. Flow diagram for isolation of gastroferrin.

Table 1
Composition of Gastric Juice Fractions

| | Fraction | Total Neutral Hexose | Protein | Iron Binding |
|---|---|---|---|---|
| I. | Pooled gastric juice (Starting Material) | 100 | 100 | 100 |
| II. | Supernatant from $Fe(OH)_3$ precipitation (Discarded) | 63 | 70 | not detected |
| III. | Excluded Fraction (Gastroferrin preparation) | 30 | 2 | 43 |

Expressed as percentage of the values for the starting material

The product obtained by the above method is not homogeneous. When examined in the Beckman Model E Analytical ultracentrifuge gastroferrin was found to give essentially one peak, but there appears to be a very small amount of a fast moving component, possibly a molecular aggregate (Fig. 2). Determination of molecular weight by sedimentation equilibrium gave an average

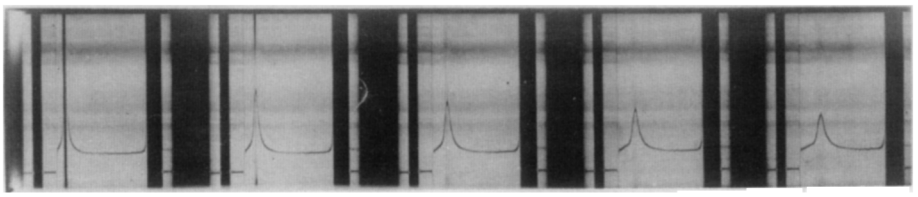

Figure 2. Sedimentation velocity of gastroferrin at 59,780 r.p.m., 20° C. The first picture was taken 16 min. after reaching maximum speed and the subsequent ones at 8 min. intervals with a bar angle of 70°. Gastroferrin concentration was 8 mg/ml of 0.01M tris buffer (pH 7.4)

value of 260,000 using 0.66 as partial specific volume, determined pycnometrically. Electrophoretic examination of gastroferrin on cellulose acetate membranes reveals the presence of several components, all of which, however, are capable of binding iron.

In spite of the inhomogeneity revealed by physical methods, chemical examination of gastroferrin revealed the presence of only five carbohydrate components. The total carbohydrate content of the preparation cited above accounted for 85% of the sample and protein for the remaining 15%. Samples of gastroferrin were submitted to acid hydrolysis and the hydrolysate was resolved into neutral and amino sugars by ion exchange chromatography. Paper chromatography of the two fractions revealed the presence of fucose, galactose, glucosamine and galactosamine. The presence of sialic acid was demonstrated colorimetrically in the material resulting from a separate mild acid hydrolysis No other sugars or sugar derivatives were observed.

Amino acid analysis of a gastroferrin preparation shows the presence of 17 amino acids. Threonine, proline and serine account for about 50% of the total amino acid present. Aromatic amino acids and amino acids containing sulfur are present only in small amounts.

The interaction of gastric juice with iron has been the subject of many recent investigations, but results are conflicting. Wynter and Williams (1968) claimed that gastric juice did not form a true complex with iron. But

their interpretation was discounted by Morgan et al. (1969) who characterized the unusual iron saturation curve shown by gastroferrin. Jacobs and Miles (1969 a and b) also confirmed the existence of gastroferrin and its ability to chelate iron and suggested it was mucoprotein in nature. Recently, Rudzki and Deller (1969) reported they had isolated and partially purified gastroferrin. Qualitative examination of their preparation showed it to consist predominently of carbohydrate residues including glucosamine, galactosamine, sialic acid, fucose and galactose.

We have reported a simple method for the isolation of gastroferrin which can be applied to large volumes of pooled gastric juice or to the small volumes obtained from a single donor. This method enables studies to be carried out with the iron-binding fraction of gastric juice and so avoid the uncertainties of previous studies which have used whole gastric juice. Further physical, chemical and immunochemical characterization of gastroferrin is in progress. In particular, the nature of iron-binding by this molecule is being investigated to help clarify its biochemical and physiological role in relation to intestinal iron absorption.

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