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The Carbohydrate Components of Hydrolysates of Gastric Secretion and Extracts from Mucous Glands of the Gastric Body Mucosa and Antrum

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1. The sugars and amino sugars of hydrolysates of gastric secretion were determined by gas–liquid chromatography. 2. All the gastric aspirations examined showed on hydrolysis the presence of fucose, galactose, mannose, glucose, galactosamine, glucosamine, *N*-acetylneuraminic acid and sulphate. 3. Galactose and glucosamine were always found in equimolar amounts, but the galactose/galactosamine ratio in different aspirations was 2:1, 3:1, 4:1 or 5:1. Repeated gastric aspirations of each subject examined showed constant ratios of these carbohydrate components. 4. Fucose and sialic acid appear to be related to glucosamine and galactosamine respectively. 5. The carbohydrate components of extracts from the mucous glands of the body mucosa and antrum did not differ from those of gastric secretion.

Previous attempts in this Laboratory to study the carbohydrate components of human gastric secretion (Schrager, 1963, 1964) provided data that suggested a quantitative relationship between these components. The adaptation of gas–liquid chromatography to measure the neutral sugars of the carbohydrate moiety of epithelial glycoproteins (Oates & Schrager, 1965) facilitated the extension of our studies.

In the present investigation the scope of the gas–liquid-chromatographic technique has been extended to include the determination of the amino sugars galactosamine and glucosamine. The method involves the ethoxycarbonylation of the amino groups in the amino sugars followed by trimethylsilylation of these derivatives together with the neutral sugars in the usual manner. The high sensitivity, specificity and accuracy of this method made possible the determination of each sugar and amino sugar in small samples of gastric secretion.

The glycoproteins in the following specimens were investigated: (1) fractional gastric aspirations from 20 people; (2) dissected mucus-secreting glands of the body mucosa and antrum of four surgical specimens.

Fucose, galactose, mannose, glucose, galactosamine, glucosamine, sulphate and *N*-acetylneuraminic acid were determined in each specimen. The data obtained are presented and discussed below.

EXPERIMENTAL

Materials

Pyridine (A.R. grade) was dried overnight with BaO and distilled. The distillate was kept over CaH_2 in an amber-coloured bottle. Trimethylchlorosilane, hexamethyldisilazane and ethyl chloroformate were commercial reagent-grade samples and were used directly. D-Galactitol, α-L-fucose, α-D-galactose, β-D-mannose, α-D-glucose, D-galactosamine and D-glucosamine (commercial reagent-grade samples) were chromatographically pure and used as supplied. Neuraminidase from *Vibrio comma* (formerly *Vibrio cholerae*) was obtained from British Drug Houses Ltd., Poole, Dorset.

De-Acidite G (7–9% cross-linking; 52–100 mesh or 100–200 mesh) was treated as follows. The resin was suspended in water, poured into a column and washed with several column volumes of 2M-Na_2CO_3 until chloride-free. Washing was continued with water until carbonate-free. Dowex 50W (X12; 200–400 mesh) was regenerated with NaOH followed by HCl and water.

Methods

Gastric secretion. The technique employed was that described by Schrager (1964) except for the following modifications. The gastric secretions were collected in ice-cooled test tubes, the patients being encouraged to expectorate the saliva and to avoid swallowing it. The two aspirations after starvation, the three post-histamine aspirations and the three post-insulin aspirations were pooled separately. The three specimens thus obtained were marked respectively 1, 2 and 3.

Gas–liquid chromatography. The separations were carried out on a Pye series 104 chromatograph equipped with a hydrogen flame detector, temperature-programming unit and a Honeywell–Brown recorder. Column packings were prepared by coating Chromosorb G (AWDMCS; 80–100 mesh) (Johns–Manville Corp., New York, N.Y., U.S.A.) with a solution of Apiezon L and neopentyl glycol adipate in benzene to give final concentrations of 2% (w/w) and 4% (w/w) respectively in the final dried packing. This material was packed into a 5ft. glass column as supplied with the series 104 chromatograph in the usual manner.

Columns were conditioned by heating to 245° for at least 48hr. with an N_2 flow of 100ml./min. and then for a further 24hr. at 210° with the same flow rate.

The analyses cited in this paper were generally achieved under the following conditions: carrier gas flow rate (N_2), 40–45ml./min.; linear temperature programming at 1·5°/min. from an initial temperature of 130° to a final temperature of 210°. Detector conditions were: H_2 flow rate, 40–45ml./min.; air flow rate, 500ml./min.; detector voltage, 45v. The amplifier attenuation was usually either 1×10^3 or 2×10^3 and the chart speed 10in./hr.

To enable the utilization of solid injection the stainless-steel injection port normally supplied with the series 104 chromatograph was drilled out to accept small glass tubes (1cm. × 0·4cm.) containing glass-wool plugs. The products of the silylation reaction were injected directly on to the glass-wool plugs and the volatile solvents rapidly removed in a stream of dry N_2. When the solvent had been completely removed, the screw cap and septum were removed from the injection port and the glass-wool plug was dropped quickly on to the top of the column packing. The screw cap and septum were then quickly replaced. The N_2 flow remained constant owing to the design of the mass-flow regulator fitted to the series 104 chromatograph; there was no need to stop the N_2 during sample addition. At the end of each run the glass tube was readily removed from the column by means of a thin wire inserted into the glass-wool plug. Peak areas were measured by multiplying the peak height by the width of the peak at half its height.

Hydrolysis of the mucopolysaccharides and preparation of the trimethylsilyl derivatives. The mucopolysaccharide solutions or suspensions were hydrolysed by making them 0·3N, 0·5N and 2N with respect to HCl by adding appropriate amounts of conc. HCl. Preliminary studies showed that the optimum hydrolytic conditions required to liberate the neutral sugars differ from those required by the amino sugars. Occasionally the best hydrolytic conditions for a particular sugar differed from one gastric secretion to another. It was therefore decided to use 0·3N-, 0·5N- and 2N-HCl for each specimen. This range covers the various requirements for both neutral and amino sugars.

Usually 5ml. of material was taken for analysis and the acidified product hydrolysed in a 25ml. screw-capped bottle at 100° for 16hr. After cooling, a suitable amount of the internal standard (D-galactitol) based on the original volume of material was added to the hydrolysate in the bottle, which was then washed out with an equal volume of water for the 0·3N- and 0·5N-HCl hydrolysates and five times its original volume for the 2N-HCl hydrolysates.

These solutions were then deacidified by passing down small columns (1cm. × 8cm.) of De-Acidite G (7–9% cross-linking; 52–100 mesh), the first 4ml. of effluent being rejected and the next 5ml. being collected in a test tube. A column of the above size could cater for about 15ml. of 0·3N-HCl hydrolysate, the pH of the effluent being 6–7. A 5ml. portion of the effluent from the De-Acidite G column was then treated with 0·3ml. of a freshly prepared saturated solution of $NaHCO_3$ followed by 0·025ml. (2 drops) of ethyl chloroformate, and the tube was immediately corked, shaken vigorously and kept at room temperature for about 1hr. The product was deionized by passing down a small column (1cm. diam.) containing a layer (2cm. high) of De-Acidite G (7–9% cross-linking; 100–200 mesh) over which was carefully placed a layer (2cm. high) of Dowex 50W (X12; H^+ form; 200–400 mesh). The first 2·5ml. of eluent was rejected and the next 2ml. collected and pipetted into a flat-bottomed phial (20ml.) equipped with a well-fitting polythene stopper. The latter half of the effluent only was collected to minimize dilution; an internal standard was present so that complete recovery of the sample was unnecessary. The analytical data for pure monosaccharides (Table 1) show that the technique was satisfactory. The aqueous solution of sugars and *N*-ethoxycarbonylated amino sugars so obtained was evaporated to dryness in a vacuum desiccator over NaOH pellets and finally silylated with the pyridine–silanes reagent (0·4ml.). This amount of reagent was ample to silylate the carbohydrate normally found in the materials being investigated. The silylated mixture was kept at room temperature for about 30min., this being ample time for complete reaction, although samples could be left much longer without any appreciable diminution of peak areas. A 10μl. Hamilton syringe was used to inject the samples (10μl. for samples from 0·3N- and 0·5N-HCl hydrolysates, 25μl. for samples from 2N-HCl hydrolysates) on to a glass-wool plug for chromatography.

The pyridine–silanes reagent was prepared by adding hexamethyldisilazane (3ml.) and trimethylchlorosilane (2ml.) to dried pyridine (10ml.). The container was stoppered immediately and the liquid swirled gently.

Identification of N-*acetylneuraminic acid.* Gastric secretions were incubated with neuraminidase from *Vibrio comma* for 24hr. at 37°. The freed sialic acid was separated from the sialic acid-free macromolecular carbohydrate fractions by passing through a column of Dowex 1 (X8; formate form; 50–100 mesh). The released sialic acid was eluted from the Dowex 1 resin with 0·3N-formic acid.

The completion of the reaction was checked by comparing the amount of free sialic acid with that released after acid hydrolysis with 0·1N-HCl at 80° for 2hr. These conditions completely cleaved sialic acid from the gastric macromolecular carbohydrate fractions.

The sialic acid eluted from the Dowex 1 column was chromatographed in *n*-butyl acetate–acetic acid–water (3:3:1, by vol.). The butan-1-ol–propan-1-ol–0·1N-HCl system for sialic acid was also used (Svennerholm & Svennerholm, 1958). The sialic acid was located on the chromatogram with the orcinol or resorcinol reagents (Klevstrand & Nordal, 1950; Svennerholm & Svennerholm, 1958). The free sialic acid proved to be chromatographically identical with *N*-acetylneuraminic acid.

Determination of N-*acetylneuraminic acid.* Each assay of *N*-acetylneuraminic acid was carried out by the thiobarbituric acid method (Warren, 1959) and the resorcinol method (Svennerholm, 1958). These two methods usually showed good agreement.

Thiobarbituric acid method. Preliminary studies showed that hydrolysis for 1hr. at 80° in 0·1N-HCl was not sufficient for maximum recovery of the sialic acid and that the maximum release of sialic acid was obtained after hydrolysis for 2hr.

Determination of sulphate. The method used was that reported by Schrager (1964). The use of Luke's (1949) reduction mixture was retained and the methylene blue method of Fogo & Popowsky (1949) used. The sulphate was digested with a reducing mixture composed of $HP(OH)_2$ and HI. The resulting H_2S was absorbed with zinc acetate solution and treated with an acid solution of *NN*-dimethyl-

phenylene-1,4-diamine followed by $FeCl_3$ solution. The methylene blue colour that developed was measured spectrophotometrically. The margin of error was within 5%.

Determination of uronic acid. The method of Dische (1947) was used.

Dissection of mucous glands of the body mucosa and antrum and the extraction of the macromolecular carbohydrate fractions. Four surgical specimens of partial gastrectomies were obtained immediately after operation. Selected areas of the body mucosa and antrum were dissected from the underlying muscular wall, divided into rectangular small pieces and frozen on to the chucks of a Cambridge rocking microtome by using solid CO_2. Sections were taken from all surfaces, stained and examined microscopically. This process was continued until an area was reached consisting entirely of the glands to be studied. Shavings were collected into small plastic bottles containing 20ml. of 0·1M-KH_2PO_4–Na_2HPO_4 buffer, pH7·4. The specimens were shaken for 20–30min. on a mechanical shaker and allowed to freeze slowly. After solidification, the temperature was decreased further by freezing in isopentane–solid CO_2 mixture for 25min. The bottles were placed at 4° and allowed to thaw slowly overnight. They were shaken again for 20–30min. and the whole process was repeated.

The repeated freezing and thawing ruptured the mucous cells effectively. The suspension was then centrifuged at 2250*g* for 15min. to pack down the cell debris and the clear supernatant was removed. The supernatant fluid was stored at −20° until required.

RESULTS

A large number of experiments were carried out to test the reproducibility and accuracy of the adopted analytical procedures. The following are representative of the experimental trials.

Quantitative analysis of pure monosaccharides by gas–liquid chromatography. A mixture of the four sugars and two amino sugars was prepared in the following concentrations: L-fucose (1·0mM), D-galactose (1·0mM), D-mannose (0·25mM), D-glucose (0·25mM), D-galactosamine (0·5mM) and D-glucosamine (1·0mM). This solution (5ml. portions) was treated with the required amount of concentrated hydrochloric acid and internal standard (0·2ml. of 10mM solution) and subjected to the full analytical technique except that the overnight hydrolysis at 100° was omitted. The results (Table 1*a*) show that excellent agreement was obtained for every sugar. The above series of experiments was repeated except that the hydrolysis step at 100° overnight was now included. The results (Table 1*b*) show that the neutral monosaccharides were recovered completely with 0·3N-hydrochloric acid hydrolysates but that with 0·5N-hydrochloric acid slight destruction occurs, leading to somewhat low recoveries of L-fucose and D-galactose. With the 2N-hydrochloric acid hydrolysis this loss was considerable. The amino sugars were recovered unchanged under all these conditions.

The results in Table 1(*a*) and Table 1(*b*) show that no loss of sugars or amino sugars occurred during the stage of ion-exchange chromatography. Table 1(*b*) also shows that, by choosing appropriate hydrolytic conditions, the freeing of sugars could be obtained without any evidence of destruction.

Release of monosaccharides from gastric secretion during acidic hydrolysis. In this series of experiments gastric secretion obtained from starved patients was subjected to hydrolysis with different normalities of hydrochloric acid at 100° overnight.

Table 1. *Quantitative analysis of a mixture of pure monosaccharides and of the monosaccharides obtained by the hydrolysis of pooled gastric secretion*

(*a*) A standard solution of L-fucose (1·0mM), D-galactose (1·0mM), D-mannose (0·25mM), D-glucose (0·25mM), D-galactosamine (0·5mM) and D-glucosamine (1·0mM) was prepared, acidified with the appropriate quantity of HCl and subjected to the usual analytical procedures as described in the text. (*b*) A similar standard solution of the six sugars was treated as described for (*a*) but hydrolysed at 100° overnight before analysis. (*c*) The gastric secretion was hydrolysed at 100° with the appropriate quantity of HCl, and samples were taken for analysis by the procedures described in the text. The results are expressed as means ±S.D., with the numbers of determinations in parentheses. —, Not determined.

	Concn. of HCl used for hydrolysis (N)	Concn. of carbohydrate component (mM)					
		L-Fucose	D-Galactose	D-Mannose	D-Glucose	D-Galactosamine	D-Glucosamine
(*a*)	0·3 or 0·5	1·01±0·02 (7)	0·99±0·03 (7)	0·25±0·005 (7)	0·25±0·01 (7)	0·50±0·01 (7)	1·01±0·02 (7)
	2	1·03±0·02 (3)	1·00±0·02 (3)	0·24±0·01 (3)	0·25±0·01 (3)	0·49±0·01 (3)	1·01±0·02 (3)
(*b*)	0·3	0·99±0·01 (4)	0·98±0·02 (4)	0·24±0·005 (4)	0·25±0·1 (4)	0·48±0·01 (4)	1·00±0·02 (4)
	0·5	0·96±0·01 (4)	0·93±0·03 (4)	0·24±0·005 (4)	0·25±0·05 (4)	0·49±0·01 (4)	1·00±0·01 (4)
	2	0·63±0·14 (4)	0·64±0·07 (4)	0·14±0·03 (4)	0·17±0·01 (4)	0·49±0·02 (4)	1·03±0·01 (4)
(*c*)	0·3	0·56±0·01 (4)	0·65±0·02 (4)	0·14±0·005 (4)	0·12±0·00 (4)	0·11±0·005 (4)	0·57±0·01 (4)
	0·5	0·49±0·02 (4)	0·61±0·02 (4)	0·13±0·005 (4)	0·11±0·005 (4)	0·16±0·005 (4)	0·67±0·02 (4)
	2	—	—	—	—	0·16±0·01 (6)	0·65±0·02 (6)

Table 2. *Release of monosaccharides from gastric secretions during acid hydrolysis*

All gastric secretions were hydrolysed overnight at 100° with the appropriate quantity of HCl. The analytical procedure was as described in the text. —, Not determined.

Concn. of HCl used for hydrolysis (N)	Concn. of carbohydrate component (mM)							
	L-Fucose (gas–liquid chromatography)	L-Fucose (thioglycollic acid)	D-Galactose	D-Mannose	D-Glucose	D-Galactosamine	D-Glucosamine	Total hexosamine (Elson–Morgan)
Gastric secretion 1								
0·1	0·22	0·23	0·31	0·07	0·08	0·12	0·25	0·48
0·3	0·23	0·22	0·32	0·07	0·07	0·15	0·30	0·47
0·5	0·22	0·22	0·30	0·07	0·09	0·13	0·29	0·47
1·0	0·11	0·13	0·22	0·06	0·07	0·13	0·27	0·47
2·0	0·07	0·10	0·12	0·03	0·04	0·14	0·28	0·42
3·0	—	—	—	—	—	0·15	0·27	0·42
Gastric secretion 2								
0·3	0·40	0·39	0·92	0·14	0·15	0·25	0·92	1·26
0·5	0·42	0·39	1·10	0·16	0·25	0·28	1·03	1·48
2	—	—	—	—	—	0·35	1·05	1·48
3	—	—	—	—	—	0·36	1·00	1·48

These results (Table 2) show that the sugars are generally released in the following order: L-fucose first, then the hexoses at approximately the same rate and finally D-glucosamine and D-galactosamine. These and other experiments (not shown in Table 2) led to the conclusions that, in general, L-fucose was released completely by 0·3N-hydrochloric acid, and very often the three hexoses were also released. With 0·5N-hydrochloric acid, L-fucose showed some loss, owing to destruction, as also did the hexoses in some cases, although in general only slightly. D-Glucosamine usually reached a maximum value at this normality of acid, and in certain cases D-galactosamine also. In many instances, however, it was necessary to use 2N-hydrochloric acid for the complete release of D-galactosamine. Under these conditions all the neutral sugars suffered destruction, making their analytical values useless. In view of these results, it was decided to standardize the 0·3N-, 0·5N- and 2N-hydrochloric acid hydrolysis conditions for all material under investigation, to obtain a complete record of every sugar. Where only amino sugars were being investigated, however, it was necessary to carry out the 2N-hydrochloric acid hydrolysis only. The reproducibility of a series of hydrolyses on the gastric secretion obtained from one individual is shown in Table 1(*c*). The agreement obtained under any one set of conditions was excellent, although the values of the various sugars vary under different hydrolytic conditions, owing to destruction on the one hand and further sugar release on the other.

Table 2 includes values for L-fucose determined by the thioglycollic acid method (Gibbons, 1955) and for total hexosamine determined by the Elson–Morgan technique (Boas, 1953) for comparison with the gas–liquid-chromatography values. Generally good agreement was obtained.

Carbohydrate and sulphate contents of gastric secretion. All gastric aspirations examined contained glucosamine, galactosamine, galactose, fucose, sulphate, *N*-acetylneuraminic acid, glucose and mannose, and the results are set out in Table 3.

Galactose and glucosamine were invariably found in equimolar amounts. The gastric aspirations investigated were divisible into groups, with reference to the galactose/galactosamine ratio, each group with a distinctive ratio that was an integral number and remained constant. These ratios did not bear any relationship to blood-group specificity.

No such constancy was noticed in the fucose, *N*-acetylneuraminic acid and sulphate contents.

Fucose appeared to be related to galactose and glucosamine, but fell short of a 1:1 ratio and varied from specimen to specimen.

The data showed the sulphate to be related to glucosamine, the ratio varying from 1:1 to 3:1, but no constancy in this ratio could be detected. The presence of sulphate-containing macromolecules in gastric secretion has been reported (Häkkinen, Hartiala & Terho, 1965).

Sialic acid was related to galactosamine in a similar manner as fucose was to glucosamine. No definite relationship was established between

Table 3. *Carbohydrate components of fractional gastric aspirations determined by gas–liquid chromatography*

Eight fractional aspirations were obtained from each person investigated: two specimens after starvation at 10 min. intervals, three post-histamine specimens at 20 min. intervals and three post-insulin specimens also at 20 min. intervals, the three groups being pooled separately and labelled 1, 2 and 3 respectively. Only the optimum release of the sugars and amino sugars is given. The results are expressed as means ± S.D., with the numbers of determinations in parentheses. Details of the relationships between the monosaccharides determined are given in the text.

	Ratio of carbohydrate component to D-galactose (= 1·00)											
	Galactose/galactosamine ratio 2:1			Galactose/galactosamine ratio 3:1			Galactose/galactosamine ratio 4:1					
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3			
D-Galactose	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00			
D-Glucosamine	0·98 ± 0·03 (3)	0·97 ± 0·01 (3)	0·99 ± 0·05 (3)	0·99 ± 0·04 (12)	0·97 ± 0·12 (12)	0·99 ± 0·05 (12)	0·96 ± 0·02 (5)	0·95 ± 0·02 (5)	0·96 ± 0·03 (5)			
D-Galactosamine	0·47 ± 0·02 (3)	0·51 ± 0·06 (3)	0·51 ± 0·05 (3)	0·33 ± 0·03 (12)	0·32 ± 0·03 (12)	0·34 ± 0·03 (12)	0·24 ± 0·01 (5)	0·24 ± 0·01 (5)	0·25 ± 0·01 (5)			
L-Fucose	0·77 ± 0·07 (3)	0·86 ± 0·05 (3)	0·73 ± 0·21 (3)	0·68 ± 0·14 (12)	0·66 ± 0·18 (12)	0·69 ± 0·18 (12)	0·59 ± 0·12 (5)	0·56 ± 0·10 (5)	0·57 ± 0·07 (5)			
Sulphate	0·55 ± 0·09 (3)	0·46 ± 0·08 (3)	0·47 ± 0·22 (3)	0·58 ± 0·34 (12)	0·53 ± 0·32 (11)	0·57 ± 0·24 (12)	0·59 ± 0·26 (5)	0·40 ± 0·10 (5)	0·43 ± 0·11 (5)			
Sialic acid	0·22 ± 0·09 (3)	0·18 ± 0·05 (3)	0·19 ± 0·06 (3)	0·22 ± 0·04 (12)	0·23 ± 0·12 (12)	0·23 ± 0·08 (12)	0·30 ± 0·09 (5)	0·30 ± 0·15 (5)	0·30 ± 0·11 (5)			
D-Glucose	0·23 ± 0·3 (3)	0·27 ± 0·15 (3)	0·30 ± 0·19 (3)	0·44 ± 0·28 (11)	0·26 ± 0·10 (11)	0·32 ± 0·30 (11)	0·22 ± 0·29 (5)	0·30 ± 0·40 (5)	0·15 ± 0·10 (5)			
D-Mannose	0·09 ± 0·11 (3)	0·14 ± 0·04 (3)	0·13 ± 0·02 (3)	0·13 ± 0·6 (12)	0·14 ± 0·04 (12)	0·15 ± 0·04 (12)	0·14 ± 0·05 (5)	0·13 ± 0·02 (5)	0·15 ± 0·02 (5)			

glucose, mannose and the other carbohydrate components.

Carbohydrate components of extracts from mucous glands of the body mucosa and antrum. The mucous glands of the body mucosa and antrum from four gastric mucosae were dissected and their carbohydrate content was measured. The glands of the body mucosa and antrum showed the same carbohydrate components, which quantitatively did not differ from those found in the gastric secretion.

Galactose and glucosamine were found in equimolar amounts in both types of glands (Table 4).

Relative release of fucose and other sugars of the gastric secretion by mild acid hydrolysis. Gastric secretions from four people were hydrolysed under various conditions of acid concentration, temperature and time (Table 5). Fucose was released under milder conditions than was any other carbohydrate component. Hydrolysis in 0·1 N-hydrochloric acid at 65° for 16 hr. freed about 89% of fucose, only 8% of galactose and only traces of glucosamine and galactosamine. Hydrolysis in 0·1 N-hydrochloric acid at 80° for 2 hr. released about 72% of fucose and only 8% of galactose. Under these hydrolytic conditions sialic acid is completely released.

Uronic acid. No uronic acid was found in any of the specimens investigated. Other workers were also unable to detect uronic acid in human gastric secretion (Häkkinen *et al.* 1965).

DISCUSSION

Examination of the amount of carbohydrate components in fractional gastric aspirations and mucus-secreting glands of the body mucosa and antrum has revealed for the first time some constant relationships between certain sugars in the macromolecular carbohydrate fraction that would assist in the determination of their structure.

Galactose and glucosamine were found in equimolar amounts in all gastric aspirations and glandular extracts. The galactose/galactosamine ratio was found to be 2:1, 3:1, 4:1 or 5:1 in different aspirations. The gastric secretions investigated were divisible into distinctive groups with reference to the ratios of the two amino sugars. The gastric secretion of each subject investigated had a constant ratio. No change in these ratios was found in repeated aspirations at 6-monthly intervals.

The clear-cut stoicheiometrical relationships between galactose and glucosamine and between glucosamine and galactosamine within each group were found in all the gastric secretions and glandular extracts with striking regularity. It is reasonable to assume that they are linked structurally. The two amino sugars and galactose form 70–80% of the carbohydrate content of the gastric secretion.

It is assumed that fucose and sialic acid occupy

Table 4. *Carbohydrate components of extracts from mucous glands of human gastric body mucosa and antrum*

Experimental details are given in the text. —, Not determined.

	Concn. of carbohydrate component (mM)						
	Sialic acid	L-Fucose	D-Galactose	D-Mannose	D-Glucose	D-Galactosamine	D-Glucosamine
Extract 1							
Body	—	0·04	0·08	0·02	0·02	0·02	0·07
Antrum	—	0·07	0·13	0·04	0·04	0·03	0·12
Extract 2							
Body	0·03	0·06	0·09	0·03	0·02	0·02	0·09
Antrum	0·05	0·13	0·32	0·04	0·03	0·10	0·34
Extract 3							
Body	—	0·19	0·20	0·10	0·39	0·07	0·21
Antrum	0·06	0·13	0·25	0·04	0·13	0·07	0·26
Extract 4							
Body	0·12	0·16	0·23	0·06	0·10	0·06	0·25
Antrum	0·16	0·17	0·33	0·06	0·31	0·09	0·34

Table 5. *Relative release of fucose and other sugars of the gastric secretion by mild acid hydrolysis*

The gastric secretions were hydrolysed under various conditions of acid concentration, temperature and time. Samples were taken for analysis by the procedure described in the text. —, Not determined.

Hydrolysis conditions			Concn. of carbohydrate component (mM)					
Concn. of HCl (N)	Temp.	Time (hr.)	L-Fucose	D-Galactose	D-Mannose	D-Glucose	D-Galactosamine	D-Glucosamine
Gastric secretion 1								
0·05	37°	16	0·06	0·03	Trace	0·06	Trace	Trace
0·5	37	16	0·18	0·06	0·01	0·10	Trace	Trace
0·05	65	16	0·35	0·04	0·01	0·07	Trace	Trace
0·1	65	16	0·66	0·07	0·01	0·09	Trace	Trace
0·2	65	16	0·67	0·15	0·01	0·11	Trace	Trace
0·3	65	16	0·68	0·18	0·01	0·11	Trace	Trace
0·5	65	16	0·72	0·28	0·01	0·11	Trace	Trace
0·1	80	2	0·54	0·07	Trace	0·10	Trace	Trace
0·4	100	16	0·75	0·87	0·16	0·26	0·17	0·64
2	100	16	—	—	—	—	0·21	0·83
Gastric secretion 2								
0·05	37	16	0·02	0·02	Trace	0·12	Trace	Trace
0·05	65	16	0·10	0·02	Trace	0·13	Trace	Trace
0·1	65	16	0·38	0·04	Trace	0·16	Trace	Trace
0·3	65	16	0·43	0·10	Trace	0·20	Trace	Trace
0·5	65	16	0·44	0·14	Trace	0·29	Trace	Trace
0·1	80	2	0·32	0·04	Trace	0·14	Trace	Trace
0·5	100	16	0·44	0·51	0·10	0·66	0·10	0·44
2	100	16	—	—	—	—	0·10	0·49

terminal positions. This can be deduced from the fact that they are removed almost totally from the macromolecule by relatively mild acid hydrolysis (Table 5) without splitting off any significant amounts of galactose, glucosamine and galactosamine.

It is difficult to reconcile these findings with the prevailing opinion that the gastric mucosa secretes a number of heterogeneous macromolecular carbohydrate fractions differing substantially in composition. The concept of heterogeneity is based mainly on results obtained by electrophoresis and ion-exchange chromatography (Glass, Rich & Stephanson, 1957; Richmond, Caputto & Wolf,

1957) and the assumption that each anatomically separated group of glands secretes a macromolecular carbohydrate fraction with a distinctive composition. The present findings for extracts from glands of the body mucosa and antrum do not support this assumption. No differences could be detected in the carbohydrate composition of the extracts from the antrum and glands of the body mucosa. The data reveal a striking similarity between the carbohydrate components of the secreted macromolecular carbohydrate fractions and those of the extracts from the antrum and body mucosa within each group. Further study of extracts from glandular elements is needed as only four specimens have so far been examined.

This investigation, however, showed that the gastric macromolecular carbohydrate fractions are polydisperse with reference to sulphate and sialic acid. Changes in the proportion of these two groups attached to these large macromolecules endows them with differing degrees of electrophoretic mobility and of absorption when fractionated on ion-exchange resin. It is therefore suggested that many of the multiple bands or fractions obtained by these separation procedures arise from variations in these two groups attached to a common basic macromolecular component rather than from major differences in its 'backbone' structure. Electrophoretic and ion-exchange separation procedures, although capable of showing up differences in charge and polarity of macromolecules, are not reliable parameters to identify major changes in the macromolecular components of gastric secretion.

The data obtained suggest that the composition of the carbohydrate moiety of the gastric macromolecular carbohydrate fraction is group-specific and that within each group the gastric macromolecular carbohydrate fractions have a common basic core. These findings tentatively indicate that the gastric macromolecular carbohydrate fractions are perhaps less heterogeneous than they have hitherto been regarded.

The above study, however, dealt only with the carbohydrate moiety. The protein composition of the macromolecular carbohydrate fraction and the composition and sequence of the amino acids have yet to be examined. Such a study may eventually reveal other differences in the protein part.

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