TABLE I. Metabolism of Glucose-U-C^{14} by Liver and Ventricular Myocardium Slices Obtained from Normal and Growth Hormone Treated Dogs.

Tissue	Treatment	Glycogen, μM glu/g	p(t)	C.P.M. in glycogen/g tissue	p(t)	C.P.M. in CO_2/g	p(t)	C.P.M./mg protein
Heart	Control	25.2 ± 2.1*	<.001	1420 ± 50	<.01	19,056 ± 472	<.01	87
	+ Growth hormone	53.8 ± 2.4		2108 ± 97		15,500 ± 204		86
Liver	Control	248 ±21.2	<.02	1780 ±135	<.05	17,038 ±1216	<.01	83
	+ Growth hormone	350 ±22.3		2652 ±258		9,990 ± 708		113

* Each figure is an average of 4 values.

on some aspects of glucose-U-C^{14} has been studied in dog. Administration of growth hormone (1 mg/kg/day) to dogs for one week was accompanied by an increase in glycogen content of the liver and ventricular myocardium. *In vitro* studies using slices of liver and heart tissue from control and growth hormone treated dogs showed a decrease in oxidation of glucose-U-C^{14} and an increased incorporation of C^{14} into glycogen by both tissues from growth hormone treated dogs. A small increase in the radioactivity of liver proteins from growth hormone treated dogs was also observed.

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Properties of Gel Mucin of Human Gastric Juice.* (29376)

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The freshly secreted epithelial mucin which forms a cohesive protective coating on the walls of the gastric mucosa, is obtained as a gel in aspirated anacid gastric juice. In normal acid juice, it is precipitated in ropey shreds. It has been customary to define this material as "visible" or "insoluble" mucin, as distinct from the soluble gastric mucins. Due to its insolubility in neutral aqueous media it has not previously been examined in detail. Canine gastric gel mucin, obtained from Heidenhain pouches following stimulation with acetylcholine, and porcine duodenal mucin both dissolve in alkali to give a complex mixture on paper electrophoresis(1) or in the ultracentrifuge (2). In an attempt to purify the gel mucin obtained from human subjects, we have discovered that the mucin, freed by very gentle methods(3) from contamination with soluble mucoproteins and from the serum proteins which occur in anacid gastric juice, can be dissolved in 8M urea to afford a macromolecular component which acts as a single entity on paper electrophoresis or in the ultracentrifuge. The use of such a mild reagent minimizes the possibility of altering the macromolecules, other than dissociating

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the hydrogen bonds responsible for gel formation. Alkali also solubilizes human gel mucin to some extent but causes more rapid and profound changes which are currently under investigation.

Gastric gel mucin comprises two-thirds by weight of the total non-dialyzable solids in gastric juice. Analysis indicated that it is a typical fucomucin of the blood group type. The specific activity of the latter depends mainly on differences in the composition and structure of prosthetic carbohydrate groups. Many reports(4,5,6) have suggested that there is a significant correlation between the occurrence of duodenal and gastric ulcers, gastric cancer, gastric atrophy and the blood group of the individual. Variations in the composition of purified gel mucin, therefore, are of particular interest and may be expected to reflect both the blood group and other factors predisposing to, or associated with gastric disease. In the present study, mucins from 11 individuals with no apparent gastrointestinal disease have been analyzed in order to establish the normal variation; 5 patients with such conditions as gastritis, gastric and duodenal ulcers have also been included.

Material and methods. Gastric juice was collected from fasting individuals. A preliminary sample was aspirated to determine resting pH and total acidity; phosphate buffer (pH 7.0, ionic strength 0.1) was then introduced to reduce total acidity(3). After 15 minutes the combined juice and buffer were aspirated and chilled. Subjects were induced not to swallow their saliva during this time. Saliva and blood samples were also taken to examine blood group and secretor status. Any frothy or heavy, sedimenting material was discarded and also bile or blood-stained juice. The whole specimen was filtered through an ultra fine G 5/3 fritted glass filter(2) in a cold room at 4°C. The dry mucin film retained on the filter was washed at least 3 times by soaking in dilute acetic acid, pH 4.0 followed by suction, until the filtrate was free from soluble nitrogen. Finally, the samples were allowed to imbibe distilled water and lyophilized. Samples of the white, rubbery solid were hydrolyzed with 1 N H_2SO_4 and neutralized with $Ba(OH)_2$ to pH 4.5. The supernatants were reduced in volume and applied to paper chromatograms. The following solvents were utilized; butanol : acetic acid : water: 5:1:4: butanol : pyridine : water: 6:4:3: and 80% phenol. The chromatograms were sprayed with aniline hydrogen phthalate for sugars, and the Morgan & Elson reagent for hexosamines. Sialic acid was detected in hydrolyzates in 0.1 N HCl at 80°C; chromatograms were sprayed with Ehrlich's reagent. Hydrolysis for amino acids was carried out in 5.7 N HCl. Two-dimensional chromatograms, developed in butanol : acetic acid : water and phenol (1% NH_3) were sprayed with ninhydrin in the usual way.

Samples of mucin dried over P_2O_5 *in vacuo* were dissolved in 8M urea. Aliquots of the solutions were analyzed for galactose(7). Appropriate amounts of protein, fucose and glucosamine were added to standard galactose solutions. Fucose was estimated by the cysteine-H_2SO_4 reaction (10 minutes)(8) : hexosamine by the Cessi(9) adaptation of the Morgan-Elson reaction; sialic acid, after hydrolysis in 0.1 N HCl at 80°C for one hour with thiobarbituric acid(10); protein by the method of Lowry *et al*(11) using Dade Labtrol serum as standard protein solution. Nitrogen determinations were carried out on dry samples by a micro diffusion technique (12). All estimations were made in triplicate. In several cases, mucin samples were obtained on different days from the same individual and both samples were analyzed separately.

Blood group activity. One per cent mucin dissolved in urea was diluted $1:10^3$, $1:2 \times 10^3$ (where possible) and $1:10^4$. Twenty μl diluted sample was allowed to react with an equal volume of diluted (1:4) standard anti-A or anti-B serum or an extract of Ulex europeus as anti-H. After 30 minutes, 20 μl 1% suspension of erythrocytes were added. The tubes were held for 1½ hours to detect inhibition of agglutination. Controls agglutinated within 5 minutes of the addition of erythrocytes.

Paper electrophoresis. A standard Beckman Spinco apparatus was utilized at room

temperature. The ionic strength of all buffers was approximately 0.1. In some cases the buffers were prepared in 8M urea. Twenty μl 1% mucin solutions in freshly prepared 8M urea were applied to Whatman No. 1 paper strips. This volume and concentration are important in reproducing results. Constant current (12 ma) was turned on for 5 hours. The strips were dried at 100°C and stained with amido black or the periodate-Schiff reagent.

Mobilities (RM) were measured from the point of application in relation to the fast moving 2:4-dinitrophenylvaline, N-β-hydroxyethyl-2:4-dinitroaniline being used to indicate electroendosmotic flow(13).

Ultracentrifuge analysis. Samples of mucin in 8M urea solution, 1% (w/v) were examined in the Spinco analytical ultracentrifuge (Model E) using an aluminum centre piece.

Results and discussion. The filtration method used to separate gel mucin from the soluble macromolecular materials present in gastric juice prevents the mechanical breakdown of the gel structure. After lyophilization the dry mucin when placed in water swells again to a slightly opaque gel. As there was no artificial stimulation of mucin production on collection, contamination of the gastric juice with cellular debris was reduced to a minimum. This was confirmed by the absence of any specific absorption due to nucleic acid derivatives at 260 mμ in the ultraviolet spectra of urea solutions.

In 8M urea, the dry mucin swells to a clear gel, and then dissolves; the gel structure is slowly but irreversibly lost. The clear urea solution passes through an ultrafine glass filter, and after dialysis to remove urea, followed by lyophilization, the white solid no longer forms a gel in water but dissolves completely to give a clear solution.

The 8M urea solutions of gel mucin from 40 normals and from 19 patients with gastric disorders, gave the same pattern on paper electrophoresis (Fig. 1_2); a single component of low mobility (RM 0.12) was detectable at pH 9.0 in borate or glycine buffers, decreasing to zero mobility at lower pH values. A sample of such a solution, when examined in the ultracentrifuge, gave a single component

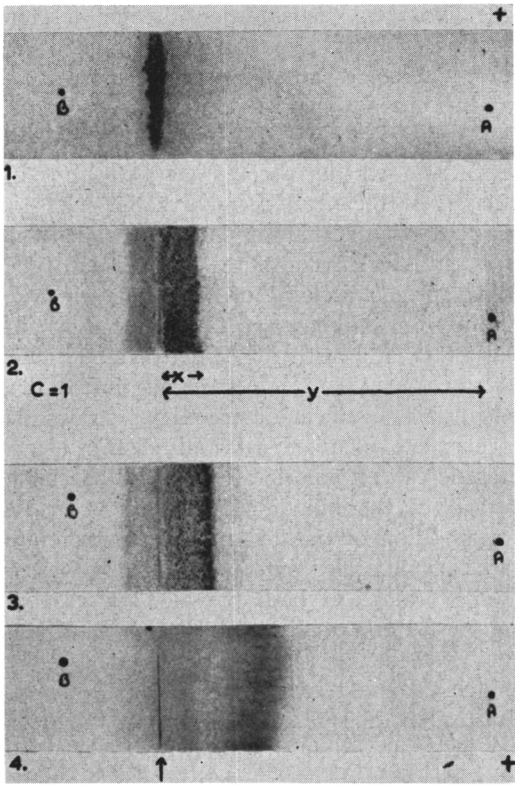

FIG. 1. Electrophoresis in borate buffer pH 9.0, I = 0.12 current 12 ma, for 5 hr. 20 μl 1% mucin solution. A = Center of marker (2:4 dinitro phenylvaline). B = Electroendosmosis (N-β-hydroxyethyl 2:4-dinitroaniline). 1) Human gastric gel mucin suspended in borate buffer. The insoluble gel remains on surface of paper. 2) Gastric gel mucin dissolved in fresh 8M urea. RM = x/y = 0.12. 3) and 4) Gel mucin in 8M urea. "Ageing" process showing development of "C = 2" component.

(C=1) with sedimentation coefficient $S^{25}_{8M\ urea} = 3.18 \times 10^{-13}$ (Fig. 2_1). However, on standing, a second component C=2 slowly appeared, which had RM 0.38 on electrophoresis at pH 9.0 (Fig. $1_{3,4}$). After about 2 months, conversion to the second component was complete (Fig. 3_1). The PAS stain and the protein stains coincided in all these experiments, indicating that the change was not due to dissociation of the protein from the carbohydrate. An ultracentrifuge analysis of the mucin C=2 had one major peak, $S^{25}_{8M\ urea} = 1.77 \times 10^{-13}$.

Mucin dissolved in 1% K_2CO_3 or N/100 sodium hydroxide, on the other hand, gave a composite pattern immediately with the major component having RM 0.4 in 1%

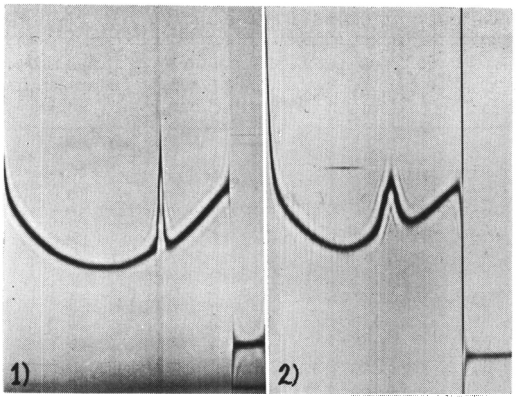

FIG. 2. Sedimentation diagrams of human gastric gel mucin in 8M urea. Concentration approximately 1% (w/v). Temperature 25°C. Top speed 59,780 rpm. 1) "C = 1" mucin (cf Fig. 1_2) 57 min after reaching top speed. 2) "C = 2" mucin (cf Fig. 3_1) 97 min after reaching top speed.

K_2CO_3 (Fig. 3_2) or 0.52 in N/100 NaOH. On further standing in alkali, further changes take place and components of greater mobility appear; these are currently under investigation.

The mechanism of "ageing" by which the faster-moving macromolecule C=2 is formed is being studied. 8M urea solutions slowly become more alkaline due to autolysis, reaching a pH of 8.5. Addition of ammonia to fresh urea solution to give pH 8.5, however, does not appear to increase the speed of formation of C=2. The change C=1→C=2 is irreversible; C=2, after dialysis and lyophylization, dissolves in water or urea to migrate at RM 0.38 with no trace of a component having RM 0.12.

The reproducibility of the electrophoretic pattern of the mucin when dissolved in urea, together with the ultracentrifuge analysis suggests that the macromolecular constituents of gel mucins from different individuals are very similar.

Chemical analysis. Fucose, galactose, N-acetyl glucosamine, N-acetyl galactosamine, N-acetyl neuraminic acid, serine, threonine, glycine, tyrosine, aspartic and glutamic acids, phenylalanine, arginine and leucine-isoleucine were identified in acid hydrolysates of gel-mucins. No glucose, mannose, uronic acids or tryptophane were detected. This composition is typical of fucomucins derived from human sources, in particular the blood group substances. Gastric gel mucin in urea solution has considerable blood group activity. This activity varies within an order of 5-fold in the blood group (Table I).

Although the behavior on paper electrophoresis suggests that the gel mucins obtained from different individuals are closely similar or identical, the percentage deviation found within each group of analyses is relatively wide (Table II). This scatter, which is outside the experimental error of the methods used, may represent a) differences in a homogeneous mucin, characteristic of the individual, or b) differences in the proportions of closely related mucopolysaccharides. Pusztai and Morgan(14) showed that highly purified blood group substances, which were substantially homogeneous according to the usual physicochemical criteria, could be further fractionated in saturated ammonium sulphate into at least two components differing slightly in their chemical composition.

Considerable differences in the proportions of the sugar units in fucomucins derived from different sites of the human body, such as the cervix, bronchus(15), and rectum(16) have been observed, but it was not reported whether these values represent analyses on single specimens or pooled samples, except in the case of intestinal plugs obtained from 2 patients with fibro-cystic disease of the

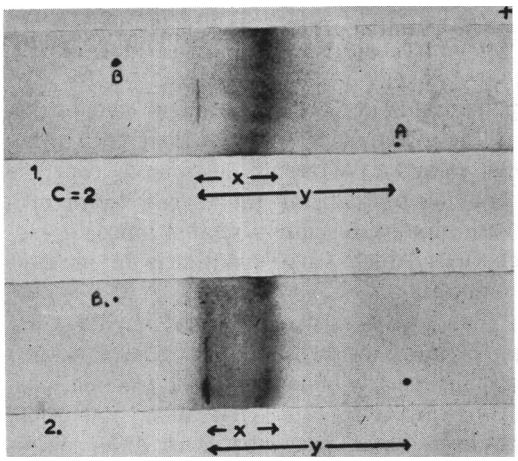

FIG. 3. Electrophoresis in borate buffer, pH 9.0, I = 0.12, current 12 ma. 1) Human gastric gel mucin in 8M urea, aged approximately 2 months. "C = 2" component RM = 0.38. 2) Human gel mucin dispersed in 1% K_2CO_3, 24 hr. RM = 0.40.

TABLE I. "Normal" Individuals (No Gastrointestinal Disease).

	Sialic acid	Total hexosamine	Fucose	Protein	Galactose	Nitrogen	Min mucin to inhibit hemagglutination, μg	Molar ratio, fucose/sialic acid
Blood Group B								
	.81	16.8	7.9	32	31	7.5	1.0	18.4
	.42	8.3	6.7	33	24	—	1.0	30.0
	.74	14.4	9.3	31	30	6.7	—	23.7
	* .72	15.1	10.7	27	32	6.3	.5	28.0
Blood Group O								
	1.25	16.9	9.9	27	35	6.5	.5	14.9
	.38	14.8	11.0	28	32	6.0	.5	25.0
	.87	12.5	8.9	33	29	6.8	.1	19.3
	*1.02	22.1	10.1	26	33	5.9	.1	18.7
Blood Group A								
	.74	11.1	8.3	30	28	—	1.0	21.1
	.32	16.2	8.7	31	29	7.2	.5	51.2
	*1.00	15.3	6.0	29	29	—	1.0	11.3
Mean	.79	14.86	8.86	29.72	30.2	6.61		
S.D.	±.18	±2.4	±1.06	±1.90	±1.98	±.37		

* Initial pH of resting juice <2.7.

All estimations expressed as mg/100 mg dried mucin. Calc. as serum protein.

TABLE II. Patients with Gastrointestinal Disease.

Sialic acid	Total hexosamine	Fucose	Protein	Galactose	Nitrogen	Min mucin to inhibit hemagglutination, μg	Diagnosis
Blood Group B							
.66	9.1	5.6	33	23	7.6	1.0	Duodenitis
Blood Group O							
.83*	20.0	10.7	23	28	6.4	.1	Gastric hem'r'ge
.92*	12.1	7.1	25	33	6.9	—	Duodenal ulcer
Blood Group A							
.61	22.5	13.0	18	30	5.2	.5	Gastric ulcer
.8	9.55	4.5	27	28	5.4	0 †	Duodenitis

* Initial pH of resting juice <2.7.

† This patient was a non-secretor.

All estimations expressed as mg/100 mg dried mucin.

pancreas(17). The percentage composition of these 2 mucins differed from each other considerably. It seems possible to conclude that mucin-producing tissues secrete an intimate mixture of closely related mucopolysaccharides which vary quantitatively in their composition.

The analysis of gastric gel mucin compares fairly closely with the pooled, water-soluble gastric mucoproteose fraction, described by Glass *et al*(18). In urea solution, however, these two substances differ markedly in their behavior on paper electrophoresis. The similarity in the proportions of carbohydrate constituents does not support the view that the mucoproteose is a product of proteolytic digestion of the insoluble gel mucin(14). In such a case it would be expected that the protein content of the gel mucin would be considerably higher, and the carbohydrate fraction correspondingly lower, as with the water insoluble gel mucin (No. 234) from ovarian cyst which was solubilized by digestion with pepsin(14).

An earlier investigation(19) showed that the gel mucin is slowly degraded by acid alone under physiological conditions *i.e.* pH 1.0-2.5, 37°C, in the absence of pepsin, liberating galactose, fucose, sialic acid and at least 2 peptides. Fucose and sialic acid usually occur in mucoproteins as terminal sugars attached to other sugar units by acid labile

bonds. Consequently, it seemed possible that gastric acidity in itself might account for a low content of both fucose and sialic acid compared with mucin from other tissues. Most of the gels described in this paper, however, were isolated from anacid juice. The few exceptions in which the initial gastric juice was acid before *in vivo* buffering indicates that there was no significant difference in the figures. It may therefore, be assumed that the mucin in the stomach had not remained in contact with acid for a prolonged period. This suggests that the action of acid and pepsin is not solely responsible for the low fucose and sialic acid content of gastric gel mucin, which may be characteristic of human gastric epithelial tissue.

Summary. "Visible" gel mucin from anacid human gastric juice has been isolated in an undegraded state by instillation of phosphate buffer into the stomach to reduce total acidity. This material has a relatively high blood group activity; its carbohydrate composition is typical of gel fucomucins from other human epithelial tissues, but differs from them in percentage composition. A smaller variation in the latter occurs among individual gastric gel mucins, which do not appear to be correlated with the blood group of the donor. Samples of mucin from 5 patients with gastrointestinal disease lie within the normal range. The mucins from a large number of individuals, including patients with gastrointestinal disease, dissolved in urea, produced the same, simple pattern on paper electrophoresis; a slow moving negatively charged "C=1 component." This material showed only one peak in the ultracentrifuge. The reproducibility of the initial C=1 electrophoretic pattern from all individuals, and the slight variation in percentage composition suggests that human gastric gel mucin may be composed of an intimate mixture of very closely related mucopolysaccharides which either vary in proportion from individual to individual or from tissue to tissue. A very slow but irreversible change occurs in all mucins in urea solution; a component C=2 with a greater negative charge appears, accompanied by a fall in sedimentation coefficient; eventually this new component represents the total macromolecular material in solution.

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