α -methyl *d*-glucosaminide (II); 2:3:4-trimethyl α -methyl *l*-fucoside (I). The direct isolation of the latter compound is of great interest since *l*-fucose must constitute a terminal residue, and it would appear to be the first instance of the isolation of a fucose derivative from an animal source. It has been isolated previously from sea-weed and from gum tragacanth (James & Smith, 1944, 1945) and also in this department from a gastric mucin polysaccharide (W. O. Cutler & S. Peat, private communication). It also appears from the direct isolation of II, that some of the *N*-acetyl glucosamine constituents are present as terminal groups. Since this investigation does not preclude the possibility that other sugar units also are present, it is evident that this stable carbohydrate residue possesses a branched chain structure of great complexity.

SUMMARY

1. Methods are described for the preparation from a wide variety of sources of polysaccharide-

amino-acid complexes having blood group A specificity.

2. Alkaline hydrolysis of the A substance from pepsin causes a degradation of the molecule and a loss of the group specificity. This loss may be due at least in part to a removal of amino-acid residues.

3. Acetylated and methylated derivatives of a relatively stable carbohydrate residue from the pepsin A substance were prepared.

4. Examination of the hydrolysis products of the methylated derivative of the stable carbohydrate residue showed that *d*-galactose, *d*-mannose, *d*-glucosamine and *l*-fucose were constituent units. Part of the *d*-glucosamine and the *l*-fucose components were present as terminal groups, and the amino group of glucosamine was naturally acetylated.

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Chemistry of Tissues

3. BLOOD GROUP SUBSTANCES FROM HUMAN GASTRIC CONTENTS

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In view of the division of the blood groups into sub-groups and of the continued discovery of new factors such as the important *Rh* factor it is highly desirable to compare the chemical properties and detailed structure of the blood group specific substances from all the groups. Inasmuch as non-human sources appeared to provide only the A substance, it was necessary, as an approach to the main problems,

to examine secretion or tissue material from humans of the appropriate groups. It appeared that gastric contents would provide a suitable means of getting the relatively large amounts of A, B and O specific carbohydrates needed for chemical study. Witebsky & Klendshoj (1940, 1941) have already examined, in a preliminary way, A and B factors prepared by Sevag's method from the gastric juice of humans in

blood groups A and B and have obtained complex carbohydrates from both secretors and non-secretors of group O.

By means of a mild saponification process we have obtained complex mucopolysaccharides from the gastric contents of humans in the three blood groups A, B and O and have attempted to purify them and to find some ready chemical method of distinguishing between them. More recently, through the courtesy and collaboration of Dr W. T. J. Morgan, we have been able to compare our products with his less degraded and more highly immunologically active A factor isolated from commercial hog mucin (Morgan & King, 1943) and to begin the chemical study of his material. Our preliminary findings are described below.

EXPERIMENTAL

Determination of activity of fractions. The activity of the various fractions described below was determined by the inhibition of isoagglutination test (cf. Bray, Henry & Stacey, 1946).

Method of preparation. Gastric contents from individuals of the same blood group (no attempt being made to differentiate between 'secretors' and 'non-secretors') were filtered in the refrigerator, heated on a water-bath for 2 hr. at 60–70°, then cooled and stored at 0° until required, a little chloroform being added as preservative. This material could conveniently be stored for several weeks. BaCO₃ or CaCO₃ (50 g./l.) was added to the liquid and the mixture evaporated to one-third volume on a water-bath at 60–70°, with frequent stirring. The solid material was then filtered off and the carbohydrate fraction containing most of the activity precipitated from the clear filtrate with ethanol. In some experiments all the active material was precipitated in one fraction, while in others several fractions were obtained by adding e.g. 2, 4 and 6 vol. of ethanol. The precipitated material was separated (centrifuge), washed with ethanol and with ether and then dried. The number of fractions and the yield of each varied somewhat owing to differences in the amount of carbohydrate material in the original gastric contents. A typical yield was 2–3 g. from 1 l. of filtered liquor. The products obtained were white powders and, even at this stage, showed considerable activity in inhibiting isoagglutination. They were purified by extraction with water at room temperature for 24 hr. When an insoluble material remained (as it did in the case of preparations made with the use of BaCO₃) it was separated (centrifuge) and the supernatant liquid dialyzed against running tap water for 24 hr. The insoluble material which was discarded contained 12–15% of ash, and a considerable amount of phosphorus-containing carbohydrate which was essen-

tially inactive and free from sulphur. After dialysis the solution was concentrated at 40° (under diminished pressure) and the carbohydrate material precipitated with ethanol (3 vol.) and isolated as before. These products had an ash content of 5–8% and at this stage were used for analysis and isoagglutination activity determinations. The ash content could readily be decreased to c. 3% by longer dialysis. The products were water-soluble white powders giving solutions which were only very slightly viscous, thus differing from the preparation of Morgan & King (1943) (see below).

This method was applied to gastric contents from individuals of blood groups A, B and O. In all cases the products were similar in appearance and general properties and no ready means was available for distinguishing between them.

Properties. The ash content (as sulphate), nitrogen content (by micro-Kjeldahl method), reducing sugar (as glucose) after acid hydrolysis (by modified Shaffer-Hartman method) (Peters & Van Slyke, 1932), specific rotation and activity in inhibiting isoagglutination were determined for each main fraction. In order to compare our method of performing the isoagglutination test with that of Morgan & King (1943), we determined the activity of one of Dr Morgan's preparations, obtaining the value of 0.15 μ g. for the minimum amount detectable, as against 0.1 μ g. obtained by them, showing that our results are reasonably comparable. On two other of their preparations, however, we obtained the value of 0.04 and 0.03 μ g. as compared with 0.1 μ g. for our best sample. In addition, the following qualitative tests were carried out using a 1% aqueous solution of the fractions: biuret, ninhydrin, Millon's, xanthoproteic, Sakaguchi (arginine), diazotized sulphanilic acid and the colour reaction for hexosamines (Elson & Morgan, 1933). In some cases the sodium fusion test for sulphur also was performed.

Of the protein reactions only the ninhydrin, Sakaguchi and diazotized sulphanilic acid reactions were always positive. The reaction for hexosamines was positive in all cases. All the fractions tested were found to be free from sulphur. A very large number of samples was examined over a period of several years and some typical analytical values for A and B preparations are given in Table 1 together with those for a polysaccharide from a group O gastric mucin for comparison. All values are corrected for ash content.

The changes shown in Table 2 were observed on heating certain of the fractions with N-HCl and with N-NaOH at 100°. Two samples of hog mucin A substance provided by Dr W. T. J. Morgan and having activities of 0.04 and 0.03 μ g. respectively were directly methylated by the method described in the previous paper (Bray *et al.* 1946). Each of Dr Morgan's samples gave a methylated derivative ($[\alpha]_D^{20}$, -21° ; OMe, 30.5%) identical with the corresponding derivative prepared from the pepsin

Table 1. *Properties of polysaccharides from gastric mucin*

Source of factor	Exp.	Reagent	Fraction	$[\alpha]_D^{18^\circ}$	Reducing sugar (as glucose) (%)	N (Kjeldahl) (%)	Minimum amount detectable by isoagglutination test ($\mu\text{g.}$)
Human gastric mucin							
A substances	1	BaCO ₃	All ppt. together	+65.5°	78.0	2.76	0.24
	2	BaCO ₃	(i)	+39.8°	61.6	2.07	0.55
			(ii)	+70.7°	70.1	3.67	0.15
	3	CaCO ₃	(i)	+14.4°	67.2	3.79	0.16
			(ii)	+26.1°	53.8	2.66	0.28
			(iii)	$\pm 0^\circ$	41.6	5.20	—
B substances	4	BaCO ₃	All ppt. together	+81.1°	82.3	2.22	2.28
	5	CaCO ₃	(i)	+42.3°	65.5	4.48	1.06
			(ii)	+44.5°	45.4	7.68	2.68
Polysaccharide from O gastric mucin	6	BaCO ₃	(i) unfractionated	+70.2°	83.5	1.75	—
Hog mucin A substance (Morgan & King, 1943)		Phenol		+11.0°	50.0	6.0	0.15 0.04 0.03 (3 samples)

Table 2. *Hydrolysis of blood-group factors*

(1% solutions in N-HCl, N-NaOH or 0.1N-Na₂CO₃ were hydrolyzed at 100° and the optical activity of samples taken after different periods of hydrolysis measured. The last value of $[\alpha]_D$ recorded is the equilibrium value.)

Fraction	Hydrolytic agent	A substance						
2 (ii)	HCl	Period (min.)	0	60	120	180	275	420
		$[\alpha]_D^{20^\circ}$	+71°	+24°	+24°	+28°	+28°	+24°
2 (ii)	NaOH	Period (min.)	0	60	120	180	300	420
		$[\alpha]_D^{20^\circ}$	+79°	+47°	+47°	+42°	+35°	+38°
3 (ii)	HCl	Period (min.)	0	70	150	270	480	
		$[\alpha]_D^{18^\circ}$	+26°	+31°	+32°	+24°	+24°	
3 (ii)	NaOH	Period (min.)	0	60	255	435		
		$[\alpha]_D^{18^\circ}$	+31°	+12°	+12°	+12°		
Morgan & King's (1943) A substance								
	HCl	Period (min.)	0	60	120	240	420	
		$[\alpha]_D^{20^\circ}$	+10°	+41°	+45°	+49°	+49°	
	NaOH	Solution was too dark for observation						
	Na ₂ CO ₃	1% solution $[\alpha]_D^{20^\circ}$, +26° treated for 2 hr. on boiling water-bath, neutralized, dialyzed for 48 hr., precipitated with ethanol and isolated in the usual way: $[\alpha]_D^{20^\circ}$, $\pm 0^\circ$.						
B substance								
5 (i)	HCl	Period (min.)	0	30	60	150		
		$[\alpha]_D^{18^\circ}$	+42°	+39°	+34°	+34°		
	NaOH	Period (min.)	0	30	60	150		
		$[\alpha]_D^{18^\circ}$	+42°	+32°	+26°	+26°		
O substance								
6 (i)	HCl	Period (min.)	0	30	60	150	210	240
		$[\alpha]_D^{18^\circ}$	+70°	+76°	+56°	+54°	+52°	+52°
	NaOH	Period (min.)	0	60	135	195		
		$[\alpha]_D^{18^\circ}$	+70°	+56°	+52°	+52°		

A substance. From each sample the fully methylated *l*-fucose derivative was obtained in *c.* 6% yield. Details of this work will be published later.

Table 3. *The properties of some blood-group factors prepared by other workers*

Description	$[\alpha]$	Reducing sugar (%)	N (%)
A substance from pseudo-mucinous ovarian cyst fluids (a)	+11°*	50	6.0
Saliva (b) (typical for all groups)	—	45–48	5.5
B substance (c) from gastric juice	—	75	1.5–1.6
O substance (d) from gastric juice	—	39.8	2.8

(a) King & Morgan (1944).

(b) Landsteiner & Harte (1941).

(c) Witebsky & Klendshoj (1940).

(d) Witebsky & Klendshoj (1941).

* λ 5461 Å.

DISCUSSION

It is abundantly clear that the mucopolysaccharide mixture from human gastric contents is highly complex and that the method of fractionation and purification by precipitation from aqueous solution by means of organic solvents is laborious and in the main is inadequate to provide pure material. Only the more elaborate physical methods such as ultracentrifugation and electrophoresis will be sufficiently precise to give a possible means of distinguishing readily between the various factors. In considering the literature and our own results on the A substance it does seem generally agreed that the most active fractions have an optical activity of $[\alpha]_D$, +10° to +20°, so that the higher preliminary values we have found for the $[\alpha]_D$ of the most active B substance and for the polysaccharide from O group mucin may eventually prove of significance. It was of interest to find that the A substance from human gastric contents (unlike the crude commercial pepsin and hog mucin which were highly laevo-rotatory, e.g. $[\alpha]_D$, -100°) contained a highly dextro-rotatory mucopolysaccharide. Inasmuch as the polysaccharide from O group mucin could not readily be fractionated it will be of interest to determine whether this is a contaminant of the A and B substances from gastric contents. The A substance is undoubtedly labile and on degradation appears to give rise to laevo-rotatory products (cf. Morgan & King, 1943) which are closely related in physical properties thus making a separation difficult. The work of Morgan & King (1943) is of great importance

as giving a new aspect on the problems concerning the nature of the blood-group factors. They have shown that a highly purified undegraded A substance, extracted by phenol from hog mucin, was split by saponification with Na_2CO_3 at 100° in such a way that one-third of it dialyzed through a colloidion membrane. The dialysate had a high nitrogen content and contained *N*-acetyl amino sugars originally linked glycosidically to amino-acid residues in the A substance. (It should be noted that the group substances prepared by Witebsky & Klendshoj (1940, 1941) and by ourselves (Tables 1 and 3) contained a significantly lower nitrogen content than did the products of Morgan & King from hog mucin and ovarian cyst fluid.) The non-diffusible portion of Morgan & King's hydrolysate was weakly laevo-rotatory ($[\alpha]_D$, -20°) and contained amino-acid constituents still attached to a carbohydrate residue, but this complex had no more than 1% of the activity of the original complex. On this point our own findings on the A substance from human gastric mucin are somewhat at variance. As shown above our fractions were prepared by a relatively vigorous saponification process and although they were less viscous and presumably more degraded than Morgan & King's A substance yet they had a dextro-rotation and the isoagglutination activity (0.15–0.1 $\mu\text{g.}$ as compared with 0.1–0.04 $\mu\text{g.}$) was not significantly less. It is possible, even with Morgan & King's preparation, that the limit of purification has not yet been reached because Landsteiner & Chase (1936) have claimed the isolation of group substances from horse saliva and pig pepsin which were detectable in quantities as minute as 0.0005 $\mu\text{g.}$ Our own activity findings on Dr Morgan's fractions agreed very closely with his and it is most likely that the discrepancy between our results and those of American workers is due to variations in technique. There may be differences among the A substances themselves and Morgan & Watkins (1944) have obtained some evidence of this from the serological point of view in investigations on the hog gastric mucin A substance (from commercial hog mucin) and on the A substance from pseudo-mucinous ovarian cyst fluid. All the active group substances we have examined so far undergo a change in optical activity when subjected to alkaline hydrolysis, e.g. with *N*-NaOH. In our experience it is unusual for any polysaccharide, e.g. starch, glycogen, dextran, etc., which is devoid of prosthetic groups, to show such a change. Thus it is possible that the effect on the group substance is due to a continuous removal of the prosthetic groups, in particular the amino-acids. The change in rotation for the A, B substances and the O mucin polysaccharide was always in the same direction, i.e. progressively less positive, thus emphasizing that all the factors are of the same type of substance. In

view of Morgan's (1944) statement that the A substance from hog mucin was shown by the method of Consden, Gordon & Martin (1944) to contain no fewer than fifteen amino-acids as part of the complex, it is not difficult to understand how changes in the number and kind of these could give rise to differences in serological specificity even as profound as those existing between the A, B and O groups. Although our present knowledge of these substances is very scanty, it is tempting to speculate that they will contain a common or closely related relatively stable polysaccharide constituent. It has been of interest and gratification to find that the two samples of undegraded and highly purified A substances from commercial hog mucin provided for us by Dr Morgan gave rise to the same methylated stable carbohydrate residue with a *l*-fucose 'end-residue' as was obtained from the pepsin A substance (Bray *et al.* 1946). These studies will be continued in collaboration with Dr W. T. J. Morgan.

SUMMARY

1. A method for preparing polysaccharides from gastric contents of individuals of blood groups A, B and O is described. This material is suitable for preliminary chemical studies of a stable carbohydrate constituent.

2. Although partially degraded, the material from A and B group individuals displays considerable activity in inhibiting isoagglutination.

3. The properties of various fractions are described, the significance of the results is discussed, and attention drawn to the complexity of structural problems in this field.

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The Fate of Certain Organic Acids and Amides in the Rabbit

1. BENZOIC AND PHENYLACETIC ACIDS AND THEIR AMIDES

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During an investigation of the fate of aromatic amino compounds in the rabbit it was observed that in some cases there was a considerable difference between the products of metabolism of an acid and its amide. Thus it was found that, whereas 45 % of a dose of anthranilic acid given to a rabbit could be recovered from its urine (after hydrolysis), only a very small amount could be obtained after giving an equivalent dose of *o*-aminobenzamide. Further, less phenolic material, as judged by the ferric chloride reaction appears to be formed from the acid than from the amide. Since there is little record in the literature of the investigation of such differ-

ences, we have planned a series of comparative studies of certain acids and their amides in order to ascertain in what instances and to what extent an acid and its amide may be regarded as metabolically interchangeable.

There are three main aspects of the problem which may be studied: (a) the hydrolysis of the amide group, (b) the modification of groups already present in the molecule and (c) the introduction of hydroxyl or other groups. The differences may, however, be quantitative rather than qualitative, and it is from the former standpoint that these investigations will in the main be conducted.