finate being considerably less stable than the *n*-propyl derivative.

2. As a previously undescribed reaction, it was found that alkyl sulfites reacted rapidly with hydrogen chloride forming alcohols and alkyl chlorides. This gas did not alter the rate of decomposition of alkyl chlorosulfinates.

3. Simultaneous addition to a refluxing solvent was found to be a convenient method for the reaction of primary and secondary alcohols with thionyl chloride to give sulfite and chlorosulfinate esters in good yields.

4. The variables of temperature, type of alcohol, and ratio of reactants have been studied in the reaction of alcohols with thionyl chloride in a refluxing solvent. The *primary* alcohol followed the expected metathesis, forming either sulfite or chlorosulfinate, depending on the molar ratio used At higher temperatures the chlorosulfinate yield decreased sharply, while olefin and alkyl chloride yields increased. The *secondary* alcohol gave an excellent yield of sulfite ester at low temperature but the chlorosulfinate yield was low and erratic under all conditions. At higher temperatures olefin or alkyl chloride formation predominated. The formation of diisopropyl ether represented a new type of reaction in which thionyl chloride was involved. The *tertiary* alcohol formed only alkyl chloride and olefin.

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[CONTRIBUTION FROM THE DEPARTMENT OF NEUROLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY AND THE NEUROLOGICAL INSTITUTE]

Immunochemical Studies on Blood Groups. V. Further Characterization of Blood Group A and O Substances from Individual Hog Stomachs[1a]

BY AARON BENDICH,[1b] ELVIN A. KABAT AND ADA E. BEZER

Gastric mucin prepared from random pools of hog stomachs has been widely employed[1c-5] as a source of the blood group A substance, despite the observation of Witebsky[6] that not all hog stomach linings showed blood group A activity. In a recent study from this Laboratory[7] in which individual hog stomach linings were used, it was found that only seven of ten stomachs examined showed blood group A activity; the remaining three were inactive in this respect. By autolysis or peptic digestion and subsequent purification (*cf.* 4, 7) products were obtained from all ten stomachs in approximately the same yield which were identical, within experimental error, in nitrogen, glucosamine, reducing sugar and acetyl content, and in relative viscosity. They could only be distinguished by their immunological and quantitative immunochemical properties,[7] seven showing a capacity to inhibit the hemagglutination of A cells by human anti-A, to precipitate anti-A from sera of humans of blood groups B and O,[5] and to induce the formation of isoagglutinins for A erythrocytes on injection into man[8]; the purified materials from the remaining three hogs were inactive in these respects.[7]

By applying the methods of quantitative immunochemistry (for reviews *cf.* refs. 9, 10, 11), six of the seven active products were found to show equal potency in precipitating anti-A and on an average, eighty-four per cent. of the glucosamine of these preparations was specifically precipitable by excess anti-A (the seventh was somewhat less active). This latter finding provides strong evidence for the high degree of purity of the active preparations on an absolute basis.

Evidence has since been obtained[12,13] indicating that the inactive products show blood group O activity; the high degree of purity of these preparations has been adduced from a quantitative immunochemical study of the cross-reaction of both the A and inactive substances with Type XIV antipneumococcal horse serum.[13] The present communication presents data showing the inactive substances previously isolated to have blood group O activity and indicates that individual hog stomach linings may contain either A substance or O substance alone and that certain hogs' stomach linings contain both A and O substances in varying proportions. It also records several additional immunological, physical, and chemical properties of the purified A and O substances and the isolation of *d*-glucosamine and of derivatives of *l*-fucose and *d*-galactose from hydrolysates. The finding of *l*-fucose confirms and extends the

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(1b) Present address: Sloan-Kettering Institute for Cancer Research, New York 21, N. Y.

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TABLE I

ASSAY OF BLOOD GROUP SUBSTANCES FROM INDIVIDUAL HOG STOMACH LININGS FOR BLOOD GROUP O ACTIVITY BY INHIBITION OF HEMAGGLUTINATION OF GROUP O ERYTHROCYTES BY CATTLE SERUM ABSORBED WITH A_1B CELLS

	0.1 ml. undiluted cow serum B (titer 4) absorbed with A_1B cells							0.1 ml. undiluted cow serum D (titer 4) absorbed with A_1B cells							
μg. substance added	20	5	2	0.5	0.2	Prop. of glucosamine precipitable by anti-A,[a] %	μg. substance added	20	10	5	2	1	0.5	0.1	Prop. of glucosamine precipitable by anti-A, %
Samples showing blood group A activity															
Hog 1	+±	+++	+++	++±	+++	83	Hog 8		++			+++		+++	81
Hog 3	+++	+++	+++	+++	+++	90	Hog 10		+			+++		+++	85
Hog 4	+++	+++	+++	+++	+++	90	Hog 16	+		+++	+++		+++		
Hog 5	+++	+++	+++	+++	+++	84									
Samples showing both blood group A and O activity															
Hog 9	–	+	++±	+++	+++	70	Hog 14		±			++		+++	
Hog 32	–	–	–	+	++±	10	Hog 32	–		–	–		+±		10
Hog 15[b]	–	±	+	++	+++		Hog 15[b]	–		±	+±		++±		
Samples showing only blood group O activity															
Hog 2	–	–	–	–	+++		Hog 13		–			–		+	
Hog 6	–	–	–	+±	+++		Hog 19		–			–		++±	
Hog 7	–	–	–	+++	+++		Hog 25		–			–		+±	
							Hog 27		–			–		++±	
							Hog 29		–			–		++	
Controls: serum, saline and cells					+++		Serum, saline and cells				+++				
Cells and saline					–		Cells and saline				–				

Cell suspension: 0.1 ml. of a 1.5% suspension of washed group O erythrocytes.

– = complete inhibition of hemagglutination

+++ = no inhibition of hemagglutination

[a] *Cf.* ref. 7. [b] Hog 15 showed 73% of the capacity of precipitating anti-A as did Hog 16.

work of Bray, Henry and Stacey[14] who first demonstrated the presence of fucose by methylation studies.

Experimental

To provide adequate quantities of the blood group substances an additional twenty individual hog stomach linings were obtained; fourteen of these showed A activity, the remaining 6 showed no A activity, the proportions of active and inactive stomachs being the same as originally observed[7] and probably not significantly different from the report that 14 of 24 hog stomachs showed A activity.[12] Purified products were obtained from 4 of the active and 6 of the inactive stomachs as described in 5, 7.

Samples of blood were obtained from each of the twenty hogs. In slide agglutination tests with the sera of each of 19 of the hogs against the red cells of all twenty hogs, no agglutination was observed.

Assays for Blood Group O Activity.—The various purified products were assayed for blood group O activity by measuring their relative capacities to inhibit hemagglutination of group O cells by cattle serum containing anti-O; before use the cattle serum was absorbed with A_1B cells to remove agglutinins for human cells other than anti-O.[15] Tests were performed by mixing 0.1-ml. portions of absorbed serum with varying quantities of substance in a total volume of 0.4 ml. After one-half hour at 37°, 0.1 ml. of a 1.5% suspension of washed group O erythrocytes was added, the contents of the tubes mixed, the tubes incubated for one hour at 37°, centrifuged lightly and the degree of agglutination determined. Table I summarizes the results obtained. The preparations fall into three groups, those showing blood group A activity only, those showing both A and O activity and those with blood group O activity only. For comparison, values for the proportion of the glucosamine of the products with blood group A activity which was specifically precipitable by an excess of anti-A are included.[7] It is noteworthy that the preparation from hog 9 which was stated[7] to be somewhat less pure than those from hogs 1, 3, 4, 5, 8 and 10 is now found to show O activity, thereby adding to the significance of the immunochemical criteria used. Hogs 15 and 32 also showed both blood group A and O activity, the latter predominantly O and the former chiefly A activity.

Forssman Antigen Studies.—The blood group substances from the ten hog stomach linings previously described[7] and 1A from gastric mucin were assayed for Forssman antigen. Assay was made of the capacity of each of the samples on preliminary incubation at 37° with antibody and complement to inhibit the hemolysis of 0.1 ml. of a 4% suspension of washed sheep erythrocytes by 10 hemolytic units of rabbit antibody to human A erythrocytes in the presence of 4 units of complement. As shown in Table II, 0.05 μg. of the preparations from those hog stomachs which showed blood group A activity[5] inhibited hemolysis completely or almost completely while 1 μg. of the purified samples from the three inactive (O) hogs,[5] 2, 6, 7, had no effect on this system. One-tenth μg. of the mixture (1A) of A and O substances was required to inhibit hemolysis. Control tests showed that even 10 μg. of the substances were not appreciably anticomplementary. It is evident, therefore, that the Forssman activity is associated exclusively with the blood group A activity.[16]

Immunization of Rabbits.—Five rabbits received a series of eight intravenous injections, twice weekly, of a solution of blood group O substance (hog 29); a total of 4 mg. of material was given. Blood samples taken seven days later showed no evidence of agglutinins for human group O erythrocytes. Three of these animals then each received an additional eight injections, 8 mg. per animal. Serum samples taken six days after the last injection showed no O agglutinins.

Two groups of four rabbits each were injected with

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(15) E. Witebsky and N. C. Klendshoj, *J. Exp. Med.*, **73**, 655 (1941).

(16) F. Schiff and L. Adelsberger, *Z. Immunitätsf.*, **40**, 335 (1924); *Zent. Bakt. (Orig.)*, **93**, 172 (1924).

TABLE II

ASSAY OF BLOOD GROUP SUBSTANCES FROM INDIVIDUAL HOG STOMACHS AND FROM GASTRIC MUCIN FOR FORSSMAN ANTIGEN

μg. substance added	1.0	0.5	0.2	0.1	0.05	0.02
Hog 1	O	O	O	O	O	C
2	C	C	C	C	C	C
3	O	O	O	O	O	C
4	O	O	O	O	O	C
5	O	O	O	O	sl	C
6	C	C	C	C	C	C
7	C	C	C	C	C	C
8	O	O	O	O	O	C
9	O	O	O	O	O	C
10	O	O	O	O	sl	C
1A	O	O	O	O	C	C

Serum used: 0.1 ml. of 1:25 dilution of a rabbit antiserum to human A erythrocytes; titer 256.

Complement: 0.1 ml. of dilution of fresh guinea pig serum; titer 40.

Cell suspension: 0.1 ml. of 4% washed sheep erythrocytes.

O = no hemolysis; sl = slight hemolysis; C = complete hemolysis

Controls: Cells + serum + complement = C
Cells + complement (no serum) = O

blood group A and O substances, respectively, incorporated in an emulsion with aquaphor, paraffin oil and heat-killed tubercle bacilli. This procedure introduced by Freund and McDermott[17] has been found to give an enhanced antibody response with a variety of antigens (*cf.* ref. 18). Each animal received three 1.0-ml. injections at weekly intervals, a total of 1.5 mg. of active or inactive substance being given. Blood samples obtained ten days and one month after the last injection showed no increase in their capacity to agglutinate group A or O erythrocytes as compared with a sample of serum obtained before immunization.

Electrophoretic Studies.—Samples of A substance from hogs 3 and 5, of O substance from hogs 2 and 6, and of 1A from gastric mucin were studied in the Tiselius electrophoresis apparatus by Dr. Dan H. Moore. Five-tenths per cent. solutions in a buffer containing 0.15 *M* sodium chloride and 0.02 *M* phosphate at *p*H 7.4 each showed a single homogenous component in the microelectrophoresis cell. The mobilities $\times 10^{-5}$ calculated from the descending boundaries were as follows

Hog 2	3	5	6	1A
−1.3	−1.4	−1.4	−1.3	−1.4

These values are probably the same within experimental error, and indicate that a mixture of A and O substances such as 1A could not be resolved by this technic. The mobilities were different from those reported by Kekwick[4] on Morgan and King's products from gastric mucin, since different concentrations of substance, different buffers and *p*H were used.

Identification of Sugar Constituents.—Hydrolyzates of the A and O substances and 1A were examined for their sugar constituents.

***l*-Fucose Diphenylhydrazone.**—Approximately 5% solutions of the substances in 54 ml. of 2 *N* sulfuric acid were hydrolyzed in sealed tubes in a boiling water-bath for two hours. After cooling, the contents were chilled, neutralized to *p*H 7 (glass electrode) with saturated barium hydroxide, and filtered through infusorial earth. 11A contained 255 mg. of reducing sugars (calcd. as glucose) of which 135 mg. was found to be glucosamine.[19] The hog 25 (O) and hog 16 (A) substances contained, respectively, 298 and 225 mg. of reducing sugars, 162 and 131 mg. of glucosamine. To each was added 0.2 ml. of glacial acetic acid, and the solutions concentrated *in vacuo* to about 1.5 ml.

Treatment of the concentrate from 11A with 60 mg. of freshly distilled diphenylhydrazine and 5 ml. of ethanol, resulted, after sixteen hours in the dark at room temperature and subsequent chilling, in the separation of 32.1 mg. of white needles (crude *l*-fucose diphenylhydrazone). The derivative, recrystallized from 3.5 ml. of 95% ethanol (Nuchar), gave 24.8 mg. which was further purified by solution in 3 ml. of pyridine and then adding 30 ml. of water. After chilling 21.0 mg. was obtained, m. p. 200.0–200.5° (dec.); mixed m. p. unchanged.

Hog 25 and hog 16 yielded 30.4 and 46.0 mg. of the crude diphenylhydrazone, respectively. By purifying as above, 16.5 mg. of *l*-fucose diphenylhydrazone, melting at 200.0–200.5° (dec.), mixed m. p. unchanged, was obtained from hog 25; 30.9 mg. of derivative melting at 199–200°, mixed m. p. 199–200° (dec.), was obtained from hog 16.

Anal. Calcd. for $C_{18}H_{22}O_4N_2$: N 8.48 $[\alpha]_D = -15.8°$,[20] mol. wt., 330.4. Found (Dumas) 11A: N, 8.43, $[\alpha]_D^{25}$ −15.4, *C* = 0.8 (pyridine); found (Dumas) Hog 25 N, 8.42; $[\alpha]_D^{24}$ −15.5°, *C* = 0.8 (pyridine); found (Dumas) Hog 16: N, 8.35, $[\alpha]_D^{24}$ −15.8°; *C* = 1.2 (pyridine).

Attempts to Isolate *d*-Mannose.—For the isolation of mannose, reported[14] to be present in samples of the blood group A substance, of unspecified purity, excess diphenylhydrazine was removed and any soluble diphenylhydrazones split by treatment of the filtrates from the fucose derivatives with benzaldehyde and benzoic acid in the customary manner. The ether extracted filtrates, concentrated *in vacuo* to about 2.0 ml. were treated with 50 mg. of freshly distilled phenylhydrazine and 0.1 ml. of 25% acetic acid according to van der Haar.[21]

The mixtures were kept at room temperature for one hour and then at 0° for four days during which time a seed crystal of *d*-mannose phenylhydrazone was added. *In none of the preparations was the slightest evidence of solid hydrazone formation observed.*

To establish that small amounts of *d*-mannose, if actually present, could indeed be isolated under the experimental conditions employed, the following experiments were carried out. The above solutions were each diluted with 3 ml. of water and to the solutions from 11A and hog 16, 15.0 mg. of *d*-mannose was added. Phenylhydrazine was then removed as above. After extraction with ether, the aqueous solutions were concentrated to 2 ml. and 40 mg. of phenylhydrazine and 0.08 ml. of 25% acetic acid added. Crystals appeared in five minutes and after one hour at room temperature and sixteen hours at 0°, the mixtures were filtered yielding pale yellow crystals amounting to 18.5 mg. from 11A and 19.3 mg. from hog 16 and corresponding to recoveries of 83 and 87% of the added *d*-mannose, respectively. The crude derivatives were recrystallized to yield, respectively, 16.7 mg. and 17.8 mg. of the typical crystals of *d*-mannose-phenylhydrazone. Both derivatives melted at 201–202° (dec.); the melting point on admixture with an authentic specimen was unchanged.

Isolation of *d*-Galactose-α-methylphenylhydrazone.—Excess phenylhydrazine was removed from the above solutions and the ether-extracted aqueous filtrates decolorized, concentrated *in vacuo* to dryness, and the residues taken up in 2 ml. of water. To the clear solution from the hog 25 preparation was added 0.1 ml. of freshly distilled α-methylphenylhydrazine, 1.5 ml. of 95% etha-

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(18) E. A. Kabat, *Ann. Rev. Biochem.*, **15**, 505 (1946).

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(20) C. A. Brown and F. W. Zerban, "Physical and Chemical Methods of Sugar Analysis," 3rd ed., John Wiley and Sons, Inc., New York, N. Y., 1941, p. 686.

(21) A. W. van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydsauren," Berlin, 1920.

nol, 0.05 ml. of 25% acetic acid and the mixture kept for two hours at 37° and for sixteen hours in the ice box.[22] Crystals amounting to 154 mg. separated out. One-hundred and nineteen mg. was obtained after 3 recrystallizations from 30% ethanol which was further purified by solution in 6 ml. of pyridine and subsequent addition of 35 ml. of water whereupon 76 mg. of glistening white rectangles slowly deposited; m. p. 190–191° (dec.). Mixed melting point was 191–192° (dec.) with a specimen of *d*-galactose-α-methyl-phenylhydrazone which melted at 192–193° (dec.).

Seventy-four mg. of crude *d*-galactose-α-methylphenyl-hydrazone was obtained from hog 16 which after purification as above yielded 52 mg.; m. p. 189.5–190.5° (dec.); mixed m. p. 190–191° (dec.). 11A yielded 75 mg. of crude derivative from which on purification 26 mg. was obtained, m. p. 191.5–192.0° (dec.); mixed m. p. 192.0–192.5° (dec.).

Conversion to the Pentaacetate.—To determine the optical activity of the galactose, the derivatives of the A and O hog substances were converted to the pentaacetate by an adaptation of the method of Wolfrom and Christman.[23] 0.77 g. of *d*-galactose-α-methyl-phenylhydrazone suspended in 9.2 ml. of dry pyridine was treated at 0° with 3.7 ml. of acetic anhydride. The mixture was kept at room temperature with occasional shaking for forty-eight hours during which time the insoluble material had completely dissolved, and then poured into 170 ml. of ice cold water. A white mass was deposited which after copious washing with chilled water and drying, weighed 1.23 g. (92%). The product was dissolved in 53 ml. of warm ethanol (Nuchar) and 65 ml. of warm water was added. On cooling 1.06 g. of the glistening white plates of *d*-galactose-α-methyl-phenylhydrazone pentaacetate deposited; m. p. 139.5–140.5° without dec., $[\alpha]^{23}D$ +26.7°, *C* 4.2, pyridine, no mutarotation; $[\alpha]^{23}D$ +37.6°, *C* 0.9, 95% ethanol, no mutarotation.

Anal. Calcd. for $C_{23}H_{30}O_{10}N_2$ (494.5): N, 5.67. Found: (Dumas) N, 5.61.

Wolfrom and Christman[23] list m. p. 138–139° and $[\alpha]^{23}D$ +27.2°, *C* = 4.0 (pyridine).

In this manner, 61.9 mg. of the purified galactose-α-methyl-phenylhydrazone from hog 25 gave 104.7 mg. of the crude, white pentaacetate (97%), yielding 93.8 mg. after one recrystallization, m. p. was 139.5–140.0°; mixed m. p. 139.0–139.5°; 42.8 mg. of the galactose derivative from hog 16 gave 72.7 mg. of the crude, white pentaacetate (98%), yielding 65.4 mg. on recrystallization; melting point and mixed melting point were 139.5–140.5°.

Anal. Calculated for $C_{23}H_{30}O_{10}N_2$: N, 5.67; $[\alpha]^{23}D$ +37.6°, *C* = 0.9 (95% ethanol); mol. wt., 494.5. Found (Dumas): Hog 25, N, 5.56; $[\alpha]^{20}D$ +37.0°, *C* = 1.0 (95% ethanol); Hog 16, N, 5.51; $[\alpha]^{24}D$ +37.5°; *C* = 1.0 (95% ethanol).

Isolation of *d*-Glucosamine Hydrochloride.—For the isolation of glucosamine, 112.2 mg. of preparation 1A from pooled hog gastric mucin was hydrolyzed at 100° for five hours with 10 ml. of 6 *N* hydrochloric acid in a sealed tube. On analysis,[19] the decolorized hydrolyzate contained 34.9 mg. of glucosamine. Excess hydrochloric acid was removed by repeated concentration *in vacuo*, and the residue adjusted approximately to 75% ethanol in a total volume of about 0.7 ml. On cooling, 21.2 mg. of colorless plates were recovered which were recrystallized from 0.6 ml. of 75% ethanol to yield 16.0 mg. of *d*-glucosamine hydrochloride. Identification was confirmed by measurement of specific rotation and Dumas nitrogen determination.

152.2 mg. of the A substance (hog 16) and 144.7 mg. of the O substance (hog 25) were each hydrolyzed with 25 ml. of 6 *N* hydrochloric acid for five hours. 34.6 mg. of the crude hydrochloride was obtained from hog 16 which on recrystallization yielded 21.3 mg.; 38.1 mg. was obtained from hog 25 which gave 31.0 mg. on recrystallization.

Anal. Calcd. for $C_6H_{13}O_5N \cdot HCl$: N, 6.50; $[\alpha]D$ (final) +72.5°; *C* 1.4 (H_2O)[24]; mol. wt., 215.6. Found (Dumas) 1A: N, 6.06; $[\alpha]^{20}D$ (final) +72.5°; *C* 0.5 (H_2O); Hog 16, N, 6.28; $[\alpha]^{21}D$ (final) +71.7; *C* 0.7 (H_2O); Hog 25, N, 6.16; $[\alpha]^{23}D$ (final) +71.3°; *C* 0.5 (H_2O).

Discussion

The present communication summarizes data thus far obtained on the composition of purified blood group A and O substances prepared from individual hog stomach linings and shown to be of high purity by several immunochemical criteria.[7,13] The importance of characterizing samples of biologically active substances by these criteria is well illustrated by the finding (Table I) that individual hog stomach linings may show either blood group A or O activity and that some stomachs may show both A and O activity as would be anticipated from genetic studies on blood groups in man. The report of Aminoff, Morgan and Watkins[12] that individual hog stomachs showed only A or O activity but not both is probably due either to accidental selection or to the use of crude alcohol precipitates of autolyzed stomach linings rather than of purified preparations in assays for activity. Obviously, for studies of the underlying chemical basis of the specificity and immunological properties of such substances, it is essential to have products of high purity that show only a single specificity. Assay of each sample for the proportion of its glucosamine specifically precipitable by anti-A has provided strong evidence for the high degree of purity of the blood group A preparations on an absolute basis.[7]

Failure to establish adequately the absolute degree of purity of certain preparations of blood group A substance is probably responsible for the report[14] that they contained *d*-mannose. As indicated above, no evidence of mannose in the A or O substances could be found although small amounts which were then deliberately added to the hydrolyzates were recovered in good yield and without difficulty.

The data presented above indicate that, thus far, no difference between the blood group A and O substances from hog stomach linings has been found apart from their immunological properties. Within experimental error, the A and O substances are identical in electrophoretic mobility at *p*H 7.4, and both contain *l*-fucose, *d*-galactose and *d*-glucosamine. Previous data[7] indicated that the A and O substances did not differ in nitrogen, reducing sugar, glucosamine and acetyl content or in relative viscosity.

With respect to their biological properties, the A and O substances did not stimulate antibody formation in the rabbit under the conditions used. As had been found by others,[16] the Forssman activity was associated exclusively with the blood group A substance.

(22) M. Ludtke, *Biochem. Z.*, **212**, 419 (1929).

(23) M. L. Wolfrom and C. G. Christman, THIS JOURNAL, **53**, 3413 (1931).

(24) J. C. Irvine and J. C. Earl, *J. Chem. Soc.*, **121**, 2370 (1922).

The failure to find any clue to explain the unique activity of the A and O substances in terms of their sugar constituents points to the need to explore further the amino acid portions of these substances. Suggestive preliminary evidence that the amino acids may be involved in the specificity of the blood group substances has been obtained by Morgan[25] who found that, when allowed to act on a mixture of A and O substances from hog gastric mucin, a crude enzyme preparation containing enzymes splitting both blood group A and O substances inactivated the A substance and liberated primary amino groups and α-amino acids in addition to causing a change from dextro to levorotation, a fall in relative viscosity and the appearance of about 10% of reducing sugars. It is possible, however, that quantitative differences or differences in configurations of the carbohydrate moiety are also determinants of the specificity of the blood group substances.

(25) W. T. J. Morgan, *Nature*, **158**, 759 (1946).

Summary

1. Individual hog stomachs have been shown to yield purified substances showing either blood group A or blood group O specificity. Certain individual stomachs showed both A and O specificity in varying proportions. The products previously isolated from this laboratory and shown to be inactive with respect to blood group A activity have been identified as the blood group O substance.

2. Forssman antigen activity was exclusively associated with blood group A activity. No evidence of antibody production to the A or O substance in rabbits was obtained.

3. Blood group A and O substances were identical in electrophoretic mobility and derivatives of *l*-fucose, *d*-galactose and *d*-glucosamine were isolated from hydrolyzates of these substances.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Steroidal Sapogenins[1a]

BY RUSSELL E. MARKER,[1b] R. B. WAGNER, PAUL R. ULSHAFER,[1c] EMERSON L. WITTBECKER,[1d] DALE P. J. GOLDSMITH[1e] AND CLARENCE H. RUOF

With the establishment of the structure of the steroidal sapogenins and the demonstration of the conversion of some of them to certain sex hormones, a search was begun in this Laboratory for naturally occurring sapogenins oxygenated in the 11-position as possible starting materials for the synthesis of various cortical hormones.

While to date no 11-oxygenated sapogenins have been isolated, we have, in an investigation of over 40,000 kilograms of plants, comprising more than 400 species collected mainly in Mexico and the Southern United States, (1) found new sources for almost all the known sapogenins, including diosgenin, the starting material for the synthesis of certain sex hormones, (2) isolated and identified twelve new sapogenins, (3) isolated and identified two new steroids which apparently are the progenitors of the sapogenins, and (4) noted a correlation between plant cycle and saponin content which suggests a biogenetic relationship.

Hitherto only notes on this work have been published.[2-7] This paper presents in more detail the results of our investigations on the new steroidal sapogenins and their biogenetic interrelationships.

Previously, we reported the isolation of steroidal sapogenins from plants belonging to the families *Liliaceae* and *Dioscoreaceae*. Steroidal sapogenins also occur in plants of the *Scrophulariaceae* family. It is noteworthy that until the present work all the known sources of these compounds were confined to these three families. Most of the sources are plants belonging to genera of the *Liliaceae* family. For example, we have shown recently that diosgenin occurs in four different genera of this family. Sarsasapogenin has been shown to occur in two more of its genera, *Smilax* and *Asparagus*. In the present work we have extended our studies to include not only plants of the above families but some from the related family, *Amaryllidaceae*. Over four hundred species in the amount of forty thousand kilograms have been obtained as a result of several extensive botanical collection trips by the senior author in Mexico and southern United

(1) (a) This is Paper 160 in the Sterol Series and 72 in the Sapogenin Series from this Laboratory. For the preceding paper see THIS JOURNAL, **65**, 1658 (1943); (b) present address: Hotel Geneve, Mexico City, Mexico; (c) present address: Ciba Pharmaceutical Products, Inc., Summit, N. J.; (d) present address: Rayon Dept., E. I. du Pont de Nemours and Co., Buffalo, N. Y.; (e) present address: Research and Development Dept., Merck and Co., Rahway, N. J.

(2) Marker, Wagner, Goldsmith, Ulshafer and Ruof, THIS JOURNAL, **65**, 739 (1943).

(3) Marker, Wagner, Ulshafer, Wittbecker, Goldsmith and Ruof, *ibid.*, **65**, 1199 (1943); *cf. ibid.*, **69**, 2242 (1947); for supplementary tables address American Documentation Institute, 1719 N Street, N. W., Washington, D. C., requesting Document 2384, and remitting 50¢ for microfilm or $2.10 for photocopies.

(4) Marker, Wagner, Ulshafer, Goldsmith and Ruof, *ibid.*, **65**, 1247 (1943).

(5) Marker, Wagner, Goldsmith, Ulshafer and Ruof, *ibid.*, **65**, 1248 (1943).

(6) Marker, Wagner, Ruof, Goldsmith and Ulshafer, *ibid.* **65**, 1434 (1943).

(7) Marker, Wagner, Ruof, Ulshafer and Goldsmith, *ibid.*, **65**, 1658 (1943).