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Immunochemical Studies on Blood Groups. XX. The Effects of *Clostridium tertium* Enzymes on Blood Group A, B and O(H) Substances and on Type XIV Pneumococcal Polysaccharide[1]

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Purified blood group A, B and O(H) substances and type XIV pneumococcal polysaccharide (SXIV) were treated with enzyme preparations from two strains of *Clostridium tertium* (Iseki and McClung, 1259). Specific precipitability of blood group A antigen by human anti-A was greatly reduced or abolished by Iseki strain enzymes, but not by those of McClung strain 1259, whereas blood group B and O(H) activities were unaffected by either enzyme preparation. The cross reactivity of all blood group substances with horse anti-SXIV was almost completely destroyed. Cross reactivity of such enzyme treated preparations could be increased above initial levels by mild acid hydrolysis. The capacity of SXIV per unit weight to precipitate anti-SXIV was substantially reduced by both enzymes. The maximum anti-SXIV precipitable by SXIV treated with Iseki strain enzymes was 82 and 87% and by SXIV treated with McClung strain 1259 enzymes 61 and 68% of that precipitable by untreated SXIV.

Introduction

Enzymes capable of altering the serologic properties of blood group antigens are finding increasing use in exploring the relationships between the structure and the antigenic specificity of these complex substances. The problem has lately been critically reviewed.[3] It has been demonstrated that loss of reactivity with rabbit anti-O(H) serum, following hydrolysis of O(H) substance with partially purified *Cl. welchii* enzyme,[4] is accompanied by the liberation of a major portion of the total fucose, some N-acetylglucosamine, galactose, amino acids and small amounts of disaccharide components.[5] Studies with extracts of snail liver, particularly from the species *Busycon*[6] rich in β-glucosamidase, have shown that reduction in blood group A activity of hog blood group A substances following enzyme treatment is accompanied by the liberation of N-acetylhexosamine and fucose. Only the latter sugar was released in significant amounts from hog O(H) substance, which retained its activity by hemagglutination inhibition assay. In conformity with the inverse relationship, established for hog blood group substances, between fucose content and capacity to precipitate anti-SXIV,[7] it was found that splitting of fucose from hog A and O(H) substances by snail enzymes, as with mild acid hydrolysis,[8] resulted in a significant increase in this cross reaction. Enzymes of *Trichomonas foetus* destroy A, B and O(H) receptors on whole erythrocytes, but partially purified enzyme from the same source has been found to be active only against O(H) substances and not against A, B, Le^a or Le^b antigens.[9] It was further found that D-galactosamine and L-fucose inhibited the action of *T. foetus* enzymes on soluble O(H) substance much more strongly than did D-galactose and D-fucose; D-glucosamine was completely inactive as an inhibitor. N-Acetylgalactosamine inhibited the action of *T. foetus* enzymes on A substance and D-galactose inhibited action on B substance.[10] It was also reported that *T. foetus* enzymes, in inactivating O(H) substance, liberated L-fucose and N-acetylhexosamine but no detectable galactose.[5b] Enzymes of *Lactobacillus bifidus* (var. Penn.) have been shown to act on various mucoids with the destruction of A, B and O(H) activities, and the liberation of N-acetylhexosamine, fucose and galactose.[11] The relationship of a number of disaccharides,[12] some of which serve as growth factors for *L. bifidus*, to blood group substances and their hydrolysis products has also received considerable attention.

Culture filtrates of *Cl. tertium* were described[13] originally as being specific for the A antigen. This has been confirmed in recent studies[14] which have shown that these filtrates have several enzyme activities including aminopeptidases, capacity to destroy human and chicken erythrocyte receptors for certain A and A′ strains of influenza virus and human erythrocyte receptors for Columbia SK virus, and render human erythrocytes panagglutinable. They also inactivate the influenza virus hemagglutinin inhibitors in several soluble inhibitory mucoids. Continued investigation into the effect of *Cl. tertium* enzymes on A, B and O(H) substances and on type XIV pneumococcal polysaccharide has disclosed that certain groupings on all four substances are attacked but that only blood group A substance and SXIV are altered in their

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reactivity with their respective specific antibodies. A, B and O(H) substances lose almost all of their cross reactivity with anti-SXIV on treatment with these enzymes. Mild acid hydrolysis restores cross reactivity of these enzyme treated products with anti-SXIV in many cases even above pre-enzyme treatment levels.

Materials and Methods

Preparation of Substrates.—Blood group A and O(H) substances were prepared from commercial hog gastric mucin (Wilson), from single hog stomach linings and from ovarian pseudomucinous cyst fluid by phenol extraction and fractional ethanol precipitation.[3,15] Blood group B substance was prepared by the same methods from the bronchial secretions of a secretor of group B. In addition to the usual peptic digestion, this preparation was also subjected to tryptic digestion (Tryptar, Armour) prior to dialysis and phenol extraction. A sample of human ovarian cyst A substance was kindly supplied by Dr. Sidney Leskowitz of the Massachusetts General Hospital. Two samples of type XIV pneumococcal polysaccharide were obtained, one (#227, lot 80320) from Mr. T. D. Gerlough of E. R. Squibb and Son, the other prepared by Mr. T. E. Gilmore in this Laboratory. All substrates were subjected to at least 24 hours of dialysis against running tap water, followed by dialysis against distilled water. These dialyzed materials were clarified by centrifugation and the supernatants lyophilized. Recovered materials were then further dried to constant weight over phosphorus pentoxide *in vacuo*.

Preparation of Enzymes. Enzymes were prepared by methods already described[14] from two strains of *Cl. tertium*. One of these strains (Iseki) was active against soluble blood group A antigen as shown by hemagglutination inhibition and quantitative precipitin determinations and destroyed A antigen on intact erythrocytes. The other strain (McClung strain 1259) was consistently without detectable activity on blood group A antigen either in mucoids or on erythrocytes. Enzymes were precipitated from 48 hour culture supernatants at full saturation with ammonium sulfate and the precipitate dialyzed against running tap water to remove ammonium sulfate and traces of free glucosamine and other dialyzable components of the medium. Enzyme preparations were stored at 4° in the lyophilized state and were freshly dissolved in $M/15$ phosphate buffer at pH 7.5 just prior to use. In some instances dialyzed culture supernatant was used.

Immunochemical Methods.—Blood group A and B antigens were estimated by inhibition of hemagglutination and determined quantitatively[16] by precipitation of anti-A and anti-B from calibrated human antisera.[17] O(H) antigen was measured by inhibition of agglutination of group O(H) erythrocytes by bovine serum containing anti-O(H) previously absorbed with A_1B erythrocytes.[18] The reactivity of SXIV and the cross reaction of A, B and O(H) substances,[5,19] with horse anti-SXIV was measured by the quantitative precipitin technique.[16 cf.20] Hexosamine was determined by the method of Elson and Morgan[21] and reducing sugar by the method of Hagedorn and Jensen[22] after hydrolysis for 2 hr. with 2 N HCl and neutralization. Methylpentose was determined by the method of Dische and Shettles[23] and nitrogen by the Markham modification of the micro-Kjeldahl procedure.[20,24] Mild acid hydrolysis was carried out by addition of dilute HCl of pH 1.5 to a solution of blood group substance to give a final concentration of 1 mg. blood group substance per ml. at pH ranging from 1.7 to 2.0. The solutions were heated for 2 hr. at 100° in sealed tubes. After hydrolysis solutions were neutralized, made up to a known volume and measured quantities of substance added to type XIV antiserum.[5]

Experimental

Table I summarizes the essential features of seven separate experiments. All preparations were completely water soluble at the outset of each experiment. The enzyme preparations used in all but the first two experiments were obtained by precipitation from culture supernatants at full ammonium sulfate saturation (FSP). In experiment 1, dialyzed lyophilized culture supernatant (DCS) was used. In experiment 2, enzyme was prepared from the full saturation precipitate by reprecipitation with ammonium sulfate between 42 and 53% of saturation (fraction III, preparation 2, Table V in ref. 14). The ratio of the weight of substrate to active enzyme was 1.4 to 6.7. It had been found that mucin blood group substance incubated for 24 to 36 hours with anything less than one fifth of its weight of such enzyme preparations (FSP) was not completely inactivated in 24 to 36 hours. The ratios of blood group substance to total enzyme used in the experiments shown were therefore somewhat lower in order to ensure the destruction of the A antigen present. Enzyme may therefore have been in excess of that needed merely to destroy cross reactivity in a similar time interval. In experiments 1 to 3, the total amount of enzyme was added in two successive portions, separated by 24 hours of incubation, since in each instance appreciable blood group A activity remained in the digestion mixture after the first addition of enzyme. All enzyme preparations were themselves devoid of detectable blood group activity. Enzyme solutions in cold $M/15$ phosphate buffer at pH 7.5 were added to the substrates in water solution. The mixtures were then incubated at 37°, under toluene, in experiments 1 and 3 for a total of 3 to 5 days, in experiments 2 and 6 for 48 hr., and in experiments 4 and 5 for 24 hr. In experiment 7, the blood group substances were incubated with each enzyme for 24 hr. Digests were dialyzed in the cold against 5 to 6 successive changes of distilled water, and the pooled dialyzates for each preparation were concentrated *in vacuo* at 48° and made up to known volume. Except in experiment 6, the non-dialyzable residues, washed from dialysis bags, were clarified by centrifugation, lyophilized and extracted with phenol, blood group substance being recovered as a phenol insoluble fraction or from phenol solution by fractional ethanol precipitation. After washing with ethanol, these materials were dried to constant weight *in vacuo* over phosphorus pentoxide and analyzed for methylpentose, nitrogen, residual blood group activity and cross reaction with horse anti-SXIV, and for hexosamine and reducing sugar. In experiment 6 with SXIV, non-dialyzable residues were made up to known volume, clarified by centrifugation and the supernatants analyzed without further purification. In experiment 7, blood group substance from a single hog stomach (hog 58 (A)), from human ovarian cyst fluid (McD) and from hog mucin (Fr. 2) were digested overnight with enzyme from McClung strain 1259. After establishing that blood group A antigen was still present in each by hemagglutination inhibition assay on a small aliquot, the digests were dialyzed against 6 changes of water and the dialyzates concentrated. To the non-dialyzable residues, enzyme from the Iseki strain was then added and the mixtures reincubated. After a second dialysis, blood group substance was reisolated from the non-dialyzable residues as already described. As controls, 83 mg. enzyme from McClung strain 1259 in buffer was incubated overnight and dialyzed. An equal amount of Iseki strain enzyme was then added, and this mixture was incubated and dialyzed. In addition, an 86-mg. sample of Iseki enzyme alone was incubated and dialyzed in the same way. The non-dialyzable residues from both enzyme controls were centrifuged, lyophilized, extracted with phenol and fractions precipitating at 16–17% and with excess ethanol as well as the phenol insoluble material were recovered for analysis. Substrate control preparations consisted of blood group substance alone (experi-

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TABLE I

EFFECT OF *Clostridium tertium* ENZYMES ON BLOOD GROUP SUBSTANCES AND TYPE XIV PNEUMOCOCCAL SPECIFIC POLYSACCHARIDE (SXIV)

Experiment no.	Substrate: Preparation	Substrate: Source	Substrate: Antigens	Substrate: Mg.	Enzyme[a]: Strain	Enzyme[a]: Mg.	% of original recovered	Reisolated substrate μg. inhibiting: A-anti-A	Reisolated substrate μg. inhibiting: B-anti-B	Reisolated substrate μg. inhibiting: O(H) anti-O(H)
1	7A[b]	Hog mucin	A + O(H)	66.0	Iseki[c]	20	52[g]	>42		1
				65.2			61[g]	1		1
2	41[d]	Hog stomach linings	A	23.1	Iseki[e]	6(heated)[f]	45[g]	0.5		>120
				36.8	Iseki[e]	14(active)	38[g]	>106		>147
	54[h]		O(H)	30.3	Iseki[e]	6(heated)[f]	67[h]			2–5
				33.1	Iseki[e]	14(active)	65[h]			2–5
3	52[i]	Hog stomach linings	A	85.9	Iseki	21	60[j]	>108[g]		
	54[h]		O(H)	74.4	Iseki	18	64[h]			2
	7A[b]	Hog mucin	A + O(H)	580.9	Iseki	85	83[k]	>113		4
4	McD[l]	Human ovarian cyst	A	34.1	Iseki	23	49[m]	>150		
	RS6[h]	Human sputum	B	125.3	Iseki	45	92[h]		1–2	
	SXIV			34.5	Iseki	23	35[m]			
5	McD		A	34.1	McClung (1259)	14	81[n]	0.5		
	RS6		B	125.3	McClung (1259)	29	83[m]		0.3	
	55[o]	Hog stomach linings	A	61.7	McClung (1259)	29	79[p]	2		
	G12[n]		O(H)	96.0	McClung (1259)	29	68[g]			1
	SXIV			34.5	McClung (1259)	14	26[m]			
6[q]	SXIV			9.1	Iseki	6				
	SXIV			9.1	McClung (1259)	5				
7	58[r]	Hog stomach lining	A	48.1	McClung (1259)	17				
					+ Iseki	17	79[j]	19[g]		
	McD		A	87.2	McClung (1259)	28				
					+ Iseki	28	58[j]	101[h]		
	Fr. 2	Hog mucin	A + O(H)	432.8	McClung (1259)	138				
					+ Iseki	138	69[g]	>114		2
Enzyme controls					McClung (1259)	83				
					+ Iseki	83	39[j]			
					Iseki	86	58[j]			

[a] Full saturation precipitate (FSP),[14] unless otherwise noted. [b] Experiment 1, fraction 10-3(8), experiment 3, fraction 8-2(8) of prep. 7A.[15d] [c] Dialyzed culture supernatant (DCS).[14] [d] Fraction 10(4).[15d] [e] Fraction III (42–53% of saturation).[14] [f] 100°, 10 minutes. [g] 16% ethanol from phenol. [h] Phenol insoluble. (The water soluble fraction of hog 54, phenol insoluble, had been dialyzed and reisolated. It was completely water soluble and had analytical properties comparable to the usual hog O(H) samples but with a somewhat lower hexosamine content). [i] Fraction 16(3).[15d] [j] Total yield of 3 fractions: phenol insoluble, 16% and excess ethanol from phenol, all devoid of blood group A activity except prep. 58, exp. 7. [k] Total yield of 3 fractions: phenol insoluble, 10% and excess ethanol from phenol, all devoid of blood group A activity. Analyses on 10% fraction. [l] 15% ethanol from phenol. [m] Water-soluble fraction of reisolated phenol insoluble material. [n] 10% ethanol from phenol. [o] Fraction 8(2).[15d] [p] Total yield of 2 fractions: 10 and 17% ethanol from phenol. Analyses on 17% fraction. [q] Substrate not reisolated after enzyme treatment. [r] 4% ethanol from phenol.

ment 1) or mixed with heat-inactivated enzyme (experiment 2, hogs 41 (A) and 54 (O(H)), and handled in the same manner as the corresponding digests in each experiment. In experiment 5, McClung (1259) enzymes, which lacked detectable blood group A splitting activity, rendered a sample of human group A erythrocytes agglutinable in plasma from an AB donor (panagglutinable). In experiment 7, the enzyme from McClung strain 1259 failed to inactivate A_1 antigen on intact erythrocytes after overnight incubation, whereas the Iseki strain enzymes rendered cells from the same donor inagglutinable by anti-A.

The amount of blood group substances recovered after enzyme treatment was usually two thirds or more of the original starting material. In a few instances (*e.g.*, SXIV in experiments 4 and 5, cyst McD(A) in experiments 4 and 7) low recoveries were due to the fact that the reisolated materials were found not to be completely water soluble. Aqueous extracts of these reisolated fractions therefore had to be made and when lyophilized, usually represented considerably less than the total reisolated substance. Phenol extraction of non-dialyzable residues of the two enzyme controls resulted in recovery of but 3% of the original enzyme material in each instance as a phenol insoluble fraction. From the phenol extracts, 22% of the combined (McClung, 1259, + Iseki) enzyme control and 34% of the Iseki enzyme control materials were recovered at 16% ethanol. From the remaining phenol-ethanol supernatants, on the addition of excess ethanol, more material was recovered which accounted for 14 and 21%, respectively, of the two enzyme control preparations.

Results

As previously demonstrated,[14] enzymes from the Iseki strain regularly inactivated blood group A antigen in hog mucin (experiments 1 and 3, hog mucin 7A; exp. 7, hog mucin Fr. 2), in material derived from single hog stomach linings (experiment 2, hog 41(A); experiment 3, hog 52(A)), and from human ovarian cyst fluids (experiments 4 and 7, cyst A preparation McD), as shown by hemagglutination inhibition (Table I). In Fig. 1, it can be seen that, with two exceptions, digestion of blood group A substance with Iseki enzymes eliminated completely the capacity of these substances to precipitate anti-A. In experiment 3, the capacity of hog 52 to precipitate anti-A was only partially reduced, while in experiment 7, hog 58 treated with both 1259 and Iseki enzymes lost little if any of its pre-

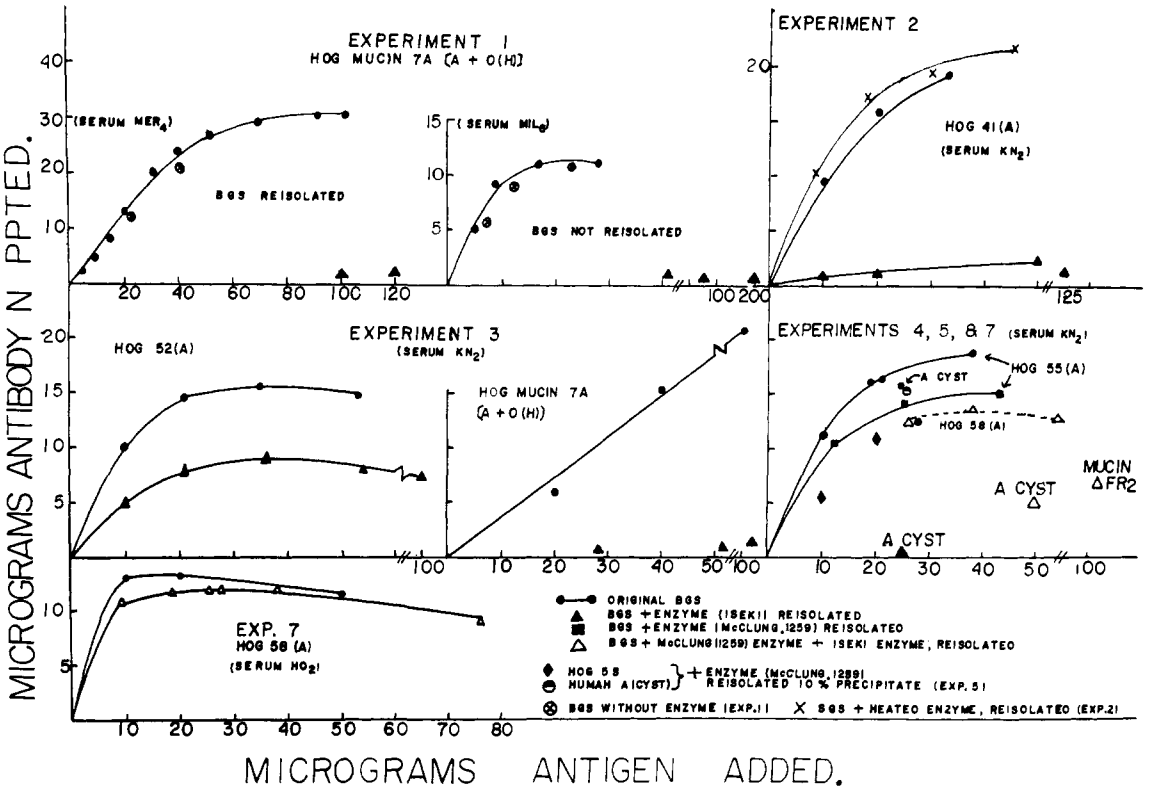

Fig. 1.—The effect of *Cl. tertium* enzymes on the capacity of blood group A antigen in hog and human blood group substances to precipitate anti-A. (Experiments 1, 2, 3 and 4, Iseki strain enzymes; experiment 5, McClung strain 1259 enzymes; experiment 7, McClung strain 1259 followed by Iseki strain enzymes.)

cipitating potency. Neither the B antigen nor the O(H) antigen was diminished significantly in its capacity to react with the corresponding hemagglutinin, and the B antigen retained its capacity to precipitate anti-B (Fig. 2) after treatment with either enzyme preparation. Quantitative O(H) precipitin studies cannot be carried out. In contrast, enzyme from McClung strain 1259 (experiment 5) produced no detectable effect on the A antigen in any of these materials, as shown by hemagglutination inhibition; capacity to precipitate anti-A was altered only slightly, if at all (Fig. 1). B and O(H) antigens were unaffected by Iseki strain enzymes with respect to reactivity with their corresponding iso-antibody. The A antigen (Hog 55) used in experiment 5, had previously been shown to be completely inactivated by Iseki strain enzymes (*cf.* Fig. 3 in ref. 14). Blood group A and O(H) activities were retained by hog substances 41 (A) and 54 (O(H)), respectively, in experiment 2 when treated with heated enzyme and by hog mucin 7A in experiment 1 without enzyme.

Cross reactivity with horse anti-SXIV of all blood group substances treated with either enzyme preparation (Fig. 3) was virtually abolished. Mild acid hydrolysis of each blood group substance reisolated after enzyme treatment restored a large measure of the cross reactivity, which in some instances greatly exceeded that of the original substance (*cf.* Fig. 3, experiments 2 and 3, hog 54 O(H) and hog mucin 7A). Cross reactivity of untreated blood group substance was also regularly increased by mild acid hydrolysis as had been shown in earlier studies.[3,8] Treatment of SXIV with either enzyme preparation resulted in marked diminution in capacity per unit weight to precipitate anti-SXIV. In Fig. 4, the highest point on each curve with enzyme-treated SXIV represents the maximum obtained with each of these altered antigens. In the

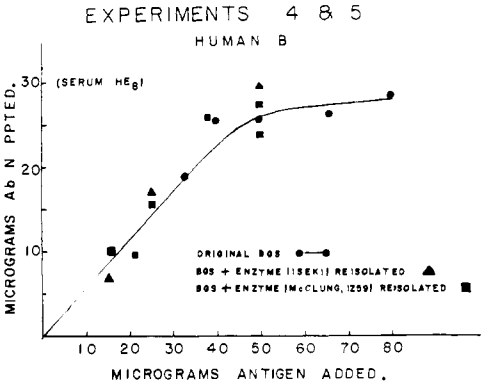

Fig. 2.—Effect of *Cl. tertium* enzymes (experiment 4, Iseki strain, experiment 5 McClung strain 1259) on the capacity of human blood group B substance to precipitate anti-B.

case of SXIV treated with Iseki strain enzymes, this maximum in experiment 4 was 82% and in experiment 6, 87% of that obtainable with untreated SXIV. Using SXIV treated with McClung (1259) enzymes, however, the maximum was significantly lower, in experiment 5 being 68% and in experiment 6, 61% of that obtainable with the un-

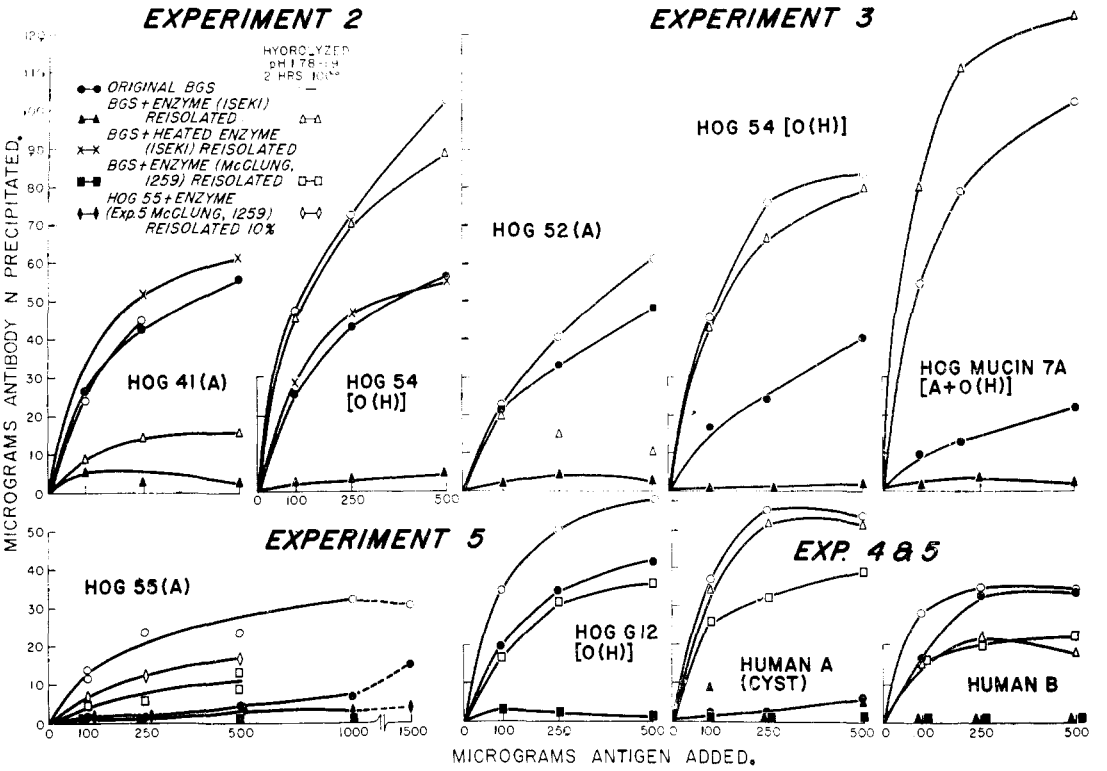

Fig. 3.—Elimination by *Cl. tertium* enzymes of cross reaction with horse anti-SXIV of blood group A, B and O(H) substances and its restoration by mild acid hydrolysis. (Experiments 2, 3 and 4, Iseki strain enzymes; experiment 5, McClung strain 1259 enzymes.)

treated polysaccharide. Reactivity of SXIV reisolated after enzyme treatment was further reduced by mild acid hydrolysis, as was the reactivity of untreated SXIV[8] (Fig. 4, experiments 4 and 5).

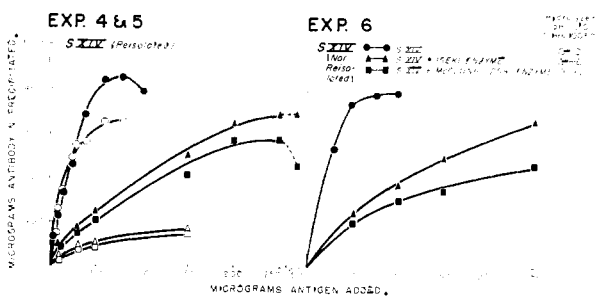

Fig. 4.—Effect of *Cl. tertium* enzymes on the capacity of SXIV to precipitate horse anti-SXIV.

Blood group substances reisolated after treatment with either enzyme preparation, or with both, as in experiment 7, gave nitrogen, methylpentose, hexosamine and reducing sugar values not significantly different from those for each corresponding untreated blood group substance[3] or for reisolated controls in two experiments (experiments 1 and 2). These analytical values are therefore not given in detail. The fraction precipitating at 16% ethanol from the phenol extract of non-dialyzable residues from the enzyme controls gave nitrogen, methylpentose, hexosamine and reducing sugar values of 5, 8, 4 and 22%, respectively, for the McClung 1259 enzymes treated with Iseki enzymes, as in experiment 7. Corresponding values for the fraction precipitable at 16% ethanol for the Iseki enzyme control were 5, 9, 7 and 26%, respectively. These enzymes therefore contain some carbohydrate material which could have survived the phenol extraction of non-dialyzable residues of blood group substance digests and have contaminated fractions precipitated from phenol at 16 or 17% ethanol. The maximum extent of contamination of these fractions could have been up to 20% for the preparation with the lowest ratio of blood group substance to enzyme (experiment 7, Fr. 2). As already noted, however, water-insoluble material in appreciable quantity, probably representing denatured enzyme, was removed from all digests after dialysis before lyophilization and phenol extraction, as well as from some of the materials subsequently precipitated with ethanol from the phenol extracts, thereby minimizing possible contamination of the reisolated blood group substances. Blood group substances reisolated as phenol insoluble fractions after enzyme treatment and dialysis are not likely to have been contaminated with residual enzyme, since negligible amounts of material (3%) were found to remain insoluble in phenol when control enzyme preparations were extracted after incubation and dialysis in the absence of blood group substance.

Discussion

Enzyme preparations from two strains of *Clostridium tertium* (Iseki and McClung, 1259) have been investigated for their effects on blood group substances and on SXIV. Inactivation of blood group A specificity in hog substances by Iseki strain en-

zymes, already demonstrated in earlier quantitative studies,[14] was amply confirmed in the present investigation, in which these enzymes also inactivated purified human A substance (ovarian cyst). These changes were unaccompanied by the appearance of O(H) specificity in the non-dialyzable residues after enzyme treatment, as reported by Iseki and Masaki.[25,26] McClung strain 1259 enzymes failed to affect the blood group A, B and O(H) antigens. The reasons for the incomplete destruction of A specificity of hog 58 (A), experiment 7, after sequential treatment with McClung 1259 and Iseki enzymes are not clear. Not only did this preparation retain some hemagglutination inhibiting potency, but the capacity of the 16% reisolated fraction to precipitate anti-A (Fig. 1) was only slightly less than that of the original material. All fractions of mucin blood group A substance and human cyst A substance reisolated in the same experiment lost all inhibiting potency, but precipitating power of the main fraction in each case, while greatly reduced, was not altogether abolished as had regularly been the case after treatment with Iseki enzymes alone (*cf.* hog mucin 7A in experiments 1 and 3; and experiment 4). This may be related to the longer times of incubation of the blood group substances with enzyme in the latter cases except for experiment 4. Thus 24 hours may have been too short an incubation period.

That both enzyme preparations effected changes in all the substances regardless of blood group specificity is revealed by the almost complete elimination of cross reactivity with horse anti-SXIV. On treatment with either enzyme, SXIV itself also showed reduced capacity per unit weight to precipitate anti-SXIV (Fig. 4) as already mentioned. As shown in Table I, the yield of water-soluble reisolated SXIV following treatment with either Iseki strain enzyme (experiment 4) or McClung strain 1259 enzyme (experiment 5) was in each instance only about one third to one quarter of the original amount of substrate introduced. In experiment 6, therefore, samples of SXIV treated with each enzyme were analyzed after dialysis, without the reisolation procedures included in experiments 4 and 5. Marked reduction in reactivity with anti-SXIV was again demonstrated with both enzymes (Fig. 4).

The increased cross reactivity of enzyme-treated blood group substances following mild acid hydrolysis indicates that in the intact blood group substance some cross reactive groupings are not accessible to enzyme or to antibody[8]; and that these groupings become available as a consequence of mild acid hydrolysis which splits off fucose and dialyzable oligosaccharides.[8,27] Cross reactivity of blood group substances has been shown to bear an inverse relationship to their fucose content.[7] The low initial cross reactivity of hog 55 in experiment 5 and that of human cyst A material in experiments 4 and 5 (Fig. 3) are therefore most likely due to the relatively high methylpentose content of these two preparations (13 and 17%, respectively) as compared with the methylpentose content of the other preparations.[3] The failure in the present studies to demonstrate appreciable change in methylpentose content of blood group substances after treatment with enzymes of *Cl. tertium* is in contrast to the findings of Iseki and Masaki.[25] These authors state not only that methylpentose was liberated from blood group substances in dialyzable form by these enzymes, but also that the non-dialyzable residues gave methylpentose values considerably less than those for untreated substances. They postulated therefore that loss of blood group A specificity was attributable to loss of L-fucose. Were this the case, however, one might expect an increase in cross reactivity with anti-SXIV rather than the decrease observed in the present study. Furthermore, it has not been possible to implicate L-fucose in blood group A specificity on the basis of hemagglutination and precipitation inhibition studies.[3]

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