Antigenicity of Chondroitin Sulfate.* (23451)

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The recent reports of Glynn and Holborow (1) in which they observed the production of complete antigens from vegetable polysaccharide haptens (agar) and the conversion of tissue polysaccharides (chrondroitin sulfate) to haptens(2) by hemolytic streptococci and other bacteria are of great interest for 2 reasons. First, because the lesions of rheumatic fever occur in connective tissue and it has been postulated that these lesions might be due to an auto-antigen, auto-antibody reaction occurring in connective tissue(3) and second, the hemolytic streptococcus is intimately involved in the pathogenesis of rheumatic fever. The findings of Glynn and Holborow would appear to be of great potential significance and every effort should be made to confirm them.

According to Meyer(4) 6 separate mucopolysaccharides have been isolated from the ground substance of connective tissue, hyaluronic acid, chondroitin sulfate A, B and C, chondroitin and keratosulfate. Chondroitin sulfate A is the main component of hyaline cartilage but it contains some chondroitin sulfate C which has also been found in heart valves, tendon, aorta and umbilical cord. None of these components has been shown to be antigenic alone. It was the purpose of this experiment to attempt to render chondroitin sulfate antigenic.

Materials and methods. Chondroitin sulfate was prepared from fresh wet human costal cartilage stored at 0°C for 2-3 months according to the method of Einbinder and Schubert (5). It was assayed by the Dische(6) glucuronic acid method with glucuronolactone as the standard and was found to be 65% pure chondroitin sulfate. Bovine chondroitin sulfate† of 70% purity was tested along with the samples. Readings were taken in a Coleman spectrophotometer at 540 mu. Strains of group A type 3 hemolytic streptococcus or hemolytic *Staphylococcus aureus* were grown for 18 hours at 37°C in beef heart infusion broth. The bacterial cells were removed by centrifugation, suspended in a 1% solution of chondroitin sulfate in 0.85% saline, incubated at 37°C for 2 hours and then heat-killed at 60°C for 75 minutes. The bacterial cells then were washed 3 times in normal saline and finally resuspended in saline. This preparation of washed, heat-killed bacterial cells *without* chondroitin sulfate, bacterial cells incubated *with* chondroitin sulfate and a 1% solution of chondroitin sulfate were the materials injected into young adult albino or chinchilla rabbits weighing from 3-5 lbs according to the dose, route and schedule outlined in Table I. The rabbits were bled at the times shown in Table I. The serum containing merthiolate at a strength of 1:10,000 was stored at 4-8°C. Tests for precipitating antibodies against chondroitin sulfate were performed using capillary tubes. An optimal proportion method was used overlaying serial dilutions of 0.1% chondroitin sulfate in normal saline with serial dilutions of each serum specimen. Each of the undiluted sera and a 1:4, 1:16, 1:32 and 1:64 dilution of each serum was tested with each of an undiluted sample and a 1:4, 1:16, 1:32 and 1:64 dilution of chondroitin sulfate. The tubes, incubated for 2 hours at 37°C, then placed in the refrigerator at 4-8°C overnight, were observed for precipitate formation both before and after incubation at 37°C and 4-8°C. Following completion of the "immunizing" series of injections, each rabbit was skin tested. One-tenth ml of 1% chondroitin sulfate was injected intradermally and observed for evidence of reaction immediately, 6 hours and 24 hours after injection.

Results. There was no evidence of the formation of precipitating antibodies against chondroitin sulfate in any of the rabbits' sera. Slight erythema, 4 mm in diameter, was seen

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† General Biochemicals Inc.

TABLE I. Dose, Route, and Date of Administration of Each Antigen to Each Rabbit.

Rabbit #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Antigen	Chondroitin sulfate + streptococci					Chondroitin sulfate		Strepto-cocci		Chondroitin sulfate + staphylococci				Staphylo-cocci	
Day of exp.	I.V.	I.V.	I.V.	I.M.	I.M.	I.V.	I.V.	I.V.	I.V.	I.V.	I.V.	I.M.	I.M.	I.V.	I.V.
	Dose of antigen in ml														
1*	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
3	.3	.3	.3	.3	.3	.3	.3	.3	.3	.3	.3	.3	.3	.3	.3
5	.6	.6	.6	.6	.6	.6	.6	.6	.6	.6	.6	.6	.6	.6	.6
8*	.8	.8	.8	.8	.8	.8	.8	.8	.8	.8	.8	.8	.8	.8	.8
10	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
12	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
15*	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
17	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
19*	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"

* Blood specimen drawn.

I.V. = intrav.; I.M. = intramusc.

about the injection site of one rabbit which received chondroitin sulfate plus streptococci and 6-8 mm erythema in a rabbit which received streptococci alone. There was no evidence of skin reactivity in any of the other rabbits.

Comments. The results of these experiments, in which there was no evidence of antigenicity of chondroitin sulfate either in the role of a hapten or as a direct antigen as measured by precipitating antibodies and skin sensitivity, are at variance with those of Glynn and Holborow(2). However, these authors have since indicated that they have been unable to repeat their original work and they suspect the precipitating antibodies observed by them were "in fact directed against blood group like substances and not against chondroitin sulphate itself."‡ It would appear at the present time that chondroitin sulfate by itself or in combination with the group A streptococcus or *Staphylococcus aureus* has not been shown to be antigenic nor to behave as a hapten. These results are similar to those of previous experiments in which hyaluronic acid proved to be non-antigenic(7).

Summary. Chondroitin sulfate extracted from human costal cartilage was prepared in 3 different ways for inoculation into rabbits, (1) a 1% solution, (2) a 1% solution incubated with heat-killed, group A, type 3, hemolytic streptococcus, and (3) a 1% solution incubated with heat-killed *Staphylococcus aureus*. These 3 preparations were injected into rabbits in an attempt to elicit antibody production. There was no evidence of the formation of precipitating antibodies against chondroitin sulfate in any of the rabbits' sera nor was there any clear indication of the acquisition of skin sensitivity by any of the rabbits.

‡ Glynn, L. E., personal communication.

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