In vivo Inhibition of Blood Agglutinins by Polysaccharides from Animal Parasites.* (20179)

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Polysaccharides were prepared from the larval form of *Trichinella spiralis* in rat muscle, and from adult *Ascaris lumbricoides* from pig. The well dried and pulverized worm material was suspended in water and placed in a boiling water bath for 30 minutes with constant stirring. The mixture was allowed to cool and then centrifuged for 15 minutes at 4000 r.p.m., the sediment being discarded. Sodium chloride was then added to the supernatant to make a 0.5% solution, followed by treatment with 4 volumes of 95% alcohol. The polysaccharide settled to the bottom after storage of the mixture at 6°C overnight. The supernatant was decanted, and the sediment centrifuged in 50 cc tubes and finally collected in one tube. Three washings, first in 95% alcohol, and 2 in ether then followed until the material remained dry. Partial purification of the polysaccharide was accomplished by resuspension in 0.5% sodium chloride, discarding the insoluble portion and reprecipitation with alcohol. This procedure was repeated at least 3 times. The final product was a clean, white powder, very soluble in water, giving a negative Biuret test for proteins.† Solutions of polysaccharides prepared from the trichina larvae and ascaris adults were injected intravenously into rabbits and monkeys. Also a commercial starch preparation from corn was injected as a control. The rabbits utilized were of both sexes and weighed from 3000 to 3500 g. Two female Rhesus monkeys were used, weighing 8 and 8.5 lb. The polysaccharide, dissolved in 0.85% sodium chloride, was injected intravenously into those animals whose serums had been previously found to have positive agglutinin titers at 37°C against human erythrocytes of Groups A_1 and A_2. The polysaccharides were also injected into animals with previously known negative titers at 37°C, so as to be able to study the effect of the polysaccharides on the agglutinins acting at 6°C. The animals were bled before, and 1, 6, 24, 48, and 96 hours after injection of the polysaccharides. The serums were always separated within 30 minutes after withdrawal of the blood. Before and after injection, serums were then tested for their content of agglutinins against human, sheep and the animals' own erythrocytes, following incubation at 37°C for one hour and after storage at 6°C overnight. The technic for the agglutination test has been described previously(1). The *human erythrocytes* utilized were obtained fresh from blood donors attending the blood bank of the School of Medicine. They were washed 3 times in 0.85% sodium chloride before testing. Erythrocytes from the same individual and from the same batch were used for the tests to be carried on for each experiment, which consisted in the testing for agglutinins within a 96-hour period. All erythrocytes were preserved at 6°C in Alsever's solution.

Results. Inhibitory effects of ascaris and trichina polysaccharides on blood agglutinins. Agglutinins at 37°C. The titers of the agglutinins in rabbit serums against human erythrocytes of Group A_2 were negative on the 1-, 6-, and 24-hour period after injection. Positive agglutination was observed, however, beginning at the 48-hour period, titer remaining lower than the one observed before inoculation. Similarly, the agglutinins in monkey serums against Group A_2 erythrocytes, were negative at the 1-, 6-, and 24-hour periods and remained low at the 48- and 96-hour periods. (See Table I). The *rabbit and monkey serums* gave positive titers against sheep erythrocytes before the inoculation of the polysaccharides, which became negative at the

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† The raw polysaccharide material from ascaris was sent by Dr. D. W. MacCorquodale, from Abbott Laboratories, Chicago, Ill.

TABLE I. Agglutinin Titers against Erythrocytes before and after Injection of Polysaccharides. Dose of ascaris, 500 mg.

Species wt, kg	Time rel. to inj.	37°C				6°C			
		A_1	A_2	Sheep cells	Own cells	A_1	A_2	Sheep cells	Own cells
Rabbit, 3.1*	Before	64	16	8	0	64	32	32	16
	+1 hr	2	0	0	0	4	0	4	4
	6	2	0	0	0	4	0	4	4
	24	8	0	0	0	16	8	8	8
	48	8	1	0	0	16	16	8	8
	96	16	2	0	0	16	16	8	8
Monkey, 3.6*	Before	256	32	8	0	4096	256	128	64
	+1 hr	16	0	0	0	256	16	8	8
	6	16	0	0	0	256	32	8	8
	24	16	0	0	0	512	64	16	16
	48	32	4	0	0	1024	64	32	32
	96	32	4	0	0	1024	64	32	32

* Results in additional rabbits and monkeys inj. with either ascaris or trichina polysaccharides were essentially the same.

1-, 6-, 24-, 48-, and 96-hour periods of examination. There was a decrease in titer of agglutinins against human erythrocytes of Group A_1, but these never became negative as observed with Group A_2 erythrocytes (see Table I). Starch did not affect the agglutinins at 37°C against any of the erythrocytes.

Agglutinins at 6°C. (Cold agglutinins). The agglutinins at 6°C against erythrocytes of all types were markedly reduced in the rabbit as well as in the monkey serums after inoculation with the ascaris and trichina polysaccharides. (See Table I). The diminution in titers was greater when a dose of 500 mg of polysaccharide was injected.

The serum from rabbits which had no agglutinins acting at 37°C, agglutinated the erythrocytes at 6°C. The polysaccharides from ascaris and trichina also reduced the cold agglutinin titers in these rabbits' serums.

Starch did not affect the agglutinins at 6°C against any of the erythrocytes.

Discussion. Polysaccharides from animal parasites have been previously reported to inhibit the isoagglutinins when added to human serums *in vitro*. The inhibition is more effective on the A_2 than on the A_1 isoagglutinins in human serums of Groups B and O. Furthermore, when animal parasites are incubated in Group B human serum the titer of the A_2 isoagglutinins are reduced to negative while the A_1 are not affected. The substance secreted by the animal parasite while incubated in the serum as well as the polysaccharide extracted from the dry worm material are, therefore, related to the A_2 isoagglutinins in the serum(1).

The intravenous inoculation of the ascaris and trichina polysaccharide into monkeys and rabbits also caused an inhibition of the A_2 isoagglutinins in the living animal. This inhibition was complete for a period of 24 hours. Positive titers against the A_2 isoagglutinins were detected on the 48- and 96-hour periods, but these were reduced as compared with the titers of the agglutinins before the animal was treated with the polysaccharide.

The A_2 or anti-O isoagglutinins are irregular agglutinins which react intensely with all erythrocytes of Group O human blood and less intensely with erythrocytes of subgroup A_2. They are sometimes responsible for agglutination of the host's own erythrocytes (autoagglutination) as observed either at 37°C or 6°C. Autoagglutination of erythrocytes, as has been observed during infection with either bacteria or animal parasites, may be partially prevented by the inoculation of the polysaccharide into the animal. The use of the polysaccharide for this purpose would have a logical explanation on the basis first; that the autoagglutinin may have developed as a result of immunization of the host with the isoagglutinogen-like substance in the infective organism and that, therefore, antibodies against the host erythrocytes cause autoagglutination; and second, that the poly-

saccharide when injected as such into animals inhibits the A_2 isoagglutinins.

Circulating autoantibodies have been reported to have a particular significance in acquired hemolytic anemias. Besides present as a circulating antibody, the autoagglutinin also attaches itself to red blood cells. The basic cause for the development of these autoantibodies is still unknown although a large majority give a history of previous infection. The serum of patients with acquired hemolytic anemia usually contains an agglutinin reacting with normal Group O human erythrocytes (2). Whether this agglutinin is the same which agglutinates the host's own erythrocytes, by reacting with some agglutinogen common to the Group O cells and the host erythrocytes, is a point of major significance. Since the anti-O behave similarly to the A_2 isoagglutinins, the role that the isoagglutinogen-like substance of infective agents may have in producing such agglutinins in patients with acquired hemolytic anemia, should be considered. The possibility of inhibiting the agglutinins by injecting the polysaccharides into the patients should be also considered.

The polysaccharides from ascaris and trichina also inhibited to a great extent the agglutinins acting at 6°C. That there was an effect on the agglutinins acting at 6°C irrelevant to those acting at 37°C, is shown by the results in the rabbits whose serum had no agglutinins at 37°C. In these the cold agglutinin titer was reduced following inoculation with the polysaccharides. The role played by cold agglutinins in certain pathological conditions is still not understood. The results of the study also suggest that there is an immunological relationship between the a_2 and cold agglutinins, and therefore, the agglutinogen responsible for both antibodies may be also related.

The trichina larvae and adult pig ascaris from which the polysaccharides can be prepared, are made easily available to the laboratory. A strain of *Trichinella spiralis* can be permanently kept in rats, from which desirable amounts of dried larval worm material can be obtained. Adult ascaris from a pig are easily available from slaughter houses.

Summary. 1. Monkeys and rabbits were injected intravenously with various amounts of polysaccharides (dissolved in 0.85% saline) prepared from the muscle stage of *Trichinella spiralis* and from adult *Ascaris lumbricoides* from pig. 2. The titer of the a_2 isoagglutinins detected at 37°C in the serums of the animals was reduced to negative for a period of 24 hours, and remained lower than the titer before inoculation for a period of 96 hours. 3. The titer of the a_1 was also reduced, but not as much as the a_2 agglutinins. 4. The titer of the agglutinins acting at 6°C against A_1, A_2 human, sheep, and the animal's own erythrocytes was also reduced after inoculation of the polysaccharide. 5. The possibility of injecting these polysaccharides to inhibit autoagglutination of erythrocytes as seen in certain pathological conditions is mentioned.

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