the crude antigen was left practically unaltered. 2) Most of this reactivity was removed by KIO_4 from the purified antigen. Such preparations became inactive or only slightly active in the CF test with either homologous or heterologous antisera since the specific activity was previously destroyed by heating. 3) Antigens possessing the heat-labile specific component as well as the KIO_4-susceptible group-reactive "component" could be rendered fairly specific by treatment with KIO_4. It appears that the group reactive "component" is not a single entity, but is made up of at least 2 antigenic constituents.

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Adsorption of A_2isoagglutinogen-like Substances of Infectious Agents on Human Erythrocytes.* (20698)

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The A_2isoagglutinogen-like substance is the term applied to a polysaccharide complex which is either extracted from or secreted by animal parasites and bacteria. This substance is characterized by the following immunological properties: 1) The polysaccharide, as isolated chemically from the infectious agent, inhibits the a_2 isoagglutinins when added to human blood serums. The agglutinin titer in the serums against erythrocytes of Group A_2 (a_2 agglutinin titer), as observed at 37°C, becomes negative. There is also a slight inhibition of the a_1 isoagglutinins, but this may be due to the fact that the a_1 and a_2 antibodies may be partially bound together(1). 2) The polysaccharide is secreted when the living infectious agent is incubated in human serums. The titer of the a_2 isoagglutinins in the serums becomes negative. There is also a slight reduction in titer of the a_1, but no effect on the β isoagglutinins(2). 3) When the polysaccharide is injected intravenously into rabbits and monkeys, the titer of the a_2 isoagglutinins, as detected at 37°C, is reduced to negative for a period of 24 hours, and remains low for a period of 96 hours. The titer of the agglutinins acting at 6°C (cold agglutinins) against human and sheep erythrocytes, is also reduced after inoculation of the polysaccharide(3).

The term A_2isoagglutinogen-like substance seems therefore, appropriate, since the polysaccharide reacts specifically with the a_2 isoagglutinins in the serums. Studies on the possible relationship of the A_2isoagglutinogen-like substance with certain pathological conditions are being continued. Attempts were made to adsorb the polysaccharide onto human red blood cells to determine whether the A_2isoagglutinogen-like property could be transmitted to the erythrocytes.

Materials and methods. The erythrocytes utilized in this study were obtained from blood donors attending the Blood Bank of the School of Medicine, at San Juan, P. R. The red blood cells were washed at least 3 times with buffered saline (Bacto Hemagglutination Buffer, Difco) before using. Polysaccharides were extracted from dry and pulverized *Ascaris lumbricoides* from pig, larval forms of *Trichinella spiralis* from rat muscle, and adult *Taenia saginata*. The method of preparation has been reported previously(3). Glycogen, prepared in the same way, and rice starch, were used as controls. Ten mg of polysaccharide were suspended in 1 cc of buffered saline, to which 0.5 cc of packed erythrocytes was then added. The mixture was incubated at 37°C, or stored at 6°C, for one hour, with frequent shakings,

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TABLE I. Isoagglutinin Titers of Human Serums before and after Addition of Erythrocytes Treated with Various Polysaccharides.*

	RBC blood group	Group O serum				Group B serum			
		37°C		6°C		37°C		6°C	
		α_1	α_2	α_1	α_2	α_1	α_2	α_1	α_2
Before treatment		128	16	128	16	64	8	64	8
After treatment with polysaccharides:									
Ascaris	O	32	0	64	0	32	0	64	0
Ascaris	B	16	0	32	0	32	0	64	0
Trichina	O	64	0	64	0	8	0	64	0
Taenia	O	64	0	64	0	16	0	32	0
Glycogen	O	64	16	64	16	64	8	64	8
Corn starch	O	128	16	128	16	64	8	64	8
None	O	128	16	128	16	64	8	64	8
None	B	128	16	128	16	64	8	64	8

* The erythrocytes of human blood groups O and B in this particular test come from the same individuals. Similar results were obtained when each of 4 additional serums of Groups O and B was treated with adsorbed erythrocytes of 4 additional Group O and 4 Group B individuals.

followed by centrifugation at 4,000 rpm for 3 minutes. The supernatant was discarded; the sedimented erythrocytes being washed 3 times with buffered saline. After washing each time the suspension was allowed to sediment in 15 cc centrifuge tubes for 5 minutes, and the erythrocytes plus the free polysaccharide which settled to the bottom of the tube during the 5 minute period were discarded. In this manner assurance was made that all excess polysaccharide was removed, since none could be observed either macroscopically or under magnification at $\times 20$. Furthermore, unseen smaller particles would be eventually washed off by the saline. This measure was taken in view of the fact that non-adsorbed polysaccharide remaining free in this suspension would have the same effect on the serums as the polysaccharide which would adsorb onto the erythrocytes. To 0.5 cc of the serums of various blood groups, 0.1 cc of the packed and carbohydrate-treated erythrocytes was added and incubated for 30 minutes at 37°C, with frequent shakings. The mixture was then centrifuged at 4,000 rpm for 3 minutes, the sedimented erythrocytes discarded, and the process repeated until the serum was treated 3 times with the polysaccharide treated erythrocytes.

Serums were also treated with erythrocytes to which no polysaccharide had been adsorbed, as control. The serums were then tested for their content of α_1, α_2, and β isoagglutinins. For these tests 0.1 cc volumes of the serums in Wassermann tubes were diluted 2-fold with buffered saline. An equal volume of a 0.5% suspension of the erythrocytes was added. Agglutination of the erythrocytes was observed under the binocular microscope, at a magnification of $\times 20$, after incubation at 37°C for one hour, and after storage at 6°C overnight.

Results. The polysaccharides from animal parasites seemed to have adsorbed onto the erythrocytes without difficulty. Treatment of the erythrocytes with the polysaccharide suspension, either at 37°C or 6°C, always gave identical results.

Inhibition of α_2 isoagglutinins by erythrocytes onto which the polysaccharides had been adsorbed. Table I presents the results of a typical experiment. Erythrocytes of human blood groups O and B, after treatment with polysaccharides extracted from the various animal parasites, inhibited the α_2 agglutinins acting at 37°C and 6°C. The erythrocytes from 5 individuals of blood group O and 5 from blood group B were each treated with the polysaccharides and each used to inhibit the agglutinins in 5 group O and 5 group B serums. In all instances the titers of the α_2 isoagglutinins were reduced to zero. Inhibition of the α_2 isoagglutinins must be attributed to the polysaccharide adsorbed onto the erythrocytes, since in the first place, none could be observed free in the suspension after the cells were washed, and second, since as previously reported, the amount of free polysaccharide re-

quired to inhibit the isoagglutinins is in the order of 20 mg per 0.5 cc which is double the amount utilized for the suspension into which the erythrocytes were added(1).

The titer of the α_1 was also reduced as compared with that of the serums treated with the erythrocytes without polysaccharide, but were not brought down to zero, as observed with the α_2 isoagglutinins. The titers of the β isoagglutinins were unaffected. No significant change in titer of all agglutinins was observed when the serums were treated with erythrocytes free of polysaccharide.

The erythrocytes treated with glycogen and corn starch did not inhibit the α_2 isoagglutinins, either at 37°C and 6°C, as observed with the erythrocytes treated with the polysaccharides from the animal parasites.

Discussion. The results of the above studies suggest that polysaccharides isolated from various species of animal parasites adsorbed onto human erythrocytes of groups O and B. Erythrocytes of blood groups A_1 and A_2 were not utilized, since these necessarily would inhibit the α_2 isoagglutinins by virtue of their homologous or related agglutinogens. The treated erythrocytes acquired characteristics of human blood group A_2, since in the serums into which they were introduced, the α_2 isoagglutinin titer was reduced to zero. No significant change in the titer was observed when the serums were treated with non-adsorbed erythrocytes.

That erythrocytes of a certain blood group can acquire characteristics of another, when polysaccharides from infectious organisms adsorbed onto their surfaces, is a finding of great interest which may have important implications. As previously pointed out, the polysaccharides *per se* have A_2isoagglutinogen-like properties, and also the infectious organisms secrete the substance when incubated in serum. During infection with organisms secreting the A_2isoagglutinogen-like substance, the erythrocytes of the host may become coated with the polysaccharide. This would introduce new antigenic groups onto the erythrocyte pattern and thus the host may produce antibodies against the new erythrocyte-polysaccharide complex and therefore against its own erythrocytes. Such antibodies may agglutinate the host erythrocytes if they are of the A_2 group, or of any other blood group which may have the polysaccharide adsorbed onto them.

Autoagglutination and hemolysis of erythrocytes, as observed in blackwater fever and other conditions in which infectious agents are involved, may be associated with such a phenomenon by which erythrocytes become coated with polysaccharides related to blood substances.

Summary. 1. Partially purified polysaccharides isolated from dry worm material from adult *Ascaris lumbricoides* (*var suum*); the larval forms of *Trichinella spiralis* and adult *Taenia saginata* adsorbed onto human erythrocytes of groups O and B, when these were incubated at 37°C or stored at 6°C in saline suspensions of the polysaccharide. 2. The treated erythrocytes were added to human serums of groups O and B and the α_2 isoagglutinin titers were reduced to zero. No significant reduction of the α_2 isoagglutinin titer was observed when control erythrocytes were added to the serums. 3. The titers of the α_1 was slightly reduced and that of the β isoagglutinins was unaffected. 4. The fact that the erythrocytes treated with the polysaccharides inhibited the α_2 agglutinins is used as evidence to show that the polysaccharides adsorbed onto the red cells. 5. Adsorption of polysaccharides with A_2 isoagglutinogen-like properties, onto erythrocytes of human groups O and B gives the cell characteristics pertaining to a different group, *i.e.*, the A_2 group. The possibility of such phenomenon occurring during actual infection, and the relationship which it may have with the development of autohemagglutinins is mentioned.

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