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Blood Agglutinins in Blackwater Fever.

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The serums from two cases of blackwater fever were tested for content of α, β, and cold agglutinins.* To 2 drops of the serum dilutions one drop of a 2% suspension of Groups A, B, and O erythrocytes was added. The suspensions in Wassermann tubes were incubated in a water bath at 37°C for 30 minutes followed by storage in the icebox at 6°C overnight. Examinations of suspensions for the presence of agglutinated erythrocytes was done after exposure to 37°C and 6°C with the aid of a dissecting microscope at a magnification of ×20.

Since both serums contained α and β agglutinins, it was necessary to absorb these in order to test for cold agglutinins. Small portions of serums were treated three times with one-half volume of packed fresh Group AB erythrocytes. During each absorption, the mixture was incubated at 37°C for 30 minutes, then centrifuged and the supernatant was treated again with fresh Group AB erythrocytes. Small portions of serum were also treated with a polysaccharide isolated from *Ascaris suum* which has been shown to inhibit specifically the α and β agglutinins in human serums.[1] Enough of the polysaccharide was added to make a 4% suspension. The mixture was incubated at 37°C for 30 minutes and then tested. The serums treated with the Group AB erythrocytes and with the ascarid polysaccharide, as well as the untreated control serums, were then tested with Group A, B, and O erythrocytes and examined after exposures to 37°C for 30 minutes and at 6°C overnight.

Table I shows that the serums from the 2 cases of blackwater fever had markedly increased α isoagglutinin titers of 1:8112 and 1:2028, respectively. After treatment of the serums 3 times with the Group AB erythrocytes, as well as after a single treatment with the ascarid polysaccharide, no α, β, or anti-O agglutinins were detected after incubation at 37°C. However, agglutination was still observed with Groups A, B, and O erythrocytes after overnight exposure to 6°C (Table I).

Discussion and Summary. A polysaccharide has been isolated from various animal parasites which inhibits the α and β agglutinins from human serums and which thus bears some relationship to the human isoagglutinogens.[2] Since the polysaccharide alone is able to incite antibody formation, immunization of the host with this substance during infection might lead to the formation of agglutinins which might act on the erythrocytes of the host. This fact may be of importance in blackwater fever. In this disease, which is associated with infection with *P. falciparum*, there is an intravascular agglutination of erythrocytes followed by extensive intravascular hemolysis. Autoagglutination may be caused by autoagglutinins resulting from the immunization of the host with the substance in the parasite related to the human isoagglutinogens. The increase in the agglutinin titer in the serums from patients suffering with blackwater fever presented in this study and the increase in the α and β agglutinin titers in the serums from patients having had repeated attacks of malaria[2] give evidence to support the view that the malarial organism is responsible for the increase in agglutinins. How these agglutinins resulting from infection may be associated with autoagglutination, however, is not evident from the present results.

The serums from the cases of blackwater fever also had agglutinins acting at 6°C.

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1 Oliver-González, José, *J. Infect. Dis.*, 1944, **74**, 81.

2 Oliver-González, José, and Torregrosa, Vicente M., *J. Infect. Dis.*, 1944, **74**, 173.

TABLE I.
The α, β, and Cold Agglutinin Titers of Serums from Two Cases of Blackwater Fever.

	Titer at 37°C for 30 min Agglutinins			Titer at 6°C overnight Agglutinins		
	α	β	anti-O	α	β	anti-O
Serum sample 1						
Untreated serum	8,112	128	0	32,448	1,024	32
Serum treated with Group AB erythrocytes	0	0	0	32	32	32
Serum treated with ascarid polysaccharide	0	0	0	32	32	32
Serum sample 2						
Untreated serum	2,028	64	0	8,112	256	32
Serum treated with Group AB erythrocytes	0	0	0	64	32	32
Serum treated with ascarid polysaccharide	0	0	0	32	32	32

These agglutinins behaved as true cold agglutinins since they agglutinated all erythrocytes regardless of blood group, and their action was reversible by changing the temperature back and forth from 6°C to 37°C. The presence of autoagglutinins in blackwater fever has been previously suggested although not proved experimentally by Dameshek.[3] As explained previously, the source of the agglutinogen may be the malarial parasite.

The possible relationship between the presence of cold agglutinins and the development of blackwater fever should also be taken into consideration. Since exposure to cold is one of the conditions known to accelerate the appearance of blackwater fever symptoms, it seems that such a relationship could profitably be studied.

[3] Dameshek, W., *J. A. M. A.*, 1943, **123**, 77.

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Acute Toxicity of Choline Hydrochloride Administered Intraperitoneally to Rats.*†

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Choline hydrochloride is not highly toxic. Mott and Halliburton[1] emphasize the low order of toxicity when they state that "We have never succeeded in killing an animal by injection of choline or choline hydrochloride." However, lethal doses for several species have been reported[1-9] as of the order of 40-60 mg per kg body weight for intravenous administration and of the order of 200-1000 mg per kg for subcutaneous administration.

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[1] Mott, F. W., and Halliburton, W. D., *Trans. Roy. Soc., Lond., B*, 1899, **191**, 211.

[2] Boehm, R., *Arch. Exp. Path. Pharm.*, 1885, **19**, 87.

[3] Asher, L., and Wood, H. C., *Zeit. Biol.*, 1899, **37**, 307.