HEMAGGLUTINATION BY THE KOCH-WEEKS BACILLUS (HEMOPHILUS AEGYPTIUS)[1]

DORLAND J. DAVIS, MARGARET PITTMAN, AND JAMES J. GRIFFITTS

Microbiological Institute, National Institutes of Health, Bethesda, Maryland

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The ability of certain viruses, notably influenza, to agglutinate red blood cells has led to a very useful immunological technique. Certain bacteria also have been reported to possess this capacity by Kraus and Ludwig (1902), Pearce and Winne (1904), Guyot (1908), and Fukuhara (1909). More recently Keogh *et al.* (1947, 1948) have shown that *Hemophilus pertussis*, *Hemophilus parapertussis*, and *Hemophilus bronchisepticus* also have the ability to clump the erythrocytes of various species of animals and have suggested that the virulence of strains of *H. pertussis* is related to their hemagglutinin content. *Shigella alkalescens* also agglutinates the erythrocytes of humans and of certain other animals, whereas some other species of *Shigella* fail to do this (Griffitts, 1948).

In connection with the study of strains of *Hemophilus* recovered from cases of acute conjunctivitis in Texas (Pittman and Davis, 1950), an investigation of the capacity of these strains to agglutinate the red blood cells of various species was made. It was found that the strains identified as the Koch-Weeks bacillus possessed a hemagglutinin whereas *Hemophilus influenzae* did not. In this paper the details of the investigation of this reaction will be described.

METHODS

The tests for hemagglutination were performed in the following way: Strains of *Hemophilus* were cultured in beef heart infusion broth or on beef heart infusion agar slants, each containing Fildes peptic digest of blood. Broth culture suspensions were used directly. Solid media cultures were washed off with sterile 0.85 per cent sodium chloride and diluted to correspond to a 500-ppm silica standard. Red blood cell suspensions were prepared from citrated whole blood by washing the cells three times and suspending them in saline. They were stored as a 10 per cent suspension at 4 C for short periods.

The test was performed by adding equal quantities (either 0.2 ml or 0.5 ml) of a 0.5 per cent suspension of red cells to a broth or saline suspension of the organisms in a 12-by-75-mm test tube. In some instances twofold dilutions of the cultures were made to determine the titer. The tubes were shaken vigorously by hand for a few minutes and allowed to stand at room temperature. Usually gross clumping occurred immediately and could be readily observed in an oblique light beam. In other instances the agglutination was less marked and could be observed, after standing, by the pattern on the bottom of the tube. Agglutinated cells formed a thin uniform blanket covering the entire bottom, whereas the

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unagglutinated cells formed a small compact disk. Specific antisera employed in this study were induced in rabbits by intravenous injections of a formalinized suspension of bacteria in increasing amounts 4 days a week for 5 weeks. In the hemagglutination inhibition test 0.25 ml broth culture of organisms (at least 4 hemagglutinating units) were added to 0.25 ml of the twofold dilution of serum in a small tube. After being shaken 0.5 ml of 0.5 per cent suspension human red cells were added and the test was completed and read as in the hemagglutination test.

RESULTS

Strains of Hemophilus tested. One hundred and four strains of *Hemophilus* were tested for their capacity to agglutinate human red cells. These included

TABLE 1

Hemagglutination by 104 strains of Hemophilus from various sources

SPECIES	SOURCE	NO. STRAINS			
		Human RBC		Chicken RBC	
		Pos.	Neg.	Pos.	Neg.
Koch-Weeks	Conjunctivitis—Texas	27	1	21	7
H. influenzae	Conjunctivitis—Texas	0	18	0	17
H. influenzae	Conjunctivitis—Maryland	0	8	0	8
H. influenzae	Meningitis, etc.—U.S.	1	43	0	44
H. suis		0	3	0	1
H. parainfluenzae	Conjunctivitis—Texas	0	1	0	1
H. parainfluenzae		0	2	0	2
H. hemolyticus		0	1	0	1

strains of Koch-Weeks bacillus and *H. influenzae* isolated from cases of conjunctivitis in Texas and *H. influenzae* isolated from cases of conjunctivitis, meningitis, and other diseases in other parts of the United States and Europe. A few strains of *Hemophilus suis*, *Hemophilus parainfluenzae*, and *Hemophilus hemolyticus* were also studied.

It is shown in table 1 that 27 of 28 strains of the Koch-Weeks bacillus agglutinated human red cells. The strain not possessing a demonstrable agglutinin, no. 178a, differed from the other 27 cultures in that it was agglutinated by anti-Koch-Weeks serum only in a low titer. Twenty-one strains agglutinated chicken red cells, and 7 did not.

In contrast to Koch-Weeks strains, 18 strains of *H. influenzae*, including 3 type-specific strains from conjunctivitis in Texas, and 8 strains of *H. influenzae* from conjunctivitis in the vicinity of Washington, D.C., did not agglutinate either human or chicken red cells. Forty-three strains of *H. influenzae*, including all 6 type-specific and non-type-specific strains, isolated from other sources but principally from meningitis did not cause hemagglutination. One strain, however, a type b from a case of meningitis, originally agglutinated human red cells but

eventually on transfer lost this property, though some of the subcultures of colonial fishings retained it. The few strains of *H. suis*, *H. parainfluenzae*, and *H. hemolyticus* tested did not cause hemagglutination.

Red cells from different animals. The second table shows the results of testing the red cells from a number of individuals of 11 mammalian and avian species with 3 strains of the Koch-Weeks bacillus. These 3 strains represented variations in the ability to agglutinate red cells of various species. Human red blood cells from a large number of individuals representing all blood groups and Rh types, and presumably other known blood antigens, were agglutinated uniformly by these strains of Koch-Weeks. Red cells of 5 goats tested were agglutinated by the 2 strains tested. Red cells from monkeys, rabbits, white mice, white rats, hamsters, and ducklings were not agglutinated. Those from guinea pigs and

TABLE 2

Agglutination of erythrocytes from different animal species by 3 strains of Koch-Weeks bacillus

SPECIES	NO. ANIMALS					
	Strain no. 15		Strain no. 172a		Strain no. 184a	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Human—all blood groups	6	0	87	0	39	0
Monkey	0	16	0	11		
Rabbit	0	16	0	16		
Guinea pig	39	0	0	39	0	5
Goat	5	0	5	0		
Cow	19	1	9	11		
Mouse	0	27	0	21		
White rat	0	15	0	15		
Hamster	0	15	0	15		
Duckling			0	14		
Chicken	20	0	0	24		

chickens were agglutinated by no. 15 but not by no. 172a. Those from cows were irregular in reaction. None of the erythrocytes from these species were agglutinated by strains of *H. influenzae*.

Characteristics of the hemagglutinin. Observations of the influence of various conditions on hemagglutination indicate that this property of the Koch-Weeks bacillus is relatively stable. Aging of the cultures did not appear to reduce the capacity of hemagglutination even though the strains soon die in culture. Suspensions of organisms from solid media 8 days old showed only slightly lower titer than a 24-hour culture. In broth cultures held at 37 C for 4 weeks there was no loss in titer even though the cultures were nonviable. Repeated weekly transfers in 0.15 per cent agar medium over a period of several months did not affect the capacity of the few strains tested to agglutinate red cells. Exposure to 37 C, 4 C, and −50 C for 4 weeks did not affect this ability. However, the property is inactivated in less than 1 minute at the temperature of boiling water,

15 minutes at 90 C, and 48 hours at 56 C. Hydrogen ion concentrations within the range of pH 6.6 to 7.6 were favorable for the reaction, but at pH 8.0 and 8.5 the readings were unsatisfactory and at pH 6.0 or below erythrocytes were usually hemolyzed. Subcultures of the strains grown on agar containing either unheated or "chocolate" rabbit blood, or unheated or "chocolate" human blood, and in Levinthal broth and rabbit blood broth possessed the property just as did the strain cultured on the usual media. *H. influenzae* cultured on these media did not acquire the property of hemagglutination.

Cultures treated with 0.5 per cent formalin or ether also retained the property.

TABLE 3

Inhibition by anti-Koch-Weeks rabbit serum of the agglutination of human red cells by the Kochs-Weeks bacillus

KOCH-WEEKS CULTURE NO.	ANTISERUM		DILUTION OF ANTISERUM*							
	Species	No.	1:20	1:40	1:80	1:160	1:320	1:640	1:128	Saline
46	K.W.	46	−	−	−	−	−	±	±	+
	K.W.	128a	−	−	−	±	+	+	+	
	K.W.	145a	−	±	±	±	±	±	+	
	K.W.	181a	−	−	−	−	−	±	+	
181a	K.W.	46	−	−	−	−	−	−	±	+
	K.W.	128a	−	−	−	−	+	+	+	
	K.W.	145a	−	−	−	−	+	+	+	
	K.W.	181a	−	−	−	−	+	+	+	
172a	*H.inf.*	162b	+	+	+	+	+			+
	H.inf.	680	+	+	+	+	+			
	K.W.	172a	−	−	−	±	±	+		
	K.W.	180a	−	−	−	−	−	−		
180a	*H.inf.*	162b	+	+	+	+	+			+
	H.inf.	680	+	+	+	+	+			
	K.W.	172a	−	−	−	±	+	+		
	K.W.	180a	−	−	−	−	−	+		

The symbols −, ±, + indicate no, partial, and complete agglutination of red cells, respectively.

* No antiserum caused hemagglutination in a dilution of 1:10 or greater; 9 samples of normal rabbit serum (diluted 1:20 and 1:40) did not inhibit hemagglutination.

Hemagglutination was not inhibited by mucin. The property was not eluted during 4 hours at 37 C after the cells were sedimented at 4 C. The susceptibility of human red cells to agglutination by the Koch-Weeks strains was not altered by exposure to suspensions of *H. influenzae*, *Micrococcus pyogenes* var. *aureus*, or a type-specific pneumococcus. It was not affected by the receptor-destroying enzyme from *Vibrio comma*, thereby differing from the influenza and certain other viruses. The property could not be washed off the organism with 3 successive saline solutions, and the washings did not contain a hemagglutinin. However, supernates and filtrates of broth cultures were active, though in much reduced titer.

Inhibition of hemagglutination. In table 3 data are presented that illustrate the specific capacity of different anti-Koch-Weeks rabbit sera to inhibit the agglutination of human red cells by four cultures of the Koch-Weeks bacillus. Two different anti-*H. influenzae* sera, however, even in low titer, failed to inhibit hemagglutination by two strains of Koch-Weeks bacillus. In tests not recorded here nine other Koch-Weeks strains were prevented from causing hemagglutination by the specific antiserum. With each antiserum the titer of inhibition corresponded closely to the bacterial agglutination titer for the particular strain; also the same serological relationship of the strains was observed as with the bacterial agglutination.

SUMMARY

Twenty-seven of 28 strains of the Koch-Weeks bacillus were found to possess an agglutinin for human red blood cells, and some strains for the red cells of other species. *H. influenzae*, from conjunctivitis or other sources, *H. suis*, *H. parainfluenzae*, and *H. hemolyticus* did not possess this ability. This property was relatively stable to aging, was not readily lost by frequent transfer of the culture, exposure to low temperatures, or treatment with formalin or ether, and was independent of the type of medium used. It could not be washed off the bacterial cell and was specifically inhibited by anti-Koch-Weeks rabbit serum. The ability of the Koch-Weeks bacillus to agglutinate human red blood cells appears to be an additional characteristic that can be used to differentiate this species from *Hemophilus influenzae*.

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