and appendage drag was conducted with methods applicable to conventional rigid bodies (*6*) in which the boundary layer is predominantly turbulent and the surfaces are smooth. Numerous measurements of Keiki were taken and photographs obtained to aid in the hydrodynamic analysis. Corrections were included for interference drag at the intersection of the body and the appendages. The calculated drag-area coefficient (drag/dynamic pressures) was 0.0644 at 29.9 km/hr (16.1 knots) where the length Reynolds number is 14.2×10^6. If his power output per unit body weight was the same as that of athletes (*7*) and his propulsive efficiency 85 percent, Keiki could travel 26.8 km/hr (14.4 knots) for 7.5 seconds, 25.8 km/hr (13.9 knots) for 10 seconds, 21.4 km/hr (11.5 knots) for 50 seconds, and 12.8 km/hr (6.9 knots) for a 24-hour day. The experimental results showed a top speed of 29.9 km/hr (16.1 knots) for 7.5 seconds, 25.2 km/hr (13.6 knots) for 10 seconds, 21.9 km/hr (11.8 knots) for 50 seconds, and at least 11.1 km/hr (6 knots) for an indefinite period. Consequently the experimental results compare closely with predicted turbulent values for this animal.

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Sialic Acid Binding Sites: Role in Hemagglutination by Mycoplasma gallisepticum

Abstract. *Hemagglutination of turkey erythrocytes by* Mycoplasma gallisepticum *was inhibited by mucoproteins containing sialic acid, by sialic acid itself, and by treatment of the erythrocytes with neuraminidase. Neuraminidase treatment of the mucoprotein-rich inhibitors reduced or abolished their inhibitory activity. The findings indicate that sialic acid on the erythrocyte surface provides binding sites for* Mycoplasma gallisepticum.

Hemagglutination of turkey red cells by *Mycoplasma gallisepticum* has been described (*1*), and we now report a study of the binding site on erythrocytes for this organism in the hemagglutination reaction.

Fresh cultures of *M. gallisepticum* were grown each day, in PPLO (pleuropneumonia-like organism) broth containing horse serum (20 percent) (*2*). The organisms were harvested by centrifugation at 4°C at 13,800*g*. They were then washed three times in Ringer solution and resuspended to a concentration 100 times that of the original culture. This procedure usually provided 10^{10} to 10^{11} organisms per milliliter. The organisms were kept at 0°C, and a fresh preparation of mycoplasma was used each day. Turkey blood was drawn from the wing vein to a syringe containing heparin; the erythrocytes were separated and washed three times in Ringer solution and suspended to make a 0.5 percent solution (by volume). In each experiment, the suspension of *M. gallisepticum*, Ringer solution, and the red cells (0.5 percent suspension) were mixed (1 : 10 : 10 by volume). Substances to be tested for inhibitory effects were incorporated in the Ringer solution and incubated with the suspensions of mycoplasmas for 10 minutes at 0°C before the erythrocyte suspension was added. Incubation of the reaction mixture was then continued at 0°C, and sample drops were withdrawn with a Pasteur pipette, placed on a slide, and observed immediately at × 100. The degree of agglutination was graded on a 0 to 4+ scale in which each 1+ represented agglutination of approximately 25 percent of the cells. Readings were made 30 minutes after the erythrocytes were added to the reaction mixtures, and at that time most preparations of mycoplasma gave a 3 to 4+ agglutination. Giemsa stains were made by diluting the reaction mixture tenfold with Ringer solution and then allowing a drop of the diluted suspension to dry on a slide. Such preparations showed many mycoplasmas adhering to erythrocytes, with a lattice of clustered mycoplasmas between clumps of red cells. Heat-killed *M. gallisepticum* did not agglutinate red cells.

Addition of serum to the reaction mixture resulted in inhibition of hemagglutination. This effect was observed with all serums tested, excluding those which themselves agglutinated turkey erythrocytes (*2a*). Thus, human, bovine, fetal bovine, rabbit, and turkey serums inhibited hemagglutination by mycoplasma. When each of these serums constituted 5 percent by volume of the reaction mixture, inhibition was complete. Each serum caused at least partial inhibition in a concentration of 1 percent. Turkey serum was slightly less effective than all others tested. Heating to 56°C for 30 minutes, 65°C for 10 minutes, or 100°C for 5 minutes did not diminish the effectiveness of the serums. Giemsa staining of reaction mixtures inhibited with serum or egg white (see below) showed a striking diminution in the number of mycoplasmas adherent to erythrocytes.

The observations that all of the serums inhibited hemagglutination in low concentrations and that heat did not diminish their effectiveness suggested that the inhibitory activity might be due to the carbohydrate moieties of their glycoprotein or mucoprotein content. When mucoprotein-rich substances such as egg white, or gastric mucin, or ovomucoid were incorporated into the reaction mixture, hemagglutination was completely inhibited. Egg white prevented hemagglutination in a concentration as low as 10 μg/ml, gastric mucin at 500 μg/ml, and ovomucoid at 1.5 mg/ml. Heating these substances at 65°C for 10 minutes or 100°C for 5 minutes did not diminish effectiveness.

Because of the high sialic acid content of the mucoprotein-rich inhibitors and the possibility that this sugar derivative determined inhibitory activity, the effect of neuraminidase was studied.

Table 1. Inhibition of hemagglutination and reversal of the effect by treatment of the inhibitor with neuraminidase (*7*). The egg white (5 mg) in 0.9 ml of Ringer was mixed with 0.1 ml of the neuraminidase preparation and incubated at 37°C for 1 hour (*8*). Fetal calf serum (10 percent) was treated similarly. Both reaction mixtures were then heated at 65°C for 10 minutes in order to inactivate the enzyme (9). Controls in the absence of egg white or fetal bovine serum showed 3 to 4+ agglutination.

Treatment	Fetal bovine serum		Egg white	
	Concn. (%)	Agglutination	Concn. (mg/ml)	Agglutination
None	2	0–tr	1	0
65°C, 10 min	2	tr	1	0
Neuraminidase	2	3+	1	2+

Treatment of serums and egg white with this enzyme greatly reduced or abolished inhibitory activity (Table 1). In giemsa preparations, neuraminidase-treated egg white showed a markedly reduced ability to prevent adherence of mycoplasmas to the surface of erythrocytes.

The substances that inhibit hemagglutination can disaggregate clumps of erythrocytes already agglutinated by mycoplasmas. This property also was markedly diminished when the substances were treated with neuraminidase before use.

Thus the substances that inhibit hemagglutination may do so by providing sialic acid sites which compete with similar sites on turkey erythrocytes for binding *M. gallisepticum*. This view is further supported in that treatment (at 37°C for 5 minutes) of turkey red cells (0.4 ml of 0.25 percent suspension) with neuraminidase (10 to 50 μl) prevents them from being agglutinated by this organism. Giemsa staining then showed a marked reduction in the numbers of mycoplasmas adherent to neuraminidase-treated erythrocytes.

When sialic acid (*N*-acetylneuraminic acid), neutralized to *p*H 6.7 ± .2 was first incubated for 10 minutes at 0°C with *M. gallisepticum* and the erythrocytes were then added to the reaction mixture, hemagglutination was inhibited. The inhibition was demonstrable only within a narrow range of concentrations of sialic acid for a limited concentration of mycoplasmas. Excessively high concentrations of sialic acid caused agglutination of the red cells in the absence of mycoplasmas. However, when such high concentrations were added to hemagglutinating suspensions of mycoplasma, mutual inhibition of hemagglutination was observed. Thus, at optimum concentrations of both organisms and sialic acid, the hemagglutinating effect of each could be cancelled (Table 2). Mycoplasmas which had been incubated with sialic acid and then washed showed no decrease in ability to agglutinate erythrocytes indicating that the association between sialic acid and mycoplasma was readily reversible. The loose association between sialic acid and the organisms was further illustrated by the finding that if the tubes were shaken vigorously hemagglutination could be induced in reaction mixtures containing unagglutinated red cells and mutually inhibiting concentrations of sialic acid and organisms. The difficulties in arranging optimum conditions for inhibition by sialic acid were similar to those described (*3*) for the inhibition of agglutination of $Rh_o(D)$ erythrocytes by specific antibody in the presence of sialic acid.

A number of substances failed to inhibit hemagglutination. The simple sugars, D-glucose, D-galactose, D-mannose and *N*-acetyl-D-glucosamine showed no inhibition (at 15 mg/ml). L-Fucose, *N*-acetyl-D-galactosamine, and *N*-acetyl-D-mannosamine (also at 15 mg/ml) showed very slight inhibition, but even this was variable. The sugars themselves did not agglutinate the erythrocytes. Heparin, in concentrations of 1 and 5 mg/ml did not inhibit. Hemoglobin, purified by electrophoresis, caused no inhibition in a concentration of 1 mg/ml.

In the preceding experiments, all reactions were carried out at 0°C in order to avoid the variable caused by the demonstrated production of hydrogen peroxide by *M. gallisepticum* at 37°C (*4*), since hydrogen peroxide itself can produce hemagglutination. However, it was found that the serums and mucoprotein-rich substances which inhibited hemagglutination at 0°C also inhibited at 37°C. Also, at 37°C, treatment of the inhibitors with neuraminidase reduced their effectiveness, and neuraminidase-treated erythrocytes were not agglutinated. Moreover, merthiolate-treated nonviable mycoplasmas retained their hemagglutinating property, as previously shown (*5*), and the observations made at 37°C with viable organisms were duplicated at 37°C with merthiolate-treated mycoplasmas.

Table 2. Inhibition of hemagglutination by sialic acid (*10*).

Content of reaction mixture		Agglutination
Sialic acid (mg/ml)	Mycoplasma	
0	Yes	3 +
0.5	Yes	1–2+
1.0	Yes	1 +
3.0	Yes	0
6.0	Yes	1–2+
3.0	No	2 +
6.0	No	2–3+

The evidence indicates that *N*-acetylneuraminic acid, or a closely related sialic acid, provides binding sites for *M. gallisepticum* at the erythrocyte surface. The situation appears analogous to hemagglutination by influenza and other myxoviruses, where neuraminic acid provides sites for virus attachment (*6*). The relation between binding sites in the hemagglutination reaction and the pathogenesis of disease caused by such organisms is not known. Possibly the location of preferred sialic acid binding sites for the organisms in special tissues may play a role in determining the host range and the localization of disease in vulnerable hosts.

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8. Egg white powder as well as the other mucoprotein-rich substances and serums were obtained commercially.
9. Enzyme heated at 65°C for 10 minutes did not itself cause agglutination of red cells nor did it inhibit hemagglutination when added to mixtures of erythrocytes and mycoplasma.
10. Purchased from K and K Laboratories, Plainview, New York.
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