TABLE II. Analysis of Variance of Salivation Scores.

Source of variation	Degrees of freedom	Variance ratio (F)* Salivation score	$\sqrt{\text{Salivation score}}$
Dogs	9	2.68†	3.43‡
Codeine *vs* diphenh. & NaCl	1	15.98‡	13.45‡
Diphenh. 4 & 8 *vs* NaCl	1	.34	.79
Diphenh. 4 *vs* diphenh. 8	1	.00	.24
Covariance§	1	17.56‡	8.57‡
Residual	26	1.00 (2.34)‖	1.00 (.4060)‖

* Reduced to accommodate uncertainties in covariance adjustments.
† $.05 > P > .01$.
‡ $P \leq .01$.
§ Salivation after treatment on salivation before treatment, independently of treatments or dogs.
‖ Actual residual variance.

ficult to assess any part played by local anesthetic concentrations of drug in pulmonary tissue, as proposed by Bucher(5) for some such agents. Any substantial role of a peripheral anticholinergic property in the test employed would seem denied by negative antitussive results‡ with the more strongly anticholinergic(7) methyl quaternary derivative. This agrees with the observation that diphenhydramine, itself, failed to influence salivation significantly in the doses used. The antitussive action of most of these agents has been predicted from effects on reflexes arising from stimulation of the superior laryngeal nerve in anesthetized animals, a situation bypassing any peripheral afferent factor. We suppose that the antitussive effect of diphenhydramine may be central in major part.

Summary. Diphenhydramine hydrochloride was estimated to be about 0.4 as potent as codeine phosphate in terms both of peak and of 2-hour total effect of subcutaneous administrations, in raising tussal thresholds to ammonia in unanesthetized dogs.

The authors are grateful to Dr. R. W. Fleming, with his long interest in benzhydryl alkamine ethers, for persistent encouragement to schedule this study.

‡ An antitussive property once inferred for the quaternary compound(6) could not be confirmed even with strongly mydriatic doses, either in terms of the ammonia tussal threshold in dogs (Rosiere and Winder, unpublished work done in 1953) or in terms of amount of coughing in dogs subjected to sulfuric acid mist (C. A. Winter, personal communication in 1953).

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Studies with Tularemia Vaccines in Volunteers.* VI. Assessment of Role of Properdin in Resistance. (27592)

HAROLD N. CARLISLE AND SAMUEL SASLAW
Department of Medicine, Ohio State University, Columbus

Recently there has been resurgence of interest in nonspecific resistance to infection (1). The role of properdin in resistance to infectious disease is not clear(2). Previous studies from this laboratory(3-7) have been concerned with clinical and serologic aspects of experimental tularemia in vaccinated and non-vaccinated volunteers after intracutaneous or respiratory challenge. Both Foshay killed (phenolized) and viable attenuated vaccine stimulated production of antibodies, but there was no correlation between inci-

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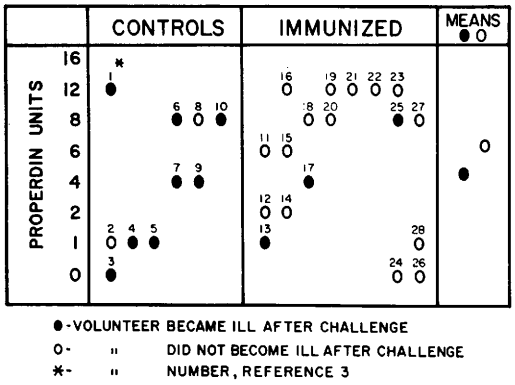

FIG. 1. Properdin levels in viable-vaccinated and non-vaccinated volunteers prior to respiratory challenge.

dence or severity of infection and prechallenge antibody titer as measured by bacterial agglutination or polysaccharide hemagglutination tests(5). Immunodiffusion studies(7) also failed to demonstrate apparent relationsnip between serum characteristics and resistance to infection. The present studies were conducted to determine if there was a relationship between prechallenge properdin level and subsequent clinical response of volunteers following aerosol exposure to *Pasteurella tularensis*.

Materials and methods. Properdin levels were determined by Pillemer's zymosan assay (8) with modifications described earlier(9, 10). *In vitro* studies of the bactericidal effect of the properdin system on *P. tularensis*, strain 38† were carried out according to methods of Wardlaw and Pillemer(11). A known properdin-sensitive strain of *Shigella dysenteriae*‡ was used for comparative purposes.

Results. Eighteen volunteers vaccinated with viable attenuated vaccine and 10 non-vaccinated controls were challenged with a *P. tularensis* aerosol(4). Properdin levels were determined on serum samples drawn prior to challenge. Three of 18 and 8 of 10 vaccinated and control subjects, respectively, exhibited constitutional signs of disease after challenge, but there was no correlation between prechallenge properdin level and whether or not the individual became ill (Fig. 1). In the immunized group, the 3 who became ill, Nos. 13, 17 and 25, exhibited serum properdin levels of 1, 4 and 8 units, respectively. Of the 15 who did not become ill, 2, 1, 2, 2, 3 and 5 had properdin levels of 0, 1, 2, 6, 8 and 12 units, respectively. In 8 non-vaccinated controls who became ill properdin titers in 1, 2, 2, 2 and 1 volunteers were 0, 1, 4, 8 and 12 units, respectively, whereas 2 controls who did not become ill showed 1 and 8 units, respectively. The mean properdin level of those who exhibited signs of disease, 4.6 units, was not significantly different from the mean of those who did not, 6.5 units ($t = 1.05$).

In addition, prechallenge serum properdin levels were determined in 4 volunteers who had received Foshay killed vaccine previous to respiratory challenge. The 2 who became ill after challenge exhibited 6 and 8 units, respectively, while the 2 who showed no signs of infection also exhibited 6 and 8 units, respectively. The 2 non-vaccinated controls who showed signs of disease had 4 and 12 units, respectively, while the 3rd control, exhibiting no illness, had 6 properdin units. Thus, in a total of 35 volunteers, prechallenge properdin determinations were not related to clinical outcome after challenge.

Four of the above subjects, 2 Foshay-vaccinated and 2 control, who exhibited constitutional signs of disease were also studied after challenge. Symptoms first appeared 5-8 days after exposure and persisted for 2-3 days. Streptomycin therapy was begun as soon as the presence of infection had been established. Properdin levels during the symptomatic period and at 15-22 days postchallenge were not significantly different from base line values.

Further evidence that the properdin system is not involved in tularemia was obtained from *in vitro* bactericidal tests with *S. dysenteriae* and *P. tularensis*. The Shigella strain was, as expected, killed rapidly by fresh normal serum but not by serum deficient in properdin. Addition of properdin to properdin-deficient serum restored bactericidal activity. With *P. tularensis*, however, the above experiments could not be duplicated in that ad-

† Kindly supplied by Dr. H. T. Eigelsbach, Fort Detrick, Md.

‡ Kindly supplied by R. J. Wedgewood, Western Reserve Univ., Cleveland, Ohio.

dition of properdin to properdin-deficient serum did not alter bactericidal effect on this organism. Thus, it would appear that *P. tularensis* is not properdin-sensitive.

Discussion. These results point to the fact that the properdin system is not involved in resistance of vaccinated or non-vaccinated humans to experimental infection by *P. tularensis*. Prechallenge properdin levels were not significantly different in volunteers who did or did not exhibit constitutional signs of disease after respiratory challenge. No significant change in properdin levels was observed following infection in 4 volunteers so studied. *P. tularensis* did not appear to be sensitive to the bactericidal effect of the properdin system *in vitro*. These findings supplement those described previously(3-7), that prechallenge agglutinin titers provided no clue as to whether the vaccinated subject would or would not become ill. Immunodiffusion studies(7) with serum samples from these same volunteers also failed to bring out a relationship between either number or pattern of precipitin lines and response of the individual after exposure. Thus, our studies have not been able to associate any particular serum component, specific or nonspecific, with immunity to experimental tularemia in volunteers.

Summary. Prechallenge properdin levels were not significantly different in 35 vaccinated or non-vaccinated volunteers who did or did not become ill after respiratory challenge with *P. tularensis*. No change was observed in properdin levels after infection in 4 volunteers so studied. *P. tularensis* was not sensitive to the properdin system *in vitro*. It would appear that the properdin system is not involved in immunity of humans to tularemia.

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Studies on Acquired Resistance of Monolayer Cell Cultures to Photodynamic Action. (27593)

ALBERT I. HOLTZ* (Introduced by C. P. Li)
Division of Biologics Standards, National Institutes of Health, Bethesda, Md.

The phenomenon of the photodynamic action of light on various biological systems has been the subject of investigation since 1899. Inhibition of photodynamic action has been described as being caused by removal of molecular oxygen, by reducing agents, and by agents which prevent the combination of the dye with the cell or other substrate(1). The photodynamic action of neutral red on cell cultures and cell culture-virus systems has been investigated in recent years in several laboratories(2,3,4). Prevention of photodynamic action in monkey kidney cell monolayers by addition of reducing agents such as cysteine and glutathione has been demonstrated by Heberling and Cheever(5). No biological system of any type was known to develop resistance to photodynamic action until Gochenour and Baron(3) reported that the photodynamic activity of neutral red-

* Present address: Nat. Heart Inst., NIH, Bethesda, Md.