isolated strains of meningococcus for white mice when the proper amount of yolk-emulsion was employed and the intraäbdominal route was used for inoculation. As little as 10 organisms were able to kill white mice in 16-48 hours. In contrast to the varying quality of gastric mucin, egg-yolk is easily obtainable and the material has been found to be uniform in pH value and in viscosity as long as the eggs were fresh. If aseptic care is taken in handling the material, no further sterilization is required. Emulsions of the desired viscosity may be prepared according to the data given.

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Immunological Studies on Proteins of *Corynebacterium diphtheriæ.*

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Previous studies[1] have shown that type-specific protein derived from the Park-Williams 8 strain of *C. diphtheriæ* may be converted into a group-specific protein by heating at 56°C for 30 minutes. This finding suggests that diphtheric proteins in general may not be a single antigen. However, direct chemical methods, such as fractional precipitations with ammonium sulfate, have, thus far, not revealed any antigenic differences. Likewise the application of the sensitive complement-fixation reaction has proven impractical since rabbit sera immunized with whole organisms are markedly anticomplementary and diphtheric proteins alone failed to produce potent antiserum.[1] If it were possible to enhance the antigenic quality of the type-specific protein in rabbits by adsorbing the protein to charcoal[2] the usual serological methods could then be applied. The results of such a study are described below.

Method. Proteins were prepared[1] from representative strains of the serological types D41 and D43 and of group x.[3] All were serologically active, giving precipitin-titers of 1:100,000 with the

1 Wong, S. C., and T'ung, T., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 824.

2 Seibert, F. B., *J. Immun.*, 1935, **28**, 425.

3 Sia, R. H. P., and Huang, C. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 348.

specific serum only. The method of preparing the charcoal for absorption was as follows: 0.2 g of animal charcoal was suspended and washed with 3 changes of sterile distilled water followed by 3 of sterile physiological saline. It was then suspended in about 30 ml of saline and left at 56°C for 2 hours. After removing the supernate by centrifugation, 15 cc of a 1% solution of the type-specific protein were added and thoroughly mixed. It was placed in a waterbath at 37°C for 2 hours with frequent agitation. Then 25 ml of sterile saline were added to the mixture and it was refrigerated. The subcutaneous route of injection was employed after preliminary trials had shown it to be the most effective. Only rabbits whose sera did not contain natural antibodies and were not anticomplementary were used. Two animals were employed for each protein. Injections were made on 2 successive days followed by 5 days of rest and a total of 8 injections, totalling 16 ml, was given to each animal. Animals were bled 7 days after the last injection.

Result. All the immune sera gave specific agglutinative reactions. The precipitin titers varied from 1:10,000 to 1:50,000 when undiluted serum was overlayered with varying dilutions of the antigen and all but one gave reaction only with the homologous protein. Type D41 immune serum, however, gave marked cross-precipitation with the group x protein. The reverse of this, namely, the reaction between group x immune serum and type D41 protein did not occur. On the other hand, when a more sensitive method such as complement-fixation was employed the sera were found to be less specific, marked cross-reactions among the various proteins and sera being found. The results showing the antigenic complexity of the diphtheric proteins are presented in Table I. An examination of the last column of the table shows that the diphtheric proteins contain at least 2 antigens, one specific, and a common one shared apparently by all types. The specific antigen is best shown with type D41 protein which gave no reaction with group x serum and only a weak reaction with type D43 serum, while the common antigen is best shown by the group x protein which reacts to the same degree with the homologous as well as heterologous immune sera. An exception to this is found in the immune serum of type D41 which reacted strongly with the type-specific protein of type D43. This may be due to the fact that rabbits were superimmunized and therefore this serum was unsuitable for the demonstration of the type-specificity. In general, however, the complement-fixation titers of all sera were higher with homologous protein than with heterologous ones. It is of interest to note that serologically inactive proteins of

TABLE I.
Antigenic Relationship of Diphtheric Proteins.

Immune serum	Protein	Precipitin-titer	Complement-fixation titer
Type D41	T41	1:10,000	1: 100,000
	G x	1:10,000	1: 100,000
	T43	Negative*	1: 50,000
Group x	T41	Negative*	Negative*
	G x	1:10,000	1: 500,000
	T43	Negative*	1: 10,000
Type D43	T41	Negative*	1: 10,000
	G x	Negative*	1: 50,000
	T43	1:50,000	1:1,000,000

*Negative in 1:1000 dilution of the antigen.

C. diphtheriæ[4] (1:1000) can not be rendered antigenic by adsorption to charcoal. The polysaccharides of *C. diphtheriæ* did not give precipitin-reactions with any of the sera but did have a complement-fixing titer of 1:10,000.

Comment. Taking the above findings in addition to those reported elsewhere[1] we may be justified in arriving at the following conclusions regarding the antigenic structure of diphtheric protein. The protein is composed of at least a heat-labile type-specific antigen and a heat-stable common antigen shared by all diphtheric organisms. The specific antigen being present in larger quantity masks the reaction of the other in the precipitative tests. The common antigen, however, may be manifested either in the serum of superimmunized animals by complement-fixation tests or by inactivating the protein with heat. The specific antigen is also lost when a diphtheric organism loses its virulence as was found in the case of the protein prepared from the avirulent organisms belonging to group x. Incidentally the method of adsorbing type-specific protein to charcoal may be useful in the production of type-specific serum since even undiluted serum did not cross-agglutinate.

Conclusion. Diphtheric protein is a complex antigen consisting of at least a type-specific antigen and a group-antigen.

[4] Unpublished results.