Most sera reacted with the homologous polysaccharides diluted 1:100,000 excepting one serum which reacted with 1:250,000 (Park 8). Corresponding titers were obtained with heterologous polysaccharides.

For further study of these group reactions, absorption-tests were carried out. Equal volumes of undiluted immune serum and the optimal dilution (1:10,000) of polysaccharide were mixed and incubated at 37°C in the waterbath for 2 hours. Then the treated serum was refrigerated overnight and centrifuged rapidly for half an hour. The supernate was tested with 1:1000 and 1:5000 dilution of the various polysaccharides. Every serum (*mitis, gravis,* or intermediate) was completely absorbed by the homologous and by each heterologous polysaccharide, including the avirulent type.

Conclusion. The polysaccharides of *C. diphtheriæ* appear to be shared by all the types studied.

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Sensitization of Guinea Pigs by a Modified Form of Seibert's Tuberculoprotein Derivative.

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Denaturation of tuberculoprotein is usually accompanied by a decrease in antigenic activity and in special cases, such as in the preparation of OT (old tuberculin) and of PPD (purified protein derivative), antigenicity is usually destroyed.[1] According to Seibert[2] the lack of antigenic properties of PPD is attributed to the use of heat and of trichloracetic acid employed in its preparation. It is here shown that a modified form of Seibert's PPD can sensitize guinea pigs.

Mycobacterium tuberculosis, H37, was grown on Wong's modification of Henley and LeDuc's synthetic medium[3] for 4 weeks. The general procedures of preparing the tuberculoprotein were essentially those used by Seibert, but 3 changes were introduced to shorten the time of preparation: (1) glycerin was not added to the tuberculin during concentration over the waterbath; (2) the concentrated tu-

1 Seibert, F. B., *Am. Rev. Tuberc.,* 1934, **30**, 713.

2 Seibert, F. B., *J. Inf. Dis.,* 1932, **51**, 383; Nat. Tuberc. Assn., 1933, 165.

3 Wong, S. C., *J. Bact.,* 1937, **33**, 451.

berculoprotein was not dialyzed through a collodion membrane but was passed through a Seitz filter and precipitated directly with 50% trichloracetic acid; (3) the precipitated protein was collected and washed with 10% trichloracetic acid until free from SO_4, about 15 washings being necessary. The PPD obtained in this manner has physical and chemical properties similar to those described by Seibert.

Cutaneous reactions of sensitive tuberculous guinea pigs to 0.005 mg were comparable to those induced by the tablet form of PPD obtained from the Mulford Laboratory. Ten to 15 mg of our PPD injected intravenously in guinea pigs weighing about 250 g produced no harmful effects. Prolonged immunization of rabbits elicited no precipitin detectable with 1:500 dilution of the antigen but complement-fixation occurred with 1:1000 dilution of antigen and serum diluted 1:5.

Twenty-four guinea pigs of various sizes were sensitized by 2 subcutaneous injections and one intraäbdominal injection of modified PPD at 5-day intervals. The total dose was 30 mg per animal. Three weeks after the last injection the animals were tested for the presence of supersensitivity by intravenous injection of varying amounts of the tuberculoprotein. The results, presented in the first 3 columns of Table I, show that 11 out of 24 sensitized animals

TABLE I.
Active Sensitization of Guinea Pigs with Modified PPD.

No. of animals	Result of 1st test	Result of 2d test	Total No. of animals
4	Death, 3 min.		
2	Severe shock, recovered after ½ to 1 hr.	Death	2
5	Mod. shock, recovered after 10-20 min.	"	3
		Mod. shock	2
13*	No response	Death	1
		Mod. shock	5
		Mild "	1
		No response	4

*2 died of intercurrent infection after the first test.

reacted to the intravenous injection of 5 to 10 mg. Typical anaphylactic death occurred in 4; no tuberculous infection was found at necropsy. The general picture of the reactions was typical of classical anaphylaxis and it is evident that this kind of purified tuberculin can actively sensitize guinea pigs. The sensitizing property of OT in guinea pigs was observed by Reichle and Goldblatt.[4]

The animals who survived the first intravenous injection of our

[4] Reichle, H. S., and Goldblatt, H., *Am. Rev. Tuberc.*, 1933, **27**, 291.

PPD were retested 3 weeks later with the same shocking dose (5-10 mg). At the same time cutaneous allergy, passive transfer of supersensitivity, and the effect of another shocking agent, tuberculopolysaccharide, were studied.

For the endermal test, 6 guinea pigs which had developed non-fatal shock in the previous trial and 2 normal controls were injected with 0.01 and 0.005 mg of the purified tuberculin contained in 0.1 cc of salt solution; none of the injected animals showed any evidence of reaction during a period of 24 hours.

For passive transfer of supersensitivity, 3 of these animals were bled and subsequently tested for the presence of sensitization by injecting intravenously 10 mg of our PPD. Since all died of typical anaphylactic shock the sera were considered suitable for use. Accordingly, each of 3 young guinea pigs weighing about 250 g received intraäbdominally 2 cc of the pooled sera. Twenty-four hours later they were tested intravenously with 10 mg of the tuberculin. Although all of them showed a moderate degree of anaphylactic shock, recovering after 10 to 15 minutes, serological examination of the same serum revealed no precipitin or complement-fixing antibody for either the purified tuberculin or the polysaccharide prepared from the same microörganism.

Six other animals were injected intravenously with 10 mg of tuberculopolysaccharide but no symptom of shock was observed. However, when they were tested a half-hour later with 5 to 10 mg of our PPD all but one reacted. The results of the retesting of the sensitivity of the 18 animals are shown in the last column of Table I. It was found that supersensitivity returned to all the 7 animals that developed non-fatal shock in the first test. As a result of the second test 5 died of acute anaphylactic shock. Of the remaining 11 animals, 7 showed varying degrees of supersensitivity.

Conclusion. Although active sensitization of guinea pigs is possible under the conditions of the experiment, it is not accomplished with the same ease and regularity as with ordinary proteins. Nevertheless the antigenic nature of our PPD is evident.