gen. This gave no skin reaction in a dilution equivalent to the original concentrated tuberculin, but gave a precipitin reaction up to a dilution of 1-40,000.

The tannic acid method, in the form so far used, has served to establish that the skin test and the precipitin test are manifestations of separate and distinct substances in the tuberculin. It, however, causes too great a loss of these substances to be used in quantity production. It was found, that by the cultivation of the organisms on synthetic media, a broth filtrate could be obtained containing both compounds in quantity not markedly inferior to meat broth cultures. From such filtrates it has proved an easy matter, by a single fractional alcohol precipitation, followed by dialysis, to prepare a substance giving the precipitin reaction in a dilution of 1-1,000,000. This material in a dilution of 1-100 gives a doubtful or negative skin test, and is now being collected for further chemical study.

The nature of the component which produces the skin reaction is also under investigation, but beyond the fact that the fraction is largely protein, no definite statement can yet be made.

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Studies on the purification of antibodies. III. Certain methods for the precipitation of pneumococcus protective antibody.

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We have tried to apply the principles developed in our work on typhoid agglutinin to the purification of pneumococcus protective antibodies. The task has turned out to be particularly difficult. The great solubility of the pneumococcus makes it impossible to get the primary extracts of sensitized bacteria as free from bacterial substances as in the case of typhoid. The variability in the resistance of mice to pneumococcus infection, and the variable virulence of pneumococcus under culture, make

quantitative observations impractical. For these reasons, we have been unable to get consistent results which could be repeated with regularity. Nevertheless, we did succeed many times in precipitating the pneumococcus protective body in a way which leaves no doubt that the same methods can be applied as were used in the purification of typhoid antibody.

Our primary material was the protective body obtained in alkaline solution by the method of Huntoon, or by a modification which consisted in extracting in N/500 NaOH instead of in ¼ percent $NaHCO_3$. Precipitation could frequently be brought about without the addition of any metallic salt, by adding hydrochloric acid to a hydrogen ion concentraction between pH 4.0 and 4.6. The following protocol is illustrative of these results:

"Isoelectric" Precipitation, and Recovery in the Dissolved Precipitate, of Pneumococcus Protective Antibody.

Ten cc. portions of an alkaline solution of pneumococcus protective antibody were brought to the indicated pH by addition of 0.15 cc. N/40 HCl, and allowed to stand in the ice box overnight. The precipitate was centrifuged, dissolved in N/400 NaOH, and placed on test. Each mouse received 0.5 cc. protective material, and 0.5 cc. 18 to 24 hours broth culture of pneumococcus Type 1, diluted 1-200, *i. e.*, 0.025 cc. of culture. Each dilution was tested on two mice.

TABLE 1.

Material.	Diluted 0		Diluted 1:10	
Original extract	72* S		64 64	
Ppt. obtained at pH 4.0	S S		64 64	
Virulence control cc. of culture	0.000,005	0.000,001	0.000,000,5	0.000,000,25
	64	64	64	64

* The figures indicate the number of hours before death of animals.
S = survival.

The addition of copper chloride, as in the case of typhoid antibody, was found to greatly facilitate precipitation of the antibody. The most favorable strength of copper chloride was about M/2200. A final concentration of M/1100 had a distinct destructive effect on the pneumococcus antibodies.

Copper Precipitation with Recovery in the Precipitate of Pneumococcus Protective Antibody.

Twenty cc. of pneumococcus antibody extract was added to 2 cc. of M/200 copper chloride, and 2.3 N/40 HCl. Precipitate formed, allowed to stand in ice box over night, centrifuged and dissolved in original volume (20 cc.) of N/200 NaOH. Each animal received 0.2 cc. protective material, diluted to 0.5 cc., with saline and the indicated amount of pneumococcus Type 1, 18 to 24 hour culture, diluted to 0.5 cc. with broth.

TABLE 2.

Material	Dose of Culture		Nitrogen per 100 cc.
	0.01 cc.	0.06 cc.	
Original extract	S 88	16 40	12 mgm.
Copper precipitate dissolved	S 88	40 88	6.6 mgm.

Virulence control cc. of culture	0.000,000,1	0.000,000,01	0.000,000,001
	40 40	40 40	S S

This method can be used for the concentration of antibodies as shown in the following protocol:

Twenty cc. pneumococcus protective antibody solution was added to 2 cc. M/200 copper chloride solution, plus varying quantities of hydrochloric acid as tabulated. Precipitates centrifuged off and dissolved in 5 cc. saline and enough normal NaOH solution (2-3 drops) to cause complete solution.

In this protocol the precipitate, dissolved in one-fourth the volume of the extract, protected against ten times the number of fatal doses as compared with the original material.

TABLE 3.

cc. N/40 HCl	Dose of Culture			Nitrogen per 100 cc.
	0.001 cc.	0.01 cc.	0.06 cc.	
1.3	90 S	48 S		
1.5	S S	S S	50 24	15 mg.
1.7	90 S	77 S	24 45	23 mg.
1.9	S S	45 S	24 24	18 mg.
2.1	S S	S S		19 mg.
2.3	S S	S S		16 mg.
2.5	S S	51 S		
3.1	45 54	24 24		
Original extract control	S S	45 69	24 45	16 mg.

Virulence control cc. of culture	0.000,000,1	0.000,000,01	0.000,000,001
	45 45	45 45	S S

Any bactericidal influence of the copper affecting the protective test was excluded by the following experiment:

Protective Effect of Copper Chloride Solutions.

Each mouse received 0.2 cc. copper solution diluted to 0.5 cc. saline, and the indicated amount of 18 to 24 hour broth culture of pneumococcus Type 1 contained in 0.5 cc. broth.

TABLE 4.

Dose Protective Material	Dose of Culture		
	0.000,01	0.000,1	0.001
0.2 cc. M/2200 copper chloride in saline	S S	40 88	25 40

Virulence control dose of culture	0.000,000,1	0.000,000,01	0.000,000,001
	40 40	40 40	S S

This protocol shows that 0.2 cc. of M/2200 copper chloride protects against 1,000 but not against 10,000 fatal doses of pneumococcus. The doses used in the antibody experiments (0.01 and 0.06 cc.) are 1,000,000 and 6,000,000 fatal doses respectively.

The material and mice (of which over 2,000 were used in these studies) were generously furnished by the H. K. Mulford Co., Glenolden, Pa., through the courtesy of Dr. F. M. Huntoon.

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Studies on the purification of antibodies. IV. The removal of extraneous material from antipneumococcus extracts at an approximately neutral reaction.

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Experiments with precipitates and supernatant fluids, obtained by bringing antibody-containing extracts of sensitized bacteria to a known range of hydrogen ion concentrations, revealed another method of purifying such antibody solutions of extraneous material, which method is different from that described in the preceding paper. It was found that at hydrogen ion concentrations between pH 7.0 and 7.6, that is, well to the alkaline side of the point of precipitation of antibody itself (about pH 4.0), pneumococcus protective antibody remained in solution, but a large amount of indifferent material, containing considerable nitrogen, was removed from solution. It was possible to remove a fraction of this indifferent material before adding copper chloride; and then, after addition of copper chloride, to remove a further fraction, as illustrated in the following protocol.

Two and a half liters pneumococcus antibody solution were brought to pH 7.0 by the addition of hydrochloric acid. A precipitate formed. This was allowed to stand in the ice box over night, and centrifuged. The supernatant was designated "M-20 C." Trial precipitations of 10 cc. portions of this supernatant by addition of copper chloride indicated complete recovery of the