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Recovery of Antigen from Type I Pneumococcus Immune Precipitate.

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In previous reports[1, 2] from this laboratory, it has been shown that the liberation of antibody from immune precipitate by the action of acid or alkali is due to a shift of equilibrium as follows:

$$B_mG_n \underset{\text{neutral}}{\overset{\text{acid or alkali}}{\rightleftharpoons}} B_{m-x}G_n \downarrow + xB$$

Where G = antigen, B = antibody and B_mG_n = immune precipitate formed in neutral solution. Upon treatment with acid or alkali, a part of the antibody, xB, is set free from the precipitate, B_mG_n, leaving behind an acid or alkaline precipitate, $B_{m-x}G_n$. Since the acid or alkaline precipitate, $B_{m-x}G_n$ has a higher antigen content than the neutral precipitate, B_mG_n, it should, when separated from the free antibody, xB, liberate some antigen upon neutralization. The purpose of the present study is to test this point.

Portions of Type I Pneumococcus horse immune precipitate (4 mg N each) were treated with dilute HCl or NaOH of different concentrations in the presence of 1% NaCl according to the technic reported in a previous communication.[2] After centrifuging, the supernatants were used for pH determinations. The acid or alkaline precipitates were evenly suspended in 2 cc of normal saline and neutralized. The neutralized suspensions were centrifuged. One cc portions of the neutralized supernatants were mixed with 1 cc of the same homologous antiserum, which was used in preparing the original immune precipitate, while the remaining portions were mixed with normal serum as control. The mixtures were incubated at 37°C for 2 hours and then chilled at 0°C overnight. Precipitation occurred in some of those tubes containing antiserum, but not in the tubes with normal serum. That the precipitation was caused by a combination of antigen and antibody and was not a reprecipitation of dissolved immune precipitate by mass action effect was shown by the fact that saline washings of Type I Pneumococcus

1 Liu, S. C., and Wu, H., *Chinese J. Physiol.*, 1938, **13**, 449.

2 Liu, S. C., and Wu, H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 144.

TABLE I.

Recovery of Antigen from Type I Pneumococcus Immune Precipitate at Different pH's in the Presence of 1% NaCl. Temperature = 23°C.

pH	2.38	2.68	3.02	3.38	3.88	—	9.72	9.86	10.22	10.65	10.92
Precipitate,* mg N	0.154	0.084	0.042	0	0		0	0	+	+	+
Polysaccharide recovered,† mg	0.20	0.10	0.05	0	0		0	0	+	+	+

*Precipitate formed by the recovered antigen with 1 cc antiserum.

† Read from a control precipitin curve.

0 = No precipitation.

+ = Definite precipitation but the amount was too small to be estimated.

immune precipitate, when mixed with the homologous antiserum, gave no precipitation under the same experimental conditions.

The precipitates were centrifuged at 0°C and washed twice with 1 cc saline also at 0°C. The N contents of the precipitates were determined by micro-Kjeldahl method. A control precipitin experiment was done by adding known amounts of the same lot of Type I Pneumococcus polysaccharide to the same lot of antiserum as that used in preparing the original immune precipitate under the same experimental conditions. The amount of precipitate N was plotted against the amount of polysaccharide. The amount of polysaccharide recovered at different pH's in the recovery experiment was then read from the control precipitin curve. The results are shown in Table I. It will be noted that antigen was recovered only in comparatively more acid solutions, where the recovery of antibody was previously shown[2] to be over 50%. When the pH was higher than 3.38, the amount of antigen recovered was too small to be estimated accurately.

Summary. Antigen was recovered from Type I Pneumococcus immune precipitate from which some antibody had been removed by treatment with acid. This experiment substantiates our previous finding that the recovery of antibody from immune precipitate by the action of acid or alkali is due to a shift in the antigen-antibody equilibrium. The present finding also suggests a possible method for the isolation of pure antigen which may be useful when it cannot be obtained otherwise.

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Further Studies on Type-Specific Protein of *Corynebacterium diphtheriae.*

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In a previous study[1] it was found that a serologically active, type-specific protein could be prepared by mild alkaline extraction at low temperature from the well-known Park 8 strain. In view of this finding it seems of interest to extend this observation to other types of *C. diphtheriæ* in order to determine (1) whether the method of

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