into the cysterna chyli. It is obvious that the pulsations of arteries would be without influence on the effective lymph flow were it not for the valves which allow the fluid to flow in only one direction. The experiments reported do not allow one to state with certainty that the arterial pulsations are an important agency in promoting the flow of lymph. However, the fact that the lymph pulsations were present even when the ducts did not appear to be distended is highly suggestive. It seems likely that the arterial pulsations are an important factor in promoting the flow of lymph when the subject is completely relaxed or sleeping. Even under these conditions, the respiratory movements aid materially. However, the effect of the respiratory movements is probably exerted in the main on large lymphatic trunks and only indirectly on most of the smaller ones, whereas the arterial pulsations probably influence directly the lymph flow throughout the body. The work of Parsons and McMaster suggests this very strongly. During activity, whether it be of the skeletal or intestinal systems, the muscular movements probably exert a major influence on the flow of lymph. The many factors which promote the flow of lymph vary quantitatively under different conditions.

Summary. Pulsations in lymphatics synchronous with those in arteries have been recorded. The possible effect of the pulse upon the flow of lymph has been discussed.

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Optimal Conditions for Recovery of Antibody from Immune Precipitate of Type I Pneumococcus.

SZU-CHIH LIU AND HSIEN WU.

From the Department of Biochemistry, Peiping Union Medical College, Peiping, China.

In a previous paper[1] we have shown that the liberation of antibody from immune precipitate of Type I Pneumococcus by the action of dilute alkali is due to a shift of antigen-antibody equilibrium as follows:

$$B_mG_n \underset{\text{neutral}}{\overset{\text{alkaline}}{\rightleftharpoons}} B_{m-x}G_n + xB$$

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

Where G = antigen, B = antibody, and B_mG_n = immune precipitate formed in neutral solution. Upon treatment with alkali, a part of the antibody, xB, is set free from the immune precipitate. A similar shift of equilibrium probably occurs in acid solution. The purpose of the present study is to determine the optimal conditions for the recovery of antibody from the immune precipitate by treatment with acid as well as with alkali.*

Recovery of horse antibody from immune precipitate. Washed precipitate was evenly suspended in water and sixteen 4 cc portions (about 1.2 mg N per cc) were transferred to a series of 15 cc graduated centrifuge tubes. Eight portions were treated with equal volumes of dilute HCl and the other 8 portions with dilute NaOH of different concentrations. The mixtures were allowed to stand 10 minutes at room temperature and centrifuged for 15 minutes. The volumes of the (acid or alkaline) precipitates were recorded and the supernates poured into 16 test tubes. The centrifuge tubes containing the precipitates were allowed to drain on a piece of filter paper.

Four cc each of the supernatants were pipetted into a series of 15-cc graduated centrifuge tubes, each containing 0.6 cc of 10% NaCl and 1 drop of 0.04% phenol red. They were neutralized with dilute HCl or NaOH, and water was added to make a volume of 6.6 cc in each tube. The tubes were centrifuged, the volume of the (neutral) precipitates were recorded, and the supernates were poured into 16 test tubes. The centrifuge tubes containing the precipitate were allowed to drain on a piece of filter paper.

The remaining acid and alkaline supernates were used for pH determinations. On the acid side, glass electrode was used. On the alkaline side, hydrogen electrode was used.

The acid and alkaline precipitates and the neutral precipitates were dissolved with 0.1 N NaOH. Aliquot portions of the resulting solutions and the neutralized supernatants were used for micro-Kjeldahl determinations. Nitrogen of the precipitate was corrected for the N of the supernate left in the precipitate by assuming the volume of fluid in the precipitate to be equal to the volume of the moist precipitate. Results are shown in Table I and Fig. 1.

The acid or alkaline precipitate represents $B_{m-x}G_n$. The neutral precipitate represents dissolved $B_{m-x}G_n$ plus some antibody which it reabsorbed upon neutralization. Nitrogen in the neutralized supernate represents the amount of antibody recovered.

It is well known that the combination of antibody with antigen

* A similar study on the recovery of antibody from the agglutinate is in progress and the results will be reported in another paper.

TABLE I.
Recovery of Type I Pneumococcal Antibody from Horse Immune Precipitate at Different pH's.
t = 23°C.

pH	N in acid or alkaline precipitates, mg	N in neutral precipitates, mg	N in neutralized supernatants, mg	Total N in immune precipitate, mg	Recovery, %
3.48	0.052	4.16	0.784	4.996	15.7
3.73	0.070	4.16	0.694	4.922	14.1
3.86	0.070	4.22	0.720	5.010	14.5
3.97	0.670	3.62	0.754	5.044	15.1
4.25	2.387	0.42	2.292	5.099	44.5
4.50	3.040	0.256	1.610	4.906	32.8
4.66	3.400	0.056	1.560	5.016	31.0
5.20	4.230	0.04	0.610	4.880	12.5
8.91	4.140	0.12	0.880	5.140	17.1
9.56	3.58	0.178	1.286	5.044	26.5
9.77	3.01	0.412	1.730	5.152	33.6
10.01	2.15	1.10	1.760	5.010	35.2
10.09	1.90	1.30	1.904	5.104	37.3
10.60	Negligible	3.72	1.240	4.960	25.1
10.69	"	3.82	1.200	5.020	23.8
10.95	"	4.36	0.784	5.144	15.3

takes place in the pH range from 5 to 9. If pH is below 5 or above 9, combination is not complete.[2] Conversely, if neutral immune precipitate is treated with acid or alkali some antibody should be liberated. However, not all the antibody which is so liberated is recoverable, because on neutralization part of the liberated antibody xB recombines with the dissolved $B_{m-x}G_n$. In solutions not far from neutrality (pH 5-6 and 8-9) where the amount of dissolved $B_{m-x}G_n$ is negligible, practically all the antibody which is set free by acid or alkali is recovered if the $B_{m-x}G_n$ precipitate is removed. In these pH ranges, however, the shift of equilibrium is slight, and the percentage recovery of antibody is low. In more acid or alkaline solutions, the shift of equilibrium is greater, but the amount of dissolved $B_{m-x}G_n$ also increases, which decreases the recovery. At a certain pH where the shift of equilibrium and the solubility of $B_{m-x}G_n$ strike a most favorable balance, the recovery is optimal. Another possible reason for the decrease of recovery in more acid or alkaline solutions is the denaturation of the antibody-protein. However, denaturation is probably not an important factor when the duration of treatment with acid or alkali is as brief as in the experiment here reported.

As shown in Fig. 1, the optimal pH is 4.25 on the acid side and 10 on the alkaline side. It has been noted that the optimal position is the same for immune precipitates prepared with different lots of polysac-

[2] Marrack, J. R., *The Chemistry of Antigens and Antibodies*, His Majesty's Stationery Office, London, 1938, pp. 129, 140.

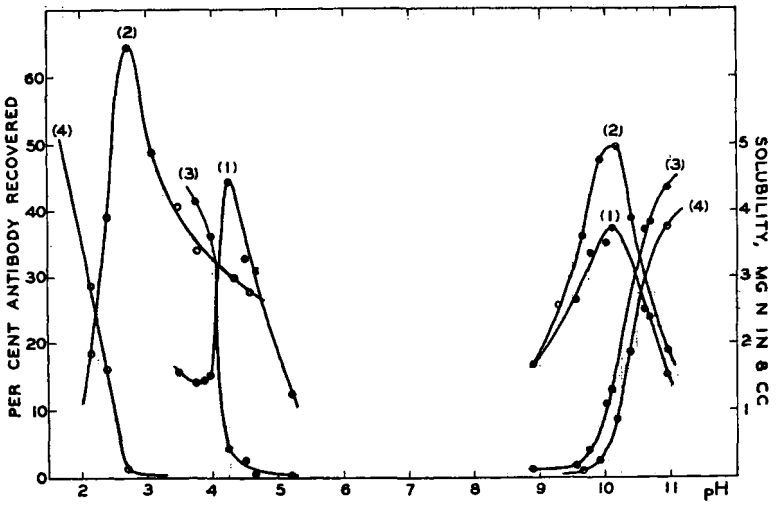

FIG. 1.

Recovery of Type I pneumococcus antibody from immune precipitate at different pH's, t = 23°C.

(1) % recovery without NaCl.
(2) " " with NaCl.
(3) Solubility of acid or alkaline precipitate without NaCl.
(4) " " " " " " with 1% NaCl.

charide, but slightly different for precipitates prepared from antisera of different horses. It has also been noted that the acid or alkaline precipitate is usually loose and translucent at the optimal pH but compact and granular at other pH's. The optimal pH can be roughly located by comparing the relative volumes of the precipitates. The significance of these findings deserves further study.

Effect of NaCl. Washed immune precipitate of Type I pneumococcus was suspended in 2% NaCl and treated with dilute acid or alkali as described above. The results are also plotted in Fig. 1. It will be noted that the solubility of $B_{m-x}G_n$ is considerably decreased by NaCl. For instance, at pH 10, the solubility of the alkaline precipitate decreases from 1.2 to 0.6 mg N in 8 cc, and the recovery increases from 38 to 50%. On the acid side, the effect of NaCl is so marked that the optimal pH is shifted from 4.25 to 2.75 and the recovery increases from 45 to 65%.

By quantitative precipitin-reaction, the purity of the antibody recovered with dilute acid, pH 4.25, and dilute alkali, pH 10, was compared with those recovered by other methods. To 4 cc portions of the antibody-solution (0.25 mg N per cc) were added 1 cc portions of polysaccharide solutions of different concentrations. The precipitates were washed and their N contents determined. Correction

was made for the polysaccharide N in the precipitate. At the point of maximal precipitation the products recovered with dilute acid and with concentrated NaCl[3] were both 91% precipitable, while those recovered with dilute alkali and with alkaline calcium phosphate[4] were 86 and 85% precipitable respectively.

Since proteins are more easily degraded by alkali than by acid, it is to be expected that the antibody recovered with alkali is less pure than that recovered with acid. While the antibody recovered with concentrated salt is as pure as that recovered with dilute acid, the latter method gives a higher yield and requires less time.

Recovery of rabbit antibody from immune precipitate. This precipitate is much more soluble than that of equine origin. In the absence of NaCl, complete solution of the precipitate occurs when the pH is above 8.6 or below 4. For this reason, the recovery is low and shows no optimal point. If the precipitate is suspended in 3% NaCl instead of water, the recovery is much improved. On the acid side there is an optimum at pH 2.70 with a recovery of 64%. On the alkaline side the optimum lies between pH 9 and 10 and the recovery varies between 30 and 50%. This variation is probably due to the fact that rabbit immune precipitate is gelatinous in alkaline solution and does not reach equilibrium as easily as in acid solution.

Summary. There is an optimal pH for the recovery of antibody from immune precipitate of Type I pneumococcus by treatment with dilute acid or alkali. In the presence of NaCl, the percentage of recovery is increased. The mechanism of the recovery is discussed. The present findings furnish the basis of a method for the isolation of antibody which is better than any of the existing methods.

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Distribution of Murine Typhus Rickettsiæ in Developing Chick Embryo.

K. H. PANG. (Introduced by Samuel H. Zia.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

Following the method of Goodpasture, Zia[1] cultivated successfully typhus Rickettsia of endemic and epidemic types in the chorioallantoic membrane of developing chick embryo. This was confirmed by

[3] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1936, **64**, 161.

[4] Felton, L. D., *J. Immunol.*, 1932, **22**, 453.

[1] Zia, S. H., *Am. J. Path.*, 1934, **10**, 211.