one involutional melancholia and 2 schizophrenics. Included in the controls were 4 normals, one chronic encephalitis and one recovered hypo-manic (manic depressive psychosis). Every effort was made to keep the experimental conditions constant. Patients with disturbing mental symptoms were not studied, the subjects used being selected on the basis of their ability to coöperate. The factor of attention was more or less uniform throughout the test periods as far as could be ascertained. The test results for 3 patients who received control tests both before and after treatment showed no significant difference from the others of the group.

Since the individuals responded in a fairly uniform manner a short tabular summary of the average results in each group is submitted:

Average difference in scores of Retests 1 and 2:

Metrazol cases	—25.1%	27.8% difference
Control I	+ 2.7%	
Control II	+ 3.5%	28.6% "

The data show that performance of the code transcription after metrazol injection resulted in a score of 27.8% lower than control tests on these same patients. The control subjects (Control II), those not receiving metrazol, showed no mean loss at all, but a small mean gain of 3.5% after the lapse of the time interval.

Although the series is small, the results are statistically reliable. The impairment in learning with metrazol would appear to be due to impaired memory. A number of factors entered into the learning procedure. The question of impairment of attention as against impairment of memory is being given further study.

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Isolation of Antibody from Agglutinate of Type I Pneumococcus by Treatment with Acid.

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In previous reports from this laboratory[1, 2] it has been shown that the liberation of antibody from immune precipitate of Type I Pneu-

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.

mococcus by the action of dilute acid or alkali is due to a shift of the antigen-antibody equilibrium as follows:

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

Where G = antigen, B = antibody, and B_mG_n = immune precipitate formed in neutral solution. The amount of antibody set free (xB) increases with acidity or alkalinity, but so also is the solubility of $B_{m-x}G_n$ which would decrease the yield by recombining with B when the solution is neutralized. Not all the antibody liberated by acid or alkali is thus recoverable, and there is in either acid or alkaline solution an optimum pH for the recovery.

The liberation of antibody from the agglutinate by the action of acid or alkali is probably due to a similar shift of equilibrium, but there is an important difference between the immune precipitate and the agglutinate. Whereas the immune precipitate and its partial dissociation product ($B_{m-x}G_n$) are more or less soluble in acid or alkaline solution, the agglutinate or its dissociation product is not soluble as long as the cells are intact. It can, therefore, be completely removed by centrifuging and all the antibody set free should be recoverable. The purpose of the present study is to test this point.

Preliminary experiments showed that Type I Pneumococcus vaccine treated with alkali (even at pH 8), gave off an appreciable amount of nitrogen, while treatment with acid (pH 5 to 1.8) caused practically no loss of nitrogen. Hence in the present study the agglutinates were treated only with acid.

Effect of pH on the dissociation of Type I Pneumococcus agglutinate. In a series of 15-cc centrifuge tubes 4 cc of immune rabbit serum, and 6 cc of washed, heat killed Type I Pneumococcus vaccine were mixed. The mixtures were incubated at 37°C for 2 hours, and then kept in ice box over night and centrifuged in the cold. The agglutinates were washed 3 times with 4 cc portions of ice cold normal saline and then suspended in 6 cc of distilled water. Equal volumes of HCl of different concentrations were added with constant stirring. After 10 minutes the mixtures were centrifuged at high speed.* The centrifuge tubes were tightly covered with tin foil and rubber band to prevent loss of water by evaporation. Water clear supernatants were obtained. The nitrogen contents of the supernatants and residues were determined and the pH's of the supernatants were measured with a glass electrode. To aliquot portions of the supernatants were added 10% NaCl solution to bring

* We used a Multispeed Attachment on Size 2 centrifuge made by International Equipment Co., Boston.

TABLE I.
Effect of pH on Liberation and Recovery of Antibody from Agglutinate of Type I Pneumococcus.

pH	A Nitrogen in vaccine, mg	B N in residue after acid treatment, mg	C N in acid supernatant, mg	D = B + C Agglutinate N, mg	E = D − A Agglutinin N, mg	F N in neutral supernatant, mg	$\frac{F}{E} \times 100$ Recovery of antibody, %
2.03	6.59	6.49	8.65	15.14	8.55	7.45	87.1
2.30	"	6.71	8.35	15.06	8.47	7.30	86.2
2.68	"	7.37	7.58	14.95	8.36	6.75	80.6
3.03	"	7.85	6.80	14.65	8.06	6.10	75.7
3.35	"	8.84	6.12	14.96	8.37	5.64	67.2
4.08	"	10.68	4.35	15.03	8.44	4.10	48.6

the concentration of NaCl to 0.9%, and then neutralized with NaOH. After standing for 20 minutes the mixtures were centrifuged. The supernatants (antibody) and the precipitates (probably acid denatured antibody) were analyzed for nitrogen. The results are shown in Table I.

It has been found in another experiment that treatment of washed vaccine with normal rabbit serum and then with HCl causes a loss of N amounting to about 12% of the total nitrogen in the vaccine. This interesting phenomenon is being studied further. In Table I the figures for vaccine N have been corrected for this loss.

From Table I it is seen that the dissociation of agglutinate of Type I Pneumococcus increases with acidity. At pH 2 the dissociation is complete, as the amount of N in the supernatant is, within the limits of error, equal to the amount of N in the agglutinin. The amount of antibody recovered after neutralization, however, is only about 87%, due probably to some denaturation.

Repeated use of the vaccine obtained from dissociated agglutinate. Thirty cc vaccine containing 16.7 mg N were mixed with 15 cc immune rabbit serum. The agglutinate was washed 3 times with cold saline and then treated with 25 cc of N/30 HCl and centrifuged. The vaccine residue left after removal of agglutinin was washed twice with N/30 HCl, suspended in 15 cc normal saline and neutralized with NaOH. This neutralized vaccine was again used for preparation of agglutinate with 15 cc immune serum. This procedure was repeated 3 more times. Microscopic examination showed that the cells were still Gram positive diplococci. The purity of the recovered antibody was determined by the method of Heidelberger and Kabat.[3] The results are shown in Table II.

It is seen that the vaccine, after repeated use, shows no decrease of antibody-combining power as shown by the amount of N in the acid supernatant. The recovery of antibody seems to show a slight improvement with repeated use of the vaccine, but this point requires further study. The degree of purity of the antibody recovered in each of the 4 successive agglutinations is at least 95%.

Summary. By treating the agglutinate of Type I Pneumococcus with dilute HCl (pH 2), all the antibody is liberated, and after removing the cells and neutralizing, as much as 87% may be recovered. The recovered antibody is at least 95% pure as shown by specific precipitation followed by agglutination. The vaccine can be used repeatedly for the isolation of the antibody. The method thus combines simplicity of procedure with good yield and high purity of product.

[3] Heidelberger, M., and Kabat, E. A., *J. Exp. Med.*, 1938, **67**, 181.

TABLE II.
Recovery of Antibody from Agglutinate Prepared from Repeatedly Used Vaccine.

No. of times the vaccine was used	Recovery of antibody			Purity of antibody				
	N in acid supernatant, mg	N in neutralized supernatant, mg	Recovery, %	Total N in antibody solution used, mg	Precipitin N, mg	Agglutinin N, mg	Total antibody N, mg	Antibody N / Total N, %
1*	22.75	17.38	76.4	.638	.522	.080	.602	94.3
				.319	—	.288	.288	90.2
2	24.50	19.47	79.5	.708	.575	.107	.682	96.3
				.354	—	.316	.316	89.3
3	25.40	21.01	82.7	.708	.581	.096	.677	95.6
				.354	—	.316	.316	89.3
4	25.40	21.93	86.4	.568	.490	.055	.545	95.9
				.284	—	.255	.255	89.7

*The vaccine contained 16.7 mg of nitrogen.