the olfactory bulbs may be useful as an indicator of the portal of entry of the virus in experimental poliomyelitis.

Practically no attention has hitherto been paid to the pathology of the olfactory bulbs in human poliomyelitis, and it is believed that their examination in the future should yield data of value to a better understanding of the epidemiology and prophylaxis of this disease.

8948 P

Chemical Studies in Bacterial Agglutination. III. A Quantitative Theory of Bacterial Agglutination.*

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It has recently been shown possible to consider the precipitin reaction as a series of competing bimolecular reactions[1] and so derive from the mass-law an expression

$$\text{mg. antibody N precipitated} = 2RS - \frac{R^2}{A} S^2$$

in which R is the ratio of antibody to hapten or antigen in the precipitate at a reference-point in the equivalence-zone, and A is the amount of antibody-N precipitated at the reference-point. This equation describes closely the behavior of a number of immune precipitating systems.

Since the agglutination reaction may be considered a precipitin reaction at the bacterial surface, it was thought that the above theory might be applied. The test involved the development of an absolute method for the micro-estimation of agglutinin[2] and the use of a single hapten at the bacterial surface and the homologous antihapten. This was realized in a freshly washed, heat-killed pneumococcus I S (Dawson "M") suspension and, for the antibody, Type I anti-pneumococcus horse-serum freed from antibodies other than type-

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1 Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1935, **61**, 563; **62**, 467, 697.

2 Heidelberger, M., and Kabat, E. A., *J. Exp. Med.*, 1934, **60**, 643; PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 595.

specific anti-carbohydrate by absorption with "C" substance and pneumococcus I R (Dawson "S") suspension.[3] Typical runs are given in Table I and show excellent agreement with the calculated curves and values. Agglutination differs from precipitation in that a maximal N:S ratio is obtained at a relatively small excess of antibody.

TABLE I.
Addition of Increasing Amounts of I S Pneumococcus (M) Suspension to 1 ml. of Serum or Antibody Solution.

Bacterial N	Equivalent SI content	Total N pptd.	Antibody N pptd.	Ratio N:S in ppt.	Total N pptd.	Antibody N pptd.	Ratio N:S in ppt.
mg.	mg.	mg.	mg.		mg.	mg.	
		H 701 0.15 N Salt 37°				H 701 0.15 N Salt 0°	
.064	.0166	.254	.18	10.8*	.187	.12	7.2*
.096	.0250	.370	.27	10.8	.290	.19	7.6*
.127	.0330	.476	.34	10.3	.376	.25	7.6*
.191	.0496	.686	.50	10.1	.534	.34	6.9*
.254	.0660	.868	.61	9.2	.722	.47	7.1
.382	.0990	1.060	.67	6.8	1.030	.65	6.5
.517	.132	1.202	.69	5.2	1.246	.74	5.6
.644	.165	1.338	.69	4.2*	1.414	.77	4.7
	Serum Salt	0.008			0.000		
		$N = 12.5\ S - 54.7\ S^2$			$N = 8.8\ S - 24.1\ S^2$		
		Maximal S = .114			Maximal S = .182		
		" N = 0.714 calcd.			" N = 0.797 calcd.		
		0.694 found			0.770 found		

*Points not considered in calculating equation.
Actual N analyses given to third decimal.
Antibody N values given to nearest second decimal place.

It would appear, therefore, that a quantitative chemical theory has been found capable of accurately describing a typical instance of bacterial agglutination. This theory makes no distinction between the initial chemical combination of multivalent antigen (or hapten) with multivalent antibody and the subsequent flocculation.[4] That this so-called second phase of agglutination is also due to the building up of large aggregates by chemical combination of antibody on the bacterial surface with antigen (or hapten) on the surface of other bacteria is indicated by the following:

Pneumococcus I M suspension is agglutinated (sensitized) with an excess of Type I antiserum, and the organisms are washed free from excess agglutinin with saline. The cells, covered with an excess of multivalent antibody, are easily resuspended in saline. A small quantity of pneumococcus I M suspension, freshly centrifuged and taken up in saline to avoid the presence of dissolved specific

[3] *Cf.* Heidelberger, M., and Kabat, E. A., *J. Exp. Med.*, 1936, **63**, 737.

[4] *Cf.*, for example, Shibley, G. S., *J. Exp. Med.*, 1926, **44**, 667.

polysaccharide, is added to the resuspended agglutinated cells and the mixture agitated for a moment. Reagglutination rapidly takes place and the entire mass of cells falls to the bottom in large clumps. Since this does not occur when pneumococcus II M, III M, or I S (formerly I R) is added, it is difficult to avoid the conclusion that chemical combination of multivalent polysaccharide on the newly added I cells takes place with multivalent antibody on the previously agglutinated and resuspended cells. Thus the entire process of agglutination may be accounted for on a chemical basis, a conclusion already reached by Topley, Wilson and Duncan[5] in a test of Marrack's views.

8949 C

Factors Influencing Nembutal Anaesthesia.

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In work recently reported from these laboratories,[1] it was found that the injection of glucose in normal-fed rabbits did not materially shorten the period of depression of nembutal anaesthesia. Fasting for 20 hours increased the duration of the anaesthesia appreciably. There was no correlation between the susceptibility to the drug and the blood sugar levels, either before the administration of the drug or at the time of greatest depression. Although the sugar level was not changed at the time of maximum depression there was a very definite drop in this level at the time of recovery.

Since the drop in the blood sugar level at the time of recovery from nembutal anaesthesia was shown only for normal-fed animals, the question arose as to the reaction of starved animals under the same conditions. It was also necessary to make a more complete study of the changes in the blood sugar level throughout the entire experimental period. The work presented in this paper is an extension of the previous work along these lines.

[5] Topley, W. W. C., Wilson, J., and Duncan, J. T., *Brit. J. Exp. Path.*, 1935, **16**, 116.

[1] Blackberg, S. N., and Hrubetz, M. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 65.