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An Experimental Test of  
the Framework Theory of Antigen-  
Antibody Precipitation

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The framework theory (lattice theory) of serological precipitation and agglutination, first proposed by Marrack<sup>1</sup>, has not been accepted by all investigators in the field, although it is supported more or less strongly by a considerable body of evidence<sup>1,2,3,4</sup>. We have now carried out some experiments which correspond so well in their results with the predictions of this theory as to leave little doubt of its correctness.

We have synthesized a substance which gives a specific precipitate with a mixture of two different antisera, but gives no precipitate with either antiserum alone. The substance contains two different haptenic groups, R and X, to which the two antisera are homologous: the anti-R serum was made by injecting rabbits with azoprotein containing R groups, and the anti-X serum by injecting with azoprotein containing X groups.

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1 J. R. Marrack, "The Chemistry of Antigens and Antibodies", Report No. 194 of the Medical Research Council, His Majesty's Stationery Office, London, 1934; Second Edition, Report No. 230, 1938.

2 M. Heidelberger and F. E. Kendall, J. Exptl. Med., 61: 559, 563; 62: 467, 697, (1935); M. Heidelberger, Chem. Rev., 24: 323, (1939).

3 L. Pauling, J. Am. Chem. Soc., 62: 2643, (1940).

4 L. Pauling, David Pressman, Dan H. Campbell, and collaborators, ibid., 64: 2994, 3003, 3010, 3015, (1942); 65: 728, (1943).

The R and X groups were respectively the p-azophenylarsonic acid group and the p-azobenzoic acid group, and the RX substance used in most of our work was 1-amino-2-p-(p-azophenylazo)phenylarsonic acid-3,6-disulfonic acid-7-p-(p-azophenylazo)benzoic acid-8-hydroxynaphthalene. Similar results were also obtained with 1,8-dihydroxy-2-p-azophenylarsonic acid-3,6-disulfonic acid-7-p-(p-azophenylazo)benzoic acid-naphthalene.

The experimental results show that in the formation of the precipitate both of the two haptenic groups of the molecule enter into specific reaction, the R group with an anti-R antibody molecule and the X group with an anti-X antibody molecule. This is shown by the fact that the RX substance precipitates only with a mixture of the two antisera, and not with either one alone; the explanation of the failure of precipitation with only one antiserum given by the framework theory is that with respect to either antiserum the RX molecule is only monohaptenic and hence only univalent, and so can not act as the link between antibody molecules in the formation of a framework.

With the effective bivalence of the precipitating antigen thus proved, knowledge of the antibody-antigen molecular ratio for the precipitate provides the value of the average valence of the antibody molecules. The molecular ratio was found by analysis to be 0.7, which corresponds to  $2/0.7 = 2.8$  for the average antibody valence<sup>5</sup>. If the antibody were

<sup>5</sup> Similar values of the antibody-antigen molecular ratio have been previously reported (Ref. 4) for precipitates of anti-R antisera and simple substances containing two or more R haptenic groups.

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univalent the molecular ratio would have the value 2, which is far greater than the experimental value. Further evidence for the effective multivalence of antibody is provided by the observation that the RX precipitate is soluble in excess of the mixed antisera; this solubility ~~cannot~~ be explained on the basis of univalent antibody.

A detailed account of this work will be published in the Journal of the American Chemical Society.

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