Pre- and Postreaction pH of two Specifically Interacting Large-Molecule Systems*

LANDSTEINER[1] believed that the attraction between oppositely charged groups, such as $-COO^-$ and $-NH_3^+$, contributed importantly to the formation of the antibody-antigen bond. If a number of such groups form part of each specific combining site, as suggested by LANDSTEINER and later authors[2], it might well be thought that some net liberation or absorption of hydrogen ions would accompany serological reactions, a possibility not entirely ruled out by the experiments of SMITH and MARRACK[3], for the antibody and antigen solutions used by them were not, by modern standards, very concentrated.

SINGER and CAMPBELL[4] however, have suggested that in some antibody-antigen systems, at least, only a single ionized carboxyl group (in antibody or antigen) with presumably a complementary $-NH_3^+$ in the corresponding site of the other reagent, is involved in each bond. Since we believe antibody to be bivalent, this would mean the liberation or absorption of at the most 2 moles of H^+ per mole of antibody, and unless the pK of the $-NH_3^+$ is considerably changed by the combination, probably much less. Furthermore, the demonstration that the specific portions of the blood group substances[5] do not contain any ionized groups seems to show that some antibody-antigen bonds do not involve the mutual attraction of $-COO^-$ and $-NH_3^+$ at all.

In view of the divergence between these more recent views and the conclusions to which LANDSTEINER[1] was led by his classical work, we felt it worth while to reexamine the question with use of modern concentrated reagents and modern equipment. The negative results are presented briefly here as setting an upper limit to the amount of H^+ liberated or absorbed in two typical reactions involving antigens.

Experimental.—As a classical antibody-antigen system we selected horse antitoxin and diphtheria toxoid. The concentrated antitoxin contained 4200 standard units per ml, nearly 4 times the strength of the preparation available to SMITH and MARRACK[3]. It contained 6·48 mg N per ml. The toxoid, containing 4200 Lf units per ml, was reported by the Massachusetts Public Health Biologic Laboratories, which supplied it, to be 100% pure. It contained 1·85 mg N per ml.

In order to obtain data from another system as different as possible, and at the same time to take advantage of other available concentrated reagents, we used blood group A substance and the plant agglutinin (lectin) from lima beans[6]. Our lectin preparation contained 2·06 mg N per ml, of which about 30% was specifically precipitable. The A substance was prepared from commercial hog gastric mucin by MORGAN's method[7]; one third of it was specifically precipitable. Since nothing derived from serum was involved in the lectin-A substance reaction, it is not strictly a serological system. The close similarity in behavior of lectins and antibodies[6], however justify its study here.

Before the reagents were mixed, they were adjusted to the same pH (studies were carried out over a range from pH 4·00 to 7·50) by the addition of the requisite amount of 0·05 *N* HCl or NaOH. The reagents were mixed in optimal proportions[8] at room temperature in amounts to give about 25 ml. The pH was determined, with the pH meter of the Cambridge Instrument Co., immediately after mixing, 15 min later, and after 24 h in the refrigerator. Six successive readings were made at each time. In the case of the antitoxin and toxoid, strict asepsis was preserved although control experiments indicated this was not necessary.

No detectable pH changes followed mixing the reagents at any pH tested.

In order to determine the limiting sensitivity of the experiments, mixtures of the reagents were titrated with 0·1 *N* HCl and NaOH, and the buffer capacity of the mixture computed at the pH where it was a minimum (pH 7 for the first system and 7·5 for the second). From the buffer capacity of the system and the observed variations in the pH readings, it could be determined, with use of FISHER's test[9] for the significance of the difference between two means, how small a release or absorption of hydrogen ions could have been detected. For the antitoxin-toxoid system this was 2·22 equivalents of H^+ per mole of antitoxin, and for the lectin-A substance system 0·95 Eq. H^+ per mole of lectin.

It would seem that our experiments are a further indication that the pH changes, if any, accompanying these large-molecule reactions are slight, in line with recent work. They probably do not exclude the possibility that if antigens containing large numbers of ionized groups were used, a pH change could be observed.

We are grateful to Dr. H. E. BOWEN of the Massachusetts Public Health Biologic Laboratories for generous gifts of antitoxin and toxoid, and to Dr. E. A. KABAT for a sample of purified A substance.

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Boston University, School of Medicine, Boston, Mass., February 5, 1957.

Zusammenfassung

Diphtherietoxoide und Antitoxin einerseits, gruppenspezifisches Pflanzenagglutinin und Blutgruppensubstanz anderseits, wurden auf verschiedenes pH gebracht und das erste und das zweite Paar gemischt, ohne dass eine pH-Änderung eintrat. Es wird eine Erklärung versucht.

* The work reported in this paper was made possible by support extended to Boston University by the National Science Foundation (G–1583) and by research grants (H–1076[C4] and RG 4104 M & G) from the National Heart Institute, National Institutes of Health, Public Health Service.

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