EFFECT OF PHOTO-OXIDATION ON ISOHEMAGGLUTINATING ANTIBODIES*

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There have been a number of reports (4, 7, 12, 19, 13, 15, and references in 15) of antibody which had the power to combine with its antigen, but which failed to bring about the expected "second stage" (precipitation, agglutination, etc). Such antibody has been interpreted, on the basis of the theory of serological reactions which is variously referred to as the "lattice," "mutual multivalence," "framework," or "alternation" theory, as univalent. That is, the hypothesis has been advanced that such antibody possesses only one specific combining group instead of the (supposed) two or more which ordinary "good" antibody has (7, 20). Experiments have also been reported which detailed treatment of antibody which resulted in modified antibody with "incomplete" or "blocking" properties (8, 2, 10, 11, 16, 18). There is no doubt that, if antibody has more than one specific combining group, as now seems likely on the basis of various experiments (*cf.* 1), the supposition of its conversion to the univalent state is the simplest explanation of the effects observed. Other interpretations are, however, possible (8), and it remains to see whether physical modification usually results in the production of "incomplete" or "blocking" antibodies before the usual antibody activity is totally destroyed. I wish to report here the results of tests of hemagglutinating sera during a process which led to their gradual destruction.

EXPERIMENTAL

As a means of modifying antibody gradually, the photo-oxidation process, as described by a number of workers, particularly Tyler (18), was adopted. Two different isohemagglutinins, an anti-A and anti-Rh serum, were selected. The anti-Rh was of particular interest, because antibodies of the "blocking" type are frequently observed to develop along with anti-Rh agglutinating antibodies (6, 14, 19). The anti-A was chosen for study because a particularly strong serum, produced by the method of Witebsky *et al.* (21), was available, and therefore a much greater range in strength, from the unmodified serum to the completely inactivated serum, was potentially available.

The sera were mixed with one-tenth the volume of sterile 2 per cent eosin Y, put in sterile square bottles with cotton plugs, and exposed to the light of 10 standard 100 watt lamps, by being placed on a glass shelf in the cold room, about 6 cm. from the tops of the lamps. An

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electric fan was employed for cooling, and the highest temperature recorded by a thermometer placed on the glass shelf about 20°C. Since the anti-A agglutinin proved to be so resistant to photo-oxidation (see below), after 49 hours the 100 watt lamps were replaced by photoflood bulbs, these being replaced, as soon as they burned out, by new photoflood bulbs. This change gave a source of illumination with much more of the energy concentrated in the blue region of the spectrum, and increased the total intensity of illumination.

Samples of the sera were taken out, with aseptic precautions, from time to time, and tested for agglutinating and "blocking" activity. Controls, also containing eosin Y, were kept in a light-tight container and tested along with the samples. Samples of the sera, exposed to the illumination, but not containing eosin Y were also tested at intervals. The activity of the controls remained essentially constant for the duration of the experiment.

TABLE I

Effect of Irradiation with Visible Light upon a Strong Anti-A Serum Sensitized by the Presence of Eosin Y

Time of irradiation	Dilution of anti-A serum tested			
	Undiluted	1:10	1:100	1:1000
hrs.				
0	++++	++++	++++	+w
1		++++	+++	+
3		++++	+++	+w
49	+±	+	+	0
56	++	+w	?	0
65	+w	0	0	0
71	0	0	0	0

Symbols indicate degrees of agglutination of group A cells, ++++ indicating the strongest reaction, +w the weakest, and 0 a negative reaction.

RESULTS

The progress of the inactivation of the anti-A antibody is shown in Table I. It will be seen that in the case of the anti-A agglutinin, an exposure of between 65 and 71 hours was necessary to destroy completely all agglutinating activity. In the case of the anti-Rh serum $15\frac{1}{2}$ hours of exposure sufficed. The difference is consistent with the presumed difference in concentration of the two antibodies, and the well known fact that anti-Rh antibodies are rather easily destroyed.

The important part of the experiment concerns the tests for "blocking" activity which might be expected to develop as a result of the photo-oxidation of antibody. Samples of the sera were tested for such inhibiting activity at various intervals by the following technics:—

To tubes containing one drop of the appropriate cell suspension (A or ORh+) there was added, in the first procedure, one drop of the irradiated serum. After mixing the contents, each tube was centrifuged, the supernatant was removed, and one drop of a good but not too strong serum (anti-A or anti-Rh) was added. The tests were then completed according to

the usual technic (mixing, centrifuging, and shaking in the case of A cells; mixing, incubating 1 hour at 37°C. for 1 hour, centrifuging at low speed, and shaking very gently in the case of the Rh cells).

Any development of inhibiting antibody of the "incomplete" or "blocker" type would have been apparent by failure of the untreated serum added in the last step to agglutinate the cells, at least if the proper concentration of irradiated serum had been employed. Nevertheless, although in the case of each sample a variety of dilutions was tried, no inhibition was ever observed.

TABLE II

Reactions of Group A Cells with Anti-A Serum after Preliminary Incubation with Two Drops of Various Dilutions of Irradiated Anti-A Serum Irradiated for 71 Hours

Dilution of irradiated serum	Dilution of anti-A (non-irradiated) serum			
	Undiluted	1:10	1:100	1:1000
Undiluted	++++	++++	+++	+++
1:10	++++	++++	+++	+
1:1000	++++	++++	+++	++
Saline (control)	++++	++++	++	++

Symbols as in Table I.

TABLE III

Reactions of Anti-Rh_0 Serum, before and after Photo-Oxidation for 15½ Hours

Type of erythrocytes	Reaction of non-irradiated serum	Reaction of irradiated serum
ORh_1	+++	0
ORh_2	+++	0
ORh_0	+++	0
ORh-negative	0	0
ORh-negative	0	0

Symbols as in Table I.

In the second procedure two drops of the irradiated serum (or dilution thereof) were added to a tube containing one drop of cell suspension, the mixture incubated at 37°C. for 1 hour, then centrifuged, the supernatant removed, and good serum added as before. This procedure also did not yield any sign that inhibiting activity was developing as the agglutinating activity was destroyed. It does not seem necessary to present the details of all these negative tests; a typical experiment is shown in Table II.

The anti-Rh serum was an anti-Rh_0 (the type originally called an "85 per cent"). Its reactions before and after 15½ hours of irradiation are set forth in Table III. It also never developed any trace of "blocking" activity.

DISCUSSION

From past experience we know that inhibiting antibodies are most easily detected in agglutinating sera, and a number of such sera have been investigated (4, 9, 16). From reports such as those of Tyler (18), for example, it would appear that photo-oxidation either sometimes converts agglutinating into inhibiting antibodies, or else, by destroying the agglutinating antibodies, leaves inhibiting antibodies already present free to exert their characteristic effects. Diamond and Abelson (5), in connection with other work on anti-Rh antibodies, have suggested the second possibility.

From the present experiments, it seems that photo-oxidation does not, in all cases at any rate, convert agglutinating antibodies for the blood group haptens A or Rh_0 into inhibiting antibodies. This finding is somewhat surprising, since there is some reason to suppose that the isohemagglutinating antibodies are bivalent (1), and since inhibiting antibodies are frequently encountered in sera from individuals sensitized to the Rh hapten. It suggests that the simplest hypothesis as to the nature of the inhibiting antibodies, namely that they represent portions of originally bivalent (or multivalent) antibodies which now possess only one specific combining group per particle, is not necessarily correct. It does not bear particularly on the controversy which has long existed (*cf.* 3) as to the mechanism of the second stage of serological reactions.

The nature of the changes produced in proteins by photo-oxidation has been discussed by Smetana and Shemin (17) and Zia, Chow, and T'ung (22).

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SUMMARY

Photo-oxidation (in the presence of eosin Y as a sensitizer) of isohemagglutinating sera destroyed their agglutinating activity gradually, but did not seem to convert any of the agglutinins studied into inhibiting ("incomplete" or "blocking") antibody.

BIBLIOGRAPHY

1. Abramson, H. A., Boyd, W. C., Hooker, S. B., Porter, P. M., and Purnell, M. A., *J. Bact.*, 1945, **50**, 15.
2. Bawden, F. C., and Kleczkowski, A., *Brit. J. Exp. Path.*, 1942, **23**, 178.
3. Boyd, W. C., Fundamentals of immunology, New York, Interscience Publishers, Inc., 1943.
4. Coca, A. F., and Kelley, M. F., *J. Immunol.*, 1921, **6**, 87.
5. Diamond, L. K., and Abelson, N. M., in preparation.
6. Diamond, L. K., and Denton, R. L., *J. Lab. and Clin. Med.*, 1945, **30**, 821.
7. Heidelberger, M., Treffers, H. P., and Mayer, M., *J. Exp. Med.*, 1940, **71**, 271.
8. Hooker, S. B., and Boyd, W. C., *Ann. N. Y. Acad. Sc.*, 1942, **43**, 107.

9. Jones, F. S., and Orcutt, M., *J. Immunol.*, 1934, **27,** 215.
10. Kleczkowski, A., *Brit. J. Exp. Path.*, 1941, **22,** 188.
11. Kleczkowski, A., *Brit. J. Exp. Path.*, 1941, **22,** 192.
12. Levine, P., and Gilmore, E. L., *Science*, 1945, **101,** 411.
13. Pappenheimer, A. M., *J. Exp. Med.*, 1940, **71,** 263.
14. Race, R. R., *Nature*, 1944, **153,** 771.
15. Ross, J., *J. Immunol.*, 1938, **35,** 351.
16. Shibley, G. S., *J. Exp. Med.*, 1929, **50,** 825.
17. Smetana, H., and Shemin, D., *J. Exp. Med.*, 1941, **73,** 223.
18. Tyler, A., *J. Immunol.*, 1945, **51,** 157.
19. Wiener, A. S., *Proc. Soc. Exp. Biol. and Med.*, 1944, **56,** 173.
20. Wiener, A. S., *Am. J. Clin. Path.*, 1945, **15,** 106.
21. Witebsky, E., Klendshoj, N. C., and McNeil, C., *Proc. Soc. Exp. Biol. and Med.*, 1944, **55,** 167.
22. Zia, S. H., Chow, B. F., and T'ung, T., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38,** 688.