Inactivation of High and Low Molecular Weight Chicken Antibodies by Mercaptoethanol.* (28299)

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Antibodies with a sedimentation coefficient of 19S are synthesized initially followed by synthesis of 7S γ-globulin antibodies in several animal species and to a variety of antigens(1-5). Delineation of these antibodies is based, in part, on dissociation of the 19S macroglobulin to 7S subunits by sulfhydryl compounds, and the relative resistance of 7S antibodies to these reagents(6). High concentrations of 19S antibody are produced in chickens following a single injection of bovine serum albumin (BSA)(4), and these antibodies, partially purified by chromatography and ultracentrifugation, were dissociated with 2-mercaptoethanol (ME)(7). It was suggested that some inactivation of anti-BSA 7S hemagglutinin might also occur(7). The purpose of the present study was to investigate in more detail the reduction of the 7S and 19S chicken antibodies by various concentrations of ME.

Materials and methods. For induction of primary responses, adult Austra hens were given a single intravenous injection of 40 mg BSA (Pentex). Booster sera were obtained by giving 4 daily intramuscular injections of 10 mg BSA each, 3-4 weeks following the primary injection. Bleedings were made 6 days following the primary and last booster injection. The sera of 15-20 chickens were pooled, and the globulins precipitated once with Na_2SO_4. Passive hemagglutination (HA) titers were determined as previously described(3). Relative amounts of low and high molecular weight antibody activities were estimated by zone ultracentrifugation in sucrose density gradients(6). Analytical ultracentrifugal examination was carried out as previously described(7). Sera were treated with various concentrations of ME for various periods at room temperature, after which they were dialyzed against pH 7.2 buffered-saline, and immediately titrated for antibody activity. In later experiments dialysis prior to titration was found to be unnecessary. For determining the antigen-binding capacity of antibody, chickens were immunized with human serum albumin (HuSA) employing the above immunization schedule, and the method described by Farr(8) was used. The I^{131} labeled HuSA‡ (I*HuSA) was diluted to contain 0.2 μg N, and sera were diluted in 1:10 normal chicken serum in borate buffer. The ammonium sulfate precipitated I*HuSA-globulin complexes were counted in a well-type gamma scintillation counter, and per cent of antigen precipitated was determined by the ratio of the counts per minute in the precipitate to total counts per minute of I*HuSA added. The usual controls were employed. Degradation with crystalline papain§ (1% by weight) was performed according to the method of Porter(9) using primary and booster anti-BSA globulins which had been concentrated by 3 precipitations with decreasing concentrations of Na_2SO_4 (7).

Results. The primary anti-BSA serum pools had hemagglutinin mainly associated with rapidly sedimenting molecules, and antibody activity in booster sera was associated with 7S globulins (Table I). Identification of the high and low molecular weight antibodies was confirmed by chromatographic fractionation(7).

Inactivation of antibody was a function of ME concentration and time. Activity completely disappeared in primary sera after 4 hours exposure to 0.1 M ME, and complete inactivation occurred within 30 minutes with

* This work was supported in part by training grant from U. S. Public Health Service.

† U. S. Public Health Service Career Development Awardee.

‡ RISA®, Abbott Laboratories, North Chicago.

§ Worthington Biochemical Co.

TABLE I. Distribution of Hemagglutinating Anti-BSA Chicken Antibodies in Fractions Separated by Density Gradient Zone Ultracentrifugation.

Fraction*	Serum	
	Primary†	Booster‡
Unfractionated	327,680§	163,840
1 (top)	0	20
2	10	20
3	80	40
4	80	320
5	160	20,480
6	80	20,480
7	160	2,560
8	10,240	2,560
9	81,920	640
10	81,920	320
11 (pellet)	40	40

* Sucrose concentrations ranging from 10 to 40%. Centrifugation carried out for 15 hr at 35,000 rpm.

† Serum pool obtained 6 days following single injection of 40 mg of BSA.

‡ Serum pool obtained following repeated immunization.

§ Reciprocal of hemagglutinin titer.

TABLE II. Precipitation of I^{131}HuSA by Mercaptoethanol-treated Primary Chicken Anti-HuSA in the Presence of 50% Saturated Ammonium Sulfate.

Reciprocal serum dilution	Per cent I^{131}HuSA precipitated with 50% sat. $(NH_4)_2SO_4$
10	43*
50	39
100	30
500	7
1,000	4
2,000	1

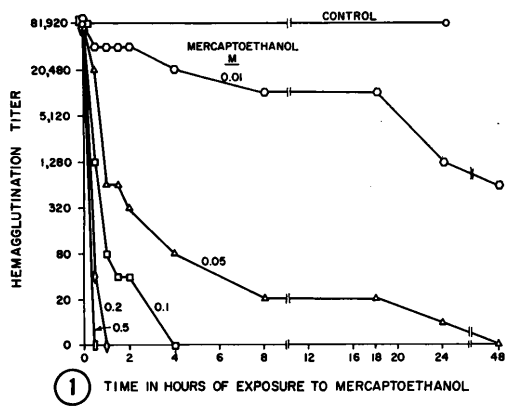
* Values obtained after subtraction of binding (7%) by normal serum controls.

0.5 M ME (Fig. 1). Although the 7S antibodies were more resistant to reduction, considerable loss of activity occurred after exposure for 120 hours to 0.1 M ME, and only slight hemagglutinin remained after 18 hours treatment with 0.7 M ME (Fig. 2). Marked turbidity of the preparations treated with the higher concentrations of ME appeared; however, loss of 7S antibody activity did not appear to be a result of precipitation of 7S globulins. Analytical ultracentrifugal analysis showed little loss in 7S globulin concentration after treatment with 0.5 M ME, and no dissociation products were detected.

The binding ability for a primary anti-HuSA serum treated with 0.1 M ME for 48 hours is shown in Table II. This serum had an HA titer of 1:819,200 and 1:80 before and after treatment, respectively. The quantity of dissociation products that might be capable of binding will be determined when purified 19S antibody becomes available. In this connection, repeated attempts to inhibit hemagglutination with ME-treated primary sera have failed, although preliminary evidence suggests that the homologous precipitin reaction may be inhibited.

Table III shows that papain destroys most of the hemagglutinin of both high and low molecular weight chicken anti-BSA. Ultracentrifugal analysis revealed a conversion of some of the globulins to 3.5S units.

Discussion. Sulfhydryl reducing agents have been used to distinguish macroglobulin



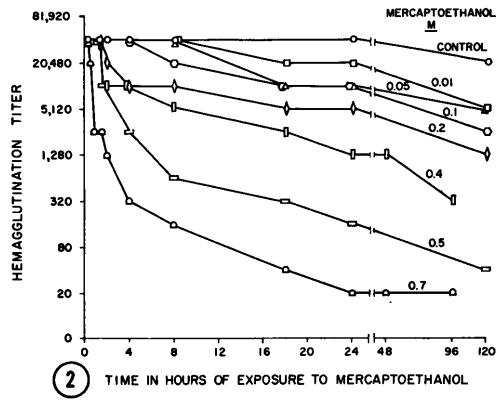


FIG. 1. Effect of different concentrations of 2-mercaptoethanol on high molecular weight chicken anti-BSA hemagglutinin.

FIG. 2. Effect of different concentrations of 2-mercaptoethanol on low molecular weight chicken anti-BSA hemagglutinin.

TABLE III. Degradation of Chicken Anti-BSA Hemagglutinating Antibody by Papain.

Serum*	Protein (μg)	Hemagglutinin titer	
		Untreated	Papain treated
Primary	38.8	40,960	8
Booster	45.0	20,480	<10

* Globulins isolated by Na_2SO_4 precipitation.

from the 7S class of antibodies; nevertheless, 7S antibodies also might be susceptible to partial inactivation. Complete inactivation of 19S chicken antibodies occurred when 0.1 M ME yielded 7S dissociation products(7). In the present study, this concentration of ME caused some loss of activity of chicken antibody of predominantly the 7S type. Higher concentrations of ME progressively inactivated 7S hemagglutinin, and little hemagglutinin remained after treatment with 0.7 M ME. Exposure to 0.5 M ME caused no apparent decrease in the molecular weight of chicken 7S globulins. These observations were consistent with those reported by Edelman and Poulik(10) that the molecular weight of human γ-globulin treated with 0.1 M β-mercaptoethylamine (MEA) in the absence of denaturing agents was the same as untreated globulin. Although there was no apparent change in molecular weight, some reduction might occur since rabbit γ-globulin treated with 0.4 M MEA in the absence of denaturing agents splits 7 disulfide bonds (11).

The mechanism by which reducing agents inactivate 7S chicken antiprotein hemagglutinin, and whether inactivated 7S antibody combines with antigen remains to be clarified. Primary sera, in which virtually all hemagglutinating capacity had been destroyed, still combined with antigen, as in the report that mercaptan dissociated saline Rh agglutinins, although showing no direct agglutinating activity, still give a positive Coomb's test(12). Whether binding was due to ME-resistant 7S antibody or to 19S dissociation products is not known.

Certain studies indicate differences in reducibility of 7S globulins from different animal species. Markus and Karush(13) reported complete reduction of bovine γ-globulin with 0.1 M MEA and 0.1 sodium decyl sulfate, whereas rabbit γ-globulin had lower reducibility under the same conditions(14). Also, slowly sedimenting frog and goldfish antibodies recently were shown to be inactivated with 0.1 M ME(5). A comparative study of reducibility of 7S globulins might place chicken antibodies in some intermediate position.

Summary. Inactivation of 7S and 19S chicken antibodies was dependent on mercaptoethanol concentration and time. Papain-degraded high and low molecular weight chicken antibodies lost agglutinating ability.

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Received January 2, 1963. P.S.E.B.M., 1963, v113.