A Method of Removal of Specific Antibody from Immunized Rabbits* (34076)

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(Introduced by L. Thomas)

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The specific removal of antibody *in vivo* might be useful for evaluation of the role of antibody as a "feedback" mechanism and for the treatment of certain antibody-associated diseases of man. One approach is to expose the blood of an immunized host to antigen that is attached to particles and in some fashion remove the particles. Murray (1) used sheep red blood cells to adsorb out the natural antibodies present in rats. This report describes the specific removal of serum antibody from rabbits by exposing their serum to an immunoadsorbent.

Two techniques were used: (1) Aliquots of blood were consecutively removed from an immunized rabbit; each aliquot was exposed *in vitro* to an immunoadsorbent which was then removed by centrifugation, and the blood returned to the animal. (2) An extracorporeal continuous flow system was established in which blood was allowed to flow into a chamber containing the immunoadsorbent before returning to the animal.

Materials and Methods. The immunoadsorbent used was bovine serum albumin (BSA) 4 X-crystallized (Nutritional Biochemical Co., Lot no. 1359) coupled to bromoacetyl cellulose (BAC) prepared according to the method of Patchornik (2), and Robbins *et al.* (3) with minor modifications. Powdered Whatman cellulose was washed with acetone and then dioxane and dried *in vacuo* over phosphorous pentoxide to a constant weight. A solution of 100 g of bromoacetic acid dissolved in 30 ml of dioxane was added to 10 g of the prepared cellulose in a volumetric flask and the mixture was vigorously stirred at room temperature for 20 hr. The flask was then connected to a sodium hydroxide trap, 75 ml of bromoacetyl bromide was added, and the mixture was vigorously stirred for 10 hr. The contents were then poured into 7 liters of deionized water and stirred overnight at 4°. The cellulose was then washed alternatively with 0.1 *M* sodium bicarbonate and water four times each and stored in water at 4°.

BSA-^{125}I was prepared according to the method of Greenwood *et al.* (4) so that 99% of the counts were precipitable by 5% trichloracetic acid (TCA) and also by anti-BSA. Six hundred mg of BSA and 0.1 mg of BSA-^{125}I (10^6 cpm/μg) were dissolved in 20 ml of 0.15 *M* phosphate-citrate buffer pH 3.8 and added to 2 g of the previously prepared BAC. The mixture was vigorously stirred at room temperature for 30 hr, centrifuged at 10,000*g* for 10 min, and resuspended in 60 ml of 0.1 *M* sodium bicarbonate–sodium carbonate buffer pH 8.9 and allowed to stand at 4° for 20 hr. The suspension was then centrifuged at 10,000*g* for 10 min and the pellet resuspended in 100 ml of 0.05 *M* 2-aminoethanol–0.1 *M* sodium bicarbonate–sodium carbonate buffer, brought to a pH of 8.9 with concentrated hydrochloric acid and

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allowed to stand at 4° for 20 hr. The suspension was then washed four times with 0.15 *M* sodium chloride until the optical density at 280 mμ of the supernatant fraction was 0.040. The sample was then suspended in 60 ml of 8 *M* urea. The urea used had been purified by recrystallization from water, washed with cold absolute ethanol, and dried in air. The suspension was stirred at room temperature for 24 hr and after centrifugation at 10,000*g* was washed with 8 *M* urea until the optical density at 280 mμ of the supernatant fraction was unrecordable. The conjugate was washed with 0.15 *M* phosphate buffer pH 7.4 three times and stored at 4° until ready for use.

It was found that an amount of immunoadsorbent which contained 13 mg of BSA bound 4.1 mg of anti-BSA. Ninety per cent of the adsorbed anti-BSA, as determined by the Farr technique (5), was recovered by incubating the BAC–BSA with 0.1 *N* acetic acid, stirring at 37° for 1 hr, followed by dialysis against 0.1 *M* sodium chloride—0.01 *M* phosphate buffer, pH 7.4, overnight.

Results. The ability of the adsorbent to remove antibody from the serum of a rabbit was tested. A 4-kg rabbit was injected intravenously with rabbit antiserum containing 16 mg anti-BSA and an anti-ϕX neutralizing titer of $k = 2700$. Blood was sampled for antibody assays 5 min and 18 hr later and the animal was anesthetized with intravenous nembutal and was heparinized. The femoral artery and vein were cannulated. Thirty-five cubic centimeter samples of blood were withdrawn from the femoral artery through a threeway stopcock. The plasma was separated from the red cells by centrifugation and incubated with aliquots of the BAC-BSA (total amount of adsorbent used contained 73 mg of BSA) for 15 min at 37°, centrifuged at 20,000*g* and the plasma and red cells were returned through the femoral vein. Five minutes later blood was drawn from the femoral artery for antibody assays. This entire maneuver involving removal and replacement of blood was repeated six times. Anti-BSA titers were determined by the Farr technique using 0.4 μg *N* BSA antigen and an ABC of 33%. The anti-ϕX titers were determined by the phage neutralization test (6).

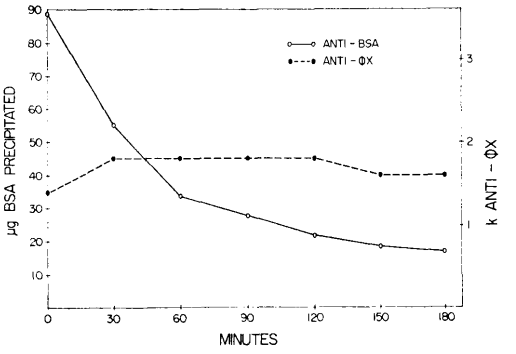

FIG. 1. Discontinuous removal of antibody from rabbit M 121. Anti-BSA determined by the Farr technique using 0.4 μg *N* BSA and an ABC-33 endpoint. Anti-ϕX determined by the phage neutralization test.

As can be seen in Fig. 1, the serum anti-BSA level was reduced to approximately one-fifth of its original value after 6 "bleedings and replacements" whereas the anti-ϕX titer remained essentially unchanged. The serum titer of anti-BSA and anti-ϕX did not change appreciably 24 hr after the experiment was concluded.

To elute anti-BSA from the BAC–BSA, the latter was extensively washed with 0.15 *M* sodium chloride until the optical density at 280 mμ of the supernatant was unrecordable, and the BAC–BSA was then suspended in 0.1 *N* acetic acid pH 2.5 for 1 hr at 37°. The suspension was then centrifuged at 20,000*g* for 20 min and the supernatant dialyzed against 0.1 *M* sodium chloride–0.01 *M* phosphate buffer pH 7.4 overnight. Some of the material in the dialyzate was rendered insoluble by this process and could only be resolubilized in strong base or acid. This material was centrifuged and the amount of anti-BSA in the supernatant fraction as determined by the Farr technique bound 770 μg BSA. Since the micrograms of BSA bound by the injected anti-BSA was known, it was determined that 64% of the removed antibody was recovered by this elution process. Attempts to elute with 8 *M* urea yielded approximately 33% of that quantity which was eluted by acetic acid.

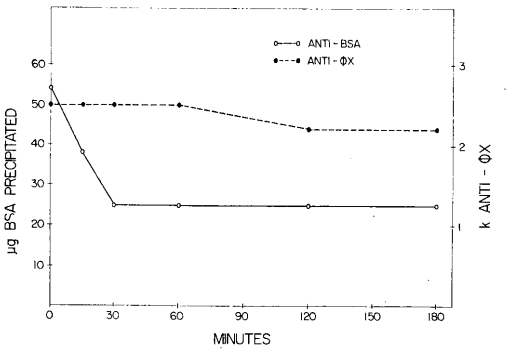

FIG. 2. Removal of antibody from rabbit M 112 using an extracorporeal circulation.

Specific antibody could also be removed from rabbits by exposing blood to immunoadsorbent through extracorporeal circulation. In order to keep the adsorbent in the chamber, BAC-BSA containing 68 mg BSA was layered onto filter paper covered with melted 2% Noble agar as it cooled before placement into the chamber. A 4-kg albino rabbit was passively immunized with anti-BSA and anti-ϕX as above. Eighteen hours later, the animal was anesthetized, anticoagulated, and the femoral vessels cannulated.

As can be seen in Fig. 2, the serum concentration of anti-BSA was reduced by approximately 50% within 30 min whereas the concentration of anti-ϕX remained essentially unchanged.

Using these two techniques, experiments were performed on an additional seven rabbits. The results are summarized in the Table. [I.] In all experiments, there was a decline in anti-BSA concentration from 32–84% whereas anti-ϕX concentration fell only 12–16% or not at all.

The next questions to arise were: (1) is antigen released from the immunoadsorbent and (2) is the immunoadsorbent dislodged from the agar during the extracorporeal procedure. Preliminary experiments with labeled BSA-^{125}I bound to BAC indicate that the BAC–BSA bond is probably not broken under the experimental conditions. Thus, after incubation for 30 min at 37° in fresh plasma, virtually no nondialyzable or TCA-precipitable label was released (<.04%). However, examination of the organs of the rabbits in the extracorporeal experiments revealed the presence of radioactivity in their lungs. It has not yet been determined whether this radioactivity represents BAC–BSA particles or free ^{125}I.

TABLE I. Specific Removal of Antibody to BSA by Bromoacetyl Cellulose BSA.

Type of procedure and animal no.	Decline in serum antibody concentration (%)[a]	
	Anti-BSA	Anti-ϕX
Discontinuous removal		
M 120	70	16
M 121	81	0
M 122	84	0
Extracorporeal circulation		
M 110	50	13
M 111	40	12
M 112	34	12
M 115	32	12
M 116	36	12
M 117	50	13

[a] Calculated on the basis of 0 time titer = 100% and the maximum fall in antibody titer 3–6 hr after removal.

These results indicate the feasibility of specific removal of antibody *in vivo* by means of exposure of immune serum to an immunoadsorbent. The use of extracorporeal circulation is particularly promising as a means of accomplishing this removal efficiently. The critical problem is to keep the immunoadsorbent in the chamber if this technique is to be useful for the study of "feedback" or for clinical purposes.

Summary. A method is described for the removal of specific antibody from immunized animals. Rabbits were passively immunized with antibody to BSA and bacteriophage ϕX. Their blood was exposed to bromoacetyl cellulose–BSA thru an extracorporeal circulation or the immunoadsorbent was mixed with large aliquots of their serum *in vitro* before returning the serum to them. Both of these methods resulted in a 32–84% reduction of circulating anti-BSA with little or no effect on anti-ϕX.

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