Cryoprecipitates and Immune Complexes: Decrease in Antibody Bound ^{131}I-Labeled BSA Antigen After Cryoprecipitation in Rabbit Serum (37226)

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Serum cryoprecipitates have been reported in animals and a variety of human diseases (1). Recent data from patients with immune-complex-mediated inflammatory disorders suggest that cryoprecipitates may contain immune complexes (2).

In prior communications, we have reported that bovine serum albumin (BSA) cryoprecipitates from serum of rabbits while immune elimination of BSA is occurring (3). We have also shown that cryoprecipitates contain antibody with specificity for BSA (4). In this study we report that immune-complexed ^{131}I-labeled BSA can be removed from rabbit serum by cryoprecipitation.

Methods. BSA was labeled with ^{131}I by the Chloramine-T method of McConahey and Dixon (5). Eleven rabbits were injected with 250 mg/kg of ^{131}I-labeled BSA (I* BSA) iv. To insure an immune response, 10 mg/kg BSA in Freund's complete adjuvant was given sc on the same day. Animals were bled from the central ear artery on Days 3, 9, 10, and 11 using a butterfly scalp vein needle. Blood was allowed to clot at 37° for 2 hr. The clotted blood was centrifuged at 2000 rpm, and serum was decanted. Serum was recentrifuged to remove all cells.

The fresh serum was divided into two parts (see Fig. 1). One part was used to determine complexed I*BSA before cryoprecipitation using the ammonium sulfate precipitation method of Farr (6). The second part was placed at 2° to allow cryoprecipitate to form. After 72 hr the refrigerated serum was spun at 5000 rpm for one hour at 2°. The Farr test was then repeated using the supernate serum. This determination gave complexed I*BSA after cryoprecipitation.

The cryoprecipitates were washed four times in cold phosphate-buffered saline (PBS), pH 7.4 by centrifugation. Cryoprecipitating I*BSA was determined by counting the entire cryoprecipitate. The precipitates were then dissolved in a small volume of 0.075 *M* barbital buffer, pH 8.8, for 4 hr at 37°. Ninety-five per cent of the radioactivity from the dissolved precipitate could

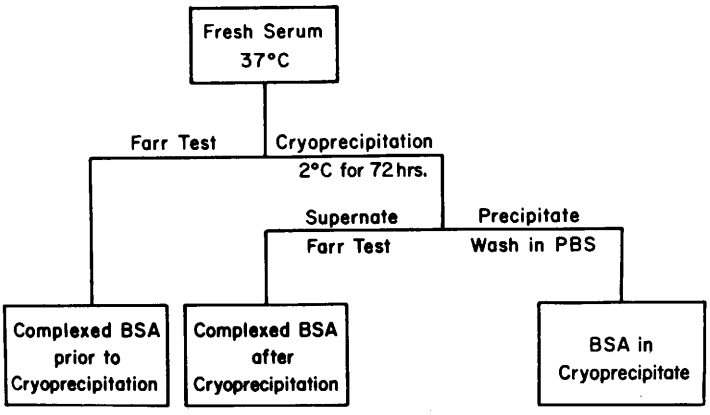

FIG. 1. Procedure for analysis of serum samples. The Farr test was done on serum before and after cryoprecipitation.

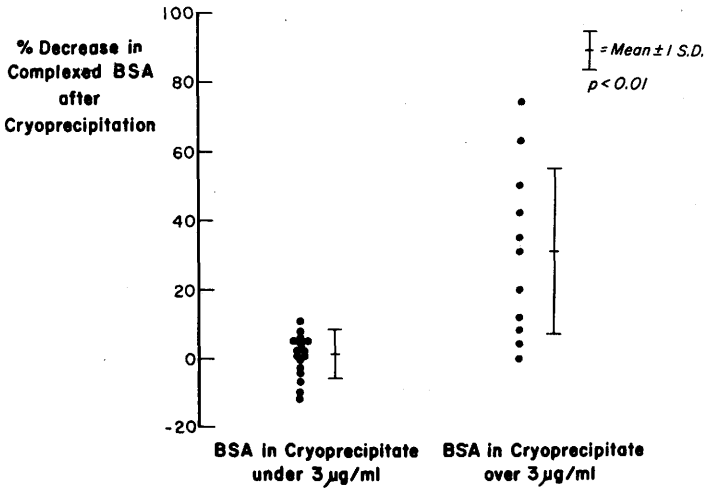

FIG. 2. Per cent decrease in complexed I*BSA in animals with cryoprecipitating I*BSA, less than 3 μg/ml and more than 3 μg/ml.

be reprecipitated by 10% trichloracetic acid. The presence of I*BSA in the redissolved cryoprecipitates was confirmed by immunodiffusion using rabbit anti BSA antiserum. I*BSA in the cryoprecipitate was calculated in micrograms per milliliter of original serum.

Serum total I*BSA was determined by counting the precipitate obtained from 1 ml of fresh serum in 10% trichloracetic acid.

All samples were counted in a Well type Packard liquid scintillation counter.

Results. Figure 2 shows the per cent decrease in serum complexed I*BSA after cryoprecipitation. Samples are divided into two groups: those with less than 3 μg/ml I*BSA in the cryoprecipitate and those with more than 3 μg/ml. Significant decreases in serum complexed I*BSA were seen in the group with cryoprecipitating I*BSA more than 3 μg/ml. The difference between the two groups is statistically significant at the 0.01 level (Student's t test).

Figure 3 shows the correlation between I*BSA in the cryoprecipitate and the decrease in serum complexed I*BSA after cryoprecipitation. Both values are expressed as per cent of complexed I*BSA measured in fresh serum. The correlation coefficient between the two variables is 0.9 ($p < 0.01$).

Table I shows the relationship between serum total I*BSA, serum complexed I*BSA, and cryoprecipitating I*BSA for samples with cryoprecipitating I*BSA over 3 μg/ml.

Discussion. The concentration of antibody bound I*BSA decreases significantly after the serum has been placed at 2° for 72 hr. The decrease in complexed I*BSA correlates with the amount of I*BSA in the cryoprecipitate (Fig. 3). Therefore, some of the I*BSA which is in immune complexes can be removed from serum by cryoprecipitation.

Since a large percentage of serum com-

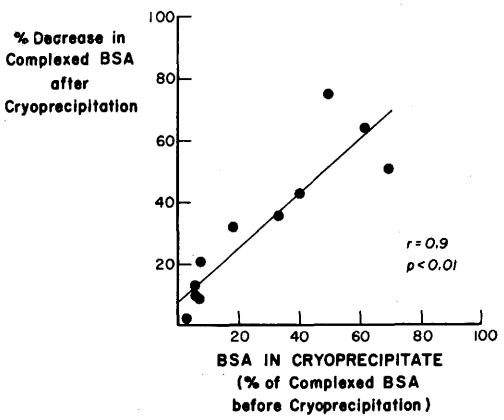

FIG. 3. Correlation between I*BSA in the cryoprecipitate and the decrease in complexed I*BSA after cryoprecipitation. Both values are expressed as per cent of complexed I*BSA measured in fresh serum.

TABLE I. Relationship Between Serum Total I*BSA, Serum Complexed I*BSA and I*BSA in the Cryoprecepitate for Samples with Cryoprecipitating I*BSA Greater than 3 μg/ml.[a]

Total serum I*BSA (μg/ml)	Serum complexed I*BSA (μg/ml)	I*BSA in cryoprecipitate (μg/ml)	Cryoprecipitating I*BSA as per cent of complexed I*BSA	Day of sample
214	61	4.0	6.6	10
28.0	11.6	5.9	51.0	11
19.5	19.9	7.9	40.0	10
13.7	9.6	5.9	61.0	11
242.0	177.0	12.5	7.1	10
97.0	50.0	9.4	18.8	11
514.0	163.0	6.0	3.7	9
199.0	134.0	8.5	6.3	10
45.6	19.0	13.6	71.6	11
86.0	56.0	5.3	9.5	11
16.0	18.0	3.9	21.7	10

[a] Values for serum complexed I*BSA were measured in fresh serum.

plexed I*BSA may cryoprecipitate, studies of circulating immune complexes should be done on fresh serum which has not been refrigerated. Studies of cryoprecipitates may aid in the detection and characterization of immune complexes in human disease. The possible pathogenetic role in serum sickness for the immune complexes which cryoprecipitate is under investigation.

Summary. Antibody-bound I*BSA was measured by the Farr technique in serum of rabbits undergoing immune elimination of I*BSA. Determinations were made before and after the serum was placed at 2° to allow the cryoprecipitate to form. Several samples showed a significant decrease in the concentration of complexed I*BSA after cryoprecipitation. The decrease in complexed BSA correlated with the amount of I*BSA in the cryoprecipitate.

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1. Moroz, A., and Rose, B., *in* "The Cryopathies in Immunological Diseases" (Max Samter *et al.*, eds.), Vol. 1, p. 459. Little Brown & Co., Boston (1971).

2. Barnett, E. V., Bluestone, R., Cracchiolo, A., Goldberg, L. S., Kantor, G., and McIntosh, R. M., Ann. Int. Med. **73**, 95 (1970).

3. Griswold, W. R., Chernack, W., and McIntosh, R. M., Immunol. Commun. **2**, No. 1 (1973).

4. McIntosh, R. M., Gross, M., LaPlante, M., Kaufman, D. B., and Kulvinskas, C., J. Exp. Med. Surg. **29**, 108 (1971).

5. McConahey, P. J., and Dixon, F. J., Arch. Allerg. Appl. Immunol. **29**, 185 (1966).

6. Farr, R. S., J. Infect. Dis. **103**, 239 (1958).

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