must be given for many years in order to accomplish bacteriologic cure, and many patients experience reactions that seriously delay treatment. The activity of capreomycin against *M. leprae* as demonstrated experimentally in this study, together with the relatively low toxicity of the drug for humans (*5, 6*), suggest controlled therapeutic trial in the human disease, perhaps in combination therapy.

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Antigen-Binding Activity of 6S Subunits of β_2-Macroglobulin Antibody

Abstract. *Direct evidence is provided for the antigen binding activity of the 6S subunits formed by reduction and alkylation of rabbit β_2-macroglobulin antibody. Binding activity of the subunits was clearly demonstrated by radioimmunoelectrophoresis with a preparation of purified rabbit antibody against the* p*-azobenzenearsonate group, which contained 40 percent of β_{2M}-antibody. The precipitate arc formed between the subunits and sheep antiserum against rabbit macroglobulin was shown to bind radioactive antigen specifically.*

Human γ_1- or β_2-macroglobulin (γ_{1M}- or β_{2M}-globulin) has been reported to dissociate to 7*S* subunits when reduced with 0.1*M* mercaptoethanol at neutral *p*H (*1*). Loss of apparent antibody function of the macroglobulin antibody by the mercaptoethanol treatment was reported in a variety of systems such as hemolysis of erythrocytes in the presence of complement (*2*), direct hemagglutination (*3–5*), hemagglutination of passively sensitized erythrocytes (*6, 7*), phage neutralization (*8*), or specific precipitation in solution or in agar (*7, 9*). However, in all these systems it was difficult to determine whether the antibody retained its capacity to combine with antigen after reduction. The results reported by Fudenberg and Kunkel (*3*) that the cold hemagglutinins studied by them lost Coombs' reactivity as well as direct agglutinating activity after dissociation are not necessarily proof of the destruction of the antigen binding sites, because the loss of Coombs' reactivity could possibly have occurred through a loss, on dissociation, of antigenic determinants capable of reacting with the particular Coombs' reagent used.

That antigen binding activity is retained on subunits of macroglobulin antibody is indicated by Chan and Deutsch (*5*) who showed that mercaptan-dissociated saline agglutinins (Rh antibody) gave a positive Coombs' test, and by Benedict *et al.* (*7*) who reported that chicken antiserum against bovine serum albumin, which contained mostly macroglobulin antibodies, lost its precipitating activity after treatment with mercaptoethanol but inhibited the specific precipitation of nontreated antibodies with the antigen. Jacot-Guillarmod and Isliker (*10*) also showed loss of agglutinating activity of isoagglutinins (anti-A and anti-B) upon mild reduction. However the reduced material inhibited agglutination by unaltered isoagglutinins. Furthermore reoxidation restored the agglutinating activity.

We now report direct evidence that rabbit β_2-macroglobulin antibody directed against the *p*-azobenzenearsonate group (R_p) still binds the antigen after reduction and alkylation to form 6*S* subunits. The antiserum was obtained from a single rabbit which had been hyperimmunized by repeated intravenous injections of bovine γ-globulin conjugated with R_p. The antibodies were absorbed on a column of an insoluble antigen (immunoadsorbent) which had been made by coupling a conjugate of R_p and rabbit γ-globulin to diazotized polyaminopolystyrene (*11*). After inert proteins were removed by washing with buffered saline, *p*H 8, the adsorbed antibodies were eluted with citrate-phosphate buffer at *p*H 4.0 in the cold.

This purified antibody preparation was examined by radioimmunoelectrophoresis (*12*) as follows. It was submitted to electrophoresis in an agar layer on a microscope slide. Then, a mixture of sheep antiserum against the whole macroglobulin fraction of rabbit serum (sheep antiserum to RMG) (*13*) and I^{131}-labeled R_p-insulin (I^{131}-R_p-insulin) was diffused from the trough. Arcs of specific precipitate formed between rabbit antibody components and sheep antiserum to RMG. The slide was washed, dried, and stained with amidoblack. The I^{131}-R_p-insulin fixed on arcs containing rabbit antibodies to R_p was revealed by radioautography.

The purified antibody preparation showed two arcs on the stained slide (Fig. 1, slide 1) which were identified as the arcs of β_{2M}- and γ-globulin (*12*). Both arcs showed antigen-binding activity as indicated by the dense arcs on radioautographs (film 1). Only the arc of β_{2M}-globulin was observed when examined with sheep antiserum to RMG previously absorbed with rabbit γ_2-globulin (slide 3 and film 3). Ultracentrifugal analysis showed that the preparation of antibody to R_p contained 39 percent of a 17.2*S* component (β_{2M}-antibody) and 61 percent of a 6.6*S* component (γ-antibody)).

This antibody preparation was treated

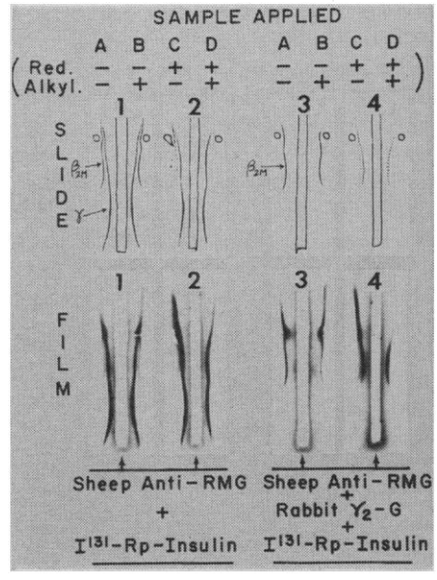

Fig. 1. Radioimmunoelectrophoresis of preparations of original and treated antibody to R_p. Drawings of stained slides and photographs of radioautographs are shown. Treatment is indicated under each sample. *Red.* refers to reduction with 0.1*M* 2-mercaptoethanol at *p*H 8.0. *Alkyl.* refers to alkylation with iodoacetamide. Samples were applied at the same protein concentration, 6.6 mg/ml. *Sheep Anti-RMG* refers to sheep antiserum against whole macroglobulin fraction of normal rabbit serum.

Table 1. Ultracentrifugal analysis before and after reduction of rabbit antibody. All samples were examined at a protein concentration of 6.6 mg/ml in 0.15*M* sodium chloride buffered with 0.02*M* borate at *p*H 8.0. Values in parentheses indicate the observed values of $S_{20,w}$.

Treatment	Relative sedimentation rate		
	Fast (%)	Intermediate (%)	Slow (%)
None	39 (17.2*S*)	0	61 (6.6*S*)
Alkylated	43 (16.3*S*)	0	57 (6.2*S*)
Reduced-nonalkylated	5 (17.1*S*)	12 (14.7*S*)	83 (6.5*S*)
Reduced-alkylated	0	0	100 (5.9*S*)*

* A small amount of component which sedimented slightly more slowly than the 5.9*S* component was observed as a slight skewing of the peaks.

in separate portions as follows: (i) For the reduced-nonalkylated antibody, it was reduced with 0.1*M* mercaptoethanol at *p*H 8.0 for 30 minutes at room temperature and then at 4°C for 15 hours; (ii) for the reduced-alkylated antibody, it was reduced as in (i) and then treated with 10 percent excess of iodoacetamide for 15 minutes in an ice bath; (iii) for the alkylated antibody, it was treated with iodoacetamide as described in (ii) but without prior reduction. All samples were treated at the same final protein concentration (6.6 mg/ml) and then dialyzed against an ice-cold solution of borate and saline, *p*H 8.0.

The results of ultracentrifugal analyses are given in Table 1. The reduced-alkylated antibody preparation showed essentially a single peak with a sedimentation coefficient of 6*S* indicating the complete dissociation into subunits. When the reduced preparation was not alkylated (reduced-nonalkylated), there appeared in addition to 6*S* components, 12 percent of 14.7*S* and 5 perecnt of 17.1*S* components. The 14.7*S* and 17.1*S* components were probably due to association of subunits through disulfide linkages. The alkylated antibody showed a pattern essentially similar to that of the nontreated antibody preparation. These results are in accord with the finding for human macroglobulin (*1*).

Antigen-binding activity of the reduced and alkylated β_{2M}-antibody was clearly shown by radioimmunoelectrophoresis (Fig. 1). Only the arcs of β_{2M}-antibody or its reduced subunits were seen on the stained slides when the sheep antibody to RMG previously absorbed with rabbit γ_2-globulin was used (slides 3 and 4). The reduced and alkylated β_{2M}-antibody subunits migrated electrophoretically to a position similar to that of the original β_{2M}-antibody (*14*). The position of this arc was closer to the center trough than that of the arc of original β_{2M}-antibody (slide 4), probably because of the faster diffusion rate of 6*S* subunits (*15*). The reduced-nonalkylated antibody preparation showed a similar but somewhat stronger arc. In addition, a very faint arc was seen at the original position of β_{2M}-antibody (slide 4). The alkylated antibody preparation showed an arc of the β_{2M}-antibody at the original position of the native β_{2M}-antibody (slide 3). The arc of γ-globulin, which was observed when examined by the use of unabsorbed sheep antibody to RMG, did not change by any of the treatment.

The binding of I^{131}-R_p-insulin on the specific precipitate of reduced-alkylated β_{2M}-antibody was definitely shown by the dense arc on the radioautograph (film 4). The density of the arc was not much different from that shown by the native β_{2M}-antibody (compare films 3 and 4). The reduced-nonalkylated preparation showed two arcs on radioautograph when the absorbed sheep antibody to RMG was used (film 4). One was dense and similar in position to the arc of the reduced-alkylated subunits. The second was weaker and was at the position of the original β_{2M}-antibody. The second arc might have been formed by reassociated components (14.7*S* and 17.1*S*) reacting with sheep antibody to RMG, but this point was not examined further. Treatment of antibody with iodoacetamide did not change significantly its radioimmunoelectrophoretic pattern.

Thus, it is clear that at least some of the 6*S* subunits produced by reduction and alkylation of β_{2M}-antibody still retain antigen-binding activity. Some of the subunits may not have antigen-binding sites because the number of antigen-binding sites on a macroglobulin antibody molecule may be less than the number of subunits produced, or the sites may not be distributed evenly on the molecule. Radioimmunoelectrophoresis as used here does not distinguish between such active and inactive subunits unless they form different arcs in immunoelectrophoresis. The formation of a single arc after reduction, as observed here (Fig. 1, slide 4), seems to indicate that either both of these subunits react similarly with sheep antibody to rabbit β_{2M}-globulin or inactive subunits do not react at all with the sheep antibody.

Thus the loss of activity upon reduction of the macroglobulin antibody that has been reported for many systems may not be due to destruction of antigen-binding activity. The loss may well be due to other changes affecting the secondary phenomena that follows the primary combination of antigen and antibody.

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13. This antiserum contained antibodies against γ-globulin, β_{2M}-globulin, and a few other components when tested with normal rabbit serum.
14. Subunits of human myeloma macroglobulin were reported by E. I. McDougall and H. E. Deutsch [*Biochem. J.* **90**, 163 (1964)] to migrate more rapidly than the intact molecule in the *p*H range, 4.5 to 8.5.
15. The staining intensity of the precipitate arc was weaker in the case of reduced subunits than with the original β_{2M}-antibody when absorbed sheep antibody to RMG was used. This is probably due to the destruction of some antigenic sites specific to β_{2M}-globulin.
16. We thank K. Mellenger, F. Maenza, and A. Trott for technical assistance. This investigation was supported in part by USPHS research grants AI-3962 and AI-5158 from the National Institute of Allergy and Infectious Diseases. One of us (K.O.) is on leave from the Department of Biochemistry, Kyushu University Medical School, Fukuoka, Japan.

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