tions at equal concentrations with Lac-HSA. The protein from the first peak and tail demonstrated a strong precipitin line, but material from the second peak did not precipitate Lac-HSA. The latter, in fact, inhibited the precipitation of the two adjacent samples. Thus, the β_{2A}-globulin antibody to Lac present in the original serum was responsible for the failure of the serum to precipitate antigen.

Ultracentrifugation of the purified nonprecipitable β_{2A}-antibody to Lac showed that the protein sedimented as a symmetrical peak with a sedimentation coefficient, $s_{20,w}$, of approximately 7*S*.

The immunologic activity of the purified β_{2A} antibody was measured by the binding of the hapten *p*-(*p*-dimethylaminobenzeneazo) phenyl-β-lactoside (Lac dye) by using the method of equilibrium dialysis at 25°C (*7*). The protein concentration was 1.37 times $10^{-5}M$, and the observed range of binding was from 1.2 to 1.8 moles of hapten bound per mole of antibody (r). The corresponding values of the free hapten equilibrium concentrations (c) were 0.16 times $10^{-5}M$ and 2.2 times $10^{-5}M$, respectively. The free hapten concentration was obtained by measurement of the optical density at 450 mμ, a molar extinction coefficient of 2.48 times 10^4 (7) being used. The molar protein concentration was determined by micro-Kjeldahl analysis on the basis of a molecular weight of 150,000. The bivalence of the antibody is obvious from the maximum measured value of r as well as from the extrapolated value of 2.0. A minimum value of the average association constant (K_A) was obtained from the extrapolated binding curve as the value of r/c corresponding to half-saturation, that is, $r = 1$ (*7*). Since the free dye concentration at this point could not be measured, the estimated value of 1 times 10^6 liter/mole for K_A represents a minimum figure.

Thus the nonprecipitable antibody in the hapten system described is a 7*S* β_{2A} immunoglobulin. This antibody has a higher affinity for the Lac hapten than precipitable antibody to Lac from the same horse (*8*) and from rabbits (*7*). As in the case of precipitable 7*S* antibodies of the same specificity, the binding results show that nonprecipitable antibody also contains two binding sites per molecule.

Therefore, neither low affinity nor univalence can account for the nonprecipitability of this antibody. This property may be the result of an arrangement of the binding sites such that the occupation of one site by a large antigen molecule sterically hinders the reactivity of the second.

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Capreomycin: Activity against Experimental Infection with Mycobacterium leprae

Capreomycin is a peptidic antibiotic with a probable equivalent molecular weight of 740 (*1–3*). It is approximately as active as streptomycin, kanamycin, and viomycin are (*1*) against *Mycobacterium tuberculosis* in mice. In cross-resistance studies Herr *et al.* (*1*) found that streptomycin-resistant tubercle bacilli were susceptible to capreomycin, and capreomycin-resistant strains were susceptible to streptomycin and viomycin. Koseki and Okamoto (*4*) reported that there was complete cross-resistance with viomycin, partial cross-resistance with kanamycin and neomycin, but no cross-resistance with streptomycin (or isoniazid).

When capreomycin was used in combination with sodium para-aminosalicylate for the treatment of pulmonary tuberculosis in humans at levels of 1 g once a day to 1 g twice a week it has been reported to have approximately the same effect as streptomycin with sodium para-aminosalicylate (*5, 6*). Toxicity to the drug was infrequent and was indicated chiefly by loss of hearing.

Shepard and Chang have described the use of the infection of mouse foot-pads with *Mycobacterium leprae* as a method for testing drugs (*7*), and have also reported results obtained with a total of 11 drugs known to be active against mycobacteria (*8*).

The activity of capreomycin against *M. leprae* in this system is reported in Table 1. The injection of 10 mg per day prevented bacillary multiplication completely. Positive results with a mouse passage inoculum containing this high percentage of solidly straining bacilli were obtained so consistently (*9*) that the failure to observe acid-fast bacteria in the treated mice is highly significant.

Although certain sulfones, especially 4, 4′-diaminodiphenyl sulfone (DDS), are efficacious in the treatment of leprosy, and are inexpensive as well, they

Table 1. Activity of capreomycin (CAM) against *M. leprae* in mouse foot-pads. Mice were injected with 5×10^3 acid-fast bacteria (AFB) containing 48 percent solidly staining bacilli from a typical strain in fifth mouse passage. Starting on the day of injection 0.1 ml of an aqueous solution of capreomycin, containing 10 mg of the drug, was given subcutaneously each day. A mouse from each group was taken at monthly intervals for histological sections. After the incubation period of 8 months AFB appeared in the sections prepared from the control animals, but after 10 months no AFB were present in sections from mice treated with capreomycin. Mice were then taken for counts of AFB in pools of foot-pad tissue (2 mice in pool of untreated animals at 14 months; all others, 4 mice per pool).

AFB in tissue sections		AFB harvested/mouse	
9* mo	10 mo	10 mo	14 mo
		CAM-treated	
0	0	$<1 \times 10^4$†	$<1 \times 10^4$†
		Untreated	
+	0	8.0×10^5	7.3×10^5

* No AFB in sections at 1–8 months. † No AFB were seen during the counting procedure. The concentration that would correspond to one AFB seen during the search was calculated and the result expressed as less than that number.

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

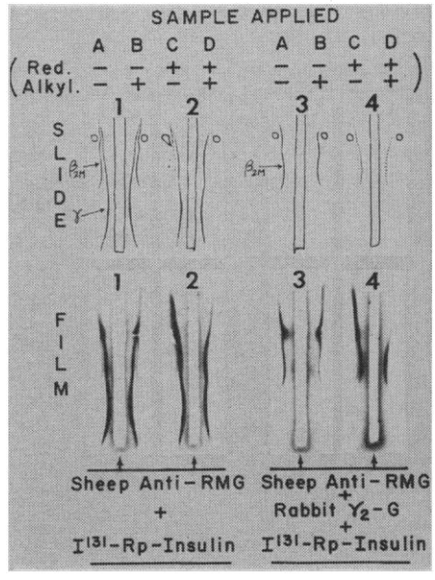

Fig. 1. Radioimmunoelectrophoresis of preparations of original and treated antibody to R_p. Drawings of stained slides and photographs of radioautographs are shown. Treatment is indicated under each sample. *Red.* refers to reduction with 0.1*M* 2-mercaptoethanol at *p*H 8.0. *Alkyl.* refers to alkylation 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.