1*B*, lower). Absorption with both γ_{2a}- and γ_{2b}-myeloma proteins yielded an antiserum specific for γ_{2c}.

γ_2-Myeloma proteins can be identified as γ_{2a}-, γ_{2b}-, or γ_{2c}-globulins with the use of these absorbed antiserums. Of 72 γ_2-myeloma proteins tested, approximately 15 percent are γ_{2a}-, 65 percent are γ_{2b}-, and 7 percent are γ_{2c}-globulins. The remaining 13 percent of γ_2-myeloma proteins are antigenic variants of still other types, indicating further complexity that will not be considered in this report.

The submolecular localization of the subclass (γ_{2a}, γ_{2b}, γ_{2c}) specific antigenic determinants was investigated in several ways. The heavy and light polypeptide chains of normal γ_2-globulin and those of γ_{2a}-, γ_{2b}-, and γ_{2c}-myeloma proteins were prepared by reduction and alkylation, and the polypeptide chains were separated on columns of Sephadex G-100 (*12*). Ouchterlony analysis with specific antiserums revealed that the specific γ_{2a}-, γ_{2b}-, and γ_{2c}-antigenic determinants were present only on the heavy polypeptide chains (Fig. 2*A*). The light chains did not react with the specific antiserums. Additional evidence that the light chains do not take part in the γ_{2a}-, γ_{2b}-, and γ_{2c}-globulin differences was obtained by examining type I and type II myeloma proteins. The subclass (γ_{2a}-, γ_{2b}-, γ_{2c}-) distribution was the same in 48 γ_2-myeloma proteins with type I light chains and 24 γ_2-myeloma proteins with type II light chains. Also, absorption of the antiserum with type I and type II Bence Jones proteins did not significantly alter immunoelectrophoretic or Ouchterlony precipitin patterns identifying γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins, which provides additional evidence that the subclass specific antigenic determinants are not located on the light chains.

Further studies of the submolecular localization of the subclass specific determinants were made with molecular fragments obtained by papain digestion (*13*). γ_{2a}- and γ_{2b}-Myeloma proteins were treated with papain in the presence of cysteine and the S (Fab) (slow) (*1*) and F (Fc) (fast) (*1*) fragments were isolated (*3*). The F fragment and the intact γ_2-myeloma protein from which it was prepared were precipitated by the antiserum while the S fragment was not (Fig. 2*B*). Thus, the distinctive antigenic characteristics of these subclasses are located on the F fragment of the heavy chain.

The F fragments of the heavy chains of γ_2-globulins determine many biologic properties of the molecules, such as complement fixation (*14*), reactivity with rheumatoid factors (*15*), placental transfer (*16*), and skin fixation (*17*). They also are the site of genetically controlled factors detected by the Gm (hereditary γ-globulin factor) hemagglutination-inhibition test system (*18*). Preliminary studies of reverse passive cutaneous anaphylaxis indicate that γ_{2b}- and γ_{2c}-myeloma protein molecules can bind to guinea pig skin, while γ_{2a}-myeloma protein molecules cannot. Differences in other biologic and genetic characteristics of γ_{2a}-, γ_{2b}-, and γ_{2c}-globulin molecules are under investigation.

After this paper was submitted, two reports (*19*) were published describing two populations of γ_2-globulin molecules detected with antiserums prepared in rabbits. Studies in our laboratory of the myeloma proteins Cr and Zu (*20*) used in these reports show that the Cr protein falls in the group of γ_2-myeloma proteins not identifiable as γ_{2a}-, γ_{2b}-, or γ_{2c}-, and that the Zu protein is antigenically related to γ_{2c}. Apparently, the rabbit antiserums used (*19*) did not distinguish the differences between γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins described in our report.

Note added in proof: Another recent paper (*20*) describes four subgroups of γ_2-globulins. γ_{2b}- and γ_{2c}-Globulins are antigenically related to the We and Vi subgroups of these authors.

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Valence and Affinity of Equine Nonprecipitating Antibody to a Haptenic Group

Abstract. *A nonprecipitable antibody to a haptenic group has been purified from equine serum. It is a 7S β_{2A} immunoglobulin with high affinity for the specific hapten and two binding sites per antibody molecule. These results rule out univalence and low affinity as explanations of the nonprecipitability of this antibody.*

Since the early descriptions of nonprecipitating equine antibody by Pappenheimer (*1*) and Heidelberger *et al.* (*2*), many explanations have been proposed for the inability of this antibody to precipitate with antigen. The two most popular explanations have been that the antibody is either of low affinity or is incomplete in the sense that it has only one antigen-combining site per molecule. In a recent review of the subject of "incomplete antibodies" (*3*) these two interpretations are discussed and several others are mentioned. Recent studies have demonstrated the frequent occurrence of nonprecipitating antibody in antiserums to protein and have indicated that these antibodies migrate on electrophoresis as β-globulins (*4*). These investigations have not, however, shed any new light on the reason for its nonprecipitability. We now describe a nonprecipitable antibody component which has been detected in a population of equine antibody molecules directed against the *p*-azophenyl-β-lactoside (Lac) haptenic group. After isolation and purification of this immunoglobulin, it has been

identified as a $\beta_{2A}(\gamma_{1A})$-globulin and its physical characteristics and hapten-binding properties have been studied. The results allow a critical evaluation of the proposed explanations for nonprecipitability.

A horse (500 kg) was immunized over a period of several months with repeated injections of Lac-hemocyanin in incomplete Freund's adjuvant. The hemocyanin, from *Limulus polyphemus*, was coupled with 20 to 25 moles of the Lac group per 100,000 grams of protein. Serum was collected from the horse at biweekly intervals and was tested for antibody to Lac by quantitative precipitation (*5*) with Lac-HSA (Lac group conjugated to human serum albumin) containing 15 to 20 moles of the Lac group per 65,000 grams of protein. The maximum antibody content of 2.14 mg of protein per milliliter of serum was reached 6 weeks after this course of immunization was initiated. The antibody remained at this concentration for 1 month. Serum collected 2 weeks after this period showed no specific precipitation when Lac-HSA was added. This serum, however, totally inhibited the precipitation of antigen by earlier serums when added in equal quantities.

A method was developed for the purification of antibody from antiserums which did not form a precipitate with Lac-HSA. In this procedure Lac-hemocyanin was used as a precipitating antigen. A copious precipitate was formed because of the high content of antibody to hemocyanin (8 to 10 mg/ml). The presence of Lac groups in the precipitate led to the coprecipitation of the antibody to Lac. After the precipitate was washed exhaustively with 0.15*M* NaCl at 37°C, the coprecipitated antibody to the Lac group was extracted with 0.5*M* lactose in 0.15*M* NaCl at 37°C. The lactose was then removed by extensive dialysis in the cold successively against solutions of 0.02*M* phosphate, *p*H 7.2, a mixture of 0.02*M* phosphate and 0.1*M* galactose, and 0.02*M* phosphate. The yield of antibody was 1.0 to 1.2 mg of protein per milliliter of serum. The preparation contained less than 2 percent of antigen and less than 5 percent of antiprotein antibody.

A precipitin test of this antibody preparation at a concentration of 140 μg of nitrogen per milliliter showed no precipitation when tested with Lac-HSA over the range of 0 to 800 μg of nitrogen per milliliter in 0.15*M* NaCl.

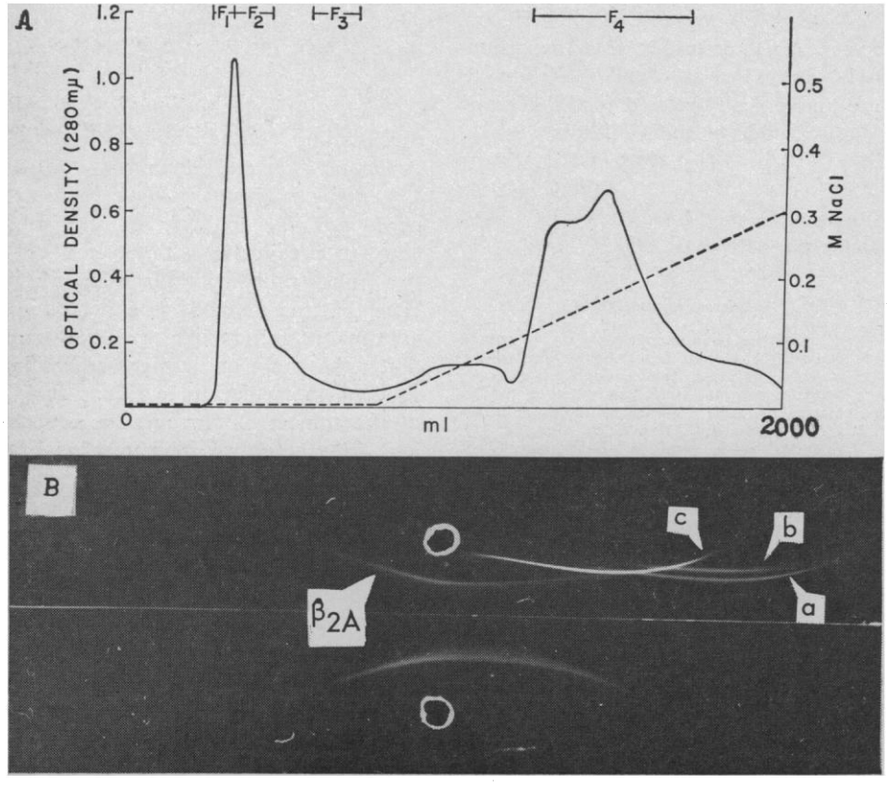

Fig. 1. *A*, Elution pattern of coprecipitated equine antibody to Lac from a DEAE-cellulose column eluted with 0.02*M* sodium phosphate buffer, *p*H 7.85 and a NaCl gradient. *B*, Immunoelectrophoresis pattern of coprecipitated antibody preparation (upper) and β_{2A}-globulin from fraction 4 (lower), both developed against rabbit antiserum to equine serum.

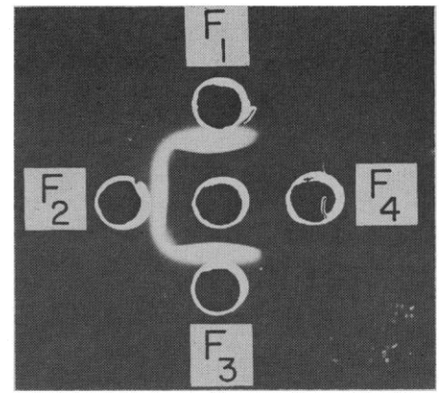

Fig. 2. Agar plate analysis showing precipitation of Lac-HSA (center well) with equal concentrations of antibody fractions from DEAE-cellulose chromatography. Precipitin lines can be seen to have formed with F_1 (7*S* γa- and γb-globulin), F_2 (7*S* γa-, γb-, and γc-globulin), and F_3 (7*S* γc-globulin), but F_4 (β_{2A}-globulin) shows no precipitation and appears to inhibit the precipitation of its neighbors.

Immunoelectrophoresis (*6*) of the purified antibody, developed with rabbit antiserums to equine γ-globulin, gave the upper of the two patterns shown in Fig. 1*B*. Four components were observed, three in the γ-globulin region (γa-, γb-, and γc-) and one in the β region (β_{2A} or γ_{1A}), each of which was antigenically distinct. Ultracentrifugal examination of the preparation showed a symmetrical peak with an approximate sedimentation coefficient, $s_{20,w}$, of 7*S*.

The antibody preparation was placed on a DEAE (diethylaminoethyl)-cellulose column in 0.02*M* sodium phosphate buffer, *p*H 7.85. A leading peak containing 20 percent of the original protein and a long tail containing an additional 20 percent were eluted with this same buffer (Fig. 1*A*). A linear sodium chloride gradient up to 0.5*M* in the initial buffer eluted a second peak containing the remaining 60 percent of the original protein. Immunoelectrophoresis of the eluted protein showed that the ascending limb of the first peak contained 7*S* γa- and γb-globulins and the descending limb contained 7*S* γa-, γb-, and γc-. The tail was mostly 7*S* γc-globulin and the second peak was almost entirely β_{2A}-globulin (lower pattern in Fig. 1*B*).

Figure 2 shows the precipitability by immunodiffusion of the various frac-

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.