acton of isoproterenol is unaltered by alpha blockade. Subsequent beta blockade (Fig. 2C) results in an almost complete attenuation of the inhibitory responses in addition to the complete loss of the phenylephrine contractile action.

In the sequence beginning with beta blockade (Fig. 3B), the phenylephrine contractile action is unchanged from control. Once again the epinephrine and norepinephrine responses become monophasic ones. In this case the loss of beta activity effectively potentiates the initial excitation. The ensuing contractions resemble pure alpha responses in that they occur rapidly and peak contraction is held as a plateau. The beta blockade can be seen to have been highly effective by the marked attenuation of isoproterenol inhibition. Following phenoxybenzamine exposure (Fig. 3C) all alpha excitatory responses are lost in addition to the previously blocked beta inhibition.

Summary. Experiments were performed on segments of terminal guinea-pig ileum utilizing differential alpha and beta adrenergic receptor site blocking techniques. Phenylephrine caused a contraction of this segment of guinea-pig intestine which resembled a pure alpha receptor site response. Both epinephrine and noreinephrine produced biphasic responses which follow a pattern of alpha excitation followed by beta inhibition while isoproterenol elicited a pure beta inhibitory action. The use of phenoxybenzamine as an alpha blocking agent abolished all excitatory responses while augmenting beta actions. Propranolol, through beta adrenergic receptor site blockade, potentiated alpha excitatory actions. The results are discussed in terms of the guinea-pig terminal ileum possessing an unique alpha adrenergic receptor mechanism which is excitatory in nature.

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Detection of Five Components Having Antibody Activity in Rat Antisera.*† (32018)

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The immunoglobulins of the rat have been described by several investigators. Precipitating antibody against bovine serum albumin (BSA) was found to reside mainly in a component designated by Arnason *et al*(1) as γA; however, these authors found the anti-BSA hemagglutinating antibody to be present largely in the γG component. Using specifically purified rat antibodies against the 2,4-dinitrophenyl (DNP) determinant, Nussenzweig and Binaghi(2) demonstrated that precipitins are present in the γ_2 component. Immunoelectrophoretic analysis of the purified antibody with one of their rabbit anti-rat antisera showed two parallel lines in the γ-globulin region. Both of these components are apparently different from the component designated as γA by Arnason *et al*(1). Nussenzweig and Binaghi(2) also detected 19 S antibody in this anti-DNP antibody preparation.

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Arnason *et al*(1) found that antibody produced in response to a particulate antigen was in the γM component. In this report radioimmunoelectrophoresis (RIEP) was utilized for detection of rat antibodies. Five radioactive bands were detected in anti-BSA and anti-BGG sera and the appearance of antibody activity in different immunoglobulins as a function of stage of immunization was determined.

Materials and methods. Animals and immunization. Rats of the Wistar strain were immunized with crystalline BSA (Armour & Co.) or bovine γ-globulin (BGG) (Armour & Co.). The animals received 5 mg of protein per injection. The protein was in saline, adsorbed to approximately 20 mg of bentonite or incorporated in complete Freund's adjuvant (FA). The immunizing emulsion, in which the oil to water ratio was approximately 1:1, was prepared from Drakeol 6-VR (Pennsylvania Refining Co., Butler, Pa.), Arlacel A (Atlas Powder Co., Wilmington, Del.) and the appropriate protein solution and contained approximately 1 mg of *Mycobacterium butyricum* and 10 mg of protein per ml. The animals received BSA-bentonite or BGG-bentonite by the intraperitoneal *(i.p.)* route or BSA-FA by either the *i.p.* or subcutaneous route. Multiple injections were given, and the animals were bled at appropriate intervals after each injection.

Anti-rat globulin antisera were prepared by injecting into rabbits partially purified rat serum globulins. Purification involved first the separations of the globulins from normal rat serum by 3 precipitations with ammonium sulfate (half saturation, pH 7). The redissolved globulins were then applied to a column of G-200 Sephadex (Pharmacia, Uppsala, Sweden) and eluted with borate buffered saline, pH 8. The first fraction and the ascending part of the second fraction were pooled and used to immunize rabbits. The animals received 5 mg of rat globulins in complete Freund's adjuvant per injection initially in the footpads and subsequently by the intramuscular route.

Radioimmunoelectrophoresis. Immunoelectrophoresis was performed according to a modification of the procedure of Scheidegger (3) and radioimmunoelectrophoresis according to the technique of Yagi *et al*(4). Antigens were labeled with I^{131} by a modification of the method of Talmage *et al*(5) or by the procedure of McConahey and Dixon (6). The immunoelectrophoretic pattern was developed with a rabbit anti-rat globulin antiserum. After removal of excess antiserum by washing, I^{131}-labeled antigen (concentrations were 1 μg BGG N/ml or 0.5 μg BSA N/ml) was added to the central trough. The I^{131}-labeled antigen was allowed to diffuse into the agar for 24-36 hours and then the slides were washed for 2-4 days, dried and wrapped with a plastic film (Saran-wrap). Radioautographs were obtained by placing x-ray film (Kodak Industrial, Type KK) in contact with the slides.

Results. Rat anti-BSA and anti-BGG sera obtained at appropriate intervals after immunization were analyzed by RIEP. A representative immunoelectrophoretic pattern of serum from a single animal immunized with BSA-bentonite is shown in Fig. 1A. At the cathodal end the γG precipitin band is split into two parts indicating the presence of two components. On the basis of electrophoretic mobility these components were arbitrarily designated as γ_1 and γ_2. In all cases the γ_1 band was much fainter than the γ_2 band. In the anodal part of the γ-globulin region an intensely stained band, which probably is transferrin, and another band were also seen. Since the position of the latter band was similar to the γM band in immunoelectrophoretic patterns of human and rabbit sera, it was called γM in the present paper (Fig. 1A).

The presence of antibodies was shown by radioautography of the immunoelectrophoretic slides. When rats were immunized with BSA adsorbed to bentonite, both γ_1 and γ_2 antibodies were detected as shown in Fig. 1B. The γM radioactive band was not detected in either pooled sera or the sera of individual rats immunized with this antigen preparation; however, it was detected in sera obtained from rats hyperimmunized with BSA-FA (Fig. 1C). In addition to the 3 antibody components, *i.e.*, γ_1, γ_2 and γM, 2 unknown radioactive bands were detected in sera from

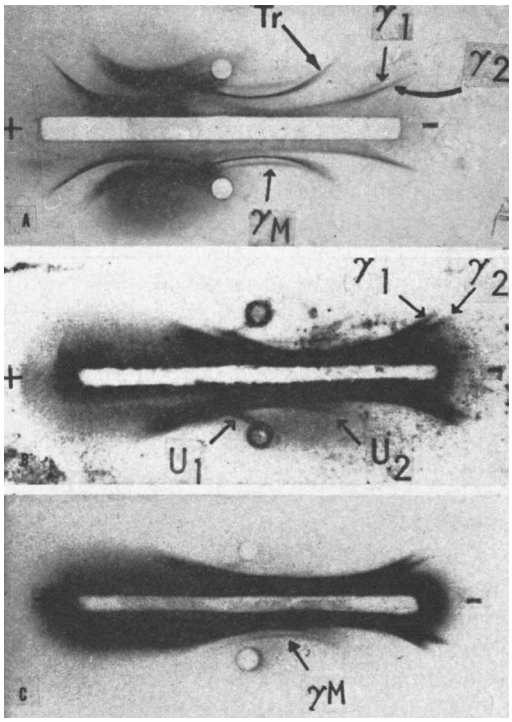

FIG. 1. Radioimmunoelectrophoresis of rat anti-BSA sera. A) Immunoelectrophoretic patterns of sera obtained from an animal immunized with BSA-bentonite. Top: 11 days after second injection, Bottom: 11 days after fourth injection. B) Radioautograph of (A). C) Pooled serum obtained from animals immunized with BSA-FA. Top: 5 weeks after third injection, Bottom: 2 weeks after fifth injection.

hyperimmunized animals (Fig. 1B). One of the bands, U_1, was located on the anodal side of the well in which the serum sample was applied. Electrophoretic mobility of the other component, U_2, was close to the γM component; however, it did not correspond to the γM precipitin band when the radioautograph was superimposed over the stained slide. Serum specimens from 6 individual rats immunized with BSA or BGG in saline were also analyzed. Only in the serum samples obtained from 2 rats (one immunized with BSA and the other with BGG) was the γ_1 radioactive band detected; the sera from the other 4 animals gave negative results. Normal rat serum also gave negative results.

To investigate whether the formation of radioactive bands is actually due to specific binding of radioactive antigen to the precipitin bands, the blocking effect of unlabeled BSA and heterologous proteins, which were human serum albumin (HSA), rabbit γ-globulin and normal rat serum, on the formation of radioactive bands was tested. Anti-BSA serum specimens which showed various radioactive bands in RIEP were subjected to immunoelectrophoresis. The slides were washed, and the appropriate unlabeled protein at a concentration of 20 to 100 μg/ml was applied to the central trough of each slide. After incubation of the slides for 24 to 36 hours at room temperature, I^{131}-BSA was added to the troughs of each slide. At a concentration of 20 μg/ml the unlabeled BSA significantly blocked the formation of all 5 radioactive bands and at a concentration of 100 μg/ml caused almost complete inhibition of the formation of the radioactive bands. The HSA even at a concentration of 100 μg/ml only slightly interfered with the formation of the radioactive bands. Use of normal rat serum or rabbit γ-globulin did not alter the radioactive band pattern. These findings indicate that the binding of I^{131}-BSA to the 5 bands, γ_1, γ_2, γM, U_1 and U_2, is due to the presence of antibody in these precipitin bands.

The sequence of the formation of antibodies in different immunoglobulins was studied by RIEP utilizing sera from individual animals and pooled sera. The radioactive intensities of various precipitin bands were scaled from negative to 3+ according to the darkness of the bands in the radioautographs. The data on serum samples from individual animals and pooled serum from animals immunized with BSA-bentonite were essentially the same. In general, the sequence of the antibody response was similar in BSA-bentonite and BSA-FA groups, and the data on pooled serum from these groups are presented graphically in Fig. 2. One week after the first injection of antigen, none of the precipitin bands showed radioactivity in any group. At 2 weeks the γ_1 radioactive band was detected in the serum obtained from animals receiving BSA-bentonite, and the γ_1 and γ_2 bands showed radioactivity at 5 weeks in both groups. After the injection of additional antigen, the intensities of radioactivity in the γ_1 and γ_2 bands increased (Fig. 2A, B). In addi-

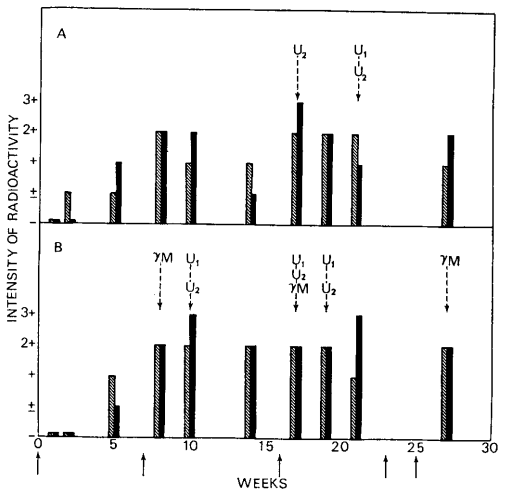

FIG. 2 Variation in levels of γ_1 and γ_2 type antibodies as measured by radioimmunoelectrophoresis and appearance of γM, U_1 and U_2 antibodies as a function of immunization. Solid arrows denote injection of antigen. Bars: shaded areas represent γ_1; solid areas represent γ_2. A) Animals received BSA-bentonite. B) Animals received BSA in complete Freund's adjuvant.

tion to the γ_1 and γ_2 antibodies, γM antibody was detected frequently at 17 weeks and occasionally at 8 and 27 weeks in serum from the group receiving BSA-FA. In both the BSA-bentonite and BSA-FA groups U_1 and U_2 were not detected before 10 weeks and usually were detected after the third injection of antigen. Two additional injections of antigen produced no radical change in the radioactivity of the γ_1 and γ_2 bands (Fig. 2A, B), and the γM, U_1 and U_2 bands were weak or not detected in these sera.

The sequence of the formation of antibodies in the animals receiving BGG-bentonite was similar to that of the BSA-bentonite group except that in the former group both the γ_1 and γ_2 radioactive bands were detected in serum obtained 1 week after the first injection of antigen. In some sera the γM radioactive band was detected at this time as well as in hyperimmune sera.

Discussion. Radioimmunoelectrophoresis of rat anti-BSA and anti-BGG sera revealed the presence of 5 radioactive bands. The formation of these bands is due to the binding of I^{131}-labeled antigen by antibody and not to nonspecific combination. This was demonstrated by specific blocking of attachment of labeled by unlabeled BSA and by negative results with normal rat serum in RIEP. That the radioactive bands actually represent the detection of antibody is supported by the fact that the intensity of radioactivity in these bands changed upon further immunization. Either very weak or no bands were detected in sera after the first immunization; whereas, with continued immunization the bands became very intense and decreased when immunization was discontinued.

In this report the 5 radioactive bands are tentatively designated as γ_1, γ_2, γM, U_1 and U_2. Although further studies are necessary to establish to which immunoglobulin classes they belong, the component labeled γ_2 is probably equivalent to what Arnason *et al*(1) called γG. It is not clear whether the γ_1 band in the present report corresponds to the component designated as γA by Arnason *et al*(1) or to the second γ-globulin band reported by Nussenzweig and Binaghi (2). If the latter alternative is correct, then the U_2 component may be γA. At present the identity of the U_1 component is undetermined. The possibility exists that the U_1 component may actually be antigen-antibody complexes rather than an immunoglobulin. However, this possibility is unlikely because the same band was observed in both anti-BSA and anti-BGG systems. In the latter case if the U_1 component were composed of antigen-antibody complexes, it should have a lower electrophoretic mobility.

The sequence of the immune response of rats immunized with BSA-bentonite, BSA-FA and BGG-bentonite seems to involve the formation of γ_1 antibody first and subsequently the appearance of γ_2 antibody. In the initial response, radioactivity of the γ_1 band was more intense that that of the γ_2 band, although the γ_1 band was much fainter in the stained slide. This indicates that the relative concentration of antibody in γ_1 was greater than in γ_2. Antibody of the γM class was found in hyperimmune serum obtained from animals immunized with BSA-FA but not in the initial response. However, occasionally γM was detected in

the initial response of animals immunized with BGG-bentonite. The unidentified components, U_1 and U_2, were only detected in serum of hyperimmunized animals. Immunization with BSA or BGG in saline produced only 2 sera from hyperimmunized animals which contained detectable antibody, and in both cases only γ_1 antibody was present. Recently, Dixon *et al*(7) reported that immunization of rats with keyhole limpet hemocyanin in saline resulted in a 19 S antibody response; whereas, administration of this antigen in incomplete Freund's adjuvant produced a predominantly 7 S antibody response. Thus it seems that the sequence of the appearance of these antibodies of different immunoglobulin class in the rat is in part dependent on the nature of the antigen and the method of immunization.

Summary. By radioimmunoelectrophoresis, antibodies associated with γ_1, γ_2 and γM globulins and 2 unidentified components, U_1 and U_2, were detected in rat antisera to BSA and BGG. When rats were immunized with these antigens adsorbed to bentonite or included in complete Freund's adjuvant, the sequence of the immune response in rats involves first the formation of γ_1 antibody followed upon further immunization by γ_2 antibody. Generally, γM and the unknown components, U_1 and U_2, only appeared in hyperimmune sera.

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Base Composition and Thermal Denaturation of DNA Isolated from *Anaplasma marginale*.* (32019)

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Although DNA and RNA occur in *Anaplasma marginale,* as confirmed by histochemical staining of infected erythrocytes (RBC)(1,2,3), quantitative data are not available on base composition and thermal denaturation. The present investigation was undertaken to isolate the nucleic acids and determine their base composition However, only DNA was found and a study of its thermal denaturation properties was also conducted.

Materials and methods. Experimental calves. Splenectomized calves were maintained and infected with *A. marginale* as previously described(4).

Collection of infected blood. When the infected RBC count reached 50 to 80%, as determined by Giemsa staining of blood smears, the calves were exsanguinated and the blood collected in heparin sodium solution (1,000 U.S.P. units/ml—0.3 ml/50 ml blood). The RBC were separated from plasma and buffy coat and washed 4 to 5 times with 0.9% NaCl solution by centrifuging at 1,060 $\times$ *g* for 20 min at 4°C.

Isolation of intact, cell-free Anaplasma *bodies.* Equal volumes of 0.9% NaCl solution containing 0.1 M sodium citrate were added to packed RBC and the suspensions, in 50-ml volumes, sonicated for 70 seconds

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