

DIFFERENCES IN THE ELECTROPHORETIC MOBILITIES
OF GUINEA PIG 7S ANTIBODIES OF
DIFFERENT SPECIFICITIES*

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Guinea pig 7S antibodies have been found to consist of two main populations, γ_1 and γ_2 , with different electrophoretic mobilities (1). A broad electrophoretic heterogeneity can be shown, however, to exist within each of these two populations, as in 7S immune globulins of other mammalian species. In contrast, myeloma proteins formed in malignant plasmocytes, which probably represent the proliferation of clones of cells, are notable for their more homogeneous physicochemical properties (2). These proteins which have many of the characteristics of individual antibodies migrate as different but distinct peaks in zone electrophoresis. It is reasonable to assign the differences in electrophoretic mobility between normal immune globulins and myeloma proteins to the cell populations involved in their synthesis and to consider that differences in electrophoretic mobility should also be detected among antibodies in relation to their specificity, if the proper antigenic stimulation were used.

In this study, the electrophoretic mobilities of guinea pig antihapten antibodies have been investigated and compared because previous studies on structure and specificity made with similar purified antibody preparations have demonstrated reproducible differences in their urea starch gel electrophoretic patterns, after reduction and alkylation (3). A number of specifically purified guinea pig 7S antibodies were compared in agar gel electrophoresis, and distinct and characteristic differences in mobility have been observed which have been shown to be related to their immunological specificities. Papain digestion (4) of these antibody preparations revealed that the electrophoretic mobility of one of the resulting fragments, which contains the antibody combining site (S fragment, reference 5), could be correlated with the mobility of the intact molecule.

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Materials and Methods

Antigens.—Ovalbumin, 2 times recrystallized, Worthington Biochemical Corporation, Freehold, New Jersey. Bovine gamma globulin ($B\gamma G$), bovine serum albumin (BSA), Armour and Company, Chicago. Poly-L-lysine (average mol wt 182,000), Mann Laboratories, New York. *Escherichia coli* (*E. coli*), 0111:B4, grown on synthetic medium as described (6). *E. coli* polysaccharide, prepared by alkaline hydrolysis and kindly provided by Dr. A. M. Staub, Institut Pasteur, Paris. Guinea pig γ_1 - and γ_2 -globulins were isolated from purified anti-DNP- $B\gamma G$ antibodies and separated by electrophoresis on geon block (7). The purity of the fractions was verified by immunoelectrophoresis.

Conjugated Antigens.—Protein conjugates were prepared according to the techniques described (8). 2,4-dinitrophenyl bovine gamma globulin (DNP- $B\gamma G$); 2,4-dinitrophenyl guinea pig albumin (DNP-GpA); 2,4-dinitrophenyl polylysine (DNP-polylysine) (9), *p*-iodobenzene-sulfonyl bovine gamma globulin (pipsyl- $B\gamma G$), benzoyl guinea pig albumin, pentachlorobenzoyl guinea pig albumin, arsanilic acid azo bovine serum albumin (AS-BSA) (10), arsanilic acid azo guinea pig albumin (AS-GpA).

Immunization.—Guinea pigs were initially immunized with the antigens emulsified in complete adjuvants (Difco Laboratories, Inc., Detroit) in the foot-pads and boosted intradermally with the antigens in saline. The dosages and immunization schedules have been described (1). A rabbit anti-whole guinea pig serum (anti-GPS) was prepared as described (1). This antiserum reacted with both γ_1 - and γ_2 -globulins. Specific antisera against guinea pig γ_1 immune globulin was prepared by injecting rabbits with the γ_1 -globulin, isolated by zone electrophoresis, emulsified in complete adjuvant.

Purification of Antibodies.—The sera were decomplexed prior to the isolation of the purified antibodies and the purification of antihapten and antiovalbumin antibodies carried out according to the techniques described (11, 12). Guinea pig anti-*E. coli* polysaccharide antibodies were isolated by precipitation at equivalence with the specific polysaccharide and the precipitate dissociated by 15 per cent NaCl (13).

Electrophoresis.—Electrophoresis in agar gel was performed as described (14) using a 1 per cent agar (bacto-agar, Difco Laboratories, Inc.) in barbital-HCl buffer, pH 8.2, 0.025 M. The same buffer, but 0.05 M, was used in the electrode jars. The separation was performed in 12-cm long plates for 3 hours at 25 ma and 100 to 150 v. Assigned differences in electrophoretic mobilities are based on runs made on the same plate or on different runs in which a standard preparation was included for comparison.

Immunoelectrophoresis.—Immunoelectrophoresis was performed by a modification of Scheidegger's technique (1, 15). Two per cent agar in a barbital buffer, pH 8.6, 0.075 M, was used.

Passive Cutaneous Anaphylaxis (PCA).—PCA was performed by the technique described (16). Each sample was injected into 3 animals, and the reactions read 20 minutes after challenge.

Hydrolysis with Papain.—The conditions were similar to those described by Porter (4). The digesting mixture contained 1 per cent antibody protein, 0.02 per cent papain in a 0.09 M Na phosphate buffer containing 0.05 M NaCl, 0.01 M cysteine, and 0.002 M sodium ethylenediamine tetracetate. The hydrolysis proceeded at 37°C for 18 hours.

FIG. 1. Electrophoretic patterns in agar gel of purified preparations of anti-DNP, anti-AS, and anti-pipsyl antibodies isolated from individual guinea pigs. Note the differences in mobility between the γ_2 and γ_1 anti-DNP and anti-AS antibodies. One preparation, AS-BSA 153, contains only the γ_2 antibody type, while another preparation, AS-BSA 146, contains mainly the γ_1 antibody type.

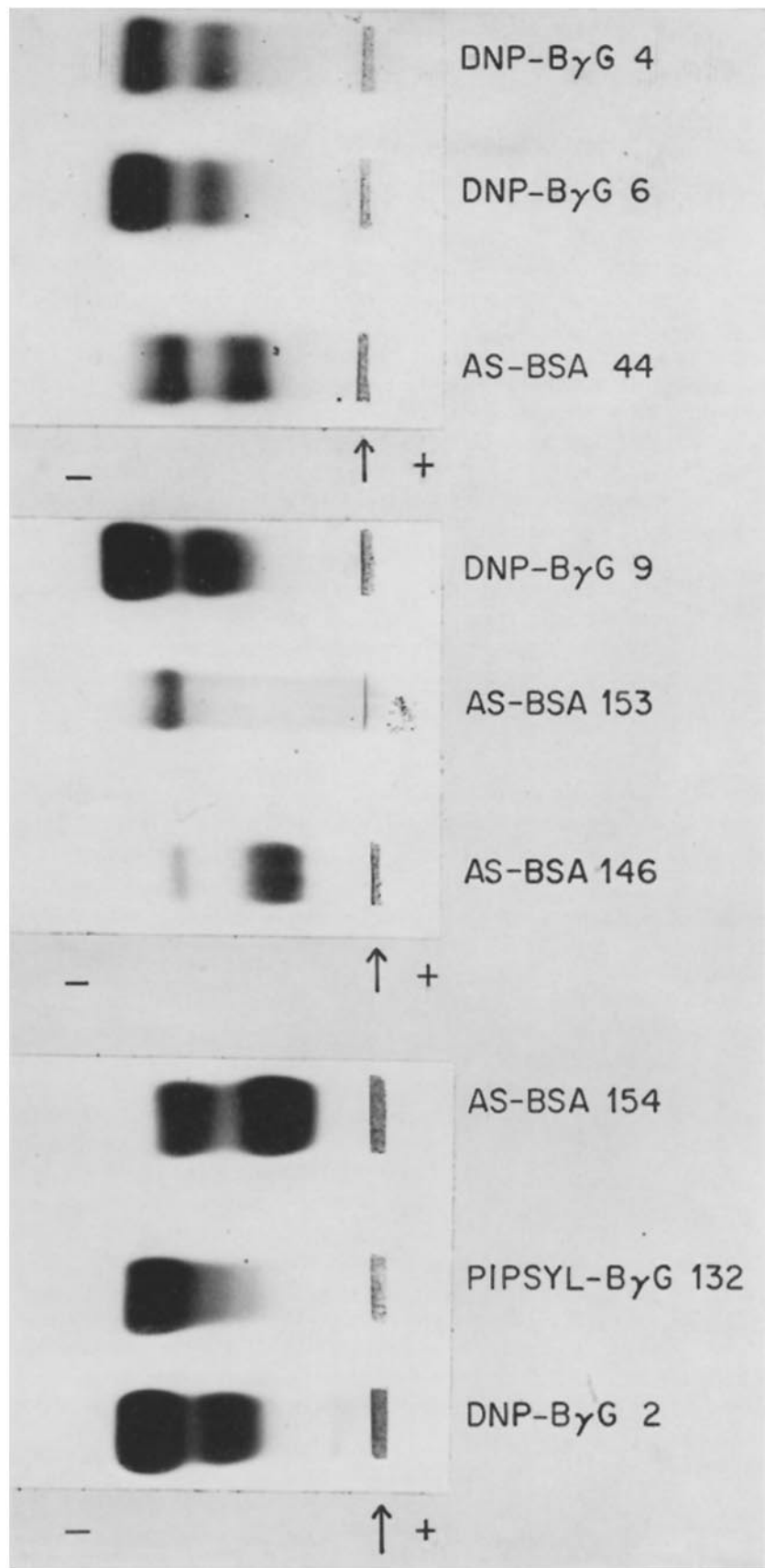


FIG. 1
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RESULTS

Electrophoretic Mobility of Antibodies of Different Specificities.—Purified antibodies bearing the same specificity, isolated from different guinea pigs, can be seen to have the same electrophoretic mobilities (Fig. 1). Some purified antibodies with different specificities vary in their electrophoretic mobilities. The experiments in Fig. 1 show that both γ_1 and γ_2 populations of anti-AS antibodies migrate more rapidly than the corresponding populations of anti-DNP antibodies. This difference in mobility has been found in every one of 9 anti-DNP and of 6 anti-AS purified antibodies examined from individual guinea pigs, irrespective of the antibody content of the original antisera. The relative proportion of γ_1 and γ_2 components in the preparations studied and in the original antisera varied. In some preparations one of the antibody types was almost absent; in such cases the mobility of the antibody type present (γ_1 or γ_2), coincided with that of the corresponding antibody population in other preparations with the same immunological specificity.

Two series of experiments were performed to ascertain whether the purification procedures could affect the mobility of the antibody preparations studied. Since the purified antibody preparations still contained some hapten used in the isolation procedure, which could not be removed by dialysis, the influence of specifically bound hapten on the electrophoretic mobilities of anti-DNP and of anti-AS antibodies was first investigated. Anti-DNP-B γ G and anti-AS-BSA guinea pig sera containing approximately 2 mg of precipitating antibody per ml, were mixed with suitable dilutions of ϵ -DNP-L-lysine and of chloracetyl tyrosine azo arsanilic acid in order to obtain a final hapten concentration of 5×10^{-5} M. This concentration is several times that required to bind all antibody sites available. Aliquots of the sera with and without hapten were subjected to electrophoresis in agar. The results are presented in Fig. 2. The migration of the band corresponding to the γ_1 antibody population has not been modified by the addition of the specific hapten. The migration of the γ_1 antibody population can be easily identified in immune sera, by comparing the electrophoretic patterns of the sera before and after precipitation with the corresponding antigen, because of the low concentration of γ_1 in normal nonimmune sera. In a second series of experiments, the mobilities of anti-AS-GpA and anti-DNP-B γ G antibodies were compared in the whole serum. As in the previous experiments, the mobilities of the γ_1 specific antibody populations only could be precisely localized, and similar to what was observed with purified antibody preparations, anti-AS γ_1 antibodies migrated faster than anti-DNP γ_1 antibodies (Fig. 3).

The electrophoretic mobilities of specifically purified guinea pig antibodies with other immunological specificities were also studied. Some antibodies such as anti-pipsyl (Fig. 1), anti-benzoyl, and antipentachlorobenzoyl had slow mobilities, comparable to those of anti-DNP antibodies, while antibodies

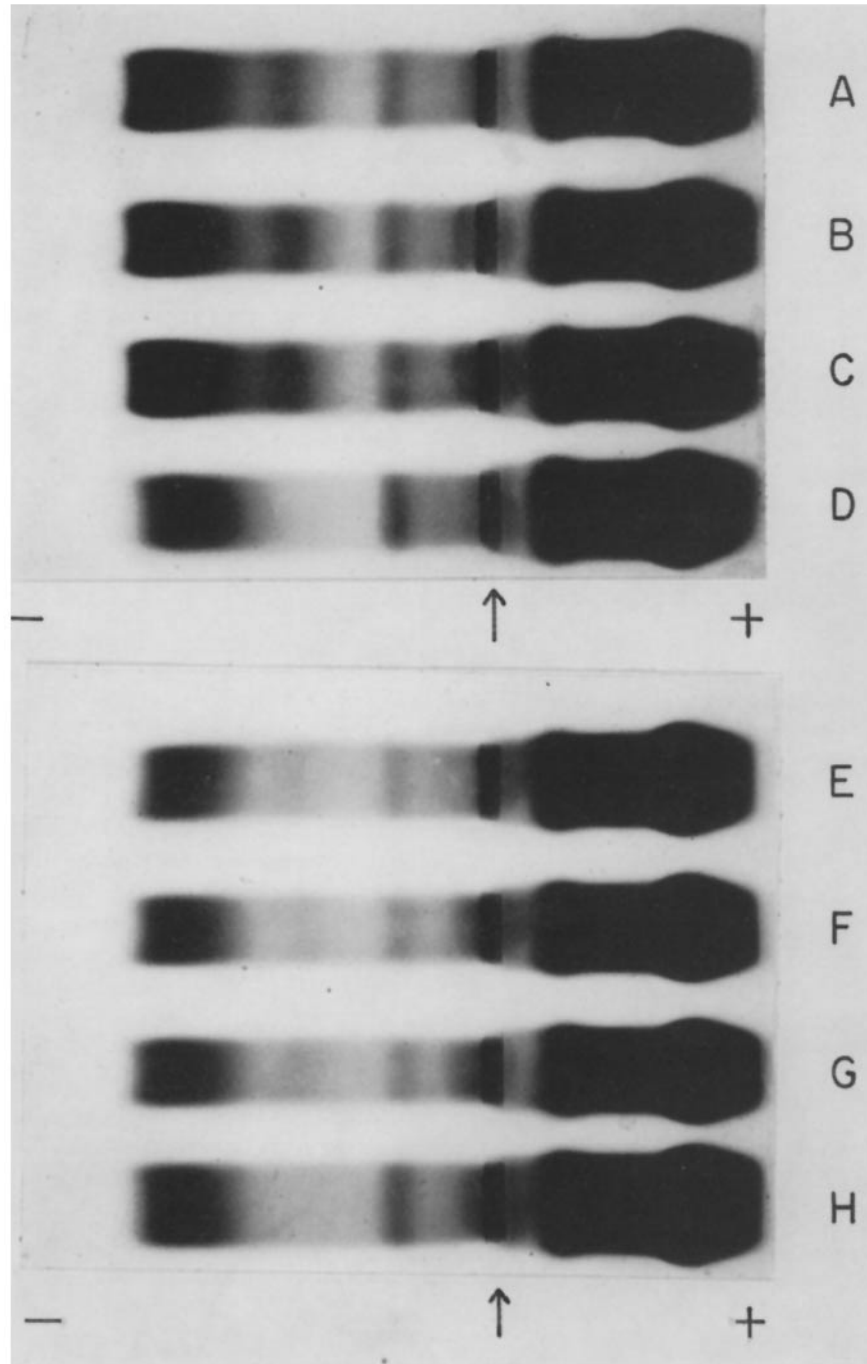


FIG. 2. Electrophoretic patterns in agar gel of guinea pig antisera. *A*, anti-DNP-B γ G 7 + chloracetyl tyrosine azoarsanilic acid, 5×10^{-5} M; *B*, anti-DNP-B γ G 7 + ϵ -DNP-L-lysine, 5×10^{-5} M; *C*, anti-DNP-B γ G 7 + saline; *D*, anti-DNP-B γ G 7 absorbed with DNP fibrinogen; *E*, anti-AS-GpA L3 + ϵ -DNP-L-lysine, 5×10^{-5} M; *F*, anti-AS-GpA L3 + chloracetyl tyrosine azoarsanilic acid, 5×10^{-5} M; *G*, anti-AS-GpA L3 + saline; *H*, anti-AS-GpA L3 absorbed with AS fibrinogen.

against the succinilanic acid hapten, or against ovalbumin, or against *E. coli* polysaccharide (Fig. 4) had a mobility similar to that of anti-AS antibodies.

Influence of the Carrier Protein on the Electrophoretic Mobility of Purified Anti-Hapten Antibodies.—Anti-DNP antibodies specifically purified from sera of guinea pigs immunized with DNP-B γ G, DNP-GpA, and DNP-polylysine have the same electrophoretic mobility (Fig. 5). Similarly, no differences in mobility could be detected between purified anti-AS antibodies prepared from anti-AS-GpA and anti-AS-BSA sera. Variations of the carrier protein appeared not to affect the mobilities of the anti-hapten antibodies.

Electrophoretic Mobility of Anti-DNP and Anti-AS γ_1 Antibodies Produced in the Same Guinea Pig.—These experiments were designed to explore whether an individual guinea pig immunized with two suitably chosen conjugated antigens produced specific antibodies with different electrophoretic mobilities. AS-GpA and DNP-B γ G were selected as antigens because previous experiments have shown that the antibodies produced against these two conjugates possessed different net charges.

Guinea pigs were initially immunized with 0.4 mg of AS-GpA emulsified in complete adjuvants injected in their four foot-pads. They were boosted twice, a week apart, with 4 intradermal injections of 0.1 mg AS-GpA. In the 3rd week the animals were immunized intramuscularly in the back of the neck, with 0.4 mg of DNP-B γ G emulsified in complete adjuvants. For the following 2 weeks they were twice boosted intradermally simultaneously with 0.2 mg of DNP-B γ G and 0.2 mg of AS-GpA. The animals were bled 6 days after the last injection.

Preliminary precipitin tests performed on the sera with both immunizing antigens showed that they contained amounts of antibodies too small to allow isolation. The electrophoretic mobilities of γ_1 anti-DNP and anti-AS antibodies were therefore investigated by assaying the fractions cut from agar gel electrophoresis separations made with these sera for their ability to transfer PCA. (17).

After the electrophoresis had been carried out, the agar was cut into fractions 2 mm wide starting at the origin. The agar cuts were placed into separate tubes and frozen in an alcohol-solid CO₂ bath for 30 minutes to break the gel. After thawing, the proteins were extracted for 24 hours at room temperature with 1 ml of 0.15 M NaCl per tube. After centrifugation and serial dilution of the eluates, the samples to be assayed were each injected intradermally into 6 guinea pigs. Three of these animals, after a 4 hour latent period of sensitization, were challenged with 250 μ g of DNP-BSA and 3 with 250 μ g of AS-GpA. The highest dilutions causing positive PCA reactions were recorded, and the PCA titer for a given fraction was considered as the average of the values obtained in 3 guinea pigs.

The results of one such experiment with one antiserum is presented in Fig. 6. A definite difference in the electrophoretic mobility of the PCA activities specific for the DNP and for the AS determinants can be observed. Since the PCA activity of a guinea pig serum is a property of the γ_1 antibody type (17), the serum investigated contained two γ_1 antibodies with different electrophoretic

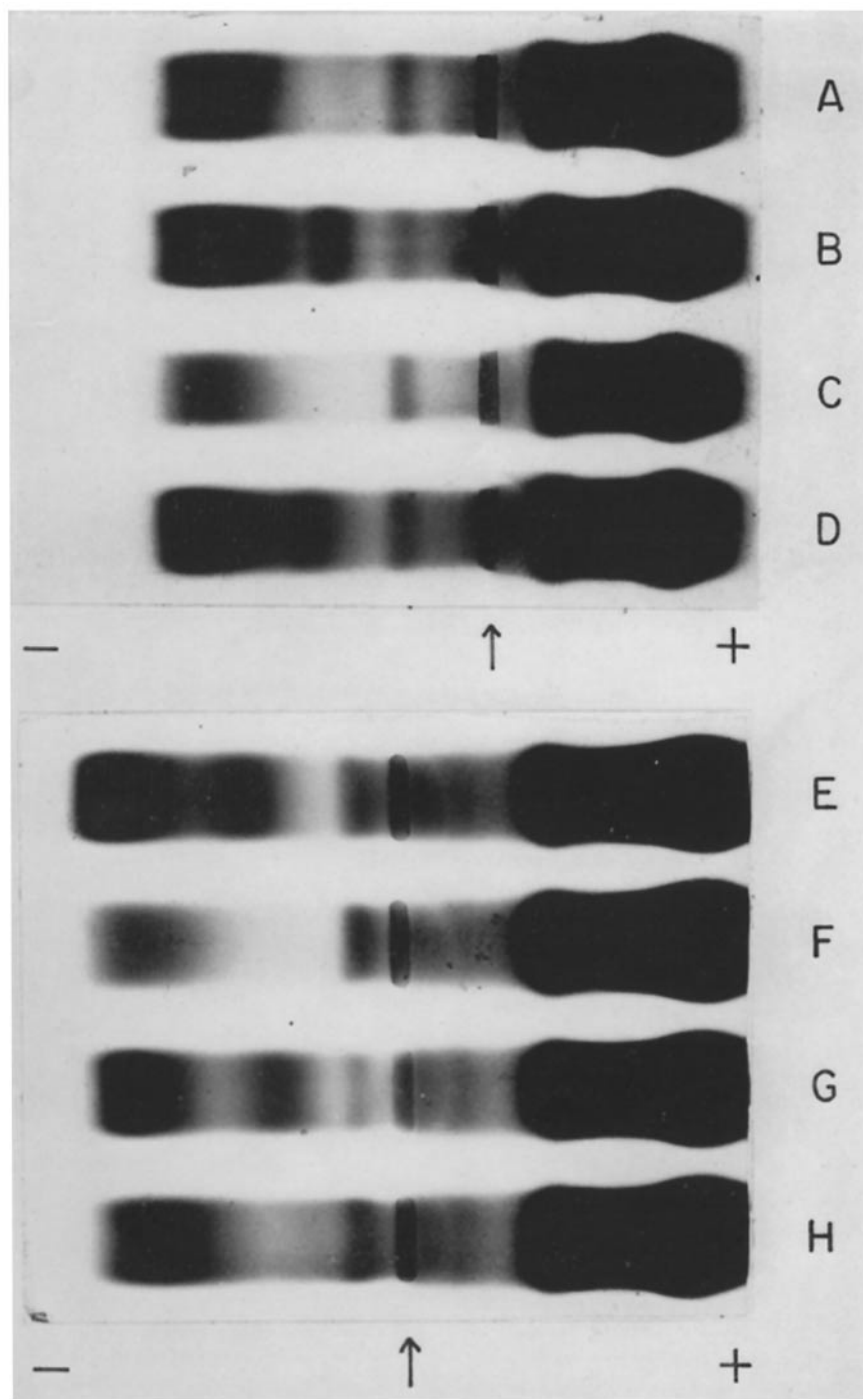


FIG. 3. Electrophoretic patterns in agar gel of guinea pig antisera. *A*, anti-AS-GpA L1 absorbed with AS fibrinogen; *B*, anti-AS-GpA L1 non-absorbed; *C*, anti-DNP-B γ G 7 absorbed with DNP fibrinogen; *D*, anti-DNP-B γ G 7 non-absorbed; *E*, anti-DNP-B γ G 11 non-absorbed; *F*, anti-DNP-B γ G 11 absorbed with DNP fibrinogen; *G*, anti-AS-GpA 16 non-absorbed; *H*, anti-AS-GpA 16 absorbed with AS fibrinogen.

mobilities. A total of three sera from different animals was examined by this technique. In two of the three experiments performed, a definite difference in mobility was observed between the γ_1 anti-DNP and anti-AS antibodies produced by an individual animal. The third experiment was not successful, the PCA activity of the serum being too low to allow a satisfactory assay.

Correlation Between the Electrophoretic Mobility of Purified 7S Antibodies and Their S Fragments Obtained by Papain Digestion.—The papain digestion of purified guinea pig antibodies, consisting of a mixture of the γ_1 and γ_2 types, yields at least three distinct products as revealed by the immunoelectrophoretic analysis (Fig. 7). One of them (S) has a slower electrophoretic mobility and is a common constituent of both γ_1 and γ_2 populations (5) and contains the antibody combining site (18). The other two components (F_1 and F_2) obtained

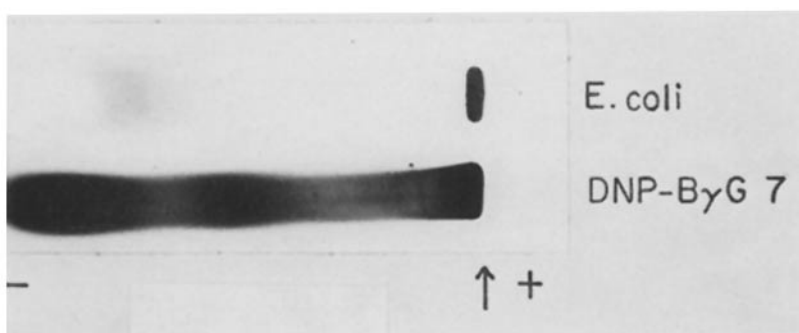


FIG. 4. Electrophoretic patterns in agar gel of a purified preparation of anti-DNP antibodies isolated from the serum of an individual guinea pig and of a preparation of anti-*E. coli* polysaccharide antibodies isolated from a pool of guinea pig anti-*E. coli* antisera.

respectively from γ_1 - and γ_2 -globulins, have distinct electrophoretic mobilities and appear to differ antigenically (18). The question arises whether the electrophoretic mobilities of the S fragments obtained from antibody of different specificity vary in their electrophoretic mobilities as do the original antibodies themselves. Fig. 7 illustrates that papain digests of anti-AS-BSA antibodies and of anti-DNP-B γ G yield S fragments with different electrophoretic mobilities. In contrast, the F_2 fragments obtained from these antibodies have the same mobilities. The difference in mobilities of the S fragments from anti-AS and anti-DNP antibodies is best observed by comparing in immunoelectrophoresis the mobilities of the S and F_2 components from antibodies of a given specificity. In the case of anti-AS antibodies, the F_2 and S components have

FIG. 5. Electrophoretic patterns in agar gel of purified preparations of anti-DNP and anti-AS antibodies isolated from pools of guinea pig sera obtained from animals immunized with DNP-B γ G, DNP-GpA, DNP-polylysine, AS-GpA, and AS-BSA.

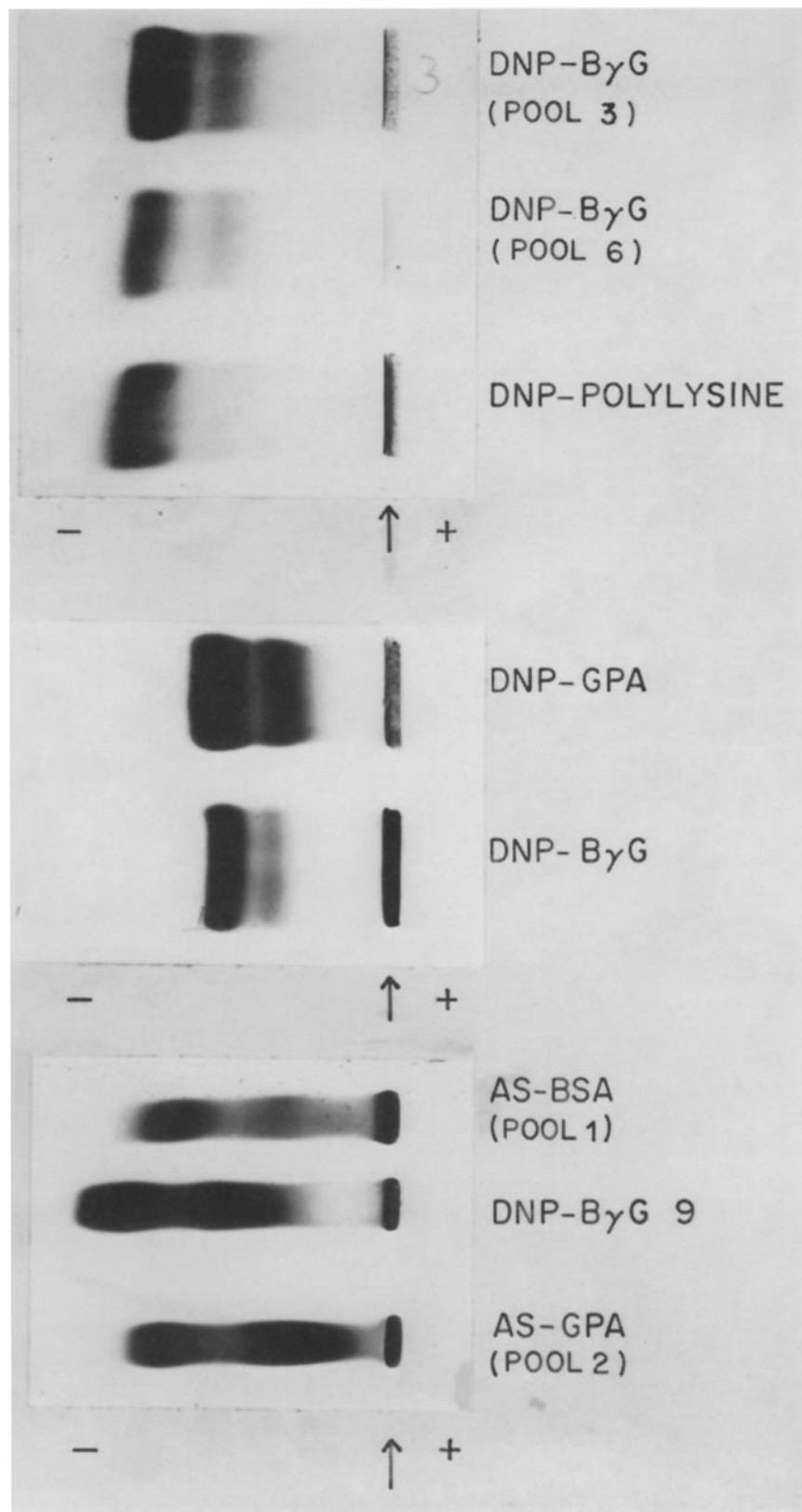


FIG. 5
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overlapping mobilities; papain digests of anti-DNP antibodies yield S and F₂ fragments with different, though overlapping, electrophoretic mobilities.

DISCUSSION

The results of these experiments illustrate the influence of the specificity of the immunizing antigen on the electrophoretic mobility of 7S guinea pig antibodies produced. Antibodies produced in several individual guinea pigs against the same hapten have the same electrophoretic mobility irrespective of the protein carrier used in immunization. This is a property of both γ_1 and γ_2

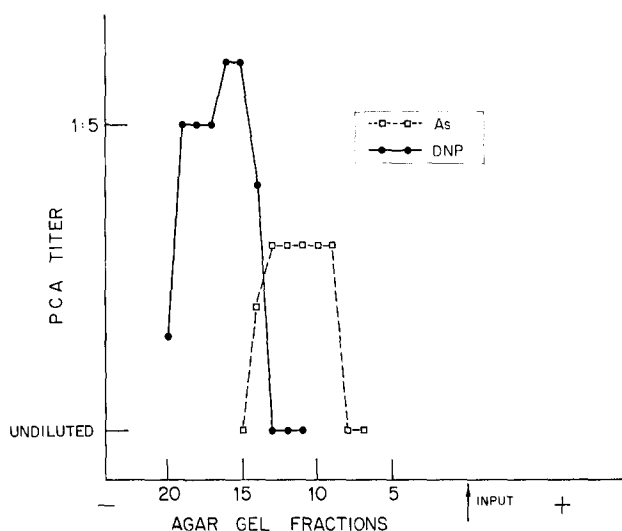


FIG. 6. PCA activities specific for the DNP and AS determinants in fractions eluted from agar gel 2-mm strips of an electrophoretic separation of an antiserum obtained from a guinea pig immunized with DNP-B γ G and AS-GpA.

guinea pig antibody types. In contrast, immunization of the same or different guinea pigs with non-cross-reacting conjugated haptens such as arsanilic acid and 2,4-dinitrophenyl induces the formation of specific antibodies with characteristically different electrophoretic mobilities. These findings are in agreement with the earlier observations of Cann *et al.* (19) who showed that antiphenylarsonate and anti-bovine gamma globulin antibodies produced in rabbits by immunization with *p*-azophenylarsonate coupled to bovine gamma globulin could be separated by electrophoretic convection. Other attempts to detect differences in net electrical charge between antibodies with different specificities and normal γ -globulin have been less successful (20, 21), possibly due to the large heterogeneity of the antibody populations studied by these investigators. Differences in electrophoretic mobility could only be demonstrated easily when antibodies directed against appropriate haptenic specificities

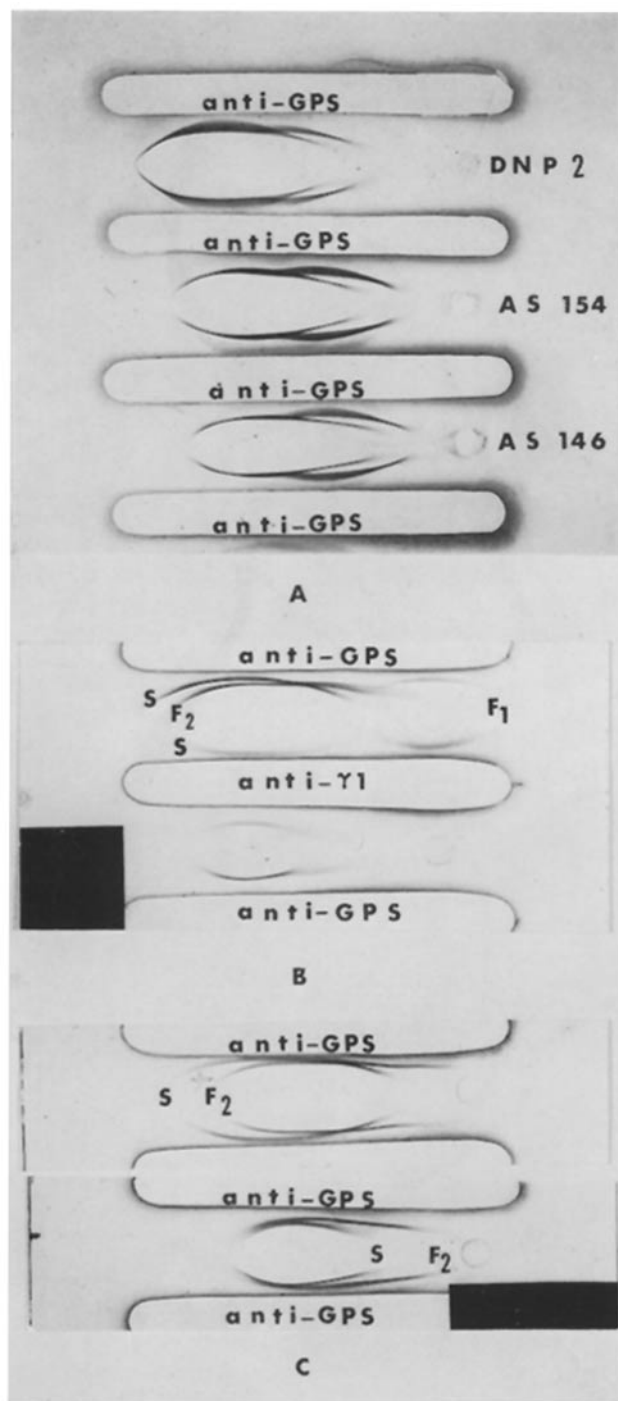


FIG. 7. Immunoelectrophoretic patterns of purified guinea pig antibody preparations before and after hydrolysis with papain. *A*, anti-AS-BSA and anti-DNP-B γ G, undigested preparations, developed with a rabbit antiserum against guinea pig serum (anti-GPS). *B*, top: anti-DNP-B γ G antibodies digested with papain. Bottom: the same preparation undigested. Immunoelectrophoretic patterns were developed with anti-GPS or with a rabbit antiserum against guinea pig γ_1 globulin (anti- γ_1). *C*, guinea pig antibody preparations digested with papain. Top: anti-DNP-B γ G. Bottom: anti-AS-BSA. Both immunoelectrophoreses were carried out on the same plate. The absence of a prominent F $_1$ line is due to the low γ_1 concentration of these preparations.

were compared with each other or with antiprotein or antipolysaccharide antibodies. But even antihapten guinea pig antibodies with a single specificity possess some degree of electrophoretic heterogeneity which can make the demonstration of these differences difficult, particularly if purified antibodies are not employed and if the separating power of the electrophoresis technique used is not very high. The separation obtained by zone electrophoresis in agar proved more efficient in this respect than the separation by starch block electrophoresis, which explains our earlier failure to detect differences in electrophoretic mobility between anti-arsanilic acid and anti-DNP guinea pig γ_1 antibodies (17).

Control experiments performed with whole antiserum in comparison with the purified antibody isolated from it, have shown that the isolation procedures used in this study did not affect the electrophoretic mobility of the antibodies. In this respect, the study of the electrophoretic mobility of the guinea pig γ_1 antibody type proved very useful, as the serum concentration of normal γ_1 -globulin is very low as compared to the amount of specific γ_1 antibody present in hyperimmune sera, which allows these antibodies to be easily identified in electrophoretic patterns made with whole sera.

The differences in the net electrophoretic charge between the antibodies with different specificities, within a given γ_1 or γ_2 type, paralleled roughly the mobility of their S fragment produced by papain digestion. This finding is in agreement with the observations made with fractions isolated from human and mouse γ -globulins in which the electrophoretic mobility of the S fragment could be closely related to the mobility of the parent molecule (22-25). It would appear therefore that the F fragment contributes primarily to the electrophoretic properties which characterize the antibody type, and the S fragment contributes to the electrophoretic heterogeneity within each type, in relation to the antibody specificity.

The differences in the mobility of the S fragments of antibodies of varying specificities could be the result of differences in their primary structure or of the folding of the L and H polypeptide chains and of the relations between these chains. The differences in amino acid composition which have been shown to exist between antibodies of different specificities (26) as well as among fractions of whole γ -globulins or fragments obtained from these fractions (27, 28), favor the first possibility.

The assignment of the electrophoretic heterogeneity within a given antibody type mainly to the S fragment, which contains the antibody combining site, indicates that the electrophoretic mobility and the antibody specificity can be determined by the same molecular structure. There is general agreement that the L chains are contained in the S fragment (3, 29, 30) and also mounting evidence that both L chains (3, 31) and a piece of the H chain present in the S fragment (30, 32) are involved in the acquisition of antibody specificity (33).

While no data is as yet available on the crucial issue of the heterogeneity of H chains obtained from antibodies of a given type but with different specificities, there are marked variations in the electrophoretic mobility and pattern of dissociated L chains from guinea pig antibodies of different specificities (3). No direct relationship could, however, be established between the electrophoretic patterns of reduced and alkylated guinea pig antibodies at pH 3.0 and the mobility of the parent molecule at pH 8.2. This relationship could hardly be expected in view of the extensive denaturation involved in the reduction process performed in 8 M urea which favors the maintenance of the unfolded configuration.

The variations of electrophoretic mobility between antibodies with different specificities, as well as the recent findings of individual antigenic specificity of isolated antibodies (34) are additional properties to be added to the extensive list of common characteristics between antibodies and myeloma proteins.

SUMMARY

The electrophoretic mobilities of guinea pig γ_1 and γ_2 antibodies bearing different specificities were compared in agar gel at pH 8.2. Specifically purified antibodies bearing the same immunological specificity showed the same electrophoretic mobility, but significant differences in mobility were observed when antibodies with certain selected different specificities were compared. The specificity of the carrier protein appeared not to affect the mobilities of antihapten antibodies. Differences in mobility have also been shown between γ_1 antihapten antibodies produced by individual guinea pigs immunized concomitantly with 2,4-dinitrophenyl bovine gamma globulin and arsanilic acid azo guinea pig albumin. The differences in the net electrophoretic charge between antibodies with different specificities roughly paralleled that of their S fragments produced by papain digestion.

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