

Evaluation of Fragmented Antibody Inhibitory Potency

Considerable insight into the structure of antibodies has been gained through the utilization of enzymatic degradation^{1,2}. The Fab immunoglobulin fragments, produced by papain digestion, are univalent possessing only one antigen combining site³. The Fc fragments do not possess antigen binding capabilities. Since the Fab fragments combine but do not precipitate in the presence of specific antigen, fragmented antibody strength can be assessed only by indirect methods. The present study concerns new approaches in evaluating the inhibitory power of fragmented antibodies. Human chorionic gonadotrophin (HCG) and its homologous antisera were utilized since immunological studies on this system are well established in our laboratory.

Materials and methods. New Zealand white rabbits were immunized with HCG (5000 IU, Parke Davis & Co.) in complete Freund's adjuvant via multiple site subcutaneous injections⁴. The serum, obtained 6-8 weeks postimmunization, will be referred to as anti-HCG whole antiserum (WA). In addition, commercial anti-HCG serum⁵ was fractionated with DEAE to obtain the IgG fraction and then digested with papain following the method of PORTER¹. The digested globulin (Fab and Fc) fragments were subjected to Sephadex G-200 gel filtration to remove undigested IgG molecules. Protein concentrations were determined by the Folin-phenol technique⁶. Immunoelectrophoretic analysis revealed the presence of a fast and a slow moving component in the γ -globulin region. As a control, normal rabbit serum was fractionated to obtain the γ -globulin fragments and digested in the same manner. The presence of antibody in the degraded product could not be detected by Ouchterlony immunodiffusion.

HCG (Antuitrin-S, 1700 IU/mg, Parke Davis) was labeled with ¹²⁵I by the method of HUNTER and GREENWOOD⁷. Sephadex G-100 gel filtration was employed for removal of the free ¹²⁵I and the resultant product rechromatographed on G-200 in accordance with the method of BAHL⁸. The radiohormone contained 2.14 μ g protein/ml and 69.9 μ C/ μ g suspended in 0.05M phosphate buffer stabilized with 0.75% bovine serum albumin. The free iodine content of the stored radiohormone was monitored periodically by thin layer radiochromatography.

The antigen combining potency of the fragmented antibody (FA) was estimated by a block titration using hemagglutination inhibition (HI). First, varying dilutions of FA were prepared ranging from 1:3 to 1:6144; then 1 drop (25 λ) of WA was added to each dilution. The WA was added at the following dilutions - 1:300, 350, 400, 450, 500, 550, 600, and 650. To each of these mixtures was added 25 λ of sheep tanned red blood cells sensitized to HCG (Parke Davis, 1700 IU/mg)⁹. After 3 h at room temperature or overnight refrigeration, the results were read and the 50% dilution endpoint (HI₅₀) estimated.

Standard radioprecipitation curves of anti-HCG WA versus ¹²⁵I-HCG were obtained by methods previously described^{10,11}. The HI₅₀ endpoint determined the WA concentration for use in radioprecipitation inhibition (RPI). Constant amounts of WA-HI₅₀ were added to varying dilutions of FA to determine the power of fragmented anti-HCG to inhibit radioantigen precipitation. To each of the mixtures was added 0.5 cm³ of ¹²⁵I-HCG (1:50 dilution) and the mixture incubated at 37°C for 1 h, then overnight at 4°C. 24 h later, 50 λ of non-diluted goat anti-rabbit γ -globulin was added to each tube. Following incubation and washing, the radioprecipitates were counted in an autowell γ -counter. Results were plotted as the inverse of the cpm in the sediment versus FA concentration.

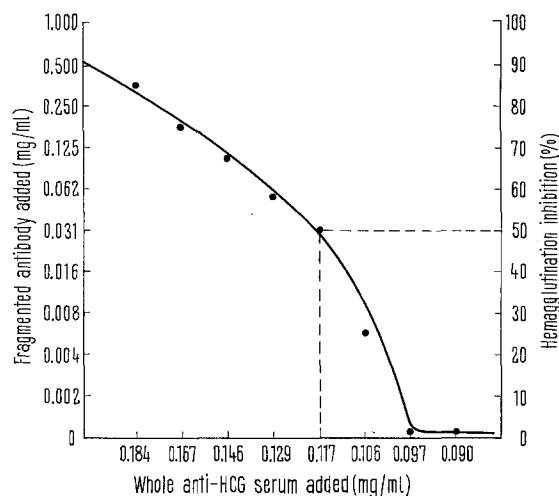


Fig. 1. The inhibitory power of fragmented anti-HCG in competition with antiserum for HCG sensitized red blood cells is demonstrated by hemagglutination inhibition. A ratio of inhibitor (fragmented antibody) to antibody (whole antiserum) of 2.6:1 was obtained at the 50% inhibition point.

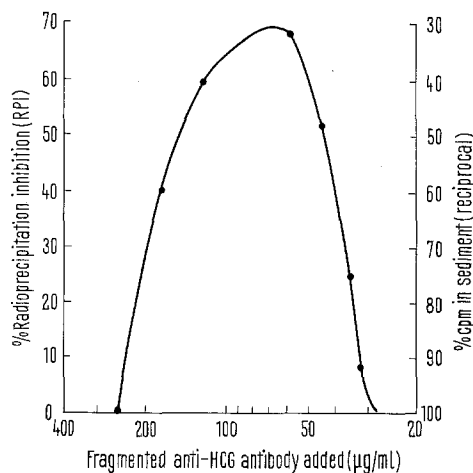


Fig. 2. The power of fragmented anti-HCG to compete with whole antiserum for radioantigen is shown by the percent of radioprecipitation inhibition (RPI). The RPI curve may represent the precipitin curve of the fragmented antibodies when plotted as the inverse of the cpm in the sediment.

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Results and discussion. The quantitative estimates of the inhibitory power of fragmented anti-HCG are shown in Figure 1. The fractionated antibody has a proportionally similar number of antigen binding sites to the intact antiserum except for dilution differences resulting from preparative procedures. Fragmented anti-HCG added to decreasing amounts of WA resulted in the competition curve shown. It can be seen that approximately proportional amounts of FA to WA were needed for inhibition of hemagglutination. The HI_{50} occurred at a whole antiserum protein concentration of 0.117 mg/ml (1:500 dilution). Assuming a minimum of 10% globulin in the whole antiserum, a ratio of inhibitor to antibody of 2.6:1 was obtained at this point.

The HI_{50} value obtained from Figure 1 dictated the WA concentration (0.117 mg/ml) for use in radioprecipitation inhibition. The power of fragmented anti-HCG to compete with WA for radioantigen is shown in Figure 2. The inverse of the cpm in the sediment relates the radioprecipitation inhibition throughout a spectrum of FA concentrations titrated against constant amounts of WA. Maximum RPI (69%) occurred at a FA protein concentration of 60 μ g/ml (1:50 dilution). In actuality, the RPI curve represents the precipitin curve of the fragmented antibodies when plotted as the inverse function. The cpm in the sediment are representative of the intact antiserum (WA) interaction with radioantigen since goat anti-rabbit globulin does not readily precipitate the fragments. Cor-

respondingly, the Fc fragments do not react with the antigen¹; hence, the curve shown in Figure 2 represents the combined precipitin curves of the 2 Fab fractions.

The inhibitory potency of fragmented antibodies may be titered by hemagglutination inhibition to determine optimum FA:WA ratios and the HI_{50} index. These data are then employed to measure the immunoprecipitation inhibition capabilities of fragmented antibody. A precipitin curve can be obtained concomitantly with a curve of the percent of inhibition. Precipitin curve analysis reveals the quantities of specific FA present in the system, the zone of equivalence, areas of antibody and antigen excess, and the molecular combining ratios between antigen and antibody. Thereby, quantitative studies involving fragmented antibodies may be more readily performed.

Zusammenfassung. Das Hemmpotential der Antikörperfragmente bei Kaninchen wird mit neuer Methode demonstriert.

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Dextran Induced Changes in the Electrophoretic Mobility of Rat Bone Marrow Erythrocytes

Large macromolecules like dextran should be expected to influence cellular electrophoretic mobility and this in turn to perhaps produce changes in the cellular metabolic activity¹. The possibility that cell charge might alter cellular activity has been previously suggested². It was of interest, therefore to determine the influence that various dextran combinations had on cellular electrophoretic mobility, and to determine if electrophoretic mobility could be correlated with an earlier work on oxygen consumption³.

Materials and methods. Male Holtzman rats, 42 days old, were used in all experiments. Animals were killed by a sharp blow to the base of the skull and cells were obtained from the bone marrow of the 2 femora and tibiae. 10 animals were used, and 10 measurements of 10 cells were made from each animal.

Dextran solutions of 1, 3, and 5% were prepared with 39,500 molecular weight dextran (lot 8, 687, Pharmacia, Sweden). One, 3, and 5% dextran solutions were prepared using 139,000 mol. wt. clinical grade H dextran (lot H 1158, Pharmachem, Pennsylvania) and 228,000 mol. wt. clinical grade HH dextran (lot HH 82262, Pharmachem). The dextrans were put into solution in Tyrode's medium. The osmolarity of the Tyrode's medium and different dextran solutions was adjusted to 310 mOsm/l with NaCl and the pH adjusted to 7.35.

Determinations of cellular electrophoretic mobility of mature erythrocytes were made using a microelectrophoresis apparatus modified from that described by BERNSTEIN et al.⁴.

Results. Electrophoretic mobility of mature erythrocytes in the various dextran solutions, isologous serum, and Tyrode's medium is shown in the Figure. Cellular electrophoretic mobility is expressed in relative practical units. Determinations of cellular electrophoretic mobility was limited to mature erythrocytes because this was the

only cell type that could be identified with certainty in unstained bone marrow cell preparations using phase and darkfield microscopy.

Erythrocytes of bone marrow suspended in isologous serum had the lowest relative electrophoretic mobility whereas 5% 39,500 molecular weight dextran medium produced the greatest cellular relative electrophoretic mobility.

Similar electrophoretic mobilities were displayed by cells in tyrodes and the 1% 228,000 molecular weight dextran suspending medium. It was found that as the molecular weight of the dextran suspending medium was increased, the cellular mobility decreased. However, as the concentration of a specific molecular weight dextran was increased the cellular mobility increased.

Discussion. The data reported here indicates that cells incubated in medium containing dextran have a greater cell mobility than do cells incubated in either Tyrode's or serum suspending medium. It is of interest to note that in the artificial media combinations the cell mobility is increased from 2 to 10 times as much as that found in serum. Most probably the cellular electrophoretic mobility of the cells in serum and in the artificial suspending medium is the result of different factors. The factors that need to be considered here are: Agglutination, sedimentation velocity, surface charge, and the dielectric of the suspending medium.

Cellular aggregation occurs with dextrans of 70,000 and greater molecular weight, whereas erythrocyte disaggrega-

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