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Immune Reactions in Polysaccharide Media

1. The Effect of Dextran on the Reaction between Iodine-125 Labelled Human Serum Albumin and γG-Globulin from Rabbit Anti-albumin Sera

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When dextran is added to a system containing ^{125}I-labelled human serum albumin and the γG-fraction from rabbit anti-albumin sera precipitation is enhanced, especially in the region of antigen excess. Simultaneously the amount of soluble antigen-antibody complexes in the supernatant is decreased. The degree to which precipitation is increased depends on the concentration and the molecular weight of the added dextran. The effect can be explained in terms of a steric exclusion of the antigen-antibody complexes from the domain of the polysaccharide molecules.

In 1961 Ogston and Phelps [1] presented data on the partition of various solutes between buffer solutions and solutions containing hyaluronic acid. They found that the solutes distributed unequally in favour of the buffer, and attributed the effect to an exclusion of the solutes from the polysaccharide domain. The degree of exclusion from hyaluronic acid depended on the molecular size of the solute and the concentration of the hyaluronic acid, but was independent of ionic strength and pH. The experiments indicated that the exclusion was essentially steric in nature.

Laurent has described this exclusion effect in a series of reports. He found [2,3] that the presence of another polysaccharide, dextran, significantly decreased the solubility of a number of proteins under salting-out and salting-in conditions. The decreases in solubility observed were correlated with the size of the protein and the concentration of dextran. The decrease in solubility of serum albumin was independent of the degree of polymerisation of dextran (mol.wt. $>$ 150 000), the pH, the absolute salt concentration, and the absolute protein concentration.

As the decrease in solubility caused by the presence of dextran depends on the size of the protein — the larger the protein, the larger the effect — the solubilities of protein complexes should be affected much more than are the

solubilities of their individual components. Antigens and antibodies can combine, to form soluble antigen-antibody complexes, especially when antigen is present in excess. The purpose of this investigation was to study the effect of polysaccharides on the behavior of soluble antigen-antibody complexes. A preliminary communication has been published earlier.[4]

EXPERIMENTAL

Antigen. Human serum albumin (lot No. Rd 0 23) was kindly supplied by AB Kabi, Stockholm, Sweden. Immunoelectrophoretic analysis showed that the albumin was contaminated with some minor components which migrated in the region of the α- and β-globulins. These were removed by vertical-column zone electrophoresis [5] with cellulose [6] as the supporting medium, as described by Björk.[7] 1.00 g of serum albumin was dissolved in 10 ml of 0.05 M tris buffer, pH 8.0, and the solution was introduced into the column 10 cm below the surface of the bed. Electrophoresis was performed in the same buffer for about 40 h at 0°C with a current of approximately 50 mA and a potential gradient of 5 V/cm. Elution was performed at 4°C with a flow rate of 30 ml/h. The eluate was collected in 7.5 ml-fractions, which were analysed at 280 mμ in a Beckman DU spectrophotometer. The protein fractions were pooled and concentrated by ultrafiltration in collodion bags [8] using a negative pressure of 500 mm Hg. The albumin was further purified by gel filtration on Sephadex G-200.[9] The concentrated main fraction from the zone electrophoresis was layered on the top of a 4 × 103 cm column, beneath a layer of buffer by means of a syringe. The buffer used was 0.1 M Tris, pH 8.0, in 0.2 M sodium chloride containing 10 % (v/v) of a saturated solution of 5,7-dichloro-8-quinolinol (a chelating agent). The flow rate was 15 ml/h and the eluate was collected in 7.5 ml fractions. Their optical densities were measured at 280 mμ. Four peaks were obtained, the last of which contained most of the material. The fractions corresponding to the main peak were pooled, concentrated, and analysed by immunoelectrophoresis. No contaminants were observed.

Iodination. The purified albumin was iodinated according to the iodine monochloride method developed by McFarlane.[10] Iodine-125 [11] free from reducing agent was used (Radiochemical Centre, Amersham, England). The McFarlane procedure introduces approximately one atom of iodine into each protein molecule. When 100 mg albumin was treated with 1 mC, iodine-125, 67 % of the latter became attached to the protein. This corresponds to one atom of iodine-125 per 3 000 molecules of iodinated albumin. The protein was separated from inorganic material used in the labelling procedure on columns of Sephadex G-25, extra course.[12] The labelled albumin behaved like the unlabelled material in gel electrophoresis and immunoelectrophoresis. The protein solution was dialysed against two changes of 0.05 M phosphate buffer, pH 7.4, containing 0.1 M sodium chloride and was then centrifuged at approximately 30 000 *g* for 60 min at 4°C in a Spinco Model L Ultracentrifuge to remove a fine turbidity. The final solution which contained 10 mg albumin/ml and 7.5 μC ^{125}I/mg albumin, was diluted to suitable concentrations with the phosphate buffer.

Preparation of immunoglobulin. Rabbits were immunized with human serum albumin (original preparation). Ten mg protein was emulgated in complete Freund's adjuvant and injected intramuscularly [13] in rabbits weighing about 2.5 kg. Injections were given every second week for two months, by which time rather big granulomas had regularly developed at the site of the injections. The animals were sacrificed three weeks after the last injection and the blood was collected. After clotting at room temperature, the blood was kept at 4°C for 12—24 h and then was centrifuged at 1 800 *g* for 20 min. The sera were tested for anti-albumin activity by gel diffusion and stored at −30°C.

The different sera were treated with DEAE-Sephadex at an ionic strength of 0.05 and a pH of 7.4 to adsorb proteins other than γ-globulins.[14] One gram of DEAE-Sephadex was added per gram of total protein in the serum, which was first diluted with 2 volumes of distilled water. After mixing at 4°C for an hour the mixture was filtered and centrifuged. The supernatant was concentrated by ultrafiltration and further purified by gel filtration on Sephadex G-200 [9] as described above. The major fraction which consisted mainly

of γG-globulin, was concentrated by ultrafiltration. The preparation showed only one protein zone when analysed by gel electrophoresis. The solution was dialysed against phosphate buffer and centrifuged as described for the iodinated albumin.

Preparation of anti-rabbit-γG-globulin serum. Guinea pigs weighing about 800 g were immunized with the purified rabbit-anti-albumin-γG-globulin emulgated in complete Freund's adjuvant. About 2 mg of protein was injected into each guinea pig every second week for two months. The animals were sacrificed and the blood was collected as described above. The sera showed three parallel bands when analysed by immunoelectrophoresis (Fig. 4). The two main bands are probably related to γG-globulin of types K and L.[15]

Determination of protein concentrations. Protein determinations on a serial dilution of the purified albumin were made by the biuret method [16] and by the modified Lowry method [17] of Eggstein and Creutz.[18] Simultaneously nitrogen was determined by the Kjeldahl method on a semimicro scale,[19] and the albumin dilutions were also analyzed at 280 mμ with a Beckman DU spectrophotometer. The concentration of albumin in each dilution was calculated using a value of 15.95 % for the nitrogen content of albumin.[20] The nitrogen analyses were used to calibrate the other methods. The $[E]^{1\,\%}_{1\,cm}$ at 280 mμ was found to be 5.2, which is in good agreement with the value found by Cohn *et al.*[21] In subsequent analyses, UV-readings were used for the determination of uniodinated albumin and the biuret or Lowry methods was used for iodinated albumin. The concentration of the immunoglobulin solution was determined as 4.3 mg/ml by Kjeldahl analysis using a value of 16.0 % for the nitrogen content of rabbit γ-globulin.[22]

Polysaccharides. Preparations of dextran (a polyglucose) and Ficoll (a polysucrose) were kindly supplied by AB Pharmacia, Uppsala, Sweden. The different dextrans had the following weight-average molecular weights: 9400 (Dextran 10); 35 000 (Dextran 35); 80 000 (Dextran 80); 450 000 (Dextran 500); 2×10^6 (Dextran 2000); and 12×10^6 (Dextran 12 000). The manufacturer had determined the degree of branching. About 94 % of the glycosidic linkages are 1 → 6. The Ficoll had a molecular weight of 1×10^6.

Stock solutions were prepared by dissolving the appropriate amount of polysaccharide at 100°C in 0.1 M phosphate buffer, pH 7.4, containing 0.2 M sodium chloride. After cooling to room temperature each solution was diluted with an equal volume of water. These stock solutions were then diluted as described with 0.05 M phosphate buffer, pH 7.4, containing 0.1 M sodium chloride. The solutions were clarified by centrifugation at approximately 30 000 *g* for 60 min in a Spinco Model L Ultracentrifuge.

Carbon-14 labelled dextran. The material was kindly supplied by dr. K. A. Granath, AB Pharmacia, Uppsala, Sweden, who also had determined the weight-average molecular weight as approximately 30 000. The radioactivity of the sample was 0.1 μC/mg. 10 mg of this dextran was dissolved in 100 μl of 0.05 M phosphate buffer, pH 7.4, containing 0.1 M sodium chloride.

Radioactivity measurements. A Baird-Atomic well type scintillation counter equipped with a gamma spectrometer was used. The liquid sample (0.1 ml) was placed as a drop in the center of the bottom of the counting tubes. Care was taken to avoid contamination of the walls.

A standard curve prepared with various dilutions of the labelled albumin was linear within the range from 5 to 200 μg albumin. As 100 μg labelled albumin containing 7.5 μC ^{125}I/mg albumin gave 171 000 cpm, the counter had an efficiency of about 10 %.

After immunoelectrophoresis of the radioactive material the gels were sliced into sections of equal size. Each gel section was placed in a counting tube in such a way that the area of the section containing the greatest activity (most precipitate) was located in the center of the bottom of the tube. To determine the absorption of radiation by the gel a series of gels were counted before and after drying in an oven at 65°C for 24 h. The number of counts was unchanged.

Autoradiograms were made by mounting the immunoelectrophoresis gels on an enveloped Ilford X-ray film. After exposure for 1 to 3 weeks the films were developed with a fine grain developer.

The carbon-14 activity was measured in a model 314EX-2 Tricarb liquid scintillation spectrometer system. The dextran samples were suspended in 5 ml of the recommended scintillation liquid.

Immunoelectrophoresis and gel electrophoresis. The LKB Immunophor Apparatus (LKB-produkter AB, Stockholm, Sweden) was used. Agarose, prepared according to

the method described by Hjertén [23] was employed as gel material to diminish the electro-endosmotic flow. The immunoelectrophoretic runs were performed by Scheidegger's [24] micromodification of the method of Grabar and Williams. The samples were applied in wells containing approximately 1 μl. The agarose concentration was 1 %. A veronal buffer, pH 8.6, with an ionic strength of 0.025 was used in most experiments. The potential gradient was about 7 V/cm. The electrophoretic step was completed after 60 min at 4°C. The immunoprecipitation was allowed to develop for 2 days at 4°C and the gels were then washed with saline and stained with thiazine red or amido black.

When supernatants obtained in the precipitin reaction were analysed, the buffer was 0.05 M phosphate, pH 7.4, containing 0.1 M sodium chloride. The wells then contained approximately 10 μl. The potential gradient was about 2.5 V/cm. The electrophoresis was performed for 9 h.

After gel electrophoresis [25] at 4°C in the above veronal buffer the preparations were fixed for 12 h in 70 % ethanol containing 2 % acetic acid and then stained with thiazine red or amino black.

Double diffusion in gel. A micromodification of the Ouchterlony technique [26] was employed. Agarose gels (1 %) were used and the test was performed on microscope slides using wells 3 mm in diameter. The buffer was 0.05 M phosphate buffer, pH 7.4, containing 0.1 M sodium chloride. Diffusion was allowed to proceed for 2 days at 4°C.

Performance of the immunoprecipitation test. 0.1 and 0.2 ml Folin pipettes, calibrated to contain 96.8 $\pm$ 2 % of the nominal volume, were used.

0.1 ml of the different polysaccharide solutions were pipetted into small test tubes which were weighed before and after addition of the sample. The variation in sample size was not allowed to exceed 2 % within an experimental series. 0.2 ml of the γ-globulin solution and 0.1 ml of the labelled albumin solutions containing 5—200 μg were then added. Precipitin series were run at different concentrations of polysaccharide. Within each series, the amount of γ-globulin (860 μg) and polysaccharide was held constant as the amount of albumin was varied.

The final concentrations of polysaccharide in the different series were calculated as follows: The density of the original polysaccharide solution was estimated from its concentration and the partial specific volume of dextran, 0.61.[27] The volume of the added polysaccharide solution was then calculated. The final polysaccharide concentration was calculated from the calibrated volumes of the pipettes used for the addition of the albumin and γ-globulin. The polysaccharide concentrations, expressed in g/100 ml, were: Dextran 10: 3.94 and 1.95; Dextran 35: 3.91 and 1.98; Dextran 80: 8.20, 4.10, 1.97 and 1.01; Dextran 500: 3.83 and 1.97; Dextran 2000: 1.96 and 0.99; Dextran 12 000: 1.87 and 0.95. The average deviation of individual measurements were within $\pm$4 %.

The additions of γ-globulin and albumin were made at 4°C. The tubes were closed with rubber stoppers and were carefully rotated to mix the solutions. The solutions were then warmed to room temperature. After 60 min at room temperature, the tubes were cooled and stored at 4°C for 7 days with mixing by rotation at 12 h intervals. After centrifugation at 600 *g* for 60 min at 4°C in a swinging-bucket rotor the supernatants were carefully decanted into similar test tubes, which were also closed with rubber stoppers. The precipitates were washed once with 0.4 ml of the corresponding polysaccharide solution and then were dissolved in 0.5 ml of 0.2 N sodium hydroxide. 0.1 ml samples of the supernatant, the washing solution, and the dissolved precipitate were analysed for radioactivity. 0.1 ml samples of the dissolved precipitates from a series containing no polysaccharide were analysed by the Lowry method.

RESULTS

To series containing polysaccharides of various molecular weights and concentrations were added a constant amount of immunoglobulins and 5-200 μg of ^{125}I-labelled human serum albumin. The immunoprecipitates were recovered by centrifugation. Lowry determinations performed on the precipitates from the series containing no polysaccharide showed that maximal

precipitation occurred at 35 μg of albumin which should thus represent the equivalence value.

Samples from the supernatants, washing solutions, and dissolved precipitates were assayed for radioactivity. The total recoveries of the isotope in these fractions were 90—95 % in the region of antibody excess, about 95 % around the equivalence zone, and 95—105 % in the region of antigen excess.

In every series of experiments, the activity found in the supernatant in the region of antibody excess corresponded to 2.5—3.0 % of the added activity. Perhaps this activity is due to free ^{125}I which has split off from the albumin molecules. Supernatants from the tubes containing 35—200 μg of albumin showed varying activities; the higher the concentration and the molecular weight of the dextran, the lower the activity found in the supernatant.

The activities in the washing solutions were usually less than 1 % of the added activity, except in the 3—4 tubes with the highest activity, where up to 2 % was observed.

The radioactivities of the precipitates have been plotted *versus* the amount of albumin added. Figs. 1, 2, 3, and 6 show that the highest activity was found in the tubes to which 40 μg of albumin has been added. This is slightly above the equivalence zone.

Effect of concentration of dextran. In four series Dextran 80 was added to approximately final concentrations of 1, 2, 4 and 8 %, respectively (Fig. 1). The activities of the precipitates from a series to which no dextran was added have also been plotted in the figure. In the region of antibody excess dextran appears to have no effect on the amount of albumin precipitated. In the equivalence zone (at 35 μg albumin) an effect can be detected. When no

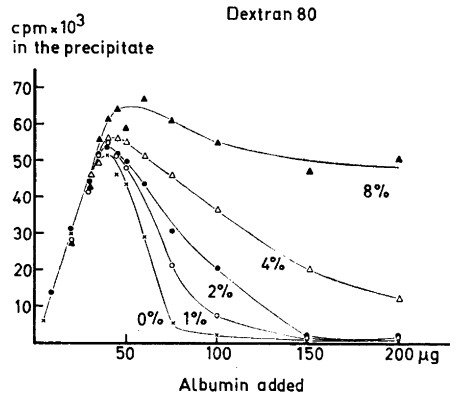

Fig. 1. The effect of different concentrations of Dextran 80 (0, 1, 2, 4, and 8 % w/v) on the precipitin reaction. In each series increasing amounts of ^{125}I-labelled human serum albumin were added to a constant amount of rabbit anti-albumin γG-globulin. The radioactivities of the precipitates are plotted *versus* the amount of albumin added.

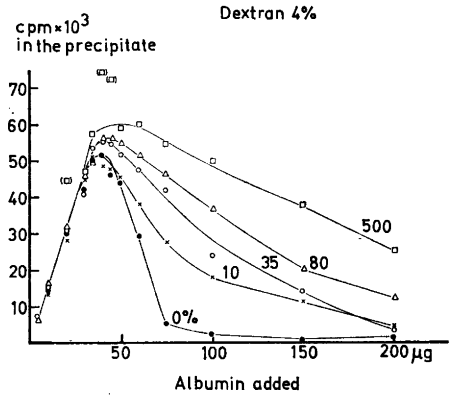

Fig. 2. The effect of dextrans of various molecular weight (Dextran 10, 35, 80, and 500) on the precipitin reaction. The polysaccharide concentration was 4 % w/v. The results are plotted as in Fig. 1.

polysaccharide is added 81 % of the activity is precipitated, when 8 % Dextran 80 is present the corresponding figure is 90 %. Fig. 1 shows that the experimental points in the region of antibody excess fall on a straight line. When "experimental losses" are considered, the line corresponds to the total amounts of albumin in the samples. When the dextran concentration is increased the region where albumin is completely precipitated extends to higher antigen concentrations. Therefore, the zone of maximal precipitation, measured from the amount of albumin precipitated varies with the dextran concentration. When no polysaccharide is added, maximal precipitation occurs at 40 μg albumin. When 4 % Dextran 80 is present it occurs at 40—45 μg, and 50—55 μg is required when 8 % Dextran 80 is present.

In the region of antigen excess the effect of Dextran 80 is striking: the amount of albumin precipitated increases sharply as the dextran concentration is raised. When 120 μg of albumin is added (*i.e.* a threefold excess of antigen) 1 %, 2 %, 4 % and 8 % dextran increase the albumin precipitate 3-, 8-, 19-, and 34-fold, respectively, as compared with that obtained in the absence of dextran.

Effect of molecular weight of dextran. The influence of the molecular weight of the dextran on the shape of the precipitin curve is illustrated in Fig. 2. The four dextrans used (Dextran 10, 35, 80, and 500) were present at the same concentration. Beyond the equivalence zone, the degree of precipitation increases with increasing molecular weight of the dextran at any given dextran concentration.

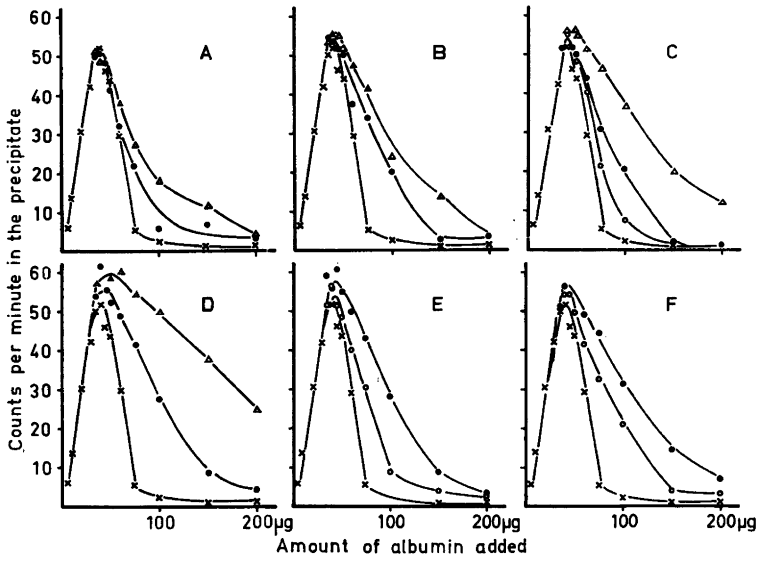

Fig. 3. The effect of dextran concentration on the precipitin reaction for dextrans of different molecular weights. The following symbols have been used: 0 % (×), 1 % (○), 2 % (●) and 4 % (△). Dextran 10 (A), Dextran 35 (B), Dextran 80 (C), Dextran 500 (D), Dextran 2000 (E) and Dextran 12 000 (F). The results are plotted as in Fig. 1.

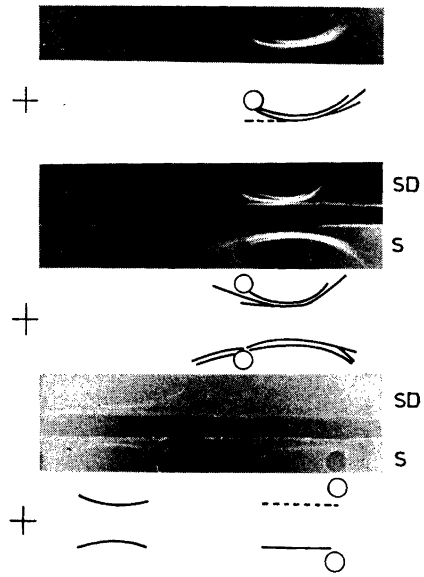

Fig. 4. Immunoelectrophoresis in veronal buffer, pH 8.6, for 60 min from wells containing approximately 10 µl. At the top, anti-albumin solution diluted 1:3 is run against 100 µl guinea pig anti-rabbit-γG-globulin serum. Three bands are visible. In the middle, supernatants from the test tubes containing 100 µg albumin and 8 % Dextran 35 (SD) and from corresponding blank tubes without dextran (S) are run against 100 µl of the same anti-γG-globulin serum. Observe the existence of precipitin lines on the anodal side of the wells! At the bottom the same supernatants are run against 50 µl of rabbit anti-human-serum-albumin serum diluted 1:3. The anodal precipitin line is present again! Note the difference between the bands from supernatants SD and S.

The influence of concentrations and molecular weights on the precipitation is also illustrated in Fig. 3. Concentrations of 1 %, 2 %, or 4 % (w/v) were used with each of the dextrans (10, 35, 80, 500, 2 000, and 12 000). A 0.4 % curve is given for comparison in each figure.

Electrophoretic analyses of the supernatants. Samples of the supernatants from the series containing Dextran 80 in various concentrations were analysed by immunoelectrophoresis and gel electrophoresis. Gel electrophoresis showed two components corresponding to albumin and γ-globulin, with a third one in between. The latter component was situated close to the γ-globulin zone (Fig. 7) and probably consisted of soluble albumin-antibody complexes.[28] A dextran zone could be made visible by fixation with 70 % ethanol containing 2 % acetic acid. It partially overlapped the γ-globulin but not the complex fraction (Fig. 7).

Immunoelectrophoresis against an antialbumin serum gave two bands corresponding to the albumin and the complex fraction (Fig. 4). Autoradiography showed that all the radioactivity was associated with these two bands. No radioactivity was detected in the dextran or the γ-globulin spot. Immunoelectrophoresis against anti-γG-globulin serum gave three parallel bands corresponding to γ-globulin and the complex fraction.

Comparison of the immunoelectrophoresis gels or the corresponding autoradiograms from runs performed with and without dextran indicated that dextran had decreased the size of the complex fraction. To confirm this, equal-sized sections containing, respectively, the albumin and the complex fractions were cut from the gels and analysed for radioactivity. Since the volumes of the wells varied with differences in the thickness along the gels

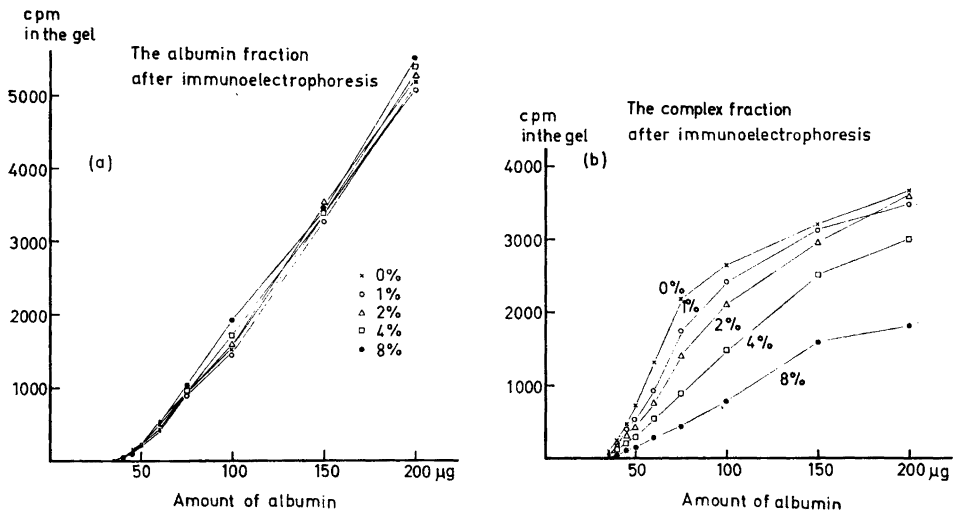

Fig. 5 The radioactivities of free albumin and albumin-antibody complexes remaining in the supernatant after the precipitin reaction. The two fractions were separated by immunoelectrophoresis (see text). The radioactivity of the free albumin is shown in Fig. 5 a, and that of the complex in Fig. 5 b. The supernatants used were from the series of experiments with Dextran 80 described in Fig. 1.

the radioactivity values obtained required correction. The measurements gave only the relative sizes of the two fractions, but since the radioactivity per 0.1 ml supernatant was known, the absolute activities of the two fractions could also be calculated. The wells were assumed to contain 10 µl. In Fig. 5 a, the corrected amount of activity found in the albumin fraction after immunoelectrophoresis has been plotted against the amount of albumin originally added. The dextran has not influenced the content of free albumin in the supernatant. The corrected activity of the complex fraction is plotted similarly in Fig. 5 b. Here the effect is pronounced. The amount of the complex fraction

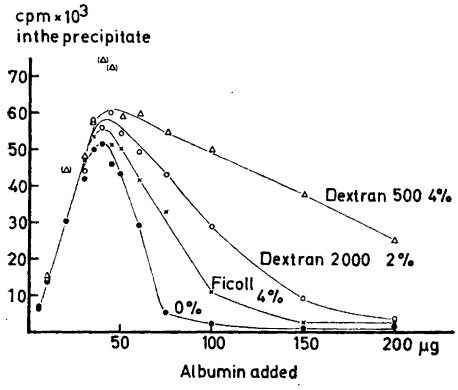

Fig. 6. The effect of Ficoll (4 % w/v) on the precipitin reaction is compared with that Dextran 2000 at 2 % concentration and Dextran 500 at 4 % concentration. The results are plotted as in Fig. 1.

remaining in the supernatant decreases as the concentration of Dextran 80 is increased.

The effect of Ficoll. The effect of Ficoll was studied for comparison. Ficoll is a polysucrose with a very compact, almost spherical structure. The preparation had a molecular weight of 1×10^6 and was used at a final concentration of 4 % (w/v). In Fig. 6 the Ficoll curve is compared with those obtained with a 4 % solution of Dextran 500 ($M = 500\,000$) and a 2 % solution of Dextran 2000 ($M = 2 \times 10^6$). Ficoll has a smaller effect than Dextran 2000 at half the Ficoll concentration. Ficoll is much less effective than Dextran 500 at the same concentration. The much smaller effect of Ficoll must be due to its globular structure. Ficoll even has a smaller effect than Dextran 10 at the same concentration.

Precipitin reaction performed in carbon-14 labelled dextran. The precipitin reaction was performed in radioactively labelled dextran in an attempt to exclude the possibility that the effect depended on complex formation between the proteins and the polysaccharide. To 100 μl of a 10 % (w/v) solution of carbon-14 labelled dextran was added 100 μl of a 0.1 % albumin solution and 200 μl of the immunoglobulin solution. A control test tube contained the same amounts of protein in the same volume without dextran. After standing for 2 days at 4°C the test tubes were centrifuged. The test tube containing the dextran solution showed much more precipitate than the control. This precipitate was washed twice with 0.4 ml of an unlabelled dextran solution containing 2.5 % of Dextran 35. After the two washings, the precipitate was dissolved in 0.4 ml of 0.1 N sodium hydroxide. Samples (10 μl) of the supernatant and the two washing solutions were analysed for radioactivity. The following activities were found: supernatant, 39 600 cpm; the first washing solution, 2367 cpm; and the second washing solution, 101 cpm. 100 μl of the dissolved precipitate gave only 240 cpm. Therefore, the precipitate contains only a minute amount of labelled dextran. Since the fraction of the added albumin represented by the precipitate is already known from the above experiments the radioactivity found here would indicate that the precipitate contains less than one molecule of dextran per molecule of albumin. Probably the activity found in the precipitate reflects inadequate washing.

The supernatant was examined in the following way: Gel electrophoresis was performed using a solution containing 2.5 % carbon-14 labelled dextran as a reference. The gel was cut into strips 2 mm broad and the activities were measured. The result is shown in Fig. 7. The regions corresponding to albumin and the complex fraction contain no significant radioactivity above the blank. If each molecule of albumin could bind one molecule of dextran, the regions corresponding to albumin and the complex fraction would contain 63 and 84 cpm above the background, respectively.

DISCUSSION

Laurent [2,3] has already shown that dextran significantly decreases the solubilities of a number of proteins. The decrease was dependent on the size of the protein and the concentration of the dextran but was independent

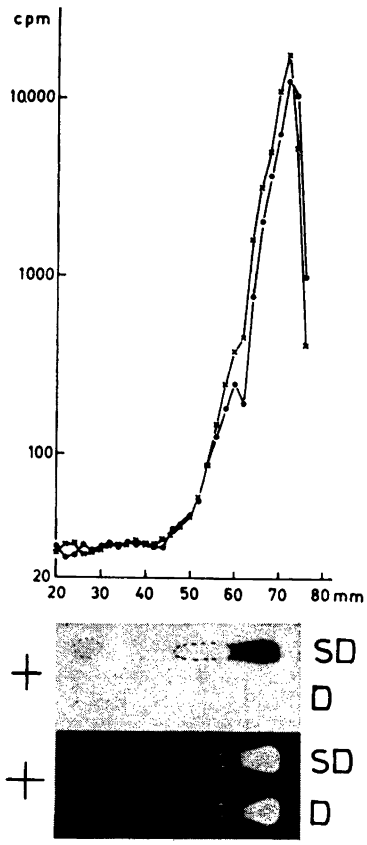

Fig. 7. Gel electrophoretic runs in veronal buffer, pH 8.6, for 60 min from wells containing approximately 10 μl. Sample SD was the supernatant from the test tube containing 100 μg albumin and carbon-14 labelled dextran, sample D was a solution of carbon-14 labelled dextran of the same concentration. The gel shown at the bottom was fixed with 70 % ethanol and 2 % acetic acid and that in the middle was stained with amido black. The gel from a similar electrophoretic run was cut directly into 2 mm strips and the radioactivities of the strips were counted. The result is shown in the diagram.

of the molecular weight of the dextran (molecular weights greater than 150 000 had been used). The effect was ascribed to a steric exclusion of the proteins from the polysaccharide domain. This hypothesis was supported strongly by experiments which showed that the solubility of a protein in a dextran solution is decreased by a factor corresponding to its degree of exclusion from a dextran gel of the same polysaccharide concentration.[29]

In this report the effect of dextran on the immunoprecipitation of serum albumin-antialbumin has been described. The presence of dextran causes an increased precipitation of the antigen. The effect is most pronounced in the region of antigen excess. The increased precipitation was shown to be accompanied by a decrease in the amount of soluble antigen-antibody complexes present in the supernatant. The amount of free albumin in the supernatant is not influenced by the presence of dextran.

The experiments indicate that dextran decreases the solubility of soluble immunocomplexes. It seems improbable that this is due to a chemical interaction between the dextran and any of the protein components. When the

reaction between albumin and its antibody was performed in a solution containing carbon-14 labelled dextran the precipitate contained only about one molecule of dextran per molecule of albumin. It is not possible to decide whether this reflects contamination or a protein-dextran complex formation. However, when the supernatant was separated by gel electrophoresis, determination of the radioactivity distribution in the gel provided no evidence of complex formation between the labelled dextran and the albumin or the immunocomplex fraction.

The immunoprecipitation effect was influenced by both the concentration and the molecular weight of the dextran. However, the molecular weight dependence was small with dextran of molecular weights over 5×10^5. The effect of the highly branched, compact molecule, Ficoll, was much less than that of dextran. These observations are very consistent with the observations of Laurent [2,3] regarding the solubility of various proteins in the presence of dextran. Therefore both effects probably depend on the same fundamental mechanism, *e.g.* a sterical exclusion of the proteins from the domain of the polysaccharide molecules.

Results similar to those described were obtained by Albertsson and Philipson [30] in studies of the reaction between phycoerythrin and its rabbit antiserum in two-phase systems containing dextran-methylcellulose or dextran-polyethylene glycol. When an antigen-antibody solution was partitioned at pH 6.8 in a phase system containing 7 % (w/w) Dextran 500 and 4.4 % (w/w) polyethylene glycol (Carbowax 6 000) a precipitate formed at the interface. This occurred even when the antigen was present in such an excess that no precipitate was formed in the absence of the polymers. The authors suggested that the adsorption of the precipitate at the interface might be due to a decreased solubility of the complexes in the phases. An increased precipitation in the region of antigen excess was observed when the immunological reaction was performed separately in solutions containing dextran or polyethylene glycol at the concentration which existed in its respective phase of the two-phase system.

Another observation that probably can be attributed to the sterical exclusion properties of chain polymers is the effect of dextran as a "completing medium" for incomplete anti-Rh-agglutination [31] and incomplete anti-A and anti-B activity.[32] Agglutination can be regarded as a precipitin reaction which occurs at the surface of large particles.[33] Some other macromolecules used for the demonstration of blocking antibodies are gelatin, gum acacia, polyvinylalcohol, and polyvinylpyrrolidon.[34] McVickar [35] has shown that polyethylene glycol (Carbowax 4000) greatly enhances the hemolytic activity of complement. At a certain dilution of complement he found 11.5 % hemolysis. When 4 % polyethylene glycol was added at the same complement dilution he found 79.5 %, an increase of nearly 700 %. Another interesting example is the observation that the activities of two nonspecific agglutinins of plant origin were higher in the presence of dextran.[36]

It seems reasonable to believe that the exclusion phenomena described above might play an important role in immuno reactions which occur in connective tissue, where the extracellular ground substance contains high concentrations of polysaccharides.

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