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Note

Effect of dextrans of different mean molecular weights on immunodiffusion reactions of serum fractions

Further investigation[1] of the observations of BECHOLD[2] regarding precipitation of soluble antigens by antibodies in gels led to the simultaneous development of double diffusion tests by OUCHTERLONY[3,4] and by ELEK[5]. Modifications of these methods now form the basis of numerous diagnostic and research techniques. Although materials such as agarose, polyacrylamide and several other gel-forming substances have been used, agar solutions remain the matrix of choice for many procedures[6–9] that are used routinely in human and veterinary medicine and in other biological sciences. Mathematical hypotheses relating to conditions that cause precipitation phenomena in agar gels have been described[10]. Failure to control adequately the conditions of diffusion in the agar matrix may result in inferior definition of precipitation arcs or even in some coalescence of adjacent arcs in multiple reactant systems, especially when whole serum is used.

Dextran may reduce the solubility of proteins such as albumin[11], cyanmethaemoglobin or beta-lactoglobin in physiological soline solutions[12,13] and gamma-globulins[14]. Low molecular weight dextran has been shown to precipitate fibrinogen from plasma[15,16]. In tube precipitation tests, the addition of neutral or acid polysaccharides of several different molecular weights[17,18] causes a decrease in the soluble antigen-antibody complexes, as a result of increased precipitation. Dextran 80 (mol. wt. 80 000) in phosphate buffer (pH 7.4) has been used extensively[18]. Similarly, the addition of Dextran 10 (mol. wt. 10 000) (prepared by Pharmacia AB, Uppsala, Sweden) has been claimed[19,20] to increase the visibility of precipitation deposits after immunoelectrophoresis.

This paper describes the investigation of gel-precipitation methods in which human immunoglobulins and three bovine serum fractions of increasing molecular weight, *viz.*, albumin, transferrin and gamma-globulin (IgG), are used concurrently in agar gels containing a series of dextrans with mean molecular weights ranging from 10 000 to 2 000 000.

Materials and methods

Antigens. Human gamma-M globulin (IgM) was obtained from Behringwerke A.G., Marburg-Lahn, G.F.R., or from Meloy Laboratories Inc., Springfield, Va., U.S.A. Crystalline bovine serum albumin was purchased from Pentex Inc., Kankakee, Ill., U.S.A.

The bovine whole serum used in experiments reported here was collected from a single steer (No. 107), although bovine serum from other sources produced similar results.

Bovine transferrin was prepared from pooled serum by precipitation with 2,5-diamino-7-ethoxyacridine lactate, followed by gel filtration chromatography of the supernatant (COCHRANE AND HYSLOP, 1972, unpublished results).

Antibodies. Antisera to bovine serum albumin and bovine IgG gamma-globulin were prepared by the immunization of rabbits or acquired from Behringwerke A.G. Antiserum to human IgM was obtained from that source.

Antisera to bovine transferrin were prepared by immunization of rabbits either with the purified transferrin product mentioned above or with commercially available bovine transferrin (Pentex Inc., Kankakee, Ill., U.S.A.).

Polysaccharides. Dextrans D10, D20, D70, D150, D500 and D2000 were purchased from Pharmacia AB, Uppsala, Sweden.

Agar gels. All gels were prepared from 1.0%. Ionagar No. 2, Batch 3471155 (Oxoid Ltd., London, Great Britain), dissolved either in 0.85% saline solution or in 0.05 *M* phosphate buffer (1.33 g K_2HPO_4 + 5.7 g NaH_2PO_4 + 5.84 g NaCl + distilled water to 1000 ml; pH 7.4). Dextrans to form a 2% (w/v) solution and 1:10000 merthiolate were added to the hot gel solution after autoclaving at 15 p.s.i.

Immunodiffusion. Gels were cast on 25 × 75 mm glass slides, which were cleaned in bichromate solution followed by several washes in alcohol, dried and then pre-coated with 0.5% agar. Warm dextran gels were poured and allowed to cool in dust-free conditions. The apparatus used for casting and punching and the immunodiffusion methods followed closely those described by OUCHTERLONY[21]. The central wells of each configuration were loaded with antigen with micro-pipettes, suitably diluted in accordance with preliminary titration against undiluted antibody: five peripheral wells were loaded with serial dilutions of antibody, while the last well contained only phosphate-buffered saline (PBS). All slides were incubated in a moist chamber at 4° for 20 or 44 h.

TABLE I

WIDTH OF PENULTIMATE PRECIPITATION ARC OF ANTIBODY DILUTION SERIES FORMED BY ANTIGEN–ANTIBODY REACTION IN AGAR GELS CONTAINING DEXTRANS OF DIFFERENT MEAN MOLECULAR WEIGHT

Antigen	*Antigen dilution*	*Antibody*[a]	*Mean arc width (mm) in agar gel*[b]							
			S/O	*P/O*	*P/D10*	*P/D20*	*P/D70*	*P/D150*	*P/D500*	*P/D2000*
Bovine whole serum	1:128	Anti-BSA	1.75	1.45	1.10	1.00	0.90	0.90	0.75	0.75
	1:256	Anti-BSA	1.45	1.20	0.90	0.70	0.60	0.55	0.50	0.50
	1:512	Anti-BSA	0.80	0.70	0.50	0.40	0.50	0.25	0.30	0.30
Bovine whole serum	1:128	Anti-T	1.40	1.30	1.05	0.85	0.50	0.50	0.50	0.50
	1:256	Anti-T	0.85	0.90	0.75	0.50	0.50	0.50	0.45	0.40
	1:512	Anti-T	0.65	0.75	0.55	0.45	0.40	0.30	0.30	0.30
Bovine whole serum	1:128	Anti-BGG	0.60	0.55	0.50	0.40	0.35	0.30	0.30	0.30
	1:256	Anti-BGG	0.45	0.45	0.35	0.35	0.30	0.25	0.25	0.25
	1:512	Anti-BGG	0.35	0.35	0.30	0.25	0.20	0.20	0.20	0.25

[a] BSA = bovine serum albumin; T = transferrin; BGG = bovine IgG.

[b] S/O = saline, no dextran; P/O = phosphate buffer, no dextran; P/D10 – P/D2000 = phosphate buffer dextran 10, 20, 70, 150, 500 or 2000.

Results

After incubation for *ca.* 20 h at 4°, precipitation arcs were clearly visible in all gels. In gels prepared with saline or with buffer, the precipitation zones were somewhat diffuse and ill-defined, but were more sharp in those containing Dextran 10. Incorporation of dextrans of higher molecular weight resulted in improvement of arc resolution (Table I), but above mol. wt. 500 000, the sharpening of the arc was slight in relation to the increase in mean molecular weight.

Precipitation zones of the large complexes formed with the higher molecular weight antigens of the gamma-globulin series were narrower than those formed with albumin.

Zone sharpening during the precipitation of serial dilutions of bovine serum albumin–anti-bovine serum albumin is demonstrated in Fig. 1. At equivalence and at all the antigen-antibody proportions tested, incorporation of dextrans improved the resolution; the effect was especially marked in the region of excess of antigen,

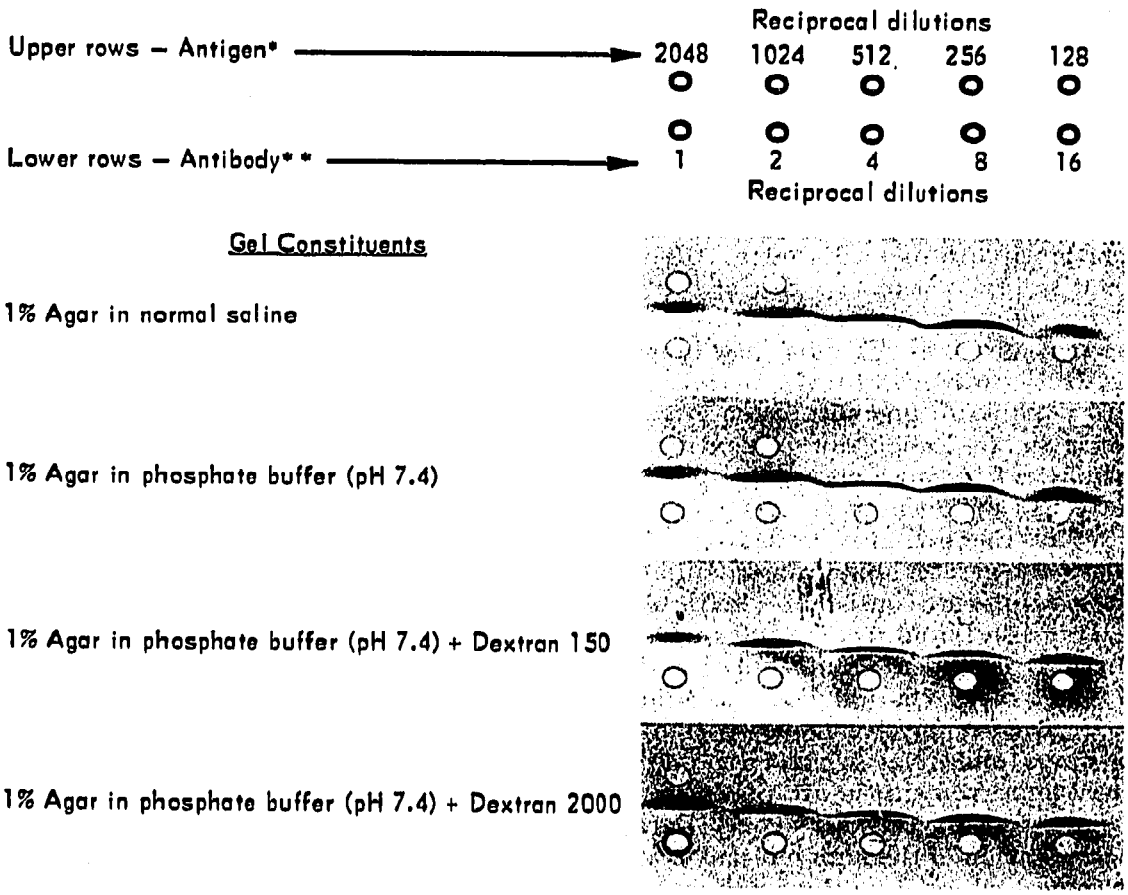

Fig. 1. Zone-sharpening effect on precipitation arcs of dextrans incorporated in the gel.

and the best definition was obtained with dextrans D150 and D500 when the entire series of dextrans was tested. Similar effects were obtained with the large complexes formed by human IgM–anti-human IgM (human IgM: mol. wt. *ca.* 900 000). Incubation at 26° increased the rate of the reaction, apparently without diminishing the influence of the dextrans.

Discussion

In tube-precipitation tests, dextran greatly enhanced immune precipitation of human albumin (HA) from phosphate buffer by rabbit antiserum to HA, particularly in the region of excess of antigen; the ratio of antibody to antigen in the precipitate was not affected by addition of the polysaccharide[17,22].

Polysucrose (Ficoll) and certain connective-tissue polysaccharides may exert similar effects on antigen–antibody systems[17,23,24]. Enhanced precipitation of albumin apparently did not result from complex formation between the polysaccharides and protein during column chromatography[25]. CEŠKA[19] used 1.5% agar in veronal buffer for immunoelectrophoretic studies on insulin–anti-insulin reactions and later excluded additional complexes from solution by repeated addition of 8% Dextran 10 to the antibody troughs. For immunodiffusion and immunoelectrophoretic reactions, the agar concentration used most frequently is 1.5%. The present series of experiments was confined to gels containing 1.0% agar; the polysaccharide was dissolved in the molten gel, which resulted in a consistency similar to that of a 1.5% agar gel.

Diffusion rates of immunoreactants in any particular gel are determined principally by the size and shape of their protein molecules, and the constitution of any precipitate is mediated by the mobility and avidity of those reactants. Consequently, it was necessary to test the influence of dextrans of increasing mean molecular weight on the formation of immune complexes by several different antigens (serum), of increasing molecular dimensions, when allowed to react with homologous antibody (principally IgG). By raising the dextran content to about 10% or greater, it is possible to precipitate the larger globulin molecules in the absence of antigen. However, the proportion of dextran (2%) incorporated in the gels to be tested was insufficient to cause observable precipitation until complexing of antigen with antibody had commenced.

In the presence of dextrans, the larger antigens, which also combine with relatively greater amounts of antibody, are precipitated selectively from the gel. These results are consistent with results of earlier investigations[11,17,18] on precipitation reactions in liquid media, and can be attributed to steric exclusion from the liquid phase. It is evident from Table I and Fig. 1 that increases in mean molecular weight of the polysaccharide were associated with concentration of precipitation into progressively narrower zones within the gel matrix. Nevertheless, great increments in mean molecular weight (10 000 to 2 000 000) resulted in disproportionately small improvements in arc resolution, especially when the mean molecular weight of the dextran exceeded 150 000. Furthermore, despite the sharpening of the arcs, incorporation of the high molecular weight Dextran D200 produced a marked opacity of the gel which, by diffusing light, made the zone boundaries somewhat less easily distinguishable to the naked eye and, when multiple antigen–antibody systems were used (*e.g.*, whole serum), tended to detract from the easy interpretation of the numerous precipitation zones.

It can be concluded that steric exclusion of large immune complexes from agar gels containing dextran is not mediated solely by the molecular weight of the polysaccharides or by that of the immunoreactants. Nevertheless, the method improves the resolution of precipitation arcs and is being used by us in immunodiffusion and immunoelectrophoretic systems other than those described herein.

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