Counterimmunoelectrophoresis for Detection of Microbial Antigens: Increased Sensitivity with Dextran-Containing Gels

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The addition of 4% dextran with a mean molecular weight of 70,000 to counterimmunoelectrophoresis gels enhanced the clarity of precipitin lines and increased the sensitivity of the procedure two- to fourfold with a variety of microbial antigens.

Counterimmunoelectrophoresis (CIE) is being widely used for the rapid detection of a variety of microbial antigens including hepatitis B surface antigen (4) and the capsular polysaccharides of *Haemophilus influenzae* type b (6, 7), pneumococci, meningococci (7), and other bacterial pathogens. The reliability of these assays in the early diagnosis of infections depends critically on the sensitivity of CIE and the clarity of the precipitin bands obtained.

Dextran has been shown to decrease the solubility of immune complexes, thereby increasing their precipitation (5). This effect has been exploited to enhance the sharpness and resolution of weak immunoprecipitin bands in agar diffusion studies using growth hormone (2) and insulin (1). We therefore investigated the effect of dextran on the sensitivity of CIE for a variety of protein and polysaccharide antigens.

CIE was performed using a Cordis REC-300-C electrophoresis unit with barbital acetate buffer (0.04 M barbital-0.02 M sodium acetate-0.1% sodium azide [pH 8.2]) in the reservoir wells. Ten-well CIE plates (4 × 8 cm) containing agarose gel with barbital acetate buffer were obtained commercially (Cordis Corp., Miami, Fla.) or prepared by pouring 10 ml of barbital acetate buffer containing 1% agarose (Indubiose A45, Fisher Scientific Co.) on the plates. For polysaccharide antigens, barbital acetate buffer containing 0.5% agarose and 0.5% Noble agar (Difco) was used. The antiserum volume applied was 20 μ l, and the antigen volumes were 40 μ l for commercial plates and 20 μ l for prepared plates. Electrophoresis was performed for 1 h at 4°C with a constant voltage of 100 V (initial current, 12 mA per plate).

Graded dextrans T10, T40, and T70 (Pharmacia, Piscataway, N.J.) with mean molecular weights of 10,000, 40,000, and 70,000 and clinical grade dextran (ICN Pharmaceuticals, Cleveland, Ohio) with mean molecular weights of 250,000 were incorporated into prepared gels. With com-

mercial plates, dextran in barbital acetate buffer (10 ml) was poured on the gel, incubated overnight at room temperature, and decanted before use. The final concentration of dextran in the gel was assumed to be one-half the concentration in the overlaid buffer.

The sensitivity of CIE with and without dextran was examined with four antigen-antibody systems: (i) bovine serum albumin (BSA) (standardized against Metrix BSA standard, Armour Pharmaceuticals, Chicago, Ill.) and anti-BSA antibody (Cappel Laboratories, Cochranville, Pa.); (ii) hepatitis B surface antigen (positive control serum, blood bank, Children's Hospital Medical Center, Boston, Mass.) and anti-hepatitis B surface antigen (lot 7N144, Ortho Diagnostics, Raritan, N.J.); (iii) *Haemophilus influenzae* type b polyribophosphate capsular polysaccharide and burro antiserum (provided by Porter Anderson, Rochester, N.Y.); and (iv) *Salmonella typhi* Vi capsular polysaccharide and rabbit antibody (provided by J. Robbins, Food and Drug Administration, Washington, D.C.).

The optimal concentration and molecular weight of dextran were determined by using the BSA-anti-BSA system. With dextran T70 at final concentrations in the gel of 0, 2, 4, and 8%, optimal resolution and clarity of precipitin bands occurred with the 4% gel. By using a final concentration of 4%, dextrans with molecular weights of 10,000, 40,000, 70,000, and 250,000 were compared. Optimal results were obtained with the 70,000-molecular weight preparation. The gels were slightly turbid with the 250,000-molecular weight preparation. Similar experiments with Vi antigen and *Haemophilus influenzae* type b capsular polysaccharide using 0, 2, 3, 4, 5, and 6% T70 dextran confirmed that a final concentration of 4% also gave optimal results with polysaccharide antigens. In subsequent experiments, dextran T70 at a final concentration of 4% in gel was used.

FIG. 1. CIE of various concentrations of *Salmonella typhi* Vi antigen with and without dextran. The sensitivity was 100 ng/ml without dextran and 25 ng/ml with dextran.

TABLE 1. Sensitivity of CIE in the detection of various protein and polysaccharide antigens^a

Plate	Minimum concn (titer) of antigen detected by CIE ^b			
	BSA (ng/ml)	HB.Ag (titer)	HIB PRP polysaccharide (ng/ml)	<i>S. typhi</i> Vi polysaccharide (ng/ml)
Commercial				
No dextran	200	1/128	0.62	100
With dextran ^c	50	1/256	0.31	25
Prepared				
No dextran	200	1/64	2.5	100
With dextran ^d	50	1/128	0.62	25

^a Comparisons with and without dextran were made during the same electrophoresis run and repeated at least twice with each antigen. Stock serial twofold dilutions of each antigen were prepared and used for all experiments.

^b HB.Ag, Hepatitis B surface antigen; HIB, *Haemophilus influenzae* type b; PRP, polyribophosphate.

^c Ten-well plates overlaid with 8% T70 dextran.

^d Ten-well plates overlaid with 8% T70 dextran or with T70 dextran incorporated at a concentration of 4% gave similar results.

With each antigen system, immunoprecipitin lines were more sharply defined and easily read when dextran was added (Fig. 1). The lowest concentration (or highest titer) of each antigen that gave a definite precipitin line after overnight incubation at 4°C is summarized in Table 1. The sensitivity was occasionally one dilution less when the plates were examined immediately after electrophoresis. With both protein and polysaccharide antigens, the sensitivity of the assay was increased two- to fourfold when dextran was

added. The sensitivity of the commercial plates was similar to that of the prepared plates with BSA and Vi polysaccharide and twofold greater than that of the prepared plates with hepatitis B surface antigen and polyribophosphate polysaccharide. This difference is probably due to the larger sample size used with commercial plates.

We conclude that the addition of dextran to CIE gels enhances the clarity of precipitin bands and increases the sensitivity of the assay two- to fourfold for a variety of protein and polysaccharide antigens.

LITERATURE CITED

1. Ceska, M. 1968. Insulin—anti-insulin precipitates in the presence of various dextrans. *Immunology* 15:837-843.

2. Ceska, M. 1968. Human growth hormone-anti-growth hormone immuno-precipitates in the presence of dextrans. *Biochem J.* 111:607-608.

3. Feigin, R. D., M. Wong, P. G. Shackelford, B. W. Stechenberg, L. M. Dunkle, and S. Kaplan. 1976. Counterimmunoelectrophoresis of urine as well as of CSF and blood for diagnosis of bacterial meningitis. *J. Pediatr.* 89:773-775.

4. Gocke, D. J., and C. Howe. 1970. Rapid detection of Australia antigen by immunoelectrophoresis. *J. Immunol.* 104:1031-1032.

5. Hellsing, K. 1969. Immune reactions in polysaccharide media. *Biochem. J.* 114:141-144.

6. Ingram, D. L., P. Anderson, and D. H. Smith. 1972. Countercurrent immunoelectrophoresis in the diagnosis of systemic diseases caused by *Haemophilus influenzae* type b. *J. Pediatr.* 81:1156-1159.

7. Shackelford, C. P., J. Campbell, and R. D. Feigin. 1974. Counter-current immunoelectrophoresis in the evaluation of childhood infections. *J. Pediatr.* 85:478-481.