Inhibition of Plaque-forming Cells by Diethylaminoethyl Dextran

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The production of hemolytic antibody by single antibody-forming cells may be measured by local hemolysis in agar as described by N. K. Jerne and co-workers (Science **140**:405, 1963; p. 109-122 in B. Amos and H. Koprowski [ed.], *Cell-Bound Antibodies*, Wistar Institute Press, Philadelphia, 1963) and by J. S. Ingraham and A. Bussard (J. Exptl. Med. **119**:667, 1964). In the technique described by Jerne et al., the use of diethylaminoethyl dextran (DEAE dextran) as an agent which inhibits the natural anticomplementary action of agar is recommended.

DEAE dextran was originally found to enhance the efficiency of viral plaque formation by encephalomyocarditis virus (H. Liebhaver and K. K. Takemoto, Virology **14**:502, 1961). It was found that a sulfated polysaccharide which is released from agar by autoclaving or heating at 100 C inhibits the formation of encephalomyocarditis plaques. DEAE dextran, a water-soluble polycation with a molecular weight of 2×10^6 , binds the sulfated polysaccharide and thus removes the inhibitor from the agar.

During preliminary experiments with Jerne's plaque-forming cell assay system, we decided to test Ionagar No. 2 (Oxoid Division, Oxo Ltd., London, England), since large molecules such as hemolytic antibodies have been reported to diffuse better in Ionagar than in Difco agar (R. M. Bechtle and G. H. Scherr, Antibiot. Chemotherapy **8**:599, 1958). A short experiment was designed to examine the effect of DEAE dextran on the appearance of hemolytic plaques in Ionagar.

LAF₁ male mice were injected with 10^8 washed sheep red blood cells. Four days later, the animals were sacrificed, and their spleens were pressed through 100-mesh stainless-steel gauze to make a single cell suspension. The cells were pooled and counted. To 0.7% Ionagar (lot 991) in Hanks' balanced salt solution were added 10^6 or 10^7 spleen cells and 0.1 ml of 20% sheep red blood cells. DEAE dextran in a final concentration of 1 mg/ml was added to half of the tubes of Ionagar. The remainder of the tubes contained no DEAE dextran. This top agar was poured over 1.4% bottom Ionagar in a petri dish, and

was incubated at 37 C for 1 hr, at which time 2 ml of a 1:5, 1:10, 1:15, or 1:20 dilution of guinea pig complement in Hanks' balanced salt solution was added. After an additional 1 hr of incubation at 37 C, the number of plaques was counted. The results of this experiment are presented in Table 1. The average numbers of plaques per 10^6 spleen cells in Ionagar with and without DEAE dextran and with various dilutions of complement are shown. In each group, there were four to six plates. A background of only 0.8 plaques per 10^6 spleen cells was observed in the

TABLE 1. Average number of hemolytic plaques in Ionagar with or without DEAE dextran and with different dilutions of complement

Dilution of complement	Plaque-forming cells/ 10^6 spleen cells ^a	
	With dextran	Without dextran
1:5	34 ± 19.5 ^b	123 ± 63.4
1:10	37 ± 20.2	92 ± 28.4
1:15	45 ± 7.5	162 ± 42.7
1:20	49 ± 23.5	105 ± 72.2

^a An average of 0.8 ± 1.3 plaque-forming cells/ 10^6 normal spleen cells was observed.

^b The number given represents the mean number of plaque-forming cells ± the standard deviation.

spleens of mice that had not been immunized. Even though the number of samples used in this experiment was small, in every case DEAE dextran inhibited the appearance of hemolytic plaques in Ionagar.

Therefore, we conclude that, depending on the source of agar used, DEAE dextran may not enhance the number of plaque-forming cells detected, but may, in fact, inhibit the number of plaque-forming cells detected.

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