A Simple Method to Remove Hemoglobin from Antiserum

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SAGISAKA, K. and IWASA, M. A Simple Method to Remove Hemoglobin from Antiserum. Tohoku J. exp. Med., 1976, 120 (1), 97-98 — Hemoglobin contamination in antiserum occurs frequently in immunohematology. The hemoglobin obscures the ring test for the observation of antigen-antibody reaction. A simple method to remove hemoglobin from antiserum is described in this paper. An appropriate amount of CM-Sephadex was added to antiserum containing hemoglobin and the mixture was packed into a cellulose tube, followed by dialysing against 0.01 M monobasic sodium phosphate solution (pH approx. 6). Equilibrating with stirring, the hemoglobin was completely adsorbed with CM-Sephadex so that hemoglobin-free antiserum was prepared without dilution of the antiserum. This method is useful for immunohematology.

Hemoglobin-contaminated antiserum disturbs the observation in precipitation. Mechanical hemolysis often results from handling of blood specimens at bleeding from immunized animals. In the case of MN blood group, hemolysis usually occurs at repeated adsorptions of antiserum with red cells which are used to remove species-specific antibodies. Although hemoglobin in antiserum matters little in agglutination, precipitin contaminated with hemoglobin is unsuitable for the ring test. In such a case as anti-fetal hemoglobin serum which was specified by adsorption with adult hemoglobin, contamination of hemoglobin is practically inevitable. The hemoglobin can be removed by gel filtration, but this procedure needs undesirable dilution of antiserum. In this paper, a simple method to remove hemoglobin without dilution of antiserum was described.

To 10 ml of human serum diluted 1:10 were added 5 mg of lyophilized human hemoglobin, and 10 mg dry weight of CM-Sephadex (Pharmacia Fine Chemicals, Sweden). The mixture was packed in cellulose tube (Visking, U.S.A.) and dialyzed against about 100 times volume of 0.01 M monobasic sodium phosphate solution, pH approximately 6, stirring on a magnetic stirrer for 2 hr. When equilibrium was established, hemoglobin was completely adsorbed with CM-Sephadex. The mixture was centrifuged at 2,000 rpm for 3 min and the supernatant was removed. The hemoglobin concentration of the supernatant was checked as follows: Spectrophotometric measurements of the adsorbed serum and the control serum which was added with only hemoglobin showed that hemoglobin was scarcely contained in the adsorbed serum (Fig. 1). The adsorbed serum was tested with anti-hemoglobin serum (precipitinogen titer 1:64,000) by the ring test. Dilution of the serum up to 8 times showed positive reaction. From this results, the hemoglobin concentration in the adsorbed serum was estimated to be approximately 0.005 mg/ml. In the same way, anti-M and -N sera containing some amount of hemoglobin were adsorbed with CM-Sephadex. This treatment was performed also on the rabbit antiserum to fetal hemoglobin which was adsorbed with adult hemoglobin. These results revealed that

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Fig. 1. Absorption spectra of antisera containing hemoglobin. Five times dilutions of the antiserum treated with CM-Sephadex (B) and non-treated antiserum (A) were measured. No absorption peak (576 nm) due to hemoglobin is noted in the treated antiserum.

serological activities of the antisera were scarcely affected by the treatment. Little change was observed on the pattern of disc electrophoretic analyses of the antisera. The adsorbed antisera were available for precipitation without adjustments of pH and molarity.

It was concluded that the CM-Sephadex treatment to remove hemoglobin from antiserum is useful in immunohematology.