SELECTIVE PRECIPTATION AND ISOLATION OF BOVINE BLOOD IMMUNOGLOBULINS BY POLYPHOSPHATE AND CHAROMATOGRAPHIC METHODS

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ABSTRACT

Mapping super-simplex optimization was applied to separation of crude immunoglobulins (Ig) from blood plasma by polyphosphate precipitation. The best conditions found were: pH 3.95, NaCl 0.132M, polyphosphate 1.04% and temperature 12.7°C. The crude Ig fraction was further purified by chromatography. Cu-loaded immobilized metal affinity chromatography yielded almost pure IgG when the crude Ig was applied after removing the residual polyphosphate by ion exchange. DEAE-Sephacel also purified the crude Ig to about same purity. The purified IgG separated from blood plasma and from cow's colostrum both unstable at temperature above 70°C and pH below 3 and almost equally degraded by pepsin and trypsin hydrolyses. The left-over plasma proteins can be used as a food ingredient.

INTRODUCTION

Recently the utilization of animal blood has been of growing interest because it contains biologically active compounds, e.g., immunoglobulins, transferrin, fibrenectin, fetuin, and heme, which are believed to play important roles in a wide variety of biological activities including passive immunity, oncogenic transformation, growth promoting function etc., (Gaillard et al., 1985) and also is a source of nutritional and functional proteins which are not alien in meat products (Crenwelge et al., 1974).

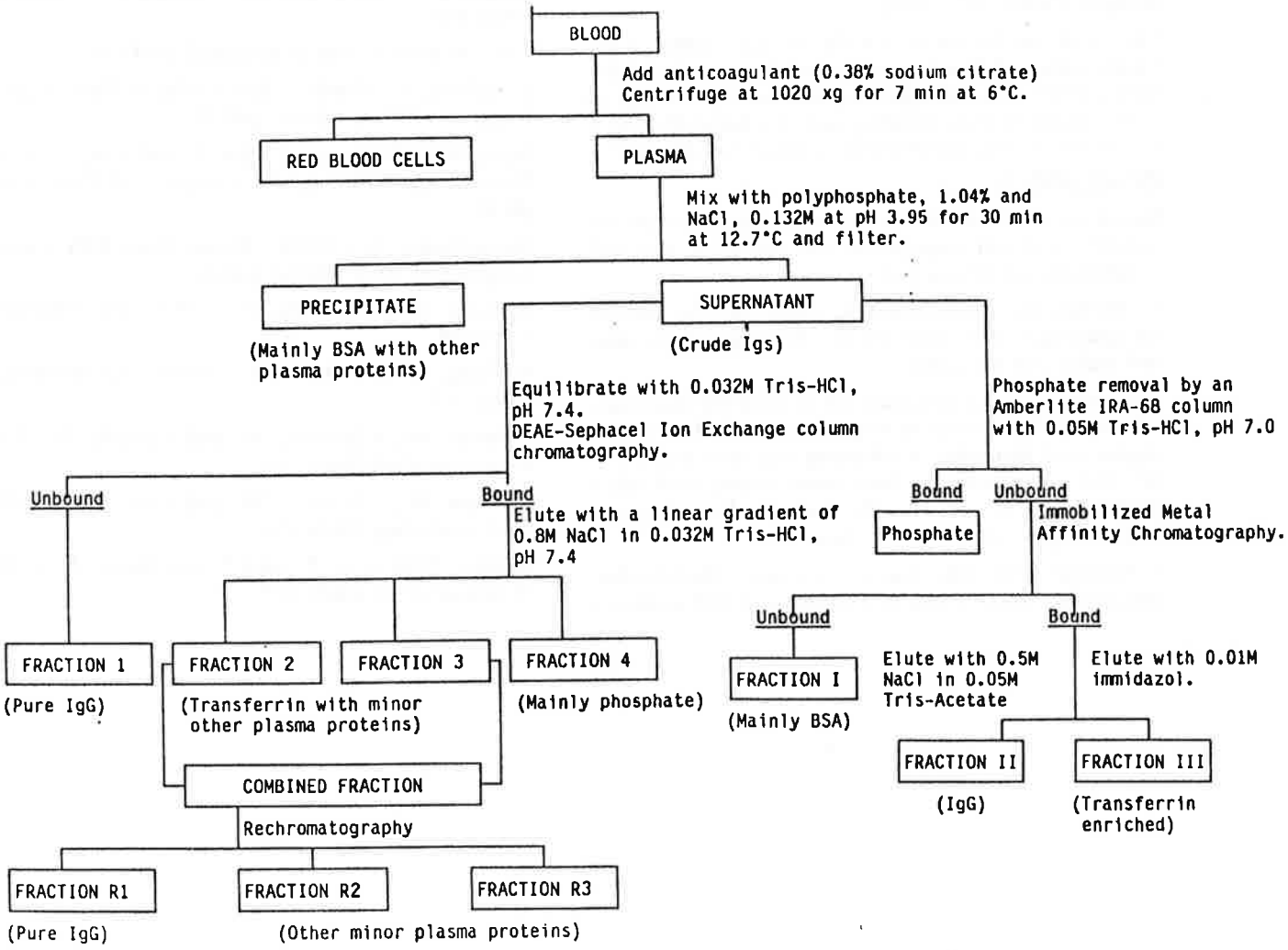

Fig. 1. Flow diagram of procedures for an preferential fractionation of plasma protein and Igs.

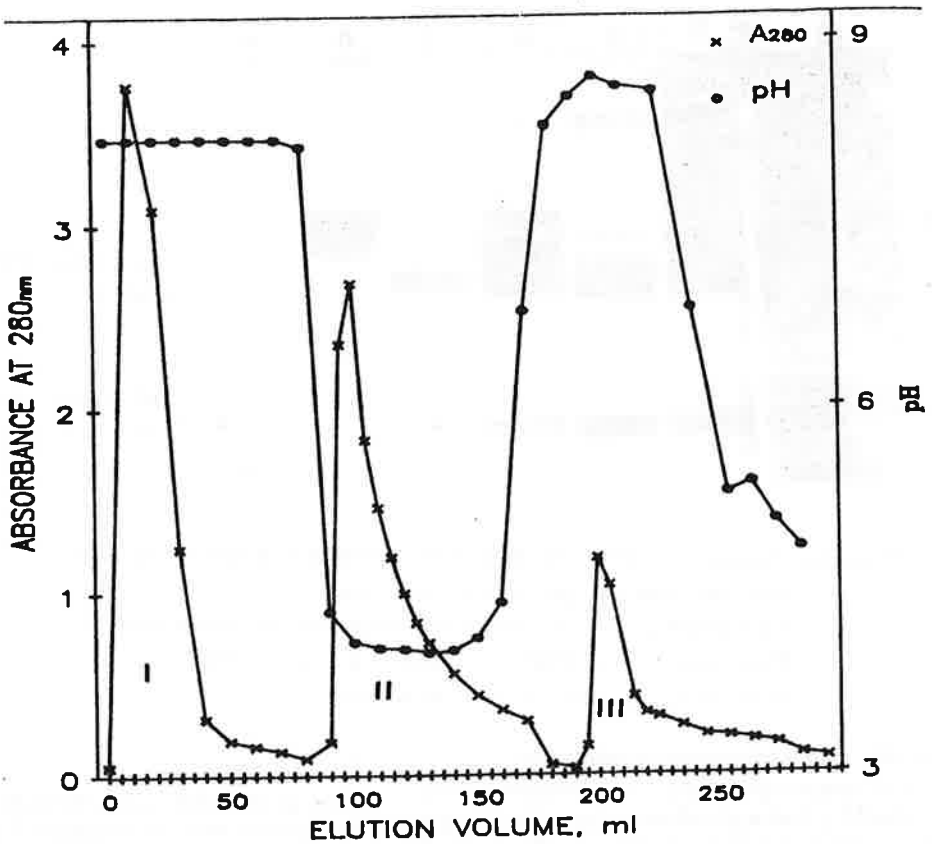

Fig. 2. Immobilized copper affinity chromatogram of the supernatant fraction from polyphosphate treated bovine blood plasma. The column was equilibrated with 0.5 M. NaCl in 0.05 M. Tris-acetate, pH 8.2; flow rate, 30 mL/h. Peak I, unbound fraction with the equilibration buffer; Peak II, eluted with 0.5 M. NaCl in 0.05 M. Tris-acetate, pH 4.0; Peak III, eluted with 0.01 M immidazol.

In animals, the protective value of orally administered immunoglobulins is well documented (Kohler et al., 1975). Weanling piglets fed blood immunoglobulins (Igs) preparations have had a faster daily weight gain, lower incidence of scours and reduced mortality (Kennelly et al., 1979), probably due to a passive immunity (McCallum et al., 1977).

Bovine blood contains approximately 18% protein and plasma contains about 6% protein. Concentrations of bovine immunoglobulins are 22.0 mg/ml, 58.8 mg/ml and 0.85 mg/ml in the serum, colostrum and milk, respectively (Butler, 1974).

The objectives of this study were to optimize polyphosphate fractionation conditions using the mapping super-simplex optimization of Nakai et al. (1984) for finding the best conditions for the separation of Igs from plasma and to use the immobilized metal affinity chromatography or the DEAE-Sephacel column chromatography for further purification of the immunoglobulins.

EXPERIMENTAL METHODS

Bovine blood was obtained from a local abattoir (Intercontinental Packers Ltd., Vancouver, B.C). plasma was prepared from freshly drawn blood containing 0.38% sodium citrate by centrifugation at 1020 x g for min at 6oC. Bovine colostrum was botained from the University herds. All other chemicals of analytical grade.

Optimization Procedure

The mapping super-simplex optimization (MSO) technique (Nakai et al., 1984) was used to find the best conditions for separation of IgG from plasm by the polyphosphate precipitation method. Withine the following ranges: pH 3.5 - 5.5; NaCl concentration, 0 to 0.7 M; polyphosphate concentration, 0.1 to 2%; and temperature, 4 to 25oC . An IBM-PC computer was used for execution of the MSO program. Experiments were performed at specified temperature by stirring mixtures for 30 minutes prior to filtration through whatman # 4 filter paper (Fig. 1).

Column Chromatography

A. Immobilized metal affinity chromatography (IMAC)

The IgG rich fraction of plasma protein obtained form the polyphosphate precipitation procedure was subjected to chromatography using IMAC column as described by sundberg and Porath (1974) and Porath and Olin (1983) (Fig.1).

B. DEAE-Sephacel ion exchange chromatography

DEAE-Sephacel was used to isolate IgG according to Friesen et al., 1985 (Fig. 1).

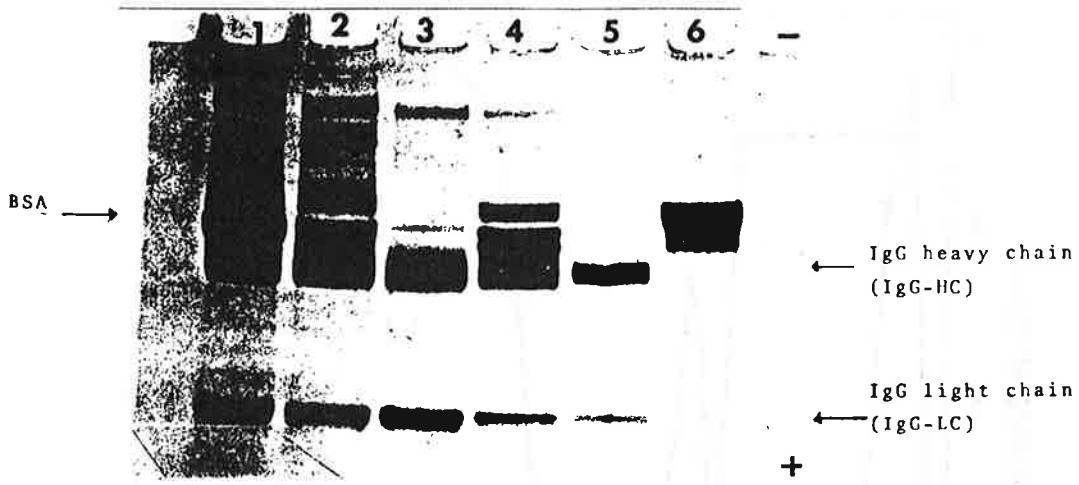

Fig. 3. Comparison of bovine plasma protein component by SDS-polyacrylamide gel electrophoresis.
1) Plasma; 2) polyphosphate supernatant fraction; 3) fraction II by IMAC; 4) fraction III by IMAC; 5) IgG standard; 6) transferrin standard.

Electrophoresis And Immunoelectrophoresis

Plasma protein samples pretreated with polyphosphate were indentified by dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). Immunoelectrophoresis and immunodiffusion were carried out by the method of Williams and Chase (1971). Immunochemical quantitative analysis of IgG was carried out py radial immunodiffustion (R.I.D.) using a kit purchased from Miles Laboratories.

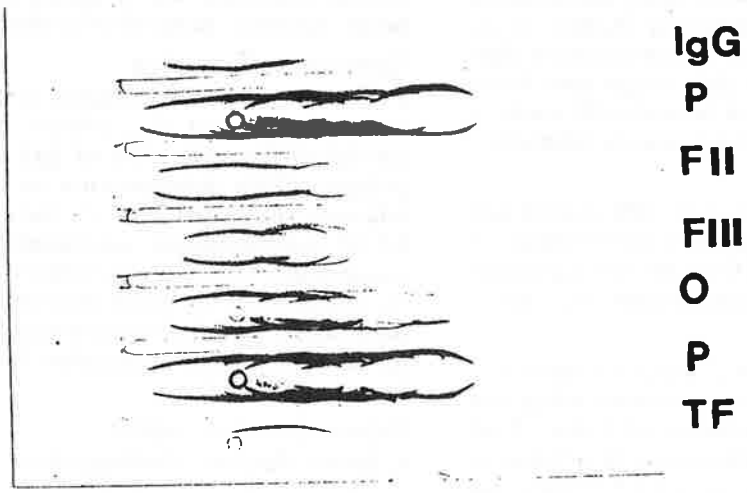

Fig.4 . Immunoelectrophoresis of bovine plasma protein. Component: IgG, Standard bovine IgG; P, bovine blood plasma; FII, IgG fraction by IMAC; FIII, transferrin fraction by IMAC; O, poly-phosphate supernatant fraction; P, plasma; TF, transferrin standard.

Calculation Of Separation Efficiency

SDS-PAGE electrophoretograms were analyzed according to the method of Ball (1986). Separation efficiency (SE) was expressed as the "Igs to BSA ratio" calculated from the optical density of Igs (OD Igs) and BSA (OD BSA).

$SE(\%) = OD_{Igs} / OD_{BSA} + OD_{Igs}) \times 100$

Enzyme Immunoassay

Immunoglobulin concentration in the plasma protein fractions was estimated by an enzyme-linked immuno-sorbent assay (ELISA) according to the method of voller et al. 91976).

In Vitro Proteolysis

Pepsin and trypsin were used to compare the resistance of bovine colostral IgG and plasma IgG to in vitro proteolysis, the remaining activity of IgG was measured by the ELISA method and the clevage of IgG was examined by SDS-PAGE.

Stability Of Bovine Igg Against Ph Chang.

To determine the esistance of bovine IgG to pH changes, 0.4% of protein was exposed to the pH 2.0, 3.0, 5.0, 6.0, 7.0 and 8.0 in a universal buffer solutions for 5 hr at 37°C. The changes in biological activity were evaluated by the ELISA method.

Thermal Stability

The immunological activity of the IgG preparation was compared by the ELISA method after 30 min-treatments at temperatures of 3, 10, 20, 30, 40, 50, 60, 70, 80, and 90°C. Also thermal transition of the IgG fractions was measured by differential scanning

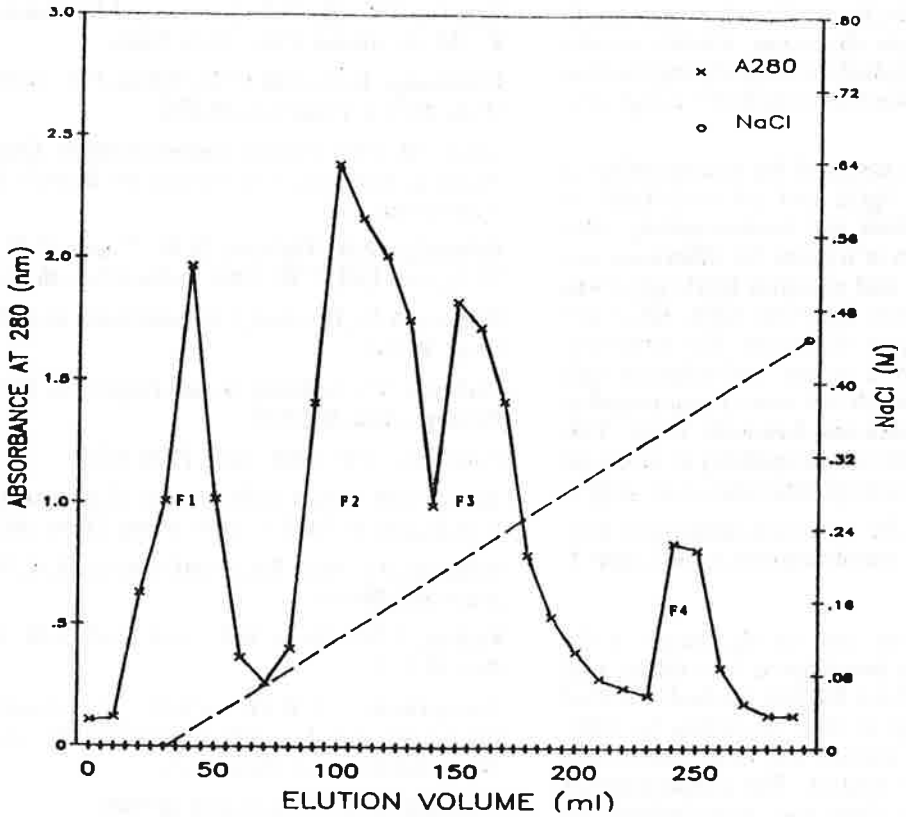

Fig. 5. DEAE-Sephacel ion exchange chromatogram of the supernatant fraction from polyphosphate treated bovine blood plasma. Compositions of peaks: F1, IgG; F2, transferrin with other plasma proteins; F3, mixed plasma proteins; F4, mainly polyphosphate. Eluent, a linear gradient of NaCl in 0.032 M. Tris/HCl, pH 7.4; flow rate, 72 ml/h.

calorimetry (DSC) by the method of ruegg et al. (1977).

Amino Acids Analysis

The sample proteins were hdrolyzed with 3N HCl at 121°C for 17 hr. Amino acids were analyzed by using a Beckman System 6300 High Performance Amino Acid Analyzer.

RESULTS AND DISCUSSION

Plasma protein fractionation using polyphosphate

In order to meet the food safety standard, an approved food additive polyphosphate was used for fractionation of immunoglobulins from bovine blood plasma. After performing 29 experiments by using mapping super-simplex optimization. The best conditions found pH 3.95, NaCl 0.132 M, polyphosphate 1.04% and temperature 12.7°C. The major protein in the plasma proteins precipitated was serum albumin, leaving IgG in the supernatant (Fig. 2 lan 2). The maximum separation efficiency (SE) of Igs in the supernatant attained was 90% immunological activity of the IgG in the superment as compared to 20% in the bovine plasma. Bovine Igs prepared by this method from plasma are presently used in feeding trials with piglets (Drew, 1987).

Purification of the crude IgG column chromatography.

A. Immobilized Copper Affinity Chromatography (IMAC)

As shown in Fig. 1, the polyphosphate in the crude IgG supernatant fraction was removed by a weakly basic anoion exchanger, Amberlite IRA-68 resin and then applied to an IMAC column.

An elution profile is shown in Fig. 2 Fraction II was rich in IgG as shown in Fig. 3, lane 3, while in Fraction III transferrin was enriched although BSA and IgG still contaminated it as shown in Fig. 3, lane 4.

Immunoelectrophoresis pattern in Fig. 4 confirms the observation made by SDS-PAGE, implicating high purity of IgG in Fraction II and intensified transferrin arc compared to the arcs for other plasma proteins.

Polyphosphate content of Fraction II was 0.14% after the anion exchanger treatment which was about 86% polyphosphate elimination. The purity of Fraction II was 99% IgG by the radial immunodiffusion method and the IgG recovery was 40.3% (not shown). There was a trend, however, of decreasing the column efficiency over time for repeating chromatography, probably due to a gradual loss of binding ability of the gels for the metal ions.

B. DEAE - Sephacel Ion Exchange Chromatography (deae)

DEAE-Sephacel was used to purify crude Igs separated by polyphosphate precipitation (Fig. 1). The elution profile is shown in Fig.5. SDS-PAGE indicated that fraction 1 was pure IgG (not shown) which was supported by the radial immunodiffusion assay indicating that is was almost 100% pure (not shown). Immunological activity of plasma fractions was compared by ELISA and fraction 1 showed the highest immunological activity. The recovery of Igs in fraction 1 was (30.4% as compared to that of IMAC process). When fractions 2 and 3 were combined, adjusted pH and ionic strength and rechromatographed on the same DEAE column. It was found that this process increased the recovery of IgG with the same purity by up to 15%.

Comparison of plasma IgG and colostral IgG

Colostrum from a cow was treated in a similar manner as for plasma for separation of Ig, i.e., DEAE-Sepphacel chromatography after polyphosphate precipitation. IgG from both sources were subjected to stability tests against proteolysis, pH changes and heating for comparison.

A. In Vitro Proteolysis And pH Changes

SDS-PAGE pattern showed no marked difference in the extent of pepsin digestion between the two IgG (not

shown); however, a slightly higher susceptibility of plasma igG observed in the heavy chain but opposite in the light chain by trypsin digestion. ELISA results indicated more intensive hydrolysis of IgG by trypsin than by pepsin and more intensive trypsin hydrolysis of plasma IgG than colostral igG.

Rham and Isliker (1977) assessed the susceptibility of bovine serum IgG1 and IgG2 and colostral IgG1 to digestion by measuring their anti-ferritin activity after digestion of Ig with pepsin or trypsin. No difference was observed between serum and colostral IgG1; igG1 was more susceptible to pepsin digestion while IgG2 was more susceptible to trypsin digestion. The structural differences between bovine serum and colostral IgG locate in the Fc region which are more pronounced in IgG2 than IgG1 (Niezgodka and Lisowski, 1980). This may explain the differences in susceptibility to digestion observed in this study between plasma and colostral IgG.

Exposure of IgG to acidic condition destroyed both plasma and colostral IgG activity equally at pH below 3.

B. Thermal Stability

The heat-induced transition and activity change of the purified plasma igG were investigated by a differential scanning colorimetry and the ELISA method. Purified bovine IgG was stable up to 70°C according to DSC; however, immunological activity was destroyed at this temperature according to ELISA. The temperature of maximum heat absorption was determined in thermogram, commonly referred to as temperature of denaturation or temperature of transition. Immunoglobulins were relatively thermostable. When immune sera were heated at temperature as high as 62°C for up to 3 min, the antibody activity of the immunoglobulins were not affected (Kwapinski, 1972). Ruegg et al. (1977) found the similar results in a calorimetric study of the thermal denaturation of bovine serum-globulin in simulated milk ultrafiltrate.

Amino acid analysis

The amino acid composition of the plasma, the crude Igs fraction (polyphosphate supernatant), fraction 1 of DEAE and fraction II of IMAC were compared (not shown). Each fraction has a similar amino acid composition to that reported for bovine immunoglobulins by Lisowski et al. (1975).

CONCLUSION

Almost pure IgG was successfully isolated by chromatographic purification of the crude IgG fraction separated from blood plasma by polyphosphate precipitation. DEAE-Sephacell was slightly superior over Cu-loaded IMAC column because of gradual loss of the binding capacity of the IMAC column during repeated chromatography.

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