Browning of Potato Tubers". *J. Sci. Food Agric.* **1963**, *14*, 673.
Mondy, N. I.; Gedde-Dahl, S. B.; Owens-Mobley, E. "Effect of Storage Temperature on the Cytochrome Oxidase and Polyphenol Oxidase Activities and Phenolic Content of Potatoes". *J. Food Sci.* **1966**, *31*, 32.
Mondy, N. I.; Owens-Mobley, E.; Gedde-Dahl, S. B. "Influence of Potassium Fertilization on Enzymatic Activity, Phenolic Content and Discoloration of Potatoes". *J. Food Sci.* **1967**, *32*, 378.
Mondy, N. I.; Koch, R. L.; Chandra, S. "Influence of Nitrogen Fertilization on Potato Discoloration in Relation to Chemical Composition". *J. Agric. Food Chem.* **1979**, *27*, 418.
Nason, A.; Oldewurtel, H. A.; Propose, L. M. "Role of Micronutrient Elements in the Metabolism of Higher Plants. I. Change in Oxidative Enzyme Constitute of Tomato Leaves Deficient in Micronutrient Elements". *Arch. Biochem. Biophys.* **1952**, *38*, 1.
Nicholas, D. J. D.; Wilson, P. T.; Heinen, W.; Palmer, G.; Bienert, H. "Use of Electron Paramagnetic Resonance Spectroscopy in Investigations of Functional Metal Components in Microorganisms". *Nature (London)* **1962**, *196*, 433.
Samotus, B.; Leja, M.; Scigalski, A. "Porownanie czterech metod Oznaczania Kwasu Askoibinowego W Owocach 1 Warywach". *Acta Agratia et Silverstria series Atratia* **1982a**, *21*, 105.
Samotus, B.; Leja, M.; Scigalski, A.; Dulinski, J.; Siwanowiez, R. J. "Determination of Tyrosine by a Modified Millon's Reaction and Its Application to Potato Tuber Extracts". *Sci. Food Agric.* **1982b**, *33*, 617.
Steel, R. G. D.; Torrie, J. A. *Principles and Procedures of Statistics*; McGraw-Hill: New York, 1980.
Tanner, P. D. "A Relationship between Premature Sprouting on the Cob and the Molybdenum and Nitrogen Status of Maize Grain". *Plant Soil* **1978**, *49*, 427.
Underwood, E. J. *Trace Elements in Human and Animal Nutrition*, 3rd ed.; Academic: New York, 1971.
Woodward, C. F.; Talley, E. A. "Review of the Nitrogenous Constitutents of the Potato". *Am. Potato J.* **1953**, *30*, 205.

Received for review September 14, 1987. Revised manuscript received February 29, 1988. Accepted April 1, 1988.

# Separation of Immunoglobulins from Bovine Blood by Polyphosphate Precipitation and Chromatography

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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 0.132 M, polyphosphate 1.04%, and temperature 12.7 °C. Cu-loaded immobilized metal affinity chromatography yielded almost pure IgG when the crude Ig was applied after residual polyphosphate was removed by ion exchange. DEAE-Sephacel also purified the crude Ig to about the same purity. The purified IgG separated from blood plasma and from cow's colostrum both became unstable at temperature above 70 °C and pH below 3 and were almost equally degraded by pepsin and trypsin hydrolyses. The leftover plasma proteins can be used as a food ingredient.

Recently the utilization of animal blood has been of growing interest because it contains biologically active compounds, e.g., immunoglobulins, transferrin, fibronectin, fetuin, and heme, that 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 that are not alien in meat products (Crenwelge et al., 1974).

In newborn pigs, the protective value of orally administered immunoglobulins is well documented (Kohler et al., 1975; Hoerlein, 1957). Weaning piglets fed blood immunoglobulin (Ig) preparations 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).

In humans, the importance of Ig in infant feeding was well proven by clinical test results in India (Narayanan et al., 1983). Successful treatment of *Escherichia coli* gastroenteritis of infants by feeding immunized cow colostrum was reported by Packard (1982) and Ballabriga (1982).

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

At present, however, little blood protein is recovered for human consumption in Canada. Most of the blood from meat- and poultry-processing plants is used in the production of blood meal or other byproducts for animal feeding (Jones et al., 1982) or as a fertilizer.

Most popular methods for plasma protein isolation entail precipitation with ammonium sulfate or ethanol. However, these precipitation methods have problems in the disposal of solvent, removal of high salt, production of heterogeneous protein mixture, and potential denaturation of proteins during isolation.

Polyphosphates have been extensively used as additives in food processing. Sofas (1986) has stated that the meat industry may use polyphosphates in low-NaCl meat formulations with the potential of improving the quality of low-salt products and of using as antimicrobial agents in meats at reduced NaCl levels. McKee and Tucker (1966) found that the metaphosphate complex of lactalbumin was useful as a substitute for milk solids in cake and cookie formulations. Also sodium hexametaphosphate has been used as an anticoagulant (Gunstone, 1980). Etheridge et al. (1981) demonstrated the functional and chemical

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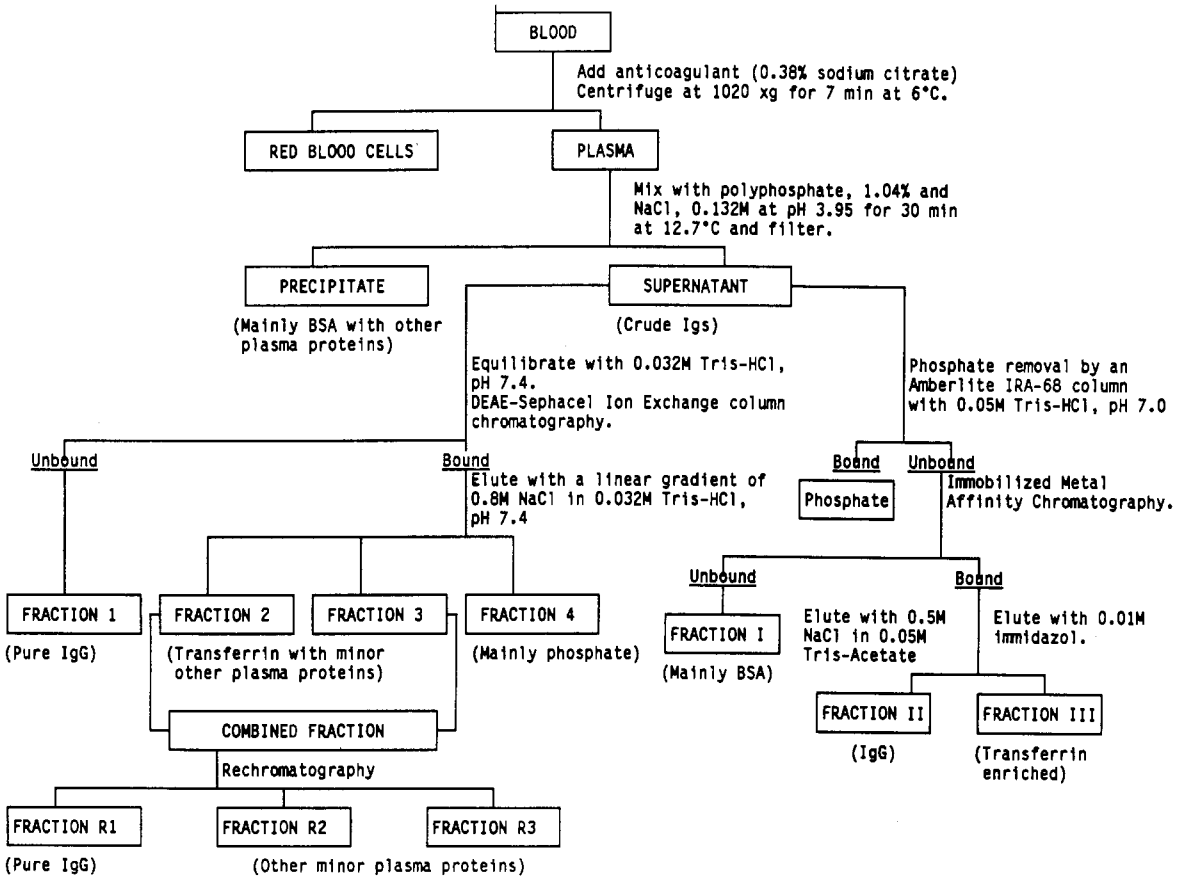


**Figure 1.** Flow diagram of procedures for a preferential fractionation of plasma protein and Ig.

characteristics of bovine plasma proteins isolated as a metaphosphate complex.

The objectives of this study were to optimize polyphosphate fractionation conditions by 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.

## MATERIALS AND METHODS

Bovine blood was obtained from a local abattoir (Intercontinental Packers Ltd., Vancouver, BC). Plasma was prepared from freshly drawn blood containing 0.38% sodium citrate by centrifugation at 1020*g* for 15 min at 6 °C. Bovine colostrum was obtained from the University herds.

Plasma protein fractions from bovine blood (IgG, albumin, transferrin) of high purity, polyphosphate (Calgon, $Na_{15}P_{13}O_{40}$–$Na_{20}P_{18}O_{55}$), rabbit anti-bovine IgG-alkaline phosphatase conjugate and disodium *p*-nitrophenyl phosphate hexahydrate, trypsin inhibitor and 2× crystallized trypsin and pepsin were purchased from Sigma Chemical Co. (St. Louis, MO). Goat anti-bovine IgG, rabbit anti-bovine IgG, and rabbit anti-bovine serum were obtained from Miles Laboratories (Elkhart, IN). DEAE-Sephacel was purchased from Pharmacia Canada Ltd. (Montreal, PQ). Microwell (Immunolon TM2, flat-bottom plate) for ELISA was purchased from Dynatech Laboratories Inc. (Alexandria, VA).

**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 plasma by the polyphosphate precipitation method. The experimental conditions (factors) for the maximum separation of immunoglobulins from bovine blood plasma were varied according to MSO within the following ranges: pH 3.5–5.5; NaCl concentrations, 0–0.7 M; polyphosphate concentrations, 0.1–2%; temperatures, 4–25 °C. The objective function used was a separation efficiency (SE) calculated from $PA_{Ig}/(PA_{BSA} + PA_{Ig}) \times 100$, where PA = peak area. An IBM PC computer was used for execution of the MSO program.

**Precipitation Procedure.** Sodium polyphosphate glass solution (20%, w/v) and 5.13 M NaCl were added to 20 mL of plasma, followed by adjustment of the pH to the desired value with 0.5 N HCl. In all experiments the final volume was adjusted to 30 mL with deionized water. Precipitation experiments were performed at the specified temperature by stirring for 30 min prior to filtration through Whatman No. 4 filter paper (Figure 1).

**Column Chromatography.** *A. Immobilized Metal Affinity Chromatography (IMAC).* The IgG-rich fraction of plasma protein obtained from the polyphosphate precipitation procedure was subjected to chromatography using an iminodiacetic acid–1,4-butanediol diglycidyl etherified (IDA–BGE) Sepharose 6B column loaded with Cu ion. Sepharose 6B was activated and cross-linked with 1,4-butanediol diglycidyl ether according to Sundberg and Porath (1974); iminodiacetic acid was coupled to the activated gel by the procedure described by Porath and Olin (1983). The upper two-thirds of a column (7.4 × 1.4 cm) packed with IDA–BGE Sepharose 6B was saturated with Cu ion by passing 0.05 M $CuSO_4$ through the column followed by washing with at least two bed volumes of the starting buffer, 0.5 M NaCl in 0.05 M Tris–acetate (pH 8.2) (Figure 1).

Removal of polyphosphate from the IgG-rich fraction prior to IMAC was necessary for better resolution of the chromatography. The residual polyphosphate was eliminated with a weakly basic anion exchanger (Amberlite

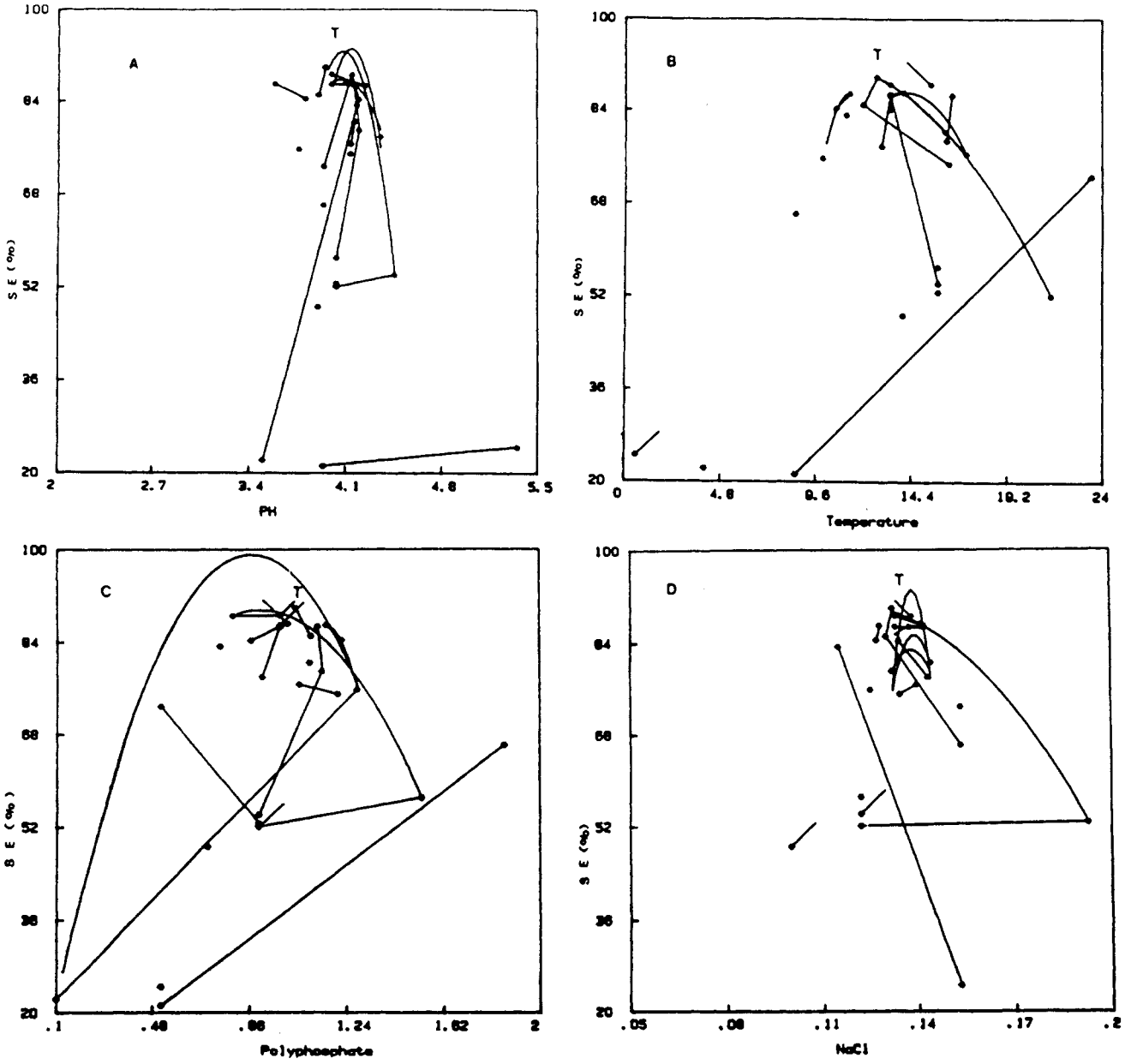


**Figure 2.** Response surface patterns for pH (A), temperature (B), and polyphosphate (C) and NaCl (D) concentrations obtained by mapping data from the simplex optimization for immunoglobulins. T indicates the target value of each factor for the subsequent simultaneous shift procedure.

IRA-68) according to the method of Boari et al. (1976). The phosphorus distribution in fractions obtained was determined according to the method of Morrison (1964).

*B. DEAE-Sephacel Ion-Exchange Chromatography.* DEAE-Sephacel was packed into a column (7.4 × 1.4 cm) and equilibrated with the first eluting buffer, 0.034 M Tris-HCl, pH 7.4 (Friesen et al., 1985). The polyphosphate-treated crude IgG fraction was applied to the column and eluted with a linear salt gradient to increase the NaCl concentration to 0.5 M (Figure 1).

**Electrophoresis and Immunoelectrophoresis.** Plasma protein samples pretreated with polyphosphate were analyzed by SDS–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 by radial immunodiffusion (RID) using a kit purchased from Miles Laboratories.

**Evaluation of Separation Efficiency.** Peak areas of Ig and bovine serum albumin (BSA) of plasma protein fractions obtained by the polyphosphate precipitation procedure on the electrophoretograms were analyzed according to the method of Ball (1986) by extracting Coomassie Blue R with 80% methanol from stained bands and then measuring the absorbance at 595 nm. Separation efficiency (SE) was expressed as the Ig to BSA ratio calculated from the peak areas of Ig ($PA_{Ig}$) and BSA ($PA_{BSA}$).

$$SE\ (\%) = PA_{Ig}/(PA_{BSA} + PA_{Ig}) \times 100$$

**Enzyme Immunoassay.** Immunoglobulin concentration in the plasma protein fractions was estimated by an enzyme-linked immunosorbent assay (ELISA) according to the method of Voller et al. (1976). A microwell plate was uniformly coated overnight at 6 °C or for 2 h at room temperature with purified rabbit anti-bovine IgG in phosphate-buffered saline (PBS; 11.7 g of $Na_2HPO_4$, 5.0 g of $KH_2PO_4$, and 2.34 g of NaCl/L, pH 7.2). The wells were washed with a detergent solution (0.05% Tween 20) in PBS. Into these wells were added aliquots of unknown plasma protein fraction samples. After the plates were allowed to stand at room temperature for 3 h, the wells

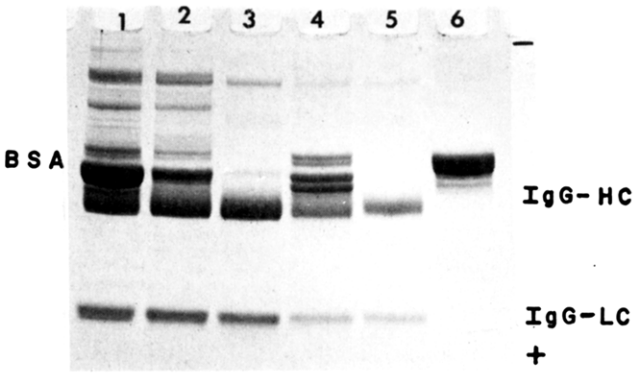


**Figure 3.** Comparison of bovine plasma protein component by SDS-polyacrylamide gel electrophoresis: (1) plasma; (2) polyphosphate supernatant fraction; (3) fraction II by IMAC; (4) fraction III by IMAC; (5) IgG standard; (6) transferrin standard.

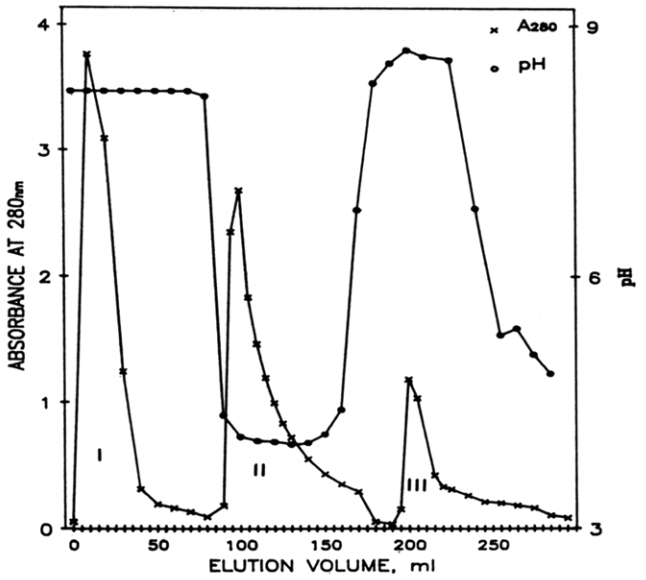


**Figure 4.** Immobilized copper affinity chromatogram of the supernatant fraction from polyphosphate-treated bovine blood plasma. The column (1.4 × 7 cm) was equilibrated with 0.5 M NaCl in 0.05 M Tris–acetate, pH 8.2; sample, 12 mL, 200 mg of protein; flow rate, 30 mL/h. Peaks: I, unbound fraction with the equilibration buffer; II, fraction eluted with 0.5 M NaCl in 0.05 M Tris–acetate, pH 4.0; III, fraction eluted with 0.01 M imidazole.

were washed with the detergent solution in PBS and a known aliquot of anti-bovine IgG linked to alkaline phosphatase was added into each well. After 2 h at room temperature, the enzyme substrate, *p*-nitrophenyl phosphate, was added to each well. Thirty minutes after the reaction was stopped by the addition of 50 μL of 5 N NaOH, the absorbance of each well was measured at 405 nm on a Titertek Multiskan photometer (Flow Laboratories, McLean, VA).

**In Vitro Proteolysis.** To compare the resistance of bovine colostral IgG and plasma IgG to in vitro proteolysis, the IgG activity was measured after reaction with trypsin and pepsin by the ELISA method. The cleavage of IgG was also examined by SDS-PAGE after reaction with the same enzymes. For trypsin digestion, after incubation of a mixture, 0.4% protein at an enzyme to substrate ratio of 1:20 in 50 mM Tris-HCl, 10 mM $CaCl_2$, pH 8.0 for 5 h at 37 °C, trypsin was inactivated by the trypsin inhibitor. For pepsin digestion, the enzyme to substrate ratio was 1:20 in 0.07 M sodium acetate buffer, pH 4.0, and the mixture was incubated for 4 h at 37 °C. Pepsin was inactivated by the addition of $Na_2CO_3$ to adjust to pH 7.4.

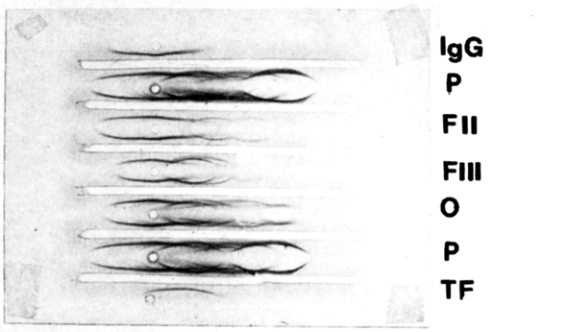


**Figure 5.** Immunoelectrophoresis of bovine plasma protein. Components: IgG, standard bovine IgG; P, bovine blood plasma; FII, IgG fraction by IMAC; F111, transferrin fraction by IMAC; O, polyphosphate supernatant fraction; P, plasma; TF, transferrin standard.

**Stability of Bovine IgG against pH Change.** To determine the resistance of bovine IgG to pH changes, 0.4% protein was mixed with the pH 2.0 (0.1 N acetic acid), 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 buffer solutions [0.1 M citric acid + 0.2 M $Na_2HPO_4$, according to Johnson and Lindsey (1939)] for 5 h at 37 °C. The changes in biological activity were evaluated by the ELISA method.

**Thermal Stability.** The IgG fraction separated by the polyphosphate precipitation procedure and purified by DEAE-Sephacel column chromatography was dissolved in pH 7.4 phosphate-buffered saline at a concentration of 0.1%. 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 calorimetry (DSC) by the method of Rüegg et al. (1977). Samples (80 mg) were sealed in aluminum pans and weighed. Thermograms within a temperature range of 20–110 °C were recorded on a Perkin-Elmer Model DSC-2 differential scanning calorimeter, equipped with the manufacturer's refrigeration accessories (Intracooler II).

**Amino Acid Analysis.** The sample proteins were hydrolyzed with 3 N HCl at 121 °C for 17 h. Amino acids were analyzed on 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.

The best conditions found by mapping super-simplex optimization were pH 3.95, NaCl 0.132 M, polyphosphate 1.04%, and temperature 12.7 °C (Figure 2). The major protein in the plasma proteins precipitated was serum albumin, leaving Igs in the supernatant (Figure 3, lane 2). The maximum separation efficiency attained was 90%. Immunological activity of the crude IgG fraction was 50%. Bovine Ig prepared by this method from plasma is presently used in feeding trials with piglets (Drew, 1987).

**Purification of the Crude IgG by Column Chromatography.** *A. Immobilized Copper Affinity Chromatography.* As shown in Figure 1, the polyphosphate in the crude IgG supernatant fraction was removed by a weakly basic anion exchanger (Amberlite IRA-68 resin) and then applied to an IMAC column.

An elution profile is shown in Figure 4. Fraction II was rich in IgG (Figure 3, lane 3) while in fraction III trans-

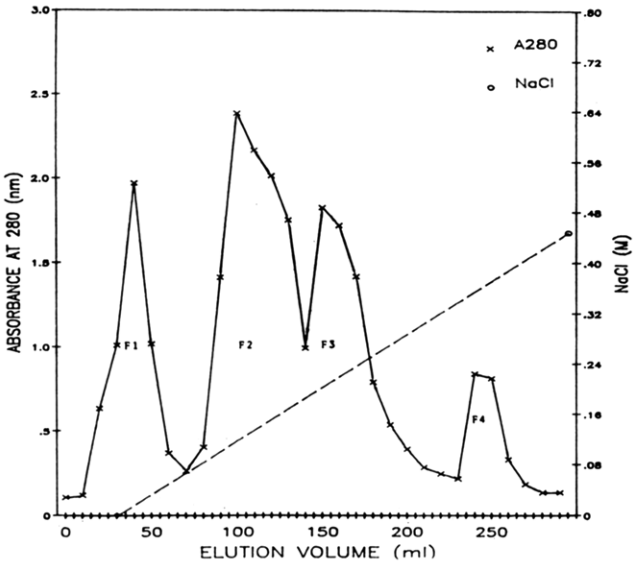


**Figure 6.** DEAE-Sephacel ion-exchange chromatogram of the supernatant fraction from polyphosphate-treated bovine blood plasma. The column (1.4 × 7 cm) was equilibrated with 0.032 M Tris-HCl, pH 7.4 sample, 15 mL, 250 mg of protein; eluent, a linear gradient of NaCl in 0.032 M Tris-HCl, pH 7.4; flow rate, 72 mL/h.

**Table I. Comparison of IgG Recovery**

| fraction | IgG purity, % | IgG rec rate, % |
|---|---|---|
| bovine plasma | 20 | |
| polyphosphate supernatant | 50 | |
| fraction II by IMAC | 99 | 40.3 |
| fraction 1 by DEAE | 100 | 30.4 |

ferrin was enriched although BSA and IgG still contaminated it (Figure 3, lane 4).

The immunoelectrophoresis pattern in Figure 5 confirms the observation made by SDS-PAGE, implicating the high purity of IgG in fraction II and intensified transferrin arc compared to the arcs for other plasma proteins.

The polyphosphate content of fraction II was 0.14% after the anion-exchanger treatment, meaning 86% polyphosphate elimination from 1% polyphosphate in the original supernatant fraction. The purity of fraction II was 99% IgG by the radial immunodiffusion method, and the IgG recovery was 40.3% (Table I).

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-Sephacel was used to purify crude Igs separated by polyphosphate precipitation (Figure 1). The elution profile is shown in Figure 6. SDS-PAGE indicated that fraction 1 was pure IgG (Figure 7), which was supported by the radial immunodiffusion assay indicating that it was almost 100% pure (Table I). Immunological activity of plasma fractions was compared by ELISA, and fraction 1 showed the highest immunological activity (Figure 8).

As the recovery of IgG in fraction 1 was low as shown in Table I, fractions 2 and 3 were combined, their pH and ionic strength were adjusted, and the resultant was 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-Sephacel chromatography after polyphosphate precipitation. IgGs from both sources were subjected to stability tests against proteolysis, pH changes, and heating for comparison.

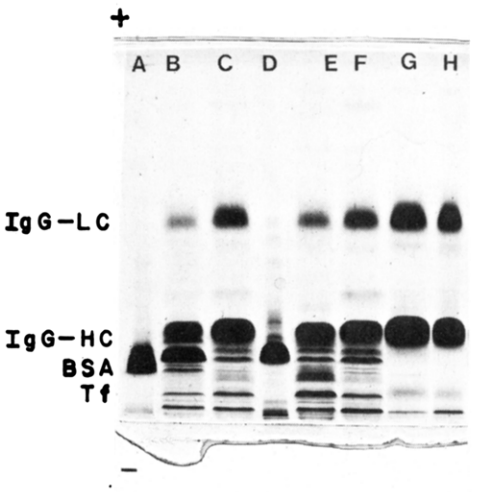


**Figure 7.** Electrophoresis of plasma protein fractions: (A) transferrin standard; (B) bovine plasma; (C) polyphosphate supernatant fraction; (D) BSA standard; (E) fraction 3 of DEAE-Sephacel chromatography (DEAE-SC); (F) fraction 2 of DEAE-SC; (G) fraction 1 of DEAE-SC; (H) IgG standard.

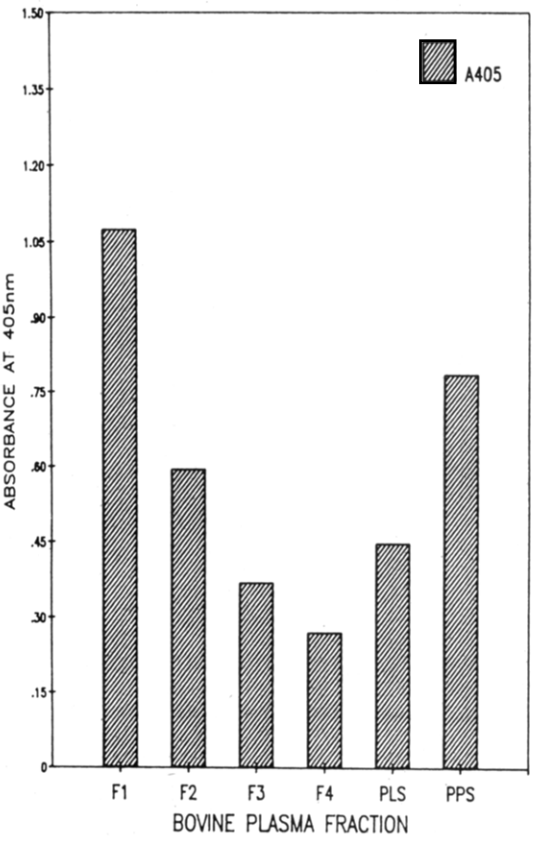


**Figure 8.** Comparison of immunological activity of bovine plasma fractions. Immunological activity was measured by an enzyme-linked immunospecific assay. Fractions F1–F4 are obtained from DEAE-Sephacel ion-exchange chromatography of the supernatant from polyphosphate-treated bovine blood plasma. Key: PLS, bovine blood plasma; PPS, polyphosphate supernatant.

*A. In Vitro Proteolysis and pH Changes.* SDS-PAGE patterns showed no marked difference in the extent of pepsin digestion between the two IgGs (Figure 9); however, a slightly higher susceptibility of plasma IgG was observed in the heavy chain but opposite in the light chain by

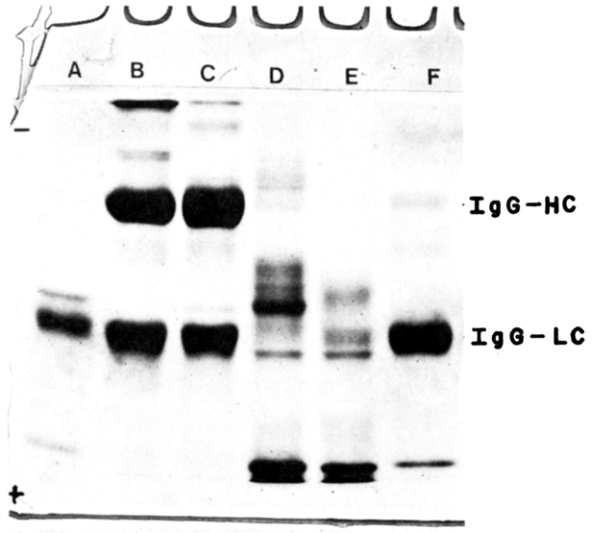


**Figure 9.** SDS-polyacrylamide gel electrophoresis of the enzyme-digested bovine immunoglobulins: (A) pepsin digests of plasma IgG; (B) intact colostral IgG; (C) intact plasma IgG; (D) trypsin digests of colostral IgG; (E) trypsin digests of plasma IgG; (F) pepsin digests of colostral IgG.

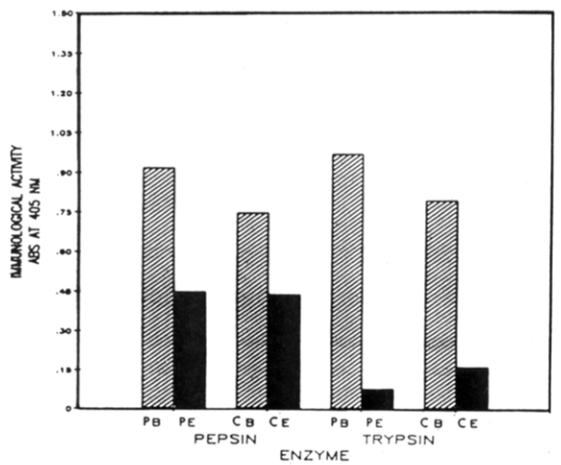


**Figure 10.** Comparison of immunological activity of the enzyme-digested bovine immunoglobulins: PB, intact plasma IgG; PE, enzyme digests of plasma IgG; CB, intact colostral IgG; CE, enzyme digests of colostral IgG. Immunological activity was measured by the ELISA method. Protein concentration in the assay was 100 ng/mL.

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 (Figure 10).

Rham and Isliker (1977) assessed the susceptibility of bovine serum $IgG_1$ and $IgG_2$ and colostral $IgG_1$ to digestion by measuring their anti-ferritin activity after digestion of Ig with pepsin or trypsin. No difference was observed between serum and colostral $IgG_1$; $IgG_1$ was more susceptible to pepsin digestion while $IgG_2$ was more susecptible to trypsin digestion. The structural differences between bovine serum and colostral IgG are located in the $F_c$ region which are more pronounced in $IgG_2$ than $IgG_1$ (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 conditions destroyed both plasma and colostral IgG equally below pH 3.

*B. Thermal Stability.* The heat-induced transition and activity change of the purified plasma IgG were investi-

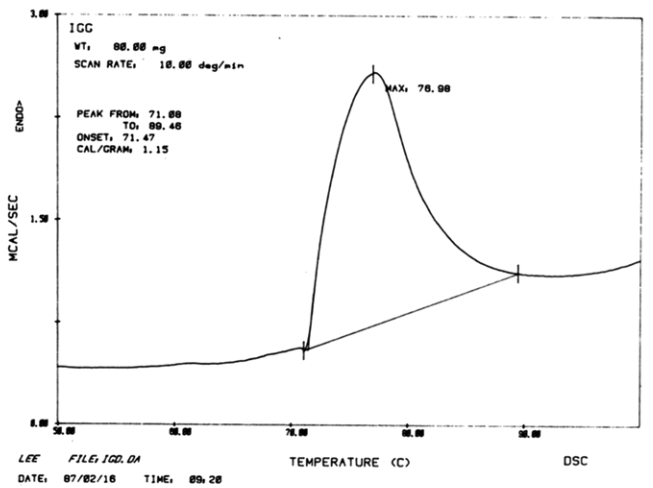


**Figure 11.** Differential scanning calorimetry thermogram of purified IgG.

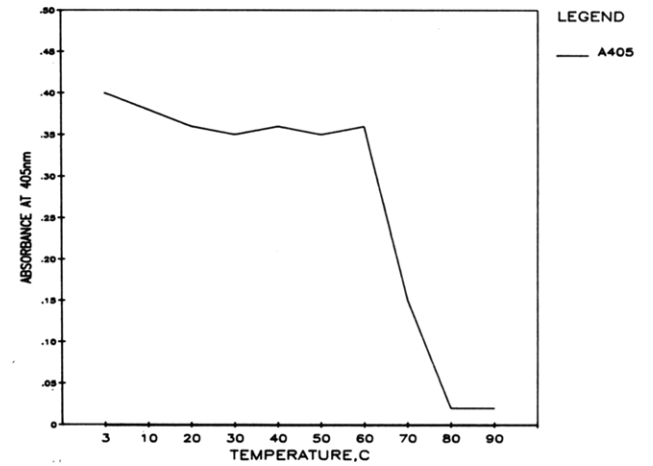


**Figure 12.** Thermal stability of purified bovine IgG. Immunological activity was measured by the ELISA method. Protein concentration in assay was 10 ng/mL.

**Table II. Amino Acid Composition of Polyphosphate Supernatant Fraction (Poly-P-SUP), Fraction 1 from DEAE-Sephacel Ion-Exchange Chromatography (DEAE-P-SUP), and Fraction II from Immobilized Copper Affinity Chromatography after Treatment with an Amberlite IRA-68 Column (IMAC/IRA-68)**

| amino acid | Poly-P-SUP | DEAE/Poly-P-SUP | IMAC/IRA-68 | bovine plasma | bovine serum[a] $IgG_1$ | bovine serum[a] $IgG_2$ |
|---|---|---|---|---|---|---|
| Asp | 9.0 | 8.8 | 9.0 | 10.0 | 7.6 | 7.5 |
| Thr | 8.2 | 9.1 | 8.3 | 6.4 | 9.6 | 8.4 |
| Ser | 9.6 | 10.9 | 10.5 | 6.4 | 13.9 | 11.0 |
| Glu | 12.2 | 10.4 | 11.5 | 13.4 | 8.0 | 7.4 |
| Pro | 6.2 | 6.1 | 6.1 | 5.3 | 7.1 | 7.8 |
| Gly | 4.5 | 4.8 | 4.6 | 3.8 | 6.3 | 7.1 |
| Ala | 4.4 | 4.0 | 4.4 | 4.6 | 5.5 | 6.0 |
| Val | 8.0 | 9.0 | 7.9 | 6.0 | 9.0 | 9.4 |
| Met | 1.3 | 1.1 | 2.7 | 1.2 | 1.2 | 0.6 |
| Ile | 2.9 | 2.8 | 2.8 | 2.8 | 2.5 | 2.4 |
| Leu | 8.7 | 7.5 | 8.3 | 9.0 | 6.9 | 7.3 |
| Tyr | 5.6 | 6.0 | 5.5 | 5.0 | 4.0 | 4.5 |
| Phe | 4.4 | 3.7 | 4.1 | 5.0 | 2.9 | 3.0 |
| His | 2.4 | 3.3 | 2.7 | 3.6 | 1.7 | 2.1 |
| Lys | 6.7 | 6.5 | 6.4 | 9.1 | 6.2 | 8.1 |
| Arg | 5.9 | 6.0 | 5.2 | 5.8 | 4.7 | 4.4 |
| Cys | ND[b] | ND | ND | 2.6 | 2.9 | 3.0 |
| TAA,[c] % | 100 | 100 | 100 | 100 | 100 | 100 |
| $NH_3$ | 1.5 | 1.5 | 1.7 | 0.8 | NT[d] | NT |

[a] Data by Lisowski et al. (1975). [b] ND = not determined. [c] TAA = total amino acids. [d] NT = not listed.

gated by differential scanning colorimetry and the ELISA method. Purified bovine IgG was stable up to 70 °C according to DSC (Figure 11); however, immunological activity was destroyed at this temperature according to ELISA (Figure 12). The temperature of maximum heat absorption was determined by use of a thermogram, commonly referred to as temperature of denaturation or temperature of transition (Figure 11). Immunoglobulins were relatively thermostable. When immune sera were heated at a temperature of 62 °C for up to 3 min, the antibody activity of the immunoglobulins was not affected (Kwapinski, 1972). Rüegg et al. (1977) found similar results in a calorimetric study of the thermal denaturation of bovine serum γ-globulin in simulated milk ultrafiltrates.

**Amino Acid Analysis.** The amino acid composition of the plasma, the crude Ig fraction (polyphosphate supernatant), fraction 1 of DEAE, and fraction II of IMAC were compared in Table II. Each fraction has a similar amino acid composition to that reported for bovine immunoglobulins by Lisowski et al. (1975).

In conclusion, almost pure IgG was successfully isolated by chromatographic purification of the crude IgG fraction separated from blood plasma by polyphosphate precipitation. DEAE-Sephacel was slightly superior to Cu-loaded IMAC columns because of the gradual loss of the binding capacity of the IMAC columns during repeated chromatography.

## LITERATURE CITED


Ball, E. H. "Quantitation of Proteins by Elution of Coomassie Brilliant Blue R from Stained Bands after Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis". *Anal. Biochem.* **1986**, *155*, 23.

Ballabriga, A. "Immunity of the Infantile Gastrointestinal Tract and Implication in Modern Infant Feeding". *Acta Paediatr. Jpn.* **1982**, *24*, 53.

Boari, G.; Liberti, L.; Passino, R. "Selective Renovation of Eutrophic Wastes Phosphate Removal". *Water Res.* **1976**, *10*, 421.

Butler, J. E. "Immunoglobulins of the Mammary Secretions". In *Lactation. A Comprehensive Treatise*; Larson, B. L., Vandegrift, V., Eds.; Academic: New York, 1974; Vol. III, p 214.

Crenwelge, D. D.; Dill, C. W.; Tybor, P. T.; Landmann, W. A. "A Comparison of the Emulsification Capacities of Some Protein Concentrates". *J. Food Sci.* **1974**, *39*, 175.

Drew, M., Department of Animal Science, University of British Columbia, Vancouver, BC, private communication, 1987.

Etheridge, P. A.; Hickson, D. W.; Young, C. R.; Landmann, W. A.; Dill, C. W. "Functional and Chemical Characteristics of Bovine Plasma Proteins Isolated as a Metaphosphate Complex". *J. Food Sci.* **1981**, *46*, 1782.

Friesen, A. D.; Bowman, J. M.; Bees, W. C. H. "Column Ion Exchange Chromatographic Production of Human Immune Serum Globulin for Intravenous Use". *Vox Sang.* **1985**, *48*, 201.

Gaillard, D.; Ailhaud, G.; Negrel, R. "Fetuin Modulates Growth and Differentiation of Ob17 Preadipose Cells in Serum-Free Hormone-Supplemented Medium". *Biochim. Biophys. Acta* **1985**, *846*, 185.

Gunstone, J. W. "Using Blood Plasma". *Natl. Prov.* **1980**, *6*, 20.

Hoerlein, A. B. "The Influence of Colostrum on Antibody Response in Baby Pigs". *J. Immunol.* **1957**, *78*, 112.

Johnson, W. C.; Lindsey, A. J. "An Improved Universal Buffer". *Analyst* **1939**, *64*, 490.

Jones, T. M.; Clark, H. R.; Beale, G. S.; Abell, L. A.; Vandegrift, V. "Characterization of Coagulant–Protein Complexes Produced by Chemical Coagulation of Industrial Whole Animal Blood". *J. Agric. Food Chem.* **1982**, *30*, 715.

Kennelly, J. J.; Ball, R. Q.; Aherne, F. X. "Influence of Porcine Immunoglobulin Administration on Survival and Growth on Pigs Weaned at Two and Three Weeks of Age". *Can. J. Anim. Sci.* **1979**, *59*, 693.

Kohler, E. M.; Cross, R. F.; Bohl, E. H. "Protection against Neonatal Enteric Colibacillosis in Sucking Pigs Orally Vaccinated Sows". *Am. J. Vet. Res.* **1975**, *36*, 757.

Kwapinski, J. B. G. *Methodology of Immunochemical and Immunological Research*; Wiley-Interscience: New York, 1972; p 484.

Laemmli, U. K. "Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4". *Nature (London)* **1970**, *227*, 680.

Lisowski, J.; Janusz, M.; Tyran, B.; Morawiecki, A.; Golab, S.; Bialkowska, H. *Immunochemistry* **1975**, *12*, 167.

McKee, J. I.; Tucke, J. W. "Food Compositions Containing Undenatured Water-Soluble Lactalbumin Phosphates". U.S. Patent 3 369 843, Aug 30, 1966.

McCallum, I. M.; Elliot, J. I.; Owen, B. D. "Survival of Colostrum-Deprived Neonatal Piglets fed Gamma-globulins". *Can. J. Anim. Sci.* **1977**, *57*, 151.

Morrison, W. R. "A Fast Simple and Reliable Method for the Microdetermination of Phosphorus in Biological Materials". *Anal. Biochem.* **1964**, *7*, 218.

Nakai, S.; Koide, K.; Eugster, K. "A New Mapping Super-simplex Optimization for Food Product and Process Development". *J. Food Sci.* **1984**, *49*, 1143.

Narayanan, I.; Prakash, K.; Verma, R. K.; Gujral, V. V. "Administration of Colostrum for the Prevention of Infection in the Low Birth Weight Infant in a Developing Country". *J. Trop. Pediatr.* **1983**, *29*, 197.

Niezgodka, M.; Lisowski, J. "Immunoglobulins of Colostrum. V. Localization of Structural Differences between Serum and Colostral Ovine and Bovine IgG1 and IgG2 Immunoglobulins". *Arch. Immunol. Ther. Exp.* **1980**, *29*, 305.

Packard, V. S. *Human Milk and Infant Formula*; Academic: Toronto, 1982.

Porath, J.; Olin, B. "Immobilized Metal Ions Affinity Adsorption and Immobilized Metal Ion Affinity Chromatography of Biomaterials. Serum Protein Affinities for Gel-Immobilized Iron and Nickel Ions". *Biochemistry* **1983**, *22*, 1621.

Rham, O. d.; Isliker, H. "Proteolysis of Bovine Immunoglobulins". *Int. Arch. Allergy Appl. Immunol.* **1977**, *55*, 61.

Rüegg, M.; Moor, U.; Blanc, B. "A Calorimetric Study of the Thermal Denaturation of Whey Proteins in Simulated Milk Ultrafiltrates". *J. Dairy Res.* **1977**, *44*, 509.

Sofas, J. N. "Use of Phosphate in Low-Sodium Meat Products". *Food Technol.* **1986**, *9*, 52.

Sundberg, L.; Porath, J. "Preparation of Adsorbents for Biospecific Affinity Chromatography. I. Attachment of Group-Containing Ligands to Insoluble Polymers by Means of Bifunctional Oxiranes". *J. Chromatogr.* **1974**, *90*, 87.

Voller, A.; Bidwell, D. E.; Bartlett, A. "Enzyme Immunoassays in Diagnostic Medicine". *Bull. WHO* **1976**, *53*, 55.

Williams, C. A.; Chase, M. W. "Immunoelectrophoretic Analysis". In *Methods in Immunology and Immunochemistry*; Academic: New York, 1971; Vol. III, p 234.


Received for review September 22, 1987. Accepted March 16, 1988.