CHAPTER IV

DISCUSSION AND CONCLUSION

Numerous methods for purification of plasma proteins were developed. Most are based upon a combination of technics such as precipitation, ion exchange chromatography, gel filtration, electrophoresis etc.

Many methods for preparation of ceruloplasmin have been published since 1947. It was isolated from plasma serum; or globulin rich fraction IV-1 of Cohn et al (29), using precipitation and/or chromatography, (17, 39, 40, 53, 54, 69, 110, 117). Broman (18) has modified the system by using DEAE-Sephadex, making a three-stage separation which yielded a highly purified ceruloplasmin. Recently the modified method employing separation on cellulose ion exchange resin and ammonium sulphate precipitation was described (36, 149).

The Stokes' method which employed only a two-stage seperation using Sephadex ion exchange chromatography was selected for this study (118). DEAE-Sephadex was found to have a high binding capacity for ceruloplasmin. In this study the glass tubing (1.7 x 30 cm) which was drawn at one end to make a small outlet was modified to make a column for chromatography. The height of DEAE-Sephadex in the column is 15 cm and it was found to be sufficient to adsorb ceruloplasmin from about 250 to 500 ml of plasma. The amount of gel required and the time for fractions collection was reduced.

According to the result, the preparation from CM-Sephadex column was still found to have traces of β-globulin contaminants (Fig. 3, p. 26). This may be caused by the column incapable of separating the ceruloplasmin from the contaminants. Rechromatography was needed to prepare pure ceruloplasmin. This could be due to overloading of the column because when the bed volume of CM-Sephadex was increased to 100 ml, pure ceruloplasmin was obtained in a high yield without any need for rechromatography.

Many methods for preparing haptoglobin have been described since 1958 (10, 30, 31, 52, 73, 126), DEAE-cellulose chromatography and Sephadex filtration were used. The method used in this study was based on the specificity of Hb-Hp reaction which is rapid and normally irreversible (89). The application of an insoluble sepharose matrix with this reaction was employed (23, 84, 96). Affinity chromatography has been extensively used in the purification of antibodies, antigen and enzymes (34, 103). Serum Hp would bind to Sepharose: Hb insoluble matrix and then dissociation of Hp from bound matrix was achived in the presence of high concentration of urea. The eluted haptoglobin was found to contain no contaminants (Fig. 10, p. 35, Fig. 11, p. 37), and the yield was high.

Although ceruloplasmin and haptoglobin obtained were shown to be pure by double immunodiffusion or immunoelectrophoresis, the antisera produced by immunization with the two purified proteins contained antibodies to IgG, and other serum proteins. The presence

of contaminant is normally evidenced by the results of immunization. These contaminating antibodies are more intense after prolonged immunization. The antisera were made monospecific to the required protein after immunoadsorption with pure IgG and eluate from DEAE-Sephadex column which contains reduced amount of ceruloplasmin, but rich in other serum proteins.

Quantitative determination of plasma proteins (ceruloplasmin and haptoglobins) are easily accomplished by radial immunodiffusion using monospecific antisera prepared.

The results of ceruloplasmin determination showed that the normal range covers a wide range (33 ± 20). This result is similar to those obtained by other investigator (12). The levels are much increased in the group having increased serum SGOT, SGPT (with or without increased serum bilirubin) and those having increased serum bilirubin. This group is classified as patients having liver diseases. The higher levels among those with liver diseases agree with previous reports (26, 86, 119). The higher levels of ceruloplasmin in hemoglobinopathies also demonstrate its lack of specificity for any disease. The level of ceruloplasmin is diagnostic particularly in Wilson's disease which shows marked decreased in ceruloplasmin level. Due to the rarity of this disease, sera of these patients were unavailable for our evaluation. The results in general supported the reliability of the antiserum prepared, and the technic used for ceruloplasmin determination.

The levels of haptoglobin among those with confirmed hemoglobinopathies generally do not overlap with those of normal ones. The levels were found to be extremely low (below 16%) in 4 cases and the level approach the lower limit of normal level in 5 cases with Hb E disease. This indicated that patients with hemoglobinopathies have decreased level of Hp most of the time and only occasionally the level reaches normal level. This study indicated that a determination of Hp is a good screening test to support the present of hemoglobinopathies. As this disease is very common in Thailand, a simple method for Hp determination, like the presence technic, is very applicable for clinical laboratories.

The immunodiffusion technic is considered to be one of the most specific method for quantitative measurement of ceruloplasmin (64, 74) and haptoglobin (106). Commercially available immunodiffusion plate is too expensive to render them suitable for general use in clinical laboratories. The demand for monospecific antibody to human plasma proteins are increasing corresponding to the interests in research in clinical immunology and protein chemistry. This study is in line with the new trend in serum protein chemistry, in which specific protein determination is replacing old form of protein investigation, such as cellulose acetate electrophoresis. This study will also be the key in purifying proteins from plasma, the production of antibody and application of the antibody in quantitative determination of such proteins. Besides ceruloplasmin

and haptoglobin, other proteins may be purified and used in a similar manner.

This study demonstrates that, with proper combination of technics, purification of many, if not all, human or animal proteins can be accomplished. This selection is based upon the availability of equipments and technical knowhow. After the purification of protein monospecific antisera can be prepared. Absorption is needed most of the time, and the choice of absorbant should be forcasted, and made available. When purified proteins and monospecific antiserum are both available, the opportunity is opened to different utilizations. Determination of such protein in biological fluids by immunodiffusion is just the simplest form of its application. It is hoped that, this work has served its limited purposes.