The disc electrophoresis advanced by Ornstein and Davis\textsuperscript{1} is the most useful method among various zone electrophoreses for separating proteins in sera. Using this method, the present author has observed and published in the previous paper\textsuperscript{2} the change of protein fraction patterns of human serum in several diseases and other biological materials.

A marked increase of amount of serum mucoid in inflammatory diseases and malignant tumors has already been noticed by many investigators. Recently Price et al.\textsuperscript{3} have emphasized the importance of serum mucoid fraction patterns divided into five types from the viewpoint of diagnosing such chronic diseases as tuberculosis, schizophrenia and malignant tumor.

The present paper presents a relationship between serum mucoid fraction patterns and blood sedimentation rates (BSR) to obtain information for differential diagnosis of pulmonary tuberculosis, gastric cancer and multiple myeloma. Goa's method\textsuperscript{4} was used here to simplify the usual tedious process as to isolation of serum mucoid.

METHOD OF STUDY

Tris(hydroxymethylaminomethane), N,N,N',N'-tetramethylenediamide manufactured by Daiichi Kagaku and Acrylamide and Bis (N,N'-methylenbisacrylamide) prepared by Canalco were used.

The whole process of disc electrophoresis was repeated from the beginning when the border between lower gel solution and water layer was ruffled or gel solution was disturbed by foam coming up from the bottom of gel tube before
the beginning of geling. The conditions of electrophoresis such as length and diameter of the gel tube, voltage of the current used and time of the electrophoresis were similar to those described in the previous papers.

In removing gel from the gel tube following electrophoresis, surface of the gel was kept being flat and smooth as far as possible. For too much breakage of sample gel may lead incorrect reading for quantitative microdensitometry. Propylene glycol is an even better lubricant than water for use in removing the gel.\(^5\)

Isolation of serum mucoid was prepared via the following steps.

\[\text{2 ml of serum + 2 ml of physiological saline solution} \]
\[\text{4 ml of mixed solution + 4 ml of 8 M perchloric acid} \]
\[\text{White precipitant, allow to stand for 5 minutes} \]
\[\text{Filtration by Toyo filter paper No. 6} \]
\[\text{3 ml of filtered solution in 2 N HCl + 1 ml of 2% phosphotungstic acid} \]
\[\text{White nubecular precipitant, centrifugation (2,000 rpm at 0-4°C)} \]
\[\text{Dissolution of precipitant with 1 or 2 drops of 0.1 N and 1 N NaOH solution} \]

This solution was diluted with upper gel solution and 20 \(\mu\)l or 32 \(\mu\)l of the diluted solution was placed on top of the upper gel depending upon the concentration of precipitant.

Staining was performed as described in the previous papers. Cautionary remark must be made, if rapid destaining is used. For it would often decomposte Peak II considered to be orosomucoid and migrate it to overlap Peak I. In destaining by allowing for stand, measurement and photographing were performed within fifty hours at least before the phenomenon mentioned above appears.

Canalco Model E microdensitometer was used for the quantitative measurements.

RESULTS

1) Pulmonary tuberculosis: 5 cases

No. 107 (47 year-old, male) and No. 108 (75 year-old, male) suffering from the disease over ten years have had cavity formations in chest X-ray film. Cultures of sputum were positive for M. tuberculosis and BSR elevated 15 mm and 35 mm in one hour respectively. Both cases were being treated with administration of PAS, INAH and SM after a clinical diagnosis of tuberculosis.

No. 139 (48 year-old, female) had no clinical symptoms, except a cavity
found in upper field of the right lung. No. 148 (27 year-old, male) had a tuberculoma-like shadow in X-ray film of the right lung with a BSR of 3 mm in one hour. No. 150 (30 year-old, male) was diagnosed as having pulmonary tuberculosis with wet pleurisy. Liver function tests of the cases were almost within normal limits. The hemoglobin content of No. 148 and 150 showed 14.1 and 13.5 g/dl respectively. These three cases may clinically be considered to be relatively mild.

Fig. 1

Fig. 2
The area ratio of $I/I+I$ for No. 107 and 108 were calculated at 0.14 and 0.11, although patterns shown in Fig. 1 measured by the microdensitometer with 2 O.D. cannot be compared with other patterns. On the other hand, No. 139, 148 and 150 indicated at 0.23, 0.36 and 0.35 as shown in Fig. 2. No. 136 of Hyland Control Serum had $I/I+I$ ratio of 0.20.

2) Gastric cancer: 2 cases

No. 133 (64 year-old, female) felt a pain over the abdomen, fatigue and dizziness, and was diagnosed by the examination of gastrointestinal tract as gastric cancer. A liver function was nearly within normal limits. However, examination of the blood disclosed a hemoglobin of 8.3 g/dl and a BSR of 32 mm in one hour.

No. 174 (68 year-old, male) had an abdominal pain and distension which admitted him to hospital. The presented a regular mucosal pattern with no evidence of a constant filling defect in X-ray finding and stools were negative for occult blood. The abdominal distension, however, was exacerbated and pathological examination revealed malignant tumor cells in ascites. The case was thus reported as having gastric cancer complicated with peritonitis. In addition, an abnormal value of Icterus Index, Thymol Turbidity Test and Cobalt Chloride Reaction was observed. Unfortunately the patient in this case died one week later.

In Fig. 3 a remarkable reduction of Peak I was shown and area ratio of $I/I+I$ was calculated below 0.03 in each case.

3) Multiple myeloma: 2 cases

No. 116 (54 year-old, female) complained of an extraction pain on back while walking and an abnormally elevated BSR three months prior to admission. After the admission the diagnosis was $\gamma$-myeloma from the results of X-ray finding and electrophoretic analysis. In addition, 0.2 serum A/G ratio, 7.7 g/dl hemoglobin, urinary protein (+ +), urinary sugar (−), Bence Jones protein (−), and 158 mm in one hour BSR were observed in laboratory studies.

No. 137 (72 year-old, male) had also complained of bilateral lumbago and back pain. A sudden fracture of the left clavicle while doing outdoor physical exercises brought him to hospital. The diagnosis was made by the result of electrophoretic analysis and blood pictures of the bone marrow. The serum A/G ratio was 0.3, the hemoglobin content was 9.2 g/dl, the urinary protein was strong positive, the urinary sugar was negative and the BSR was 148 mm in one hour.
Fig. 4 shows a reduction of area ratio \( I/I+II \) of No. 116 and 137 at 0.05 and 0.03. As seen in Fig. 5, a relationship between BSR and the area ratio of \( I/I+II \) in chronic diseases mentioned above was drawn on logarithmic (both abscissa and ordinate) paper.

**DISCUSSION**

The classifications of glycoproteins were essentially based on the chemistry of the component sugars and on the structural details of the carbohydrates group, although many authors still define glycoproteins in their own ways resulting in a great confusion of the meaning of the term.\(^5\)\(^,\)\(^7\) According to a general belief, human serum mucoid is characterized by its solubility in perchloric acid and insolubility in phosphotungstic acid. The results obtained from the present study are considered to indicate fraction patterns of serum mucoid. The identification of each fraction is not yet clear, but at least Peak II showing a strong positive PAS reaction is probably a band of orosomucoid.

The increase that occurs in the total amount of serum mucoid has been known in chronic diseases,\(^8\)\(^,\)\(^9\)\(^,\)\(^10\) however, little information is available as to its pattern change. In this respect, the importance of the paper by Price *et al.* is certainly noted. The pattern change of serum mucoid which was recognized by
Price can hardly be regarded in this study. The problem should be discussed after further investigations concerning the isolation technique in each study.

The significance of the data shown in Fig. 5 must be emphasized. In case of pulmonary tuberculosis, area ratio of I/I+II seems to be reduced on a straight line in disproportion to the elevation of BSR. The two dots obtained from the cases of multiple myeloma can be failed on the same line. In contradiction, the dots in case of gastric cancer falls off the line because the reduction of area ratio of I/I+II is not accompanied by the corresponding elevation of BSR. The result may be explained by the electrophoretic patterns which show a marked increase in Peak II in multiple myeloma and a marked reduction in Peak I in gastric cancer. In other words, the increase of Peak II by the marked elevation of BSR is one possibility. However, the pathophysiological mechanism of the low Peak I and high Peak II are still to be remained as an unknown problem. At the same time, the identification of the substances would be an important research work.

CONCLUSION

The change of serum mucoid fraction patterns in chronic diseases such as
pulmonary tuberculosis, gastric cancer and multiple myeloma was examined using disc electrophoresis. The results obtained were as follows:

1) In pulmonary tuberculosis, area ratio of I/I+II was reduced with the development of the disease.

2) In gastric cancer and multiple myeloma, the remarkable reduction of area ratio of I/I+II was illustrated, but the details are a little different from each other.

REFERENCES

5. Disc electrophoresis newsletter, #6, October, 1964.