PHOTOELECTRIC EMISSION SPECTROMETRIC ANALYSIS OF THE METAL ELEMENT CONTENT IN STARCH BLOCK ELECTROPHORETICALLY SEPARATED HUMAN SERUM PROTEINS

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Kang Chong Nah

1964

PHOTOELECTRIC EMISSION SPECTROMETRIC ANALYSIS OF THE METAL ELEMENT CONTENT IN STARCH BLOCK ELECTROPHORETICALLY SEPARATED HUMAN SERUM PROTEINS

By

Keng Cheng Nah

A THESIS

Submitted to
Michigan State University
in partial fullfilment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Biochemistry

1964

ABSTRACT

PHOTOELECTRIC EMISSION SPECTROMETRIC ANALYSIS OF THE METAL ELEMENT CONTENT OF STARCH BLOCK ELECTROPHORETICALLY SEPARATED HUMAN SERUM PROTEINS

By Keng Cheng Nah

This investigation was carried out to extend a series of studies on the metal element content of electrophoretically separated normal human blood serum proteins. The objectives were to adapt new and improved methods of establishing the results on a more quantitative basis and to find out about the nature and distribution of the metal elements associated with proteins in a biological fluid system.

The separation of the serum proteins was accomplished by employing starch block electrophoresis and subsequent elution with physiological saline. Each eluate was treated with FCL reagent and measured spectrophotometrically to obtain data for a protein distribution curve which indicated the number and quantity of fractions separated.

All eluates found to be part of the same protein fraction were combined, concentrated, evaporated to dryness, and ashed. Each ash sample from a protein fraction was analyzed in the photoelectric spectrometer and thus the metal elements associated with the albumin, alpha-, beta-, and gamma-globulin fractions were determined. The final data provided an estimate of the distribution of the following metal elements among the four protein fractions separated: Ca, Mg, Mn, Fe, Cu, Zn, Mo, and Al. The results indicated the presence of all elements, but not to the same extent, in the serum fractions.

VITA

The author was born September 12, 1938, in Amoy, China. His secondary education was completed in 1956 at Chung Cheng High School, Singapore, Malaysia. In 1960, he was graduated from Nanyang University, Singapore, with a Bachelor of Science degree. He was admitted to the Graduate School of Michigan State University in September, 1962, and has been in attendance since that time.

ACKNOWLEDGMENT

The author wishes to express appreciation and gratitude to: (1) Professor H. A. Lillevik for guidance and counsel; (2) the Asia Foundation for the Scholarship which enabled the author to achieve the participation and accomplishment of this study; (3) Professor A. L. Kenworthy of the Department of Horticulture, Michigan State University, for the services of the photoelectric emission spectrometer; and (4) Dr. Robert B. Foy of Edward W. Sparrow Hospital, Lansing, Michigan, for the supply of human blood serum specimens.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

A great many proteins have become well-known in ability to form complexes with metal ions. Among them are the human blood serum proteins. Being in trace amounts in blood serum, the cations, especially the transition metals, have many potential bonding orbitals, and can interact with different electron donor groups on proteins through chelation and coordinate covalent bonding. One kind of cation may form several types of complexes with different kinds of proteins and one protein may form different complexes with different cations. Some types of bonding between different functional groups on proteins and different cations have already been known, but by and large, the interaction is not yet properly understood. Nevertheless, no matter what kind of biological function each metal ion possesses, its binding to proteins involves highly specific activities in biochemical processes and is of great significance in both physiological and pathological aspects.

In spite of their fulfillment of some criteria of purity, quite a few human serum proteins, like other serum proteins, have not been truly separated, purified, or completely identified. It is more convenient to consider them in terms of groups of proteins based on certain common properties which are consequently characterized by methods of separation such as salt-fractionation, density gradient ultracentrifugation, chromatography, and other techniques. Electrophoresis is also

one of the principal techniques frequently used on blood serum protein separation and the names of the fractions obtained have been most commonly designated as alpha, beta, gamma, and subfractions thereof.

It was the purpose of this study to observe both the distribution of protein-binding metal ions and the quantity of each as they occur in the fractions of normal human blood serum as separated by starch block electrophoresis. Such results would serve as reference for a study on the variation of these ions due to diseases or other abnormal physiological conditions. Inasmuch as the affinity of proteins for sodium and potassium ions is rather small because of their low valency, the distribution of these ions has not been included in this work.

II. HISTORICAL

Several preceding reports have summarized various aspects of the historical development of the chemistry of metal proteins and metalloproteins in human blood serum. These include those submitted by LeDuc (29), Foy (13), and Long (33).

In addition, the proceedings of a conference on "Biological Aspects of Metal-Binding" held at Pennsylvania State University in 1960 has been published with Johnson (11) as editor. A chapter included in a textbook written by Martin (36) this year presents a summary discussion of the role of metal ions in biological systems by giving an introduction to formation constants, metal ion specificity, protein binding, and ligand field theory.

Some of the more direct and pertinent past investigations relative to major metal ions examined in this study are reviewed as follows.

A. Calcium

Since it was suggested in 1911 that calcium is bound to serum proteins (44), there have been many reports concerning this relationship (14, 38, 51, 47, 37). Copeland (8) and Carr (5) observed that more calcium was bound to albumin than to the globulin portion in serum. According to LeDuc (29), two-thirds of the calcium was distributed equally between albumin and the gamma-globulins in human serum. Prasad and Flink (40, 41, 42) determined that 43 to 55 percent of all calcium in serum was protein bound, and also concluded that 50 to 55 percent of the non-ultrafiltrable calcium was bound to albumin. They further claimed that the main portion of globulin bound calcium was in the

beta-globulin fraction and that only a slight amount was bound to the alpha- and gamma-globulins. Previously, in 1956, Laurell, et al. (28) demonstrated that human serum calcium was bound to the alpha- and beta-globulins. Pathological evidences showed that the beta-2-A fraction of the gamma-globulins might be one of the proteins that bound calcium (17). It was reported by Lillevik, et al. (31) that calcium was associated with proteins in all electrophoretic fractions of human serum. A similar distribution pattern of protein serum calcium was also supplied by Foy (13) who stated that 45 to 55 percent of the bound calcium was associated with albumin. Long (33), however, concluded that calcium was bound only to the alpha-1- and alpha-2-globulin fractions.

Owing to differences of experimental conditions, techniques used, and possible variation of serum protein concentration in different samples, the chances for discrepancies in the foregoing results can be more readily understood.

B. Magnesium

By means of an ultrafiltration method, magnesium in serum which was protein bound was shown by Watchorn and McCance (51) to be one-fourth of the total human serum magnesium. Cantarow and Scheparts (3), in their textbook, report this value to vary from 15 to 30 percent. It was similarly observed by Copeland and Sunderman (8) that the amount of magnesium bound by albumin was greater than that by globulins. The distribution of protein bound magnesium in plasma was found by Carr (4) to be similar to that for calcium but, on the other hand, its presence among all protein fractions of human serum was reported by Lillevik,

et al. (31). The occurrence of magnesium in all electrophoretic fractions was found similarly by Foy (13), and yet Long (33) observed that it was present only in the alpha-globulin fractions as was the occurrence of serum calcium.

C. Iron

Since the 1920's, serum (or plasma) iron has been distinguished from hemoglobin iron (12). Later, Barkan (2) showed that serum iron was non-dialyzable at the pH of blood. Vahlquist (48) in 1941 found that both albumin and globulins could bind iron but that the main portion was in the alpha- and beta-globulins. Holmberg and Laurell (18) showed that iron was also firmly bound by serum to form a salmon pink colored compound. That this compound involved the formation of an iron-beta-globulin complex was confirmed by Schade and Caroline (45), Koechlin (23), and Cohn, et al. (6). The specific iron binding beta-globulin to which the major part of serum iron was bound was named "transferrin" by Laurell (27). LeDue (29) also ascertained that serum iron was associated with the beta-globulins.

It was found by Crosby and Demeshek (9) and Maier (34) that a small amount of hemoglobin normally existed in plasma or serum. This was because of haptoglobin, a protein that binds hemoglobin. Thus, in the alpha-2-globulin fraction where haptoglobin occurs, protein bound iron was observed. Foy (13) and Long (33) reported that iron was present in all electrophoretic fractions of the human serum proteins. They also emphasized that the higher content of iron was present in the alpha- and beta-globulin fractions.

D. Copper

In 1927, Abderhalden and Moller (1) showed that copper in serum was non-dialyzable, but on acidification, Warburg (50) found that it was released. Eisler, et al. (10) regarded copper as being bound to the albumin fraction after observing its migration with this serum protein in electrophoresis. In 1948, Holmberg and Laurell (19) showed that more than 90 percent of the serum copper was firmly bound to an alpha-2-globulin called "ceruloplasmin" which also possessed oxidase activity. It was determined that one mole of this protein (molecular weight = 151,000) combined with eight atoms of copper. A proportionate relationship was found between serum copper level and the oxidase activity which indicated that the serum copper level greatly depended upon the ceruloplasmin content (20, 21). Lahey, et al. (25) also noted the correlation between copper level and the amount of alpha-2- and beta-globulin content.

In 1953, Gubler, et al. (15) found that, other than the firmly ceruloplasmin-bound copper, there was present a small amount of relatively loosly bound copper which probably represented this element in transport. By means of oral administration of Cu^{64} they were able to demonstrate that copper was incorporated by albumin and that later, through indirect exchange, it was turned over to ceruloplasmin.

LeDuc (29) noticed that copper migrated with the gamma-globulin fraction upon paper electrophoresis. Foy (13) concluded that copper was bound by all protein fractions but occurred principally in albumin and beta-globulin. Long (33) showed by means of starch block electro-

phoresis that copper was associated with the albumin and alpha-globulin fractions.

E. Zinc

Since the demonstration by Pauli and Schon (39) in 1924, zinc has been regarded to be protein bound. Gurd and Goodman (16) observed the reversible combination of human serum albumin and zinc ions. They provided good evidence for showing that zinc ions were bound by the imidazole group. Vikbladh (49) reported that zinc existed in at least two forms of linkage to protein in plasma. These involved a firmly bound type (30 to 40 percent) and a loosely bound form at physiological pH. Cohn (6) observed that zinc reacted reversibly with different human plasma proteins.

Ressler, et al. (43) first precipitated albumin and gamma-globulins from human serum with zinc ions. By means of oral administration of Zn^{65}, Wolff, et al. (52, 53) noted that radioactive zinc largely appeared in the albumin and alpha-globulin fractions of canine plasma. The ratio between loosely and firmly bound zinc in dog plasma was similar to that seen for human serum, and the greatest amount of zinc was associated with the alpha-globulin fractions. LeDuc (29), on the other hand, found zinc mainly present in the gamma-globulins. Occurrence of zinc in all fractions was seen by Foy (13), and isomigration of zinc with the alpha-fractions was shown by Long (33).

III. EXPERIMENTAL METHODS

A. Apparatus

1. Starch Block Electrophoresis Apparatus.

The instrument used was a product of the E-C Apparatus Co., 538 Walnut Lane, Swarthmore, Pennsylvania. Constructed of lucite, each buffer tank at the ends was subdivided into three compartments with electrodes so that three simultaneous runs could be made. The starch block bed was placed on a 1/8 inch thick flat lucite sheet under which was the lower water cooling plate. During the performance of experiments, a sponge pressure pad was put on top of the starch block and under the upper cooling plate. Finally the whole assemblage was clamped together with the lower cooling plate. To achieve better separation and requirement for less amount of sample, a compartment one-third the width of the original was constructed with the aid of the following lucite strips:

No. of Strips	Direction of Use	Dimensions (cm.)
2	Parallel to sides	45 x 1.5 x 0.6
2	Transverse at outer ends	6 x 1.5 x 0.6
2	Transverse across top to clamp sides together	7 x 1.5 x 0.6

The other two-thirds of the compartment could be either used as a single compartment of twice the new size, or else as two more parallel compartments of the same new size.

The E-C power supply unit had a range of 0-1000 volts and could supply a current varying from 0-200 milliamperes.

2. Photoelectric Emission Spectrometer (22).

The emission spectrograph used was a 1.5 meter photoelectric spectrometer (commercially referred to as the "Quantograph"), with a grating containing 981 lines per mm. and manufactured by the Applied Research Laboratory, Inc., Glendale, California. Its availability was a service of the Department of Horticulture at Michigan State University. It had a spectrographic system equipped with automatic recording instrument. It was designed for the analysis of the following ten elements: Al, B, Ca, Cu, Fe, Mg, Mn, Mo, P, and Zn. Cobalt was utilized as an internal standard.

The excitation conditions were obtained from a "Multi-source" unit, made by the same manufacturer. During excitation, it produced a spark discharge from 230 volts input, 900 volts output, 2 microfarads capacitance, 50 microhenries inductance, and residual resistance.

The electrodes were composed of high purity graphite with the counter electrode being 1/8 inch diameter and shaped to a hemispherical point. The revolving disc-shaped electrode had a diameter of 0.492 inch and a thickness of 0.200 inch. The electrode disc revolved in the ash solution in question and transferred it to the spark.

3. The Spectrophotometers.

The models DU and B manufactured by the Beckman Instrument Co. were used for absorbancy measurement at 280 and 660 mμ, respectively.

B. Reagents and Solutions

1. Starch Suspension.

The purification of raw potato starch (Fischer, No. S-513) was carried out by suspending 500 g. in 1 liter of 0.1 N HCl with constant

stirring for 15 min. The supernatant was decanted and the suspending, stirring, settling, and decanting of starch were all repeated once again with another liter of 0.1 N HCl solution. After decantation, the starch was then suspended in two volumes of distilled water, stirred, allowed to settle, and the wash water was poured off. This washing was repeated until the starch suspension came to a pH value the same as that for distilled water when Hydrion paper was used. Washing once more was carried out with glass redistilled water. The suspension was filtered on a large Buchner funnel and the starch was allowed to dry under suction. The semi-dried starch was then washed two or three times with 50 ml. portions of veronal buffer solution (vide infra) containing a few drops of CCl_4. The buffer solution with CCl_4 aided in the removal of smaller particles (fines) and impurities and helped to avoid conductivity and pH changes during electrophoresis (2). Lastly, the washed starch was suspended in an equal volume of veronal buffer and kept in the cold room until ready for use.

2. Veronal Buffer Solution.

The solution was prepared by dissolving 0.24 mole (44.20 g.) of anhydrous veronal (diethylbarbituric acid, Merck and Co.), and 0.2 mole of sodium hydroxide in two liters of redistilled water. It was made to an ionic strength of 0.1 and a pH value of 8.6.

3. Folin-Ciocalteau-Lowry Reagent.

This reagent consists of two solutions referred to as A and B.

a. Solution A was simply the Folin-Ciocalteau reagent commercially available from Scientific Products Co., Evanston, Illinois. It is a bright yellow solution containing sodium tungstate, sodium molybdate,

lithium sulfate, phosphoric acid, and hydrochloric acid in amounts as given by Litwack (32). It was kept from light and diluted just before use.

b. Solution B was an alkaline composition made up of the following contents:

Compound	Concn. (% w/v)	Ratio (by vol.)
Sodium carbonate	4	100
Cupric sulfate	2	1
Sodium potassium tartrate	4	1

The sodium carbonate solution was filtered before mixing with the other two solutions.

4. Physiological Saline.

This was an 0.85 % (w/v) NaCl solution prepared by dissolving 17 g. of C. P. sodium chloride into distilled water until two liters of solution are obtained.

5. Internal Standard Solution for the Spectrograph.

The internal standard solution (22) contained 0.04 % of Co (II) ion, 0.5 % of Li ion, and 1.0 % of K ion acidified with 1.8 N HCl (1500 ml. conc. HCl per 10 liters).

6. The Metal Ion Stock Standard Solution for Calibration.

This solution was composed of a mixture of salts containing the ten metallic elements to be analyzed. After evaporation to dryness and mixing with the internal standard solution, it was used for the calibration of the "Quantograph." The following substances and amounts for each is as follows [illegible].

Compound [1]	Quantity (g./10 l.)
$CaCO_3$	50.06
H_3BO_3	0.287
$CuSO_4.5H_2O$	0.156
$FeSO_4.7H_2O$	0.6203
$ZnSO_4.7H_2O$	1.097
$MnCl_2.4H_2O$	0.713
$AlCl_3.6H_2O$	2.193
NaCl	4.0879
MoO_3	0.012
$MgSO_4$	34.68
H_3PO_4 (85.0-87.0 %)	7.708 ml.

7. Human Blood Serum Samples.

Pooled human serum samples collected from subjects were kindly supplied by Dr. R. B. Foy of the Clinical Chemical Laboratory at the Edward W. Sparrow Hospital, Lansing, Michigan.

C. Procedure

1. Preparation of the Starch Block.

A 1/8 inch thick lucite sheet was placed over the lower cooling plate of the apparatus. Four lucite strips, all 1.5 cm. high, two of which are 45 cm. long and two others which are 6 cm. long, were placed in such a way that a rectangular compartment one-third as wide as the original resulted. Two other strips of 7 cm. length were placed across the top of the ends and held down with C-clamps so as to hold the four sides of the new compartment rigid.

---

[1] An adaptation of the recommendations given by Mathis (35).

The starch suspended in veronal buffer was well stirred and poured into the new compartment until the suspension became about 1 cm. high. To prevent leaking of the starch, filter paper strips were used to pack the sides of the box. As starch settled, the supernatant was gradually decanted as much as possible by the use of a medicine dropper. The remainder of the supernatant buffer solution could further be removed by blotting with filter paper strips. At the same time, while blotting, the starch block was gently pressed to exclude occluded air bubbles and thus continued until the block was dry enough to cut a slit with a sharp spatula. When ready, the resulting starch block was approximately 0.5 cm. high. If this height was exceeded, then a greater smearing effect was noticed upon electrophoresis of serum.

2. Starch Electrophoresis of Normal Human Blood Serum.

At a position 11 cm. from the cathodic end of the block, a rectangular section of damp starch 1 cm. wide and a length across of within 2 mm. of the edges was cut out. This excised wet starch was replaced by an equal volume of dried starch made from the previously described starch-buffer suspension. One ml. (or up to 2 ml.) of human blood serum was evenly streaked across the dry starch with a 1 ml. pipette. Each electrode compartment was filled with about 500 ml. of veronal buffer solution and allowed at least 30 secs. for equalization of the levels. Both ends of the starch block were connected to the electrode compartments with 6 cm. wide Whatman No. 1 filter paper strips placed above and below the starch bed. The whole block was then encased with a sheet of wax paper, followed by putting on the pressure pad and

the upper cooling plate. The electrical circuit was attached and the current was immediately turned on.

It is recommended that the current be switched on as rapidly as possible after applying the serum sample on the block as this will prevent broadening of the sample band due to the diffusion of serum. A constant voltage of 450 volts was observed to be the best to maintain. With a current of 20 to 24 milliamperes (the magnitude of the current depended upon the degree of moistening of the starch), a period of 22 to 24 hrs. was required for separation. Whether or not electrophoresis was accomplished could be detected visually by looking for the position of the pale yellowish brown color of the albumin zone, the fastest moving protein fraction, and thus over-run could be avoided.

3. Elution of Serum Proteins.

After electrophoresis, a sharp stainless steel spatula was used to cut the starch block into transverse slices 1 cm. apart. The two slices of starch that covered the filter paper strips on each end were discarded. Each of the other slices was placed into a consecutively numbered test tube containing 5 ml. of physiological saline solution. After shaking for 1 to 2 min., the starch was allowed to settle. Each supernatant was poured into a centrifuge tube and rotated at 3/4 full speed in the International Clinical Centrifuge for 3 to 4 min. This procedure was repeated five or six times or until no additional protein could be washed out from the starch. Completeness could be detected when no further foam formation occurred in the solution during shaking.

4. Determination of Protein Composition in Serum.

From each tube containing eluate, 1 ml. was pipetted into 5 ml. of alkaline solution B of the Folin-Ciocalteau-Lowry reagent (32). This mixture was kept at 40°C in a water bath for 15 min. Into each tube was next added 0.5 ml. of freshly diluted (1:1) Folin-Ciocalteau-Lowry solution A, and the mixture was allowed to stand at room temperature for 30 min. Determination of the absorbancy of each sample was made at a wavelength of 660 mμ with a Beckman Model B Spectrophotometer. A graph (see Fig. 1) of the absorbancy readings versus tube number was plotted and the protein fractions under each peak of the resulting curve could be identified. A simpler method of determining these amounts was to sum up the absorbancy readings of each eluate fraction and to divide this sum by the total of absorbancy readings in all tubes. An ultraviolet absorption determination at 280 mμ was also carried out on the elements by using a Beckman Model DU Spectrophotometer to confirm the separation of the protein fractions. However, the areas under the peaks of the curve obtained was only proportional to protein aromatic amino acid content and not the true proportion of all the protein fraction.

5. Ashing.

a. Ashing of the protein fractions.

The eluates from the tubes of the same protein fraction were combined and the volume of each fraction was reduced to about 10 ml. by free flame evaporation. The concentrated solutions were then transferred to separate crucibles and further evaporated in an electric air heated oven maintained at 105°C. After the contents were dry, the crucibles

were placed into a muffle furnace and ignited at 500-550°C for 12-15 hrs.

b. Ashing of whole serum.

Normal human blood serum was pipetted into a crucible and likewise dried and ignited as above.

6. Photoelectric Emission Spectrometric Determination of Metal Ions.

a. Calibration.

Calibration was carried out according to Kenworthy (22) by using synthetic standard solutions of seven different concentrations for ten elements. These solutions were prepared for sparking by dissolving into ca 1.8 N HCl the residues of the standard solution mixtures after they had been evaporated to dryness. As an internal standard, enough cobalt chloride was added to this solution to make a 0.02% (w/v) concentration. The spectral lines used were in the second order spectrum except that for Mo which was in the first order and that for zinc, in the third order.

b. Analysis of ashed serum protein samples.

The ash from whole and each fraction of serum protein was dissolved by pipetting 5 ml. of the internal standard solution into the crucible. (The volume of 5 ml. was arbitrarily chosen to be used when 0.5 g. of the protein sample was ashed. It could be varied to suit samples of different weight.) An aliquot of the solution was then transferred into the boat for spectroscopic analysis from which an amount was brought into ignition by the rotating disc electrode. The result was recorded automatically on a graph and the data calculated therefrom. The results are given in Tables II, III, IV, V, and VI.

IV. RESULT AND DISCUSSION

A. Serum Protein Distribution in the Starch Block Electrophoretically Separated Fractions

Figs. 1 and 2 show how the electrophoretic patterns appeared as obtained by the starch block technique. The solid curves were obtained by measurements using the Folin-Ciocalteau-Lowry method for protein analysis. The area under each peak indicates the protein content in each fraction. The dotted lines were drawn from ultraviolet absorbancy readings of the eluates at 280 mμ wavelength. They do not indicate the true protein concentration but rather show the aromatic amino acid residue content in each fraction. They are included for supporting evidence in judging the points of separation of the serum proteins.

It can be easily noticed, from the figures, that there is no sharp demarcation between peaks for the alpha-1- and alpha-2-globulin fractions. Thus, in subsequent analysis for metal element content they were combined and are grouped as from only one fraction -- the alpha-globulin.

Table I shows a quantitative distribution of the proteins in human blood serum as separated by starch block electrophoresis. The protein concentrations in the fractions are reported in total absorbancy units for combined eluates of the same fraction. The actual total absorbancy value corresponding to unseparated serum was obtained by using whole human blood serum of the same amount as that applied for a starch block electrophoresis run. This served as a reference base for estimating the percentage of recovery shown in Part B of Table I.

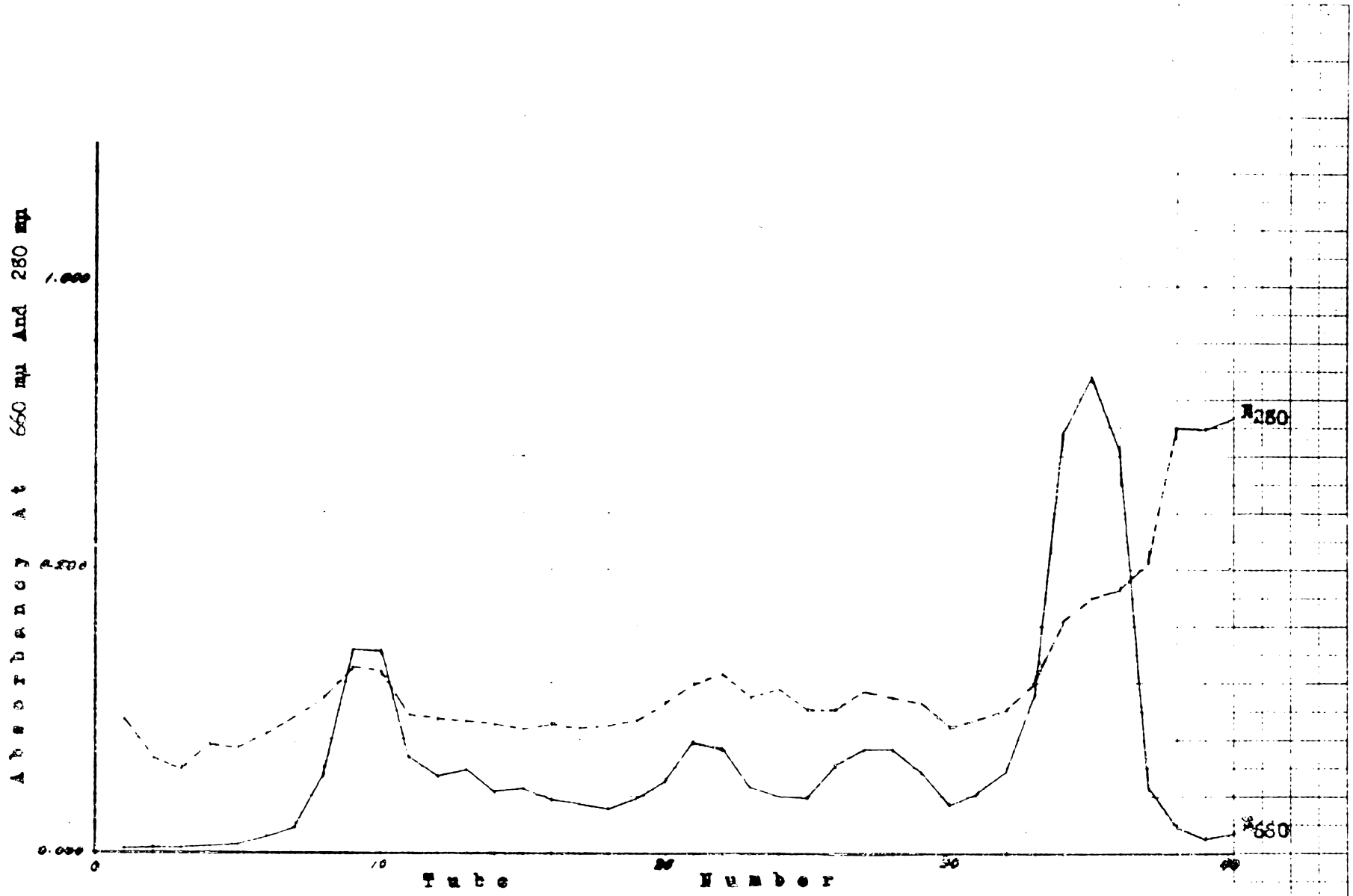

Fig. 1. Human Serum Protein Distribution Curve Obtained By Starch Block Electrophoresis Of 1.0 Milliliter Of Applied Sample.

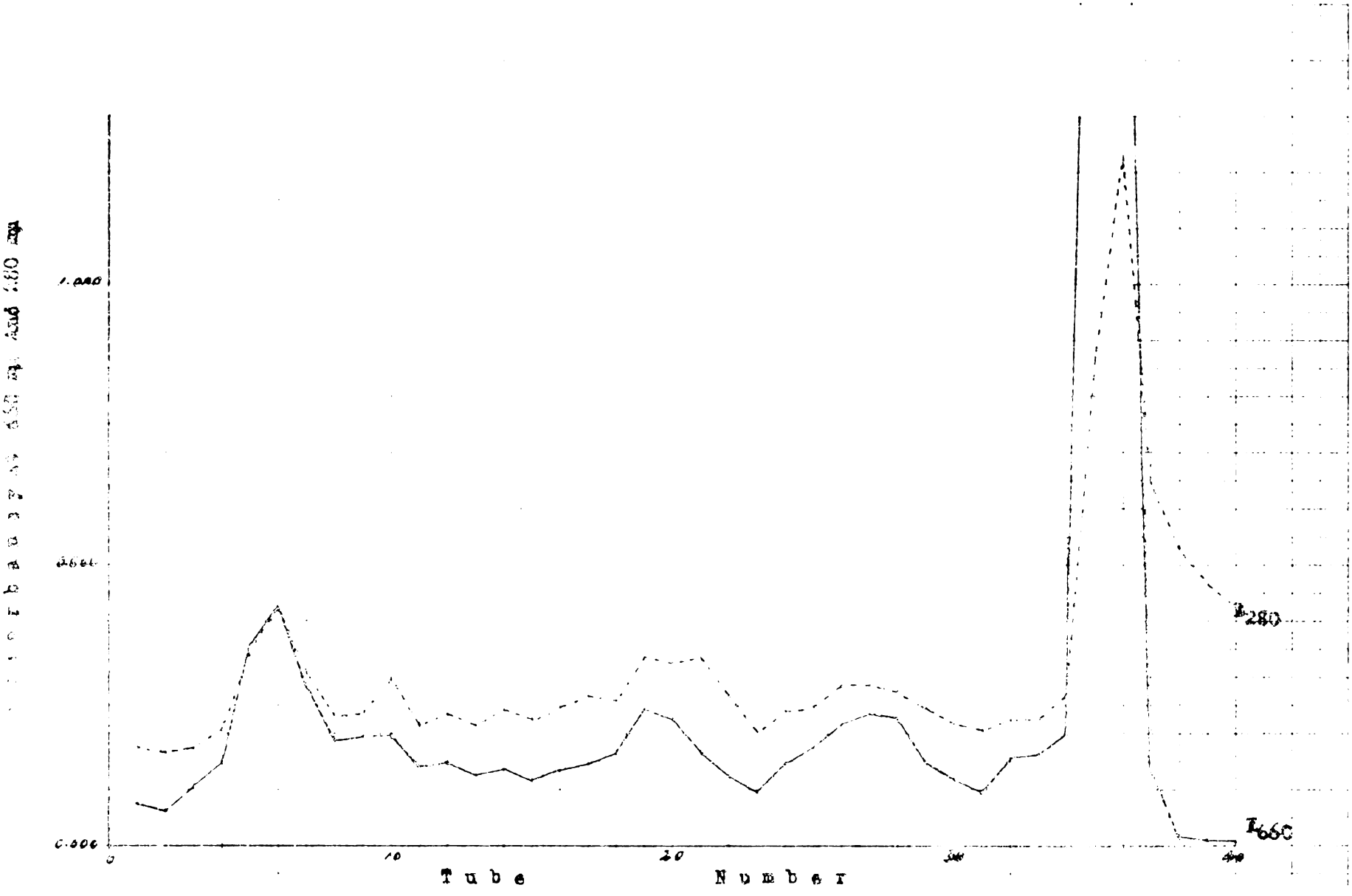

Fig. 2. Human Serum Protein Distribution Curve Obtained By Starch Block Electrophoresis Of 2.0 Milliliters Of Applied Sample.

TABLE I. Distribution of Proteins in Human Blood Serum Separated by Starch Block Electrophoresis[1].

Protein Fraction	1	2	3	4	5	6
			A. Absorbancy Units			
Albumin	2.478	3.038	2.476	3.038	2.371	5.239
Alpha-globulins	1.149	0.845	0.553	0.624	0.543	1.359
Beta-globulins	0.864	0.812	0.627	0.518	0.386	1.296
Gamma-globulins	2.048	1.884	1.027	1.377	0.939	2.698
Total (above fractions)	6.539	6.579	4.683	5.557	4.239	10.592
Original Protein Content[2]	9.240	7.080	5.200	5.400	4.880	10.920
			B. Percent in Each Fraction			
Albumin	38.2	46.2	52.9	54.7	55.9	49.5
Alpha-globulins	16.5	12.8	11.8	11.2	12.8	12.8
Beta-globulins	14.6	12.3	13.4	9.3	9.1	12.2
Gamma-globulins	30.7	28.6	21.9	24.8	22.2	25.2
Recovery[3]	71.0	92.9	90.1	102.9	86.9	97.0

[1]For samples 1-5, 1 ml. of eluate from each tube was taken, and for sample 6, 2 ml. was used.

[2]Arrived at by dilution of whole serum sample and subsequent multiplication by dilution factor. See Appendix A for calculation.

[3]See Appendix A.

The results of the present study are comparable to values obtained by similar experimental procedures reported from other investigators and are thus listed in Table II. The slightly low albumin content and slightly high globulin quantity are likely due to incomplete elution of the albumin fraction from the starch block medium. The process might be made more efficient by using smaller quantities of saline to furnish more times of elution and yet give the same final volume of eluate.

B. Trace Element Composition of Whole Serum

The emission spectrometer or "Quantograph," is structurally set up for the analysis of only the following ten elements: P, Ca, Mg, Mn, Fe, Cu, B, Zn, Mo, and Al. Of these ten elements, only the eight metals were of interest in this study.

The emission spectrograph readings of the various metal elements present in whole human serum are given in Table III. Table IV gives in Part A the correct percentage of each element calculated from data and indicates in Part B the amount of each element in terms of mg. per 100 ml. of serum. The results show good agreement among different samples of serum. Only the percentage of iron has a greater variation among them. The cause for the high readings of aluminum is unknown. According to Seibold (46), the amount of aluminum in human serum is about 17.2 gamma-percent. Finally, the reading of manganese is too small to be significant, and the value for molybdenum is not too reliable because of the interference of other elements such as those due to the high concentration of sodium in the serum.

TABLE II. Comparison of Protein Distribution by Starch Block Electrophoresis of Human Blood Serum with Other Results.

Method	Ref.	Protein Fractions, %				
		Albumin	Globulins			
			Alpha-1-	Alpha-2-	Beta-	Gamma-
Starch Block Electrophoresis	This Study	46.2-55.9	11.2	12.8	9.1-13.4	21.9-28.6
Paper Electrophoresis	(7)	47 -71	2.7-5.8	5.1-12	4.5-15.7	11.3-24
Paper Electrophoresis	(13)	66.4(av.)	3.4(av.)	6.4(av.)	9.6(av.)	14.2(av.)
Paper Electrophoresis	(24)	57.9(av.)	5.1(av.)	7.68(av.)	10.89(av.)	18.53(av.)
Paper Electrophoresis	(26)	51.4-67.6	3.1-7.3	6.0-10.7	9.2-14.6	9.8-20.2
Paper Electrophoresis	(30)	63.2(av.)	3.4(av.)	7.4(av.)	11.3(av.)	14.7(av.)
Free Boundary Electrophoresis	(7)	56 -62	4 -7	9 -11	11 -15	12 -16

TABLE III. Emission Spectrograph Readings for Metal Elements Present in Whole Human Blood Serum[1,2].

Metal Element	Units	Sample No. 1	2	3	4
Ca	%	0.22	0.19	0.46	0.49
Mg	%	0.04	0.04	0.11	0.11
Mn	p.p.m.	0	0	12	12
Fe	p.p.m.	69	22	69	95
Cu	p.p.m.	46.7	65.8	101.0	106.2
Zn	p.p.m.	26	24	67	60
Mo	p.p.m.	1.2	1.0	2.2	2.2
Al	p.p.m.	--[3]	--[3]	--[3]	--[3]

[1]Samples 1 and 2 involved the use of 5 ml. and samples 3 and 4 involved the use of 10 ml. of whole serum.

[2]Samples were ashed at 600°C for 17 hrs.

[3]Value exceeded range of spectrographic instrument.

TABLE IV. Metal Element Content in Whole Human Blood Serum Samples.

Metal Element	Units	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
		Part A			
Ca	%	0.02	0.02	0.02	0.02
Mg	%	0.00(+)	0.00(+)	0.01(-)	0.01(-)
Mn	p.p.m.	00	0	0.6	0.6
Fe	p.p.m.	6.7	1.2	3.4	4.2
Cu	p.p.m.	4.6	6.7	5.0	5.2
Zn	p.p.m.	2.6	2.4	3.3	2.9
Mo	p.p.m.	0.1	0.1	0.1	0.1
Al	p.p.m.	--[1]	--[1]	--[1]	--[1]
		Part B			
Ca	mg. %	20.34	20.34	20.34	20.34
Mg	mg. %	4.07	4.07	5.09	5.09
Mn	mg. %	0	0	0.06	0.06
Fe	mg. %	0.68	0.12	0.35	0.43
Cu	mg. %	0.47	0.68	0.51	0.53
Zn	mg. %	0.26	0.24	0.34	0.29
Mo	mg. %	0.01	0.01	0.01	0.01
Al	mg. %	--[1]	--[1]	--[1]	--[1]

[1]Value exceeded range of spectrographic instrument.

C. The Distribution of Metal Elements with the Protein Fractions Obtained by Starch Block Electrophoresis of Several Samples of Human Blood Serum.

Table V reveals the metal element content present in the protein fractions of six samples of human blood serum subjected to starch block electrophoresis. At first glance they appear to be quite random. This may be attributed to the binding of protein to the cations. As is well known, there are two kinds of protein binding combinations for cations: loosely bound and tightly bound. The cause of the inconsistent results may be due to the loosely bound portions which may not retain the same amount of cations throughout the run of electrophoresis. Of course, artifacts due to procedure may be a great factor in producing the noted deviation. In Table VI is shown a comparison of emission spectrograph reading values for metal element content between serum protein fractions and their corresponding blanks. It indicates that the reagents also contribute significant interference to the results. But above all, the diminution of characteristic radiation of other ions by the excessive sodium content is probably the main consideration that causes such imprecise results. It might be partially overcome by preparing an internal standard solution containing excess amount of sodium ions to serve as a radiation buffer.

Nevertheless, from these results, an outline form or profile of the distribution pattern of each metal element in the serum protein fractions of human blood can be discussed as follows:

TABLE V. The Distribution of Metals Associated with the Protein Fractions Obtained by Starch Block Electrophoresis on Various Samples of Human Blood Serum.

Metal Sample	Concn. Units	Protein Fractions: Albumin	Globulins: Alpha-	Beta-	Gamma-
		Human Blood Serum Sample No. 1			
Ca	%	0.20	0.22	0.22	0.30
Mg	%	0.00	0.00	0.00	0.02
Mn	p.p.m.	13.2	15.3	15.3	17.3
Fe	p.p.m.	98.2	41.7	44.9	39.7
Cu	p.p.m.	20.4	58.0	16.8	21.9
Zn	p.p.m.	322	71.2	71.2	211
Mo	p.p.m.	2.7	2.8	2.8	3.3
Al	p.p.m.	600[1]	736[1]	—[2]	—[2]
		Human Blood Serum Sample No. 2			
Ca	%	0.27	0.19	0.16	0.30
Mg	%	0.01	0.00	0.00	0.03
Mn	p.p.m.	17.3	13.2	11.2	11.2
Fe	p.p.m.	37.1	33.6	27.0	39.7
Cu	p.p.m.	81.3[2]	87.4[2]	10.9	28.2
Zn	p.p.m.	163	80.6	83.5	144
Mo.	p.p.m.	3.1	2.7	2.3	2.9
Al	p.p.m.	—[2]	—[2]	—[2]	575[1]
		Human Blood Serum Sample No. 3			
Ca	%	0.18	0.20	0.27	0.27
Mg	%	0.02	0.01	0.02	0.03
Mn	p.p.m.	9.7	13.2	17.3	13.2
Fe	p.p.m.	24.0	25.4	36.4	31.5
Cu	p.p.m.	21.0	11.3	13.8	12.7
Zn	p.p.m.	168	44.2	72.3	92.6
Mo	p.p.m.	2.3	2.3	2.8	2.7
Al	p.p.m.	637[1]	359	455[1]	480[1]

TABLE V (Continued)

Metal Sample	Concn. Units	Protein Fractions			
		Albumin	Globulins		
			Alpha-	Beta-	Gamma-
		Human Blood Serum Sample No. 4			
Ca	%	0.47	0.09	0.12	0.22
Mg	%	0.07	0.02	0.02	0.03
Mn	p.p.m.	22.4	6.1	9.7	13.2
Fe	p.p.m.	28.6	9.2	12.7	18.3
Cu	p.p.m.	20.7	5.6	9.0	11.8
Zn	p.p.m.	215	11.2	18.8	29.1
Mo	p.p.m.	5.2	1.8	2.0	2.8
Al	p.p.m.	173	58	112	115
		Human Blood Serum Sample No. 5			
Ca	%	0.37	0.21	0.22	0.24
Mg	%	0.04	0.02	0.02	0.04
Mn	p.p.m.	21.3	13.3	15.2	11.2
Fe	p.p.m.	36.8	20.9	30.0	15.8
Cu	p.p.m.	25.9	13.3	18.3	17.8
Zn	p.p.m.	337	80.6	195	67.2
Mo	p.p.m.	3.7	2.6	2.6	2.7
Al	p.p.m.	194	131	456[1]	342
		Human Blood Serum Sample No. 6			
Ca	%	0.12	0.12	0.13	0.16
Mg	%	0.00[3]	0.00[3]	0.00[3]	0.01
Mn	p.p.m.	10.0	10.0	8.9	8.9
Fe	p.p.m.	14.8	15.5	20.6	36.9
Cu	p.p.m.	11.3	9.7	7.4	12.5
Zn	p.p.m.	74.5	19.7	68.6	62.6
Mo	p.p.m.	1.6	1.6	1.6	1.5
Al	p.p.m.	186	246[1]	193	258[1]

[1]Extrapolated value from curve for that particular element on spectrographic instrument.

[2]Values exceeding the range of spectrographic instrument.

[3]Magnesium occurs, but values became too small after calculation.

TABLE VI. Emission Spectrograph Reading Values for Metal Element Content of Human Blood Serum Fractions Separated by Starch Block Electrophoresis and Corresponding Blank Run[1].

Metal Sample	Concn. Units	Protein Fractions			
		Albumin	Globulins		
			Alpha-	Beta-	Gamma-
A. Of Serum Protein Fractions					
Ca	%	0.46	0.46	0.49	0.58
Mg	%	0.02	0.01	0.02	0.05
Mn	p.p.m.	38	38	34	34
Fe	p.p.m.	56	59	78	140
Cu	p.p.m.	42.4	37.0	28.0	47.6
Zn	p.p.m.	283	75	260	237
Mo	p.p.m.	6.0	5.8	6.0	5.6
Al	p.p.m.	705	932[2]	735	980[2]
B. Of Corresponding Blank Run Fractions					
Ca	%	0.27	0.43	0.52	0.58
Mg	%	0.00	0.00	0.01	0.00
Mn	p.p.m.	22	42	46	42
Fe	p.p.m.	50	72	78	130
Cu	p.p.m.	25.1	33.0	38.0	45.6
Zn	p.p.m.	158	108	164	146
Mo	p.p.m.	4.2	5.6	6.4	6.7
Al	p.p.m.	660	922[2]	1116[2]	1135[2]

[1]The Starch Block Electrophoresis was run simultaneously in two parallel compartments; one containing applied serum sample, the other buffer only.

[2]Extrapolated values from curve for that particular element on spectrographic instrument.

1. Calcium.

From the result obtained and given in Table V, calcium occurs almost in even amount in each serum protein fraction. This is in contrast to many previous reports. Prasad and Flink (41) determined that 50 to 55 percent of bound calcium was in the albumin fraction. Foy (13) gave a similar conclusion based upon paper electrophoresis; namely, that 45 to 55 percent of the bound calcium was held by albumin. Neither does it agree with LeDuc's result (29), that an equal amount of calcium was bound by albumin and the gamma-globulins and that this contributed to two-thirds of the total bound calcium. However, Foy and LeDuc substantiated the observation that calcium occurred in every fraction of human serum. In Long's paper (33), calcium was reported present in only the alpha-globulin fractions in spite of the use of starch block electrophoresis as employed in this study.

2. Magnesium.

It can be noticed from Table V that the distribution of protein bound magnesium is similar to that for protein bound serum calcium in that it occurs in every fraction. In the subparts of Table V, the percentage of magnesium in some of the fractions is recorded as zero. This does not mean that there is an absolute absence of magnesium in these fractions, but because the "Quantograph" was calibrated to read only in percent, the small amount of magnesium present was out of this range of measurement. It appears that more magnesium is associated with the albumin and gamma-globulin fractions than in the globulins. Previous studies by Lillevik et al. (31) and Foy (13) also supported the finding of magnesium in all electrophoretic fractions of human blood serum. However, Long (33), using the starch block technique as in this study,

presented a varying conclusion in that only alpha-globulin fractions appeared to contain magnesium.

3. Iron.

Foy (13) and Long (33) previously observed that iron was present in all fractions of human serum proteins after electrophoresis which is the same result found indicated in Table V. However, the contention that the main portion of iron was found in the alpha- and beta-globulins as reported by Foy is not substantiated by the results. Instead, the albumin fraction seems to contain a slightly higher iron content than the others. High iron content in the beta-globulin fraction due to the binding of transferrin has generally been reported in various publications (see "Historical" section), but was not observed in any of the experiments from this investigation.

4. Copper.

Protein bound copper was also found as seen in Table V to be present in all electrophoretic fractions of human blood serum. Among the various previous reports, only the experiments of Foy (13) had a similar result. Long (33) indicated that the albumin and alpha-globulin fractions combined with copper ions. LeDuc (29) demonstrated the isomigration of copper with gamma-globulin fraction. Although Laurell (27) stated that more than 90 percent of serum copper was firmly bound by ceruloplasmin (one of the alpha-2-globulin proteins), there is not very strong evidence of such a degree of complex formation in this study.

5. Zinc.

From the data acquired (Table V), zinc is found to be present in all serum fractions with a higher concentration among the albumin and gamma-globulin fractions, especially the former. Foy (13) also noted that zinc occurred in all fractions of human serum, whereas Long (33) reported that zinc was present in the alpha-globulin fractions and LeDuc (29) showed the existence of zinc with the gamma-globulins. Previously, in 1954, Ressler et al. (43) precipitated albumin and gamma-globulins from human serum with zinc ions.

V. SUMMARY AND CONCLUSIONS

A quantitative study of the metal elements associated with the albumin, alpha-, beta-, and gamma-globulins of human blood serum was carried out by electrophoretic separation on the starch block and quantitative emission spectrographic analysis of each eluted protein fraction for the presence of Cu, Ca, Fe, Zn, Mn, Al, Mg, and Mo. The experimental technique was adapted and modified to investigations on human serum proteins, and the following specific developments were noticed:

A. The electrophoretic pattern shown by starch block electrophoresis is comparable and similar to that obtained by other zone electrophoretic methods.

B. The recovery of separated proteins from starch block segments was found quite efficient by elution with physiological saline solution.

C. Calcium was found present in all the serum protein fractions and in about an even distribution.

D. Magnesium was found in higher concentration with the albumin and gamma globulin fractions.

E. Iron and copper were found to be present in about equal amounts in all fractions.

F. Most of the zinc associated with protein was found present in the albumin and gamma globulin fractions.

G. The results and experience of this study suggest that in future researches of this nature and technique, the addition of excess sodium to the internal standard solution be included. This might then serve as a radiation buffer to minimize interference with intensity of radiation of the other metal constituents.

H. Another consideration suggested for future study is that the atomic absorption spectra method of analysis be adapted to determine the metal element content of the separated serum protein fractions.

VI. BIBLIOGRAPHY

1. Abderhalden, E., and Moller, P., Untersuchungen uber den Gehalt des Blutserum en Eisen, Kupfer und Mangan. Z. physiol. Chem., 176, 95-100 (1928).

2. Barkan, G., Die Verteilung des leicht abspaltbaren Eisens zwischen Blutkorperchen und Plasma und sein Verhalten unter experimentellen Bedingungen. Z. physiol. Chem., 171, 194-221 (1927).

2a. Boemendal, Zone Electrophoresis in Blocks and Columns, Edsevier Publishing Co., New York 17, N.Y. (1963), pp. 13-14.

3. Cantarow, A., and Schepartz, B., Chapter 22, Inorganic Metabolism, Philadelphia, W. B. Saunders Co. (1954), p. 848.

4. Carr, C. W., Competitive Binding of Calcium and Magnesium with Serum Albumin. Proc. Soc. Exptl. Biol. Med. 89, 546 (1955).

5. Carr, C. W., and Woods, K. R., Studies on the Binding of Small Ions in Protein Solutions with the Use of Membrane Electrodes. V. The Binding of Magnesium Ions in Solutions of Various Proteins. Arch. Biochem. Biophys. 55, 1 (1955).

6. Cohn, E. J., Gurd, F. R. N., Surgenor, D. M., Barnes, B. A., Brown, R. K., Deronaux, G., Gillespie, J. M., Kalirt, F. W., Lever, W. F., Lin, C. H., Mittleman, D., Moulton, R. F., Schmid, K., and Uronia, E., A System for the Separation of the Components of Human Blood: Quantitative Procedures for the Separation of the Protein Components of Human Plasma. J. Am. Chem. Soc., 72, 465-474 (1950).

7. Cooper, G. R., Electrophoretic and Ultracentrifugal Analysis of Normal Human Serum, Chapter 3, The Plasma Proteins, Vol. 1, ed. by F. W. Putnam, Academic Press, New York 3, N. Y. (1960), pp. 51-103.

8. Copeland, B. E., and Sunderman, F. W., The Magnesium Binding Property of the Serum Proteins. J. Biol. Chem., 197, 331-341 (1952).

9. Crosby, W. H., and Dameshek, W., The Significance of Hemoglobinemia and Associated Hemosideriuria, with Particular Reference to Various Types of Hemolytic Anemia. J. Lab. Clin. Med. 38, 829 (1951).

10. Eisler, B., and Resdahl, K. 3., untersuchungen uber die Zustandenform des Kupfers in Blutserum mit Kilfe der Kataphorese. Biochem. Z., 286, 435-438 (1936).

11. Fed. Proc., 20, No. 3, Part II, Biological Aspect of Metal Binding, ed. by Johnson, pp. 1-273 (1961).

12. Fontes, G., and Thiovolle, L., Surla teneur du serum en fer non hemoglobinque et sur sa dimunition as course de l'anemie experimentale. Compt. rend. soc. biol., 93, 687-689 (1925).

13. Foy, R. B., "The Binding of Metal Ions by Proteins in Normal and Abnormal Human Blood Serum," A Thesis for the Degree of Ph.D., Michigan State University (1960), 81 numbered leaves.

14. Greene, C. H., and Power, N. H., The Distribution of Electrolytes between Serum and the In-Vivo Dialysate. J. Biol. Chem., 91, 183-202 (1931).

15. Gubler, C. J., Lahey, M. E., Cartwright, G. E., and Wintrobe, M., Studies on Copper Metabolism. IX. The Transportation of Copper in Blood. J. Clin. Invest. 32, 405 (1953).

16. Gurd, F. R. N., and Goodman, D. W., Preparation and Properties of Serum and Plasma Proteins. XXII. The Interaction of Human Serum Albumin with Zincoins. J. Am. Chem. Soc., 74, 670-675 (1952).

17. Heremans, J., Acta Med. Scand. (1960). Cited in Putnam, Plasma Proteins, Academic Press, New York 3, N.Y. (1960), pp. 374,376.

18. Holmberg, C. G., and Laurell, C. B., Studies on the Capacity of Serum to Bind Iron. The Regulation of Mechanism of Serum Iron. Acta Physiol. Scand. 10, 307-319 (1945).

19. Holmberg, C. G., and Laurell, C. B., Investigations on Serum Copper. II. Some of its Properties. Acta Chem. Scand., 2, 550-556 (1948).

20. Holmberg, C. G., and Laurell, C. B., Oxidase Reactions Caused by Coeruloplasmin. Scand. J. Clin. and Lab. Invest. 3, 103-107 (1951).

21. Hornykiewicz, O., and Niebauer, G., Die Polyphenoloxydase des menschlichen Blutserums. Arch. exptl. Pathol. Pharmakol. Naunyn-Schmiedeberg's, 218, 448 (1953).

22. Kenworthy, A. L., Photoelectric Spectrometer Analysis of Plant Materials. Proc. of Thirty-Sixth Annual Meeting Council on Fertilizer Application, 39-50 (1960).

23. Koechlin, B. A., Preparation and Properties of Serum and Plasma Proteins. XXVIII. The Beta Metal-Combining Protein of Human Plasma. J. Am. Chem. Soc., 74, 2649-2653 (1952).

24. Kopteva, I. A., Blood Serum Protein Fraction in Normal Persons. Tr. Kuibyshevik. Med. Inst., 17, 212-214 (1961). Cited in Chem. Abstr. 59, 11900a (1963).

25. Lahey, M. E., Gubler, C. J., Brown, D. M., Smith, E. L., Jager, B. V., Cartwright, G. E., and Wintrobe, M. M., Copper Metabolism. VIII. Correlation between the Serum-Copper Level and Various Serum-Protein Fractions. J. Lab. Clin. Med., 41, 829-835 (1953).

26. Laue, D., The Practical Value and the Limits of Paper Electrophoretic Serum Protein Fractionation. Internist, 4, 521-529 (1963).

27. Laurell, C. B., Metal-Binding Plasma Proteins and Cation Transport. Chapter 19, The Plasma Proteins, Vol. 1, ed. by F. W. Putnam, Academic Press, New York 3, N.Y. (1960), pp. 369-378.

28. Laurell, C. B., Laurell, S., and Skoog, N., Buffer Composition in Paper Electrophoresis. Considerations on its Influence, with Special Reference to the Interaction Between Small Ions and Proteins. Clin. Chem., 2, 99-111 (1956).

29. LeDuc, H. M., "Equilibrium Dialysis, Moving Boundary and Paper Electrophoresis Studies on the Binding of Human Serum Proteins with Calcium, Magnesium, Iron, and the Copper Ions." A. Ph.D. Thesis, Michigan State University (1956), 77 numbered leaves.

30. Levanov, Yu. M., Electrophoretic Study of Protein Fractions of Blood Serum of Donors. Zdravookhr. Kazakhstana, 1962, 45-47. Cited in Chem. Abstr. 59, 14380c (1963).

31. Lillevik, H. A., LeDuc, H. M., Ewing, D. T., and Morse, P. F., Moving Boundary and Paper Electrophoresis Studies on the Binding of Human Serum Proteins with Metal Ions, Fed. Proc., 16, 910 (1957).

32. Litwack, G., Experimental Biochemistry, John Wiley and Sons, Inc., New York (1960), pp. 147-148.

33. Long, M. J., "Metal Ion Analysis by Emission Spectroscopy of Starch Block Electrophoretically Separated Human Serum Proteins." A Thesis for M.S. Degree, Michigan State University (1961), 33 numbered leaves.

34. Maier, C., "Hamolyse und Hemolytische Krankheiten." Huber, Bern (1950).

35. Mathin, W. T., Spectrochemical Analysis of Plant Material Using Spark Excitation, Anal. Chem., 25, 943-947 (1953).

36. Martin, R. B., Introduction to Biophysical Chemistry, McGraw-Hill Book Co., New York (1964), pp. 335-356.

37. McClean, F. C., and Hastings, A. B., Am. J. Med. Sci. 189, 601 (1935). Cited in Putnam, F. W., The Plasma Proteins (1960), pp. 372,377.

38. Nicholas, H. C., Diffusible Serum Calcium by High Pressure Ultrafiltration. J. Biol. Chem., 97, 457-463 (1932).

39. Pauli, W., and Schon, M., Untersuchungen an Electrolyt frein Wasserloseichen Proteinen. Biochem. Z., 153, 253,284 (1924).

40. Prasad, A.S., and Flink, E.B., Effect of Carbon Dioxide on Concentration of Calcium in an Ultrafiltrate of Serum Obtained by Centrifugation. J. Appl. Physiol., 12, 103-107 (1957).

41. Prasad, A.S., and Flink, E.B., Base-Binding Property of the Serum Proteins, with Respect to Calcium. J. Lab. Clin. Med., 51, 345-364 (1958).

42. Prasad, A.S., Studies on Ultrafilterable Calcium. A.M.A. Arch. Internal Med. 105, 560-573 (1960).

43. Ressler, N., Uhl, H., and Boyle, A.J., The Relation Between the Second Ionization Potential of a Metal to its Interaction with Serum, J. Colloid Sci., 9, 112-131 (1954).

44. Rona, P., and Takahashi, D., Uber das Verhalten des Calciums in Serum und uber den Gehalt der Blutkorperchen an Calcium. Biochem. Z., 31, 336-344 (1911).

45. Schade, A.L., and Caroline, L., An Iron-Binding Component in Human Plasma, Science, 104, 340-341 (1946).

46. Seibold, M., Determination of Aluminum in Blood Serum with Ion-exchangers and Eriochrome Cyanine R. Klin. Wochschr. 38, 117-119 (1960).

47. Todd, W. R., The Diffusible Serum Calcium in Normal Individuals and Hyparathyroid Patients Treated with Parathormone, Dihydrotachysterol and Vitamin D. J. Biol. Chem., 140, cxxxiii (1941).

48. Vahlquist, Bo C:son, A Pediatric-Clinical and Experimental Study, Acta Paediat. 28, Suppl. V, 374 pp. + 68 pp. of Tables and Case Histories (1941).

49. Vikbladh, I., Studies on Zinc in Blood II. Scand. J. Clin. and Lab. Invest. 3, Suppl. 2, 9-74 (1951).

50. Warburg, O., and Krebs, H. A., Uber locker gebundenes Kupfer und Eisen im Blutserum. Biochem. Z., 190, 143-149 (1927).

51. Watchorn, E., and McCance, R. A., Inorganic Constituents of Cerebrospinal Fluid. II. The Ultrafiltration of Calcium and Magnesium from Human Serum. Biochem. J. 26, 54-64 (1932).

52. Wolff, H.P., Lang, N., and Knell, M., Untersuchungen mit Cu^{64} uber die Bindung des Kupfer an Serumeiweisskorper. Klin. Wochschr., 33, 186-187 (1955).

53. Wolff, H.P., Schmidt, J.G., Althans, G., and Knedel, M., Z. ges. exptl. Med. 127, 362 (1956). Cited in Putnam, F.W., The Plasma Proteins (1960), pp. 370-378.

APPENDIX A

A. Calculations for the Composition of Human Blood Serum and the Percent of Recovery (See Table I).

1. Calculation of Composition of Human Blood Serum.

$$\text{The percent of each fraction in human blood serum} = \frac{\text{The sum of } E_{660} \text{ readings for each eluate of a fraction}}{\text{the sum of } E_{660} \text{ readings of all eluates}} \times 100$$

Example:

The sum of the absorbancy readings for all eluates in the albumin fraction of sample 1 = 2.478, and

the sum of absorbancy readings for all eluates = 6.539

$$\text{the percent of albumin} = \frac{2.478}{6.539} \times 100 = 38.2$$

2. Calculation of Percent of Recovery.

An absorbancy reading expressing the quantity of original blood serum was obtained by: (1) dilution with physiological saline of the same quantity of serum as used for electrophoresis to a volume equal to that of the combined eluates, and (2) multiplying the absorbancy reading of this diluted solution by the number of tubes.

$$\text{The percent of recovery} = \frac{\text{the sum of the } E_{660} \text{ readings of all eluates}}{\text{the absorbancy reading expressing the quantity of original blood serum}} \times 100$$

Example:

The sum of absorbancy readings for all eluates in sample 2 = 6.579, and

the absorbancy reading expressing the quantity of the original blood serum sample = 7.080

The percent of recovery = $\frac{6.759}{7.080} \times 100 = 92.9$

B. Calculation for the Metal Element Content in a Sample of Whole Blood Serum (See Tables III, IV, and V).

The emission spectrograph readings in Table III were obtained by dissolving the ash of serum sample in 5 ml. of 1.8 N HCl acid solution. If the ash of 0.5 g. of sample was used, the emission spectrograph was calibrated such that the readings would be a direct metal element content of the sample. When more than 0.5 ml. of serum sample was used, the readings obtained were corrected to true percentage of p.p.m. as indicated by the amount of serum sample used,, shown in the footnote of Table III.

1. Calculation of the Metal Element Content of Human Blood Serum in Percent or p.p.m. Units.

$$\text{the percent of metal element in human blood serum} = \frac{\text{the emission spectrograph reading}}{\text{weight of serum sample}} \times 0.5$$

Example:

Calculation of the percentage of calcium in sample 1 from Table III: the emission spectrograph reading = 0.22 ( ).

Weight of 5 ml. of serum sample = 1.017 x 5 = 5.085 g. (The weight of 1 ml. of whole serum was determined to be 1.017 g.)

$$\text{the percent of metal element in human blood serum} = \frac{0.22}{5.085} \times 0.5 = 0.02\ \%$$

2. Calculation of Metal Element Content in Human Blood Serum in mg. per 100 ml. Serum.

$$\text{Metal element content} = \frac{\text{the percent of metal element in human blood serum}}{100} \times 100 \times 1000 \times 1.017$$

Example:

The percent of Ca in Sample 1 = 0.02

$$\text{Metal element content} = \frac{0.02}{100} \times 100 \times 1000 \times 1.017 =$$

20.34 mg. per 100 ml. serum.

C. Calculation of the Metal Element Content in the Electrophoretic Fractions of Human Blood Serum (See Table V).

Let R = Emission spectrograph reading,

W_s = Weight of serum sample applied in Starch Block Electrophoresis,

W_f = Weight of each serum protein fraction,

W_m = Weight of each metal element,

N_m = Metal element content, percent of total serum,

V_f = Total volume of eluate for each fraction obtained, and

V = Volume of eluate in each fraction used for ashing.

$$W_m = W_f \times \frac{R}{W_f} \times 0.5 \times \frac{V_f}{V} = \frac{RV_f}{2V}$$

$$N_m = \frac{W_m}{W_s} = \frac{RV_f}{2W_sV}$$

Example:

Ca in albumin of sample 1 (Table V):

R = 0.38 ( )

W_s = 1.017 g.

V_f = 30 ml.

V = 28 ml.

$$N_m = \frac{0.38 \times 30}{2 \times 1.017 \times 28} = 0.20$$