METAL ION ANALYSIS BY EMISSION SPECTROSCOPY OF STARCH BLOCK ELECTROPHORETICALLY SEPARATED HUMAN SERUM PROTEINS

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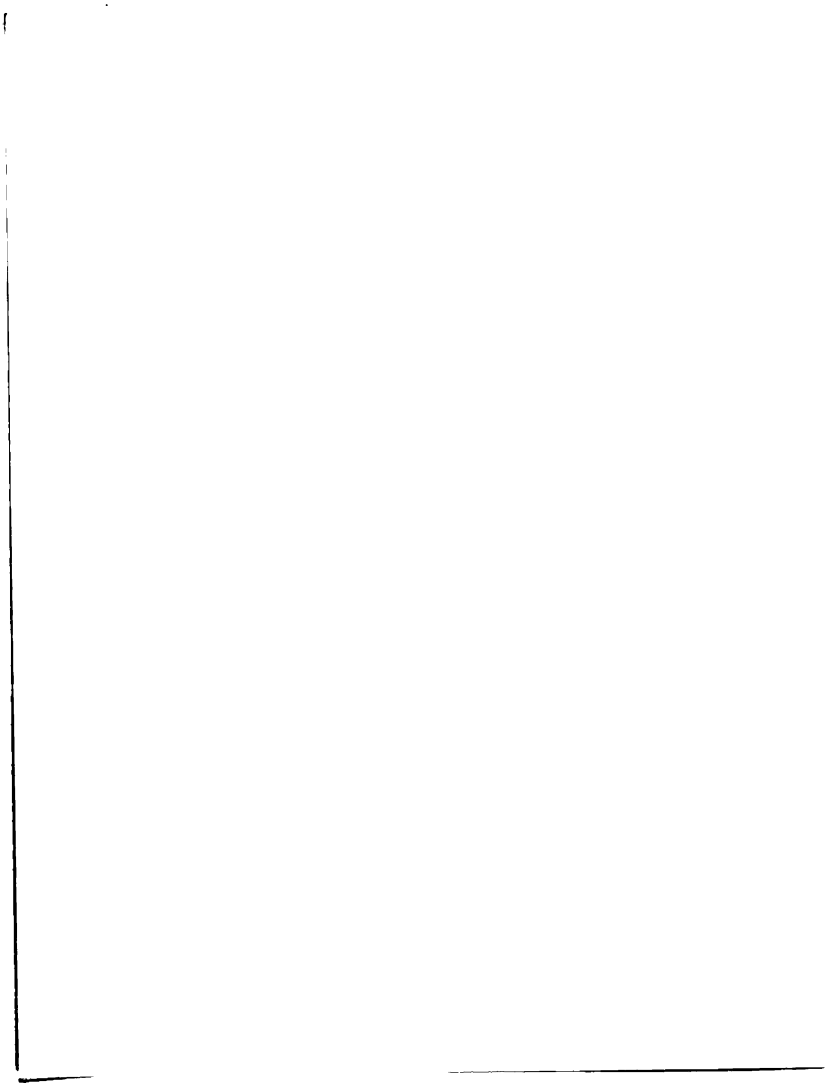

ABSTRACT

METAL ION ANALYSIS BY EMISSION SPECTROSCOPY OF STARCH BLOCK ELECTROPHORETICALLY SEPARATED HUMAN SERUM PROTEINS

by Mary Jean Long

This study was undertaken to extend investigation in the metallic elements associated with normal human serum albumin and the alpha, beta and gamma globulins. Such new information is of value in connection with more completely understanding the nature and function of metallo-protein complexes in normal blood serum.

Starch block electrophroesis was employed to separate the normal serum protein fractions. The fractions were then eluted from the starch segments with physiological saline solution and protein content identified by measuring absorbancies with the Beckman DU spectrophotometer. These readings were plotted versus section number. Biuret determinations were performed on the saline eluted proteins also, and readings were plotted versus section number. The plots revealed the eluates to be combined to give the respective blood serum protein fractions.

The results of this study lead to the major conclusion that emission spectroscopy applied to human serum protein fractions separated by starch block electrophoresis shows promise of greater precision and accuracy in analysis for their metallic element content than heretofore achieved.

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By

Mary Jean Long

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VITA

The author was born March 28, 1914 in Duluth, Minnesota, and her secondary education was completed in 1931 at East Grand Rapids High School, East Grand Rapids, Michigan. In 1935 she graduated from Michigan State University with a Bachelor of Science degree. She was admitted to the School for Advanced Graduate Studies of Michigan State University in September 1959 and has been in attendance since.

Her employment experience has included that of Laboratory Technician with Park, Davis and Company, Detroit, Michigan, and as Medical Technologist 1954-1959 at Blodgett Memorial Hospital, Grand Rapids, Michigan. She is a member of the American Society of Medical Technologists, the Michigan Society of Medical Technologists and the Western Michigan Society of Medical Technologists.

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I. INTRODUCTION

One of the oldest and most important problems in biochemistry is the study of protein-metal complexes. In all biological systems, proteins are found in combination with or associated with metal ions. Human blood serum is no exception. Here are found various metals acting as cofactors in enzyme systems, copper forming a complex with serum albumin and iron chelating with various protein fractions (notably beta globulin), and calcium playing a role in the clotting mechanism.

It was the purpose of this study to identify the metal ions of human blood serum protein fractions after separation by electrophoresis on a starch block. Emission spectroscopy was the method of metal identification employed.

II. HISTORICAL

A. Emission Spectroscopy

The emission spectrograph is often used in other than metallurgical problems although that is one of its main applications. It has been used extensively to determine the contents of alkali and alkaline earth metals in plant and animal tissues, including such materials as blood serum. The early workers in this field employed flame methods. They ashed the material to be examined, folded it in a filter paper and burned the entire packet in a flame while recording the emissions on a photographic plate (37).

Le Duc (23) attempted spectroscopy on serum protein fractions separated by paper electrophoresis. She reported that examination of the spectrophotographic plate showed no significant differences between the eluted protein fractions, paper, and electrodes. She presumed that the cause of her failure was due to the minute quantities of metals present in the 0.2 ml. of serum applied to the paper strip. Even 1 ml. of serum applied on heavier paper produced similar inconclusive results. Assuming that the cause of her failure was due to the metal impurities in the paper, this study was carried out with spectrographic analyses of protein fractions prepared by electrophoresis on a starch block.

Judd Lewis (37) in 1912 was the first to apply spectroscopy as a regular means of analysis in industrial problems. By 1936 he described a method for preparing samples of animal or vegetable matter for the emission spectrograph. In 1928 Lowe (37) demonstrated success with the use of carbon electrodes. He recommended placing the liquid sample in the cavity of the lower electrode and included the arc spectrum of the carbon electrode for comparison. Lowe's method was adopted in this investigation.

B. Electrophoresis

Electrophoresis is defined as the migration of charged particles in an electric field toward the oppositely charged electrode. Many types of supporting media have been investigated. Tiselius after his development of the moving boundary apparatus in 1933 demonstrated that human blood serum separated by paper electrophoresis at pH 8.6 in 0.05 to 0.10 M Veronal buffer resulted in five apparently different components (4).

Kunkel and Slater (22) in 1952 carried out a series of experiments involving zone electrophoresis on various types of media in a comprehensive search for an ideal support free of the disadvantages of filter paper. Of all the materials studied, the starch block was found to produce the most satisfactory results. The starch block was made from raw, insoluble, unhydrolyzed starch granules. Around 1955 Smithies (31, 32) introduced the use of starch gel for zone electrophoresis. This starch was hydrolyzed with heat to form the gel, which at the completion of the electrophoretic preparation could be stained, washed and preserved similar to paper. Mitchell and Herzenberg (25) successfully tried foam rubber sponges for the supporting material in the preparative electrophoretic separation of small amounts of protein and pointed out that the advantage of this medium is the ease with which the protein fractions could be recovered.

This study was commenced on paper to familiarize the investigator with the technique and apparatus for serum protein separation, continued on starch block for the major part of the investigation, and for comparison one electrophoretic run was tried on foam rubber sponges.

III. EXPERIMENTAL

A. Equipment

Electrophoresis Apparatus (Figure 1). -- The E-C Apparatus manufactured and sold by the E-C Apparatus Company, 538 Walnut Lane, Swarthmore, Pennsylvania, was used. It is constructed of lucite and at each end contains three separate compartments with platinum electrodes. A water cooled lucite bed is provided to support the paper or starch medium and a water cooled lucite upper plate is clamped over a pressure pad. A 2000 volt power supply unit was also provided by the E-C Apparatus Company. Paper strips were first used in the apparatus to observe the behavior of normal human serum samples under varying conditions of pH, time and voltage. These strips were heavy 3MM Whatman paper 46.5 x 6.5 cm. Foam rubber sponges 83x13x13 mm. were also used as supporting medium. Lucite strips of the dimensions of the starch block-bed were made and clamps to hold them in position obtained. These lucite strips, one, 45 x 1.5 x 0.6 cm. and two strips 6.5 x 1.5 x 0.6 cm., were necessary to subdivide the lucite bed into smaller starch block sizes.

Beckman DU Spectrophotometer, Power Supply and Hydrogen Lamp. --The instrument manufactured by the Beckman Instrument Company, Fullerton, California, was used to estimate the amount of protein in saline elutes.

Beckman Model B Spectrophotometer. --This instrument was also used to carry out the Biuret determinations on the above mentioned protein solutions.

Bausch and Lomb Medium Quartz Emission Spectrograph. -- Emission spectroscopy was carried out on this instrument made by Bausch and Lomb Incorporated, Rochester, New York.

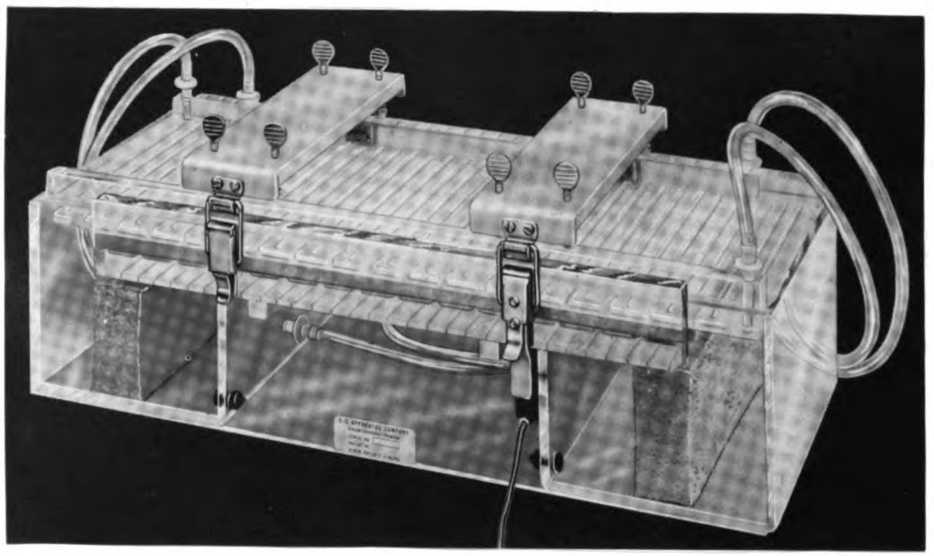

Figure 1. E-C Pressure Plate Electrophoresis unit: transparent plastic with non wettable migration surfaces and platinum electrodes; migration path direct-contact cooled; 2500 ml. volume.

Figure 1a. E-C Power Supply: input 110-120 v.; output adjustable 0-1000 v. at 200 ma. or less; two units can be connected in series for 2000 v. output.

Bausch and Lomb Littrow Spectrograph.--This instrument was also used to supplement the other spectrograph and is manufactured and sold by the same company.

Arc and Spark Stand.--Electrode holders were carried on porcelain insulators attached to rack and pinion movements by which they were raised or lowered as indicated or desired. A voltage of 110 volts, 4.5 amperes direct current was employed to supply the arc.

Special Graphite Spectroscopic Electrodes.--The electrodes used in this investigation were obtained from the National Carbon Company, Cleveland, Ohio.

Kodak Spectrum Analysis Plates.--Number 1 Kodak Spectrum Analysis Plates were used throughout the experimental work. They were obtained from the Eastman Kodak Company, Rochester, New York. They measured 4 x 10 inches and were 1 mm. thick.

Rocker Type Plate Developer.--The plate developer was also a product of the Eastman Kodak Company.

B. Materials and Reagents

Starch.--The supply of starch used was labelled Fisher Potato Starch No. 5-513 and obtained from Fisher Scientific Company, Fair Lawn, New Jersey. It was purified by suspending 500 grams in 2 liters of 0.1 M HCl, stirring well and allowing to settle. The supernatant was decanted and the washing procedure was repeated six times with distilled water or until the starch was neutral to pH-Hydrion paper indicating pH 7.0. The final wash was made with glass distilled water. Before pouring the starch into the lucite block-form the water was decanted and the starch resuspended in an approximately equal volume of 0.1 M Veronal buffer of pH 8.6.

Buffer.--The Veronal buffer used was prepared by dissolving 4.00 g. sodium hydroxide pellets and 20.26 g. Veronal in distilled water and diluting to 1 liter.

Biuret Reagent.--The Biuret reagent was prepared by dissolving 1.5 g. of crystalline cupric sulfate pentahydrate, and 6.0 g. sodium potassium tartrate in about 500 ml. distilled water in a 1 liter volumetric flask. To this was added while swirling, 300 ml. freshly prepared, carbonate free, 10% sodium hydroxide. The mixture was diluted to 1 liter with distilled water and stored in a polyethylene bottle.

Solutions for Developing Plates.--Three solutions were used for developing the spectroscopic plates. The developer, Kodak K-19 was made according to the directions furnished by the suppliers. The stop solution was 1% acetic acid. The fixer solution was sodium bisulfite dissolved in water to make a solution of 20%.

Physiological Saline.--The saline used to elute the protein from the starch sections was prepared by dissolving 17 g. sodium chloride in 2 liters of distilled water.

Human Blood Serum Samples.--Serum samples were supplied from the Clinical Laboratory of the Edward W. Sparrow Hospital, Lansing, Michigan.

Digestion Solution.--The solution used to digest the protein and non-protein nitrogenous materials in the samples preparatory for spectroscopy, was made from 16 M nitric acid and 60% perchloric acid in the proportion of two to one.

C. Methods

Electrophoresis on Starch Block.--A wax paper box was prepared by taking a piece of paraffin impregnated paper 57 x 10 cm., folding it

in 6 cm. at each end and leaving a base 45 cm. long. The sides were folded in 18 mm. each leaving a bottom 6.5 cm. wide. This paper box was placed on the lower lucite bed. A 45 cm. long lucite strip was placed along one side of the wax paper box for support. Two 6.5 cm. long pieces of lucite strip were placed on the inside of the box at the ends to form square corners. Two extra lucite strips were placed across the corners of the box where the long and the short lucite strips met and held in place with metal C-clamps.

The electrode vessels were filled with buffer and allowed to come to the same level. Starch, previously purified, was suspended in 0.1 M Veronal buffer pH 8.6 and poured into the paper box-form to cast a block about 0.5 cm. thick. After the starch settled for ten minutes, the supernatant buffer was blotted off with strips of absorbent paper cut to size. The starch block was allowed to air dry until a test slit made with a spatula maintained its shape. This time varied from one to two hours depending on the relative humidity. The cooling plate water supply was not turned on because it was found that water condensing on the plate from the atmosphere during humid weather kept the block wet thereby delaying the drying process considerably.

When the barely moist block could be cut easily, a 1 cm. wide transverse section was removed 10 cm. from the right edge of the lower lucite plate at the point of sample application. This section of starch was placed in a clean petri dish and 1 ml. of human blood serum for electrophoresis was applied. The serum and starch were thoroughly mixed. A stirring rod covered with tygon tubing and sealed at one end to form a flat straight surface was used to transfer the starch serum mixture quantitatively from the petri dish into the space from which the section of starch was originally removed.

After the serum-starch mixture had been returned, the two metal clamps and the lucite strips were removed, the ends of the paper box

torn off and contact with the electrode vessels established by means of two paper strips previously moistened with buffer. The paper strips were laid on the starch at each end of the block for a distance of five centimeters and allowed to dip down into the buffer in the electrode chambers. The use of cellulose sponges provided for this purpose was discarded since they caused the buffer to appear cloudy.

The entire block was now covered with a plastic sheet, the pressure pad was placed on top of the sheet, and the top lucite cooling plate clamped over the whole assembly. The power supply of the E-C Apparatus was connected. The current was adjusted to 13 milliamperes under a potential of 450 volts and allowed to run for twenty-four hours with the cold water running at 18°C. through the cooling plates.

At the completion of a run, the current was turned off and the upper layers of pad and plastic film removed. Using a clean sharp spatula, the whole starch block was cut into 1 cm. transverse sections. Each section was placed into a test tube containing 5 ml. physiological saline. Each tube was thoroughly mixed by shaking, allowed to settle, its supernatant poured into a 15 ml. centrifuge tube and centrifuged for five minutes at nearly full speed. About 3-4 ml. clear supernatant was decanted into a clean test tube and used for determining its absorbancy at 280 mμ in the Beckman DU spectrophotometer. The result gave a measure of the protein content in each starch section. These solutions were saved and then Biuret determinations performed on them. The Biuret absorbancies were read on the Model B Beckman instrument at 540 mμ. The two sets of absorbancy values were plotted on graph paper against tube number to compare the results which are shown in Figures 2 and 3.

From the graph (of Figures 2 and 3) the peaks representing the five serum protein fractions were identified. The eluates could then be combined into their various fractions or discarded according to the

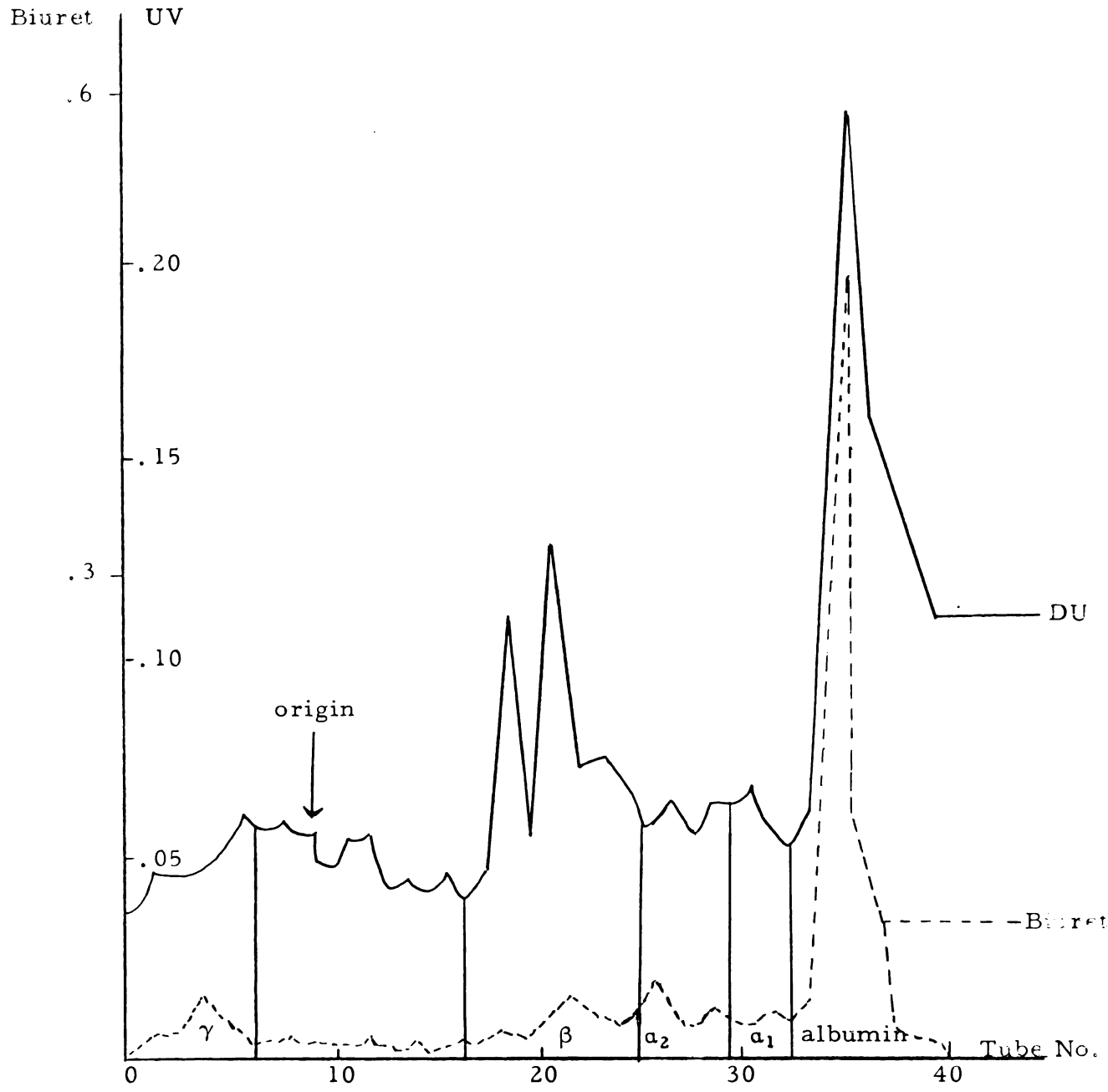

Figure 2. Comparison of protein analysis by E_{280} Absorbancy and Biuret Method of Analysis.

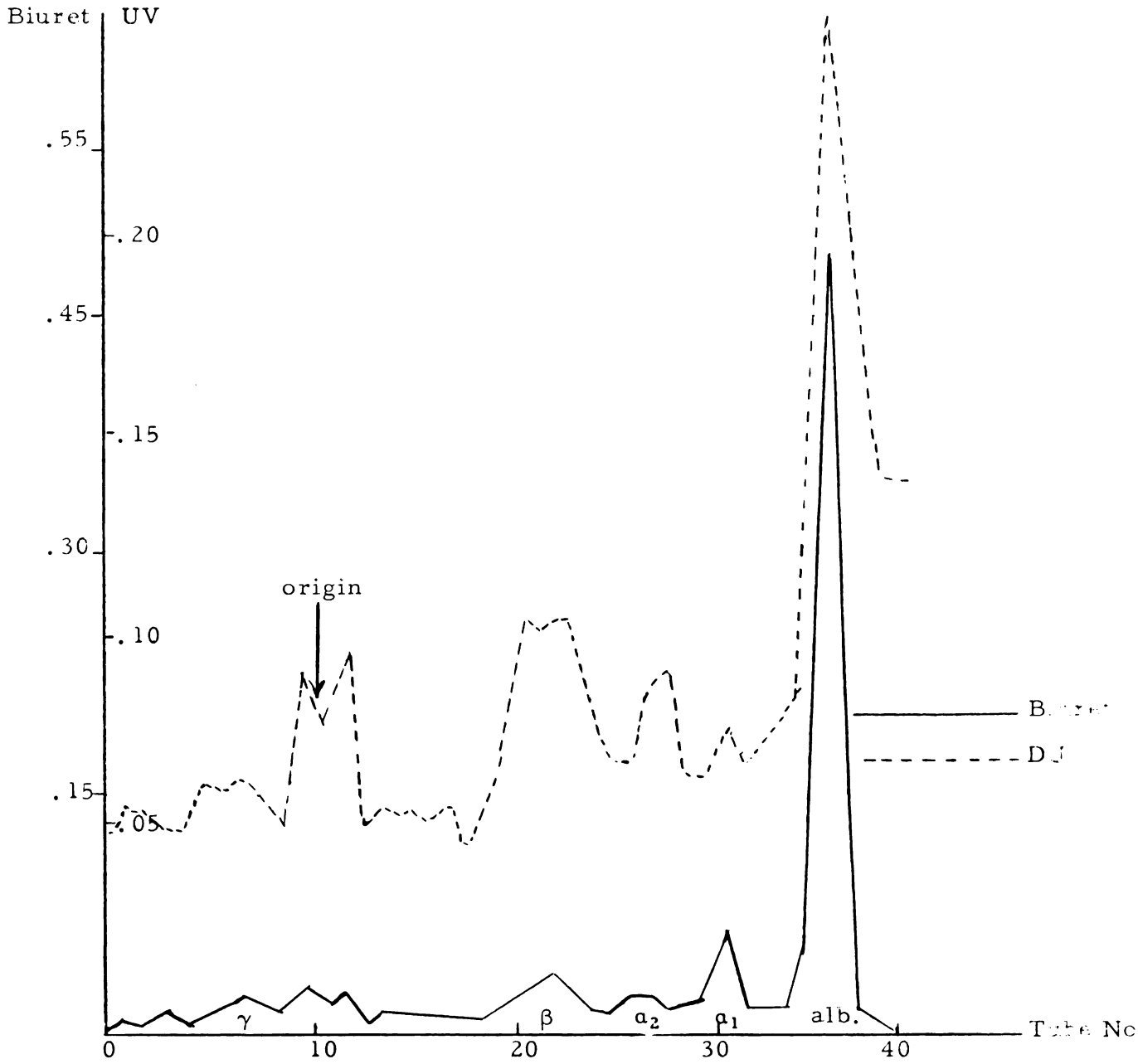

Figure 3. Comparison of protein analysis by E_{280} Absorbancy and Biuret Method of Analysis.

information obtained from the analytical results and the graph. Each solution of combined eluate for each fraction was gently evaporated to approximately 2 ml. on an electric hot plate. The method of Boyle, Whitehead et al. (5) for oxidizing the protein with a nitric acid-perchloric acid mixture was used by adding ten ml. of the acid mixture to each solution of concentrated protein fraction. The fractions were thus prepared for spectroscopy. They were stored in the refrigerator at 4 degrees C. between analyses.

Spectroscopy of Protein Fractions.--New electrodes were prepared before each spectrographic analysis (7). By placing 2-3 drops of the concentrated protein fraction into the cup of the lower electrode and passing a direct current arc, spectra were photographed and developed. Spectrographic studies were also made concurrently by using salts of the metals of interest to the investigation and obtaining their spectra on the same plate. These spectra included physiological saline; starch alone; carbon, copper and iron electrodes; cobalt carbonate, manganese chloride, zinc carbonate, magnesium oxide and magnesium carbonate, and calcium chloride (Tables I, III).

The wavelengths of unknown lines were determined by marking the plates while scanning each pair of spectra with a magnifying lens, then measuring the unknown lines and interpolating their respective wavelengths from a dispersion curve, Figure 4. The dispersion curve was previously prepared by running spectra of copper or iron, measuring recognizable lines as found in the standard tables prepared by Harrison (21) and plotting these measurements against their known wavelengths. Marking was carefully done with waterproof India ink, always labelling the spectra in proper order and using the lines of the copper and iron spectra as reference standards. The exact points where a match occurred was marked, or where a line appeared which was neither carbon, from

the electrodes, nor sodium, from the physiological saline. These lines were then identified using Brode's tables (6). The results are shown in Tables II, IV, V, VI, VII and VIII. Additional runs were made on unoxidized protein and data compiled as before. This data is presented in Tables V and VI.

Table I. Materials Sequence of Spectra, Medium Quartz Spectrograph, Figure 8,[1] see page 26, Plate 1, Unoxidized Proteins.

Exposure	Material	Exposure	Material
1	Cu electrodes	11	Alpha 1 globulin 2
2	Fe electrodes	12	Alpha 2 globulin 1
3	MgO and $MgCO_3$	13	Alpha 2 globulin 2
4	Zn CO_3	14	Beta globulin 1
5	$Ca_3(PO_4)_2$	15	Beta globulin 2
6	Albumin 1	16	Physiological saline
7	Albumin 2	17	Carbon electrodes
8	Gamma globulin 1	18	Cu electrodes
9	Gamma globulin 2	19	Fe electrodes
10	Alpha 1 globulin 1		

[1]Current 4 amps; exposure 40 sec.

Table II. Metal Identification Data Obtained from Plate 1.

Protein Fraction	Observed Line at cm.	Approximate Wave length	Brode
Albumin	13.3	2940	2940 Mn
	13.2	2935	2936 Fe
	11.55	2760	2766 Cu
Beta globulin	8.7	2540	2543 Fe
	11.55	2760	2766 Cu
	13.2	2935	2936 Fe
	13.3	2940	2940 Mn
Alpha 1 and 2 globulins	10.55	2715	2712 Zn
	10.73	2720	2721 Ca
	10.97	2700	2698 Mg
	11.4	2750	2750 Fe
	11.55	2760	2766 Cu
	12.65	2860	2852 Mg
	13.2	2935	2936 Fe
	13.25	2937	2937 Fe
	13.7	2980	2975 Mg
	14.22	3040	3040 Fe
	15.05	3180	3179 Ca
	16.25	3420	3417 Co
	16.4	3460	3455 Co
	17.9	3855	3856 Fe
	17.95	3860	3859 Fe
	18.5	4060	4059 Zn
Gamma globulin	11.56	2760	2766 Cu
	13.67	2995	3000 Fe
	14.87	3340	3345 Zn

Table III. Materials Sequence of Spectra, Medium Quartz Spectrograph, Figure 8, see page 26, Plate 2, Oxidized Proteins.

Exposure	Material	Exposure	Material
1	Cu electrodes	8	Albumin
2	Fe electrodes	9	Alpha 1 globulin
3	Carbon electrodes	10	Alpha 2 globulin
4	Starch	11	Beta globulin
5	Zn CO_3	12	Gamma globulin
6	MgO and $MgCO_3$	13	Starch
7	$Ca_3(PO_4)_2$	14	Carbon electrodes

[1]Current 4-4.5 amps, time 60 sec.

Table IV. Metal Identification Data Obtained from Plate 2.

Protein Fraction	Observed line at cm.	Approximate Wave length	Brode
Albumin	10.7	2660	2661 Fe
	14.1	3060	3060 Fe
	15.68	3300	3307 Cu
Beta globulin	10.7	2660	2661 Fe
	14.35	3080	3079 Mn
	15.68	3300	3307 Cu
Alpha 1 and 2 globulins	12.8	2900	2901 Fe
	13.8	3020	3018 Zn
	14.96	3198	3199 Fe
	15.1	3200	3200 Fe
	15.5	3260	3260 Co
	15.68	3300	3307 Cu
	16.1	3400	3405 Co
	16.6	3500	3495 Co
Gamma globulin	6.2	2340	2341 Co
	6.25	2360	2363 Co
	9.5	2580	2576 Mn
	10.6	2660	2661 Fe
	13.05	2920 (triplet)	2923 Fe (triplet)
	14.4	3100	3100 Fe
	15.1	3220	3228 Mn

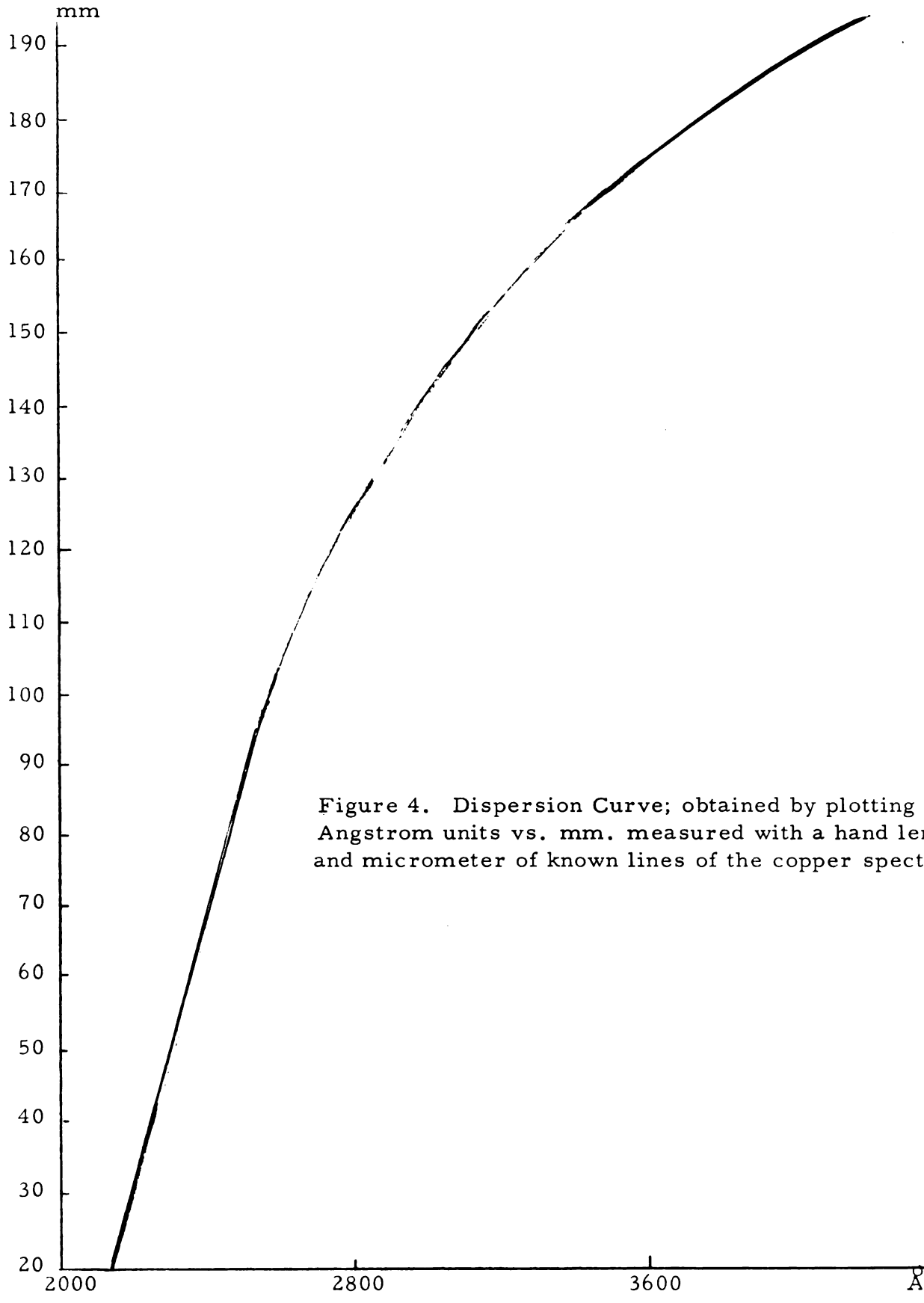

Figure 4. Dispersion Curve; obtained by plotting Angstrom units vs. mm. measured with a hand lens and micrometer of known lines of the copper spectrum.

Table V. Metal Identification Data Obtained from Plate 3.

Protein Fraction	Observed Line at cm.	Approximate Wave length	Brode
Albumin	9.45	2570	2572 Mn
	11.55	2760	2766 Cu
	13.2	2935	2936 Fe
	13.3	2940	2940 Mn
	16.35	3440	3440 Fe
	17.5	3740	3740 Zn
Alpha 1 and 2 globulins	9.3	2560	2559 Co
	10.55	2715	2712 Zn
	10.73	2720	2721 Ca
	10.97	2700	2698 Mg
	11.4	2750	2749 Fe
	11.55	2760	2766 Cu
	12.65	2860	2852 Mg
	13.2	2935	2936 Fe
	13.25	2937	2937 Fe
	13.7	2980	2981 Fe
	14.22	3040	3040 Fe
	15.05	3180	3179 Ca
	16.25	3420	3417 Co
	16.4	3460	3455 Co
	17.9	3855	3856 Fe
	17.95	3860	3859 Fe
	18.5	4060	4057 Zn
Beta globulin	8.7	2520	2521 Co
	9.3	2560	2559 Co
	11.55	2760	2766 Cu
	13.2	2935	2937 Fe
	13.3	2940	2941 Fe
	14.9	3180	3179 Ca
	16.35	3440	3440 Fe
Gamma globulin	9.3	2560	2559 Co
	11.56	2760	2766 Ca
	13.67	2995	3000 Fe
	14.87	3340	3345 Zn
	16.35	3440	3440 Fe

Table VI. Metal Identification Data Obtained from Plate 4.

Protein Fraction	Observed Line at cm.	Approximate Wave length	Brode
Albumin	1.9	2140	2139 Fe
	2.7	2180	2178 Cu
	3.95	2240	2238 Cu
	5.8	2330	2331 Fe
	8.7	2520	2522 Fe
	9.8	2595	2592 Mn
	12.45	2860	2862.5 Fe
	triplet		2863.4 Fe
			2863.8 Fe
	13.8	3025	3025 Fe
Alpha 1 globulin	8.75	2525	2527 Fe
	10.00	2620	2618 Mn
	12.3	2860	2862 Fe
Beta globulin	8.75	2525	2522 Fe
Gamma globulin	10.0	2620	2618 Cu

Table VII. Metal Identification Data from Plate 1 by Visual Comparison

Protein Fraction	Wavelength	Brode
Alpha 1 and 2 globulins	2392	2392 Cu
	2618	2618 Cu
	2244	2246 Cu
	2766	2766 Cu
	3500	3502 Co
	2442	2442 Fe
	2824	2824 Cu
	2411	2410 Fe
Gamma globulin	2824	2824 Cu
Albumin	2825	2824 Cu
	2600	2599 Fe

Table VIII. Composite of Tables II, IV, V, VI, VII.

Protein Fraction	Fe	Cu	Mn	Mg	Co	Zn	Ca
Albumin	14	6	4			1	
Beta globulin	7	3	2		2		1
Alpha 1 and 2 globulin	21	9		6	10	6	4
Gamma globulin	6	4	2		3	2	

*Incidence of lines of the spectra of metal ions occurring in the spectra of the protein fractions.

IV. DISCUSSION

Several conclusions may be drawn from the results of these experiments involving starch block electrophoresis and emission spectroscopy.

A. The Biuret Method of Protein Analysis versus E_{280} Absorbancies

As demonstrated in Figures 2 and 3 the Biuret method of analysis for human serum protein content is preferable when quantitative protein distribution results are essential. The absorbancies at E_{280} are sufficient in the case of the starch sections to designate which eluted portions to combine for each protein fraction, but examination of the graphs readily demonstrates that these readings were not the true proportionate value for the quantity of protein present in each fraction. This is verification of a fact that such values represent the proportion of aromatic amino acid residues rather than true protein content. However, Tomb, Souter, and MacLagan (36) contend that spectrophotometric determination of proteins at E_{210} is a reliable quantitative method if the protein to be investigated does not contain large amounts of aromatic amino acids. In human serum fractions one should take into account the proportions of tyrosine and tryptophan particularly in relating this to actual protein content.

B. The Use of Three Milliliter Samples

Although it is possible to run up to 3 ml. samples of serum for electrophoresis by the starch block method, Figure 5 shows that for accurate results with the E-C Apparatus, it is advantageous to use one ml. sample at a time. The difficulty appears to be due to diffusion of serum into the starch in the first strip (compartment one) while loading the serum onto the other strips, and diffusion of serum in strips one

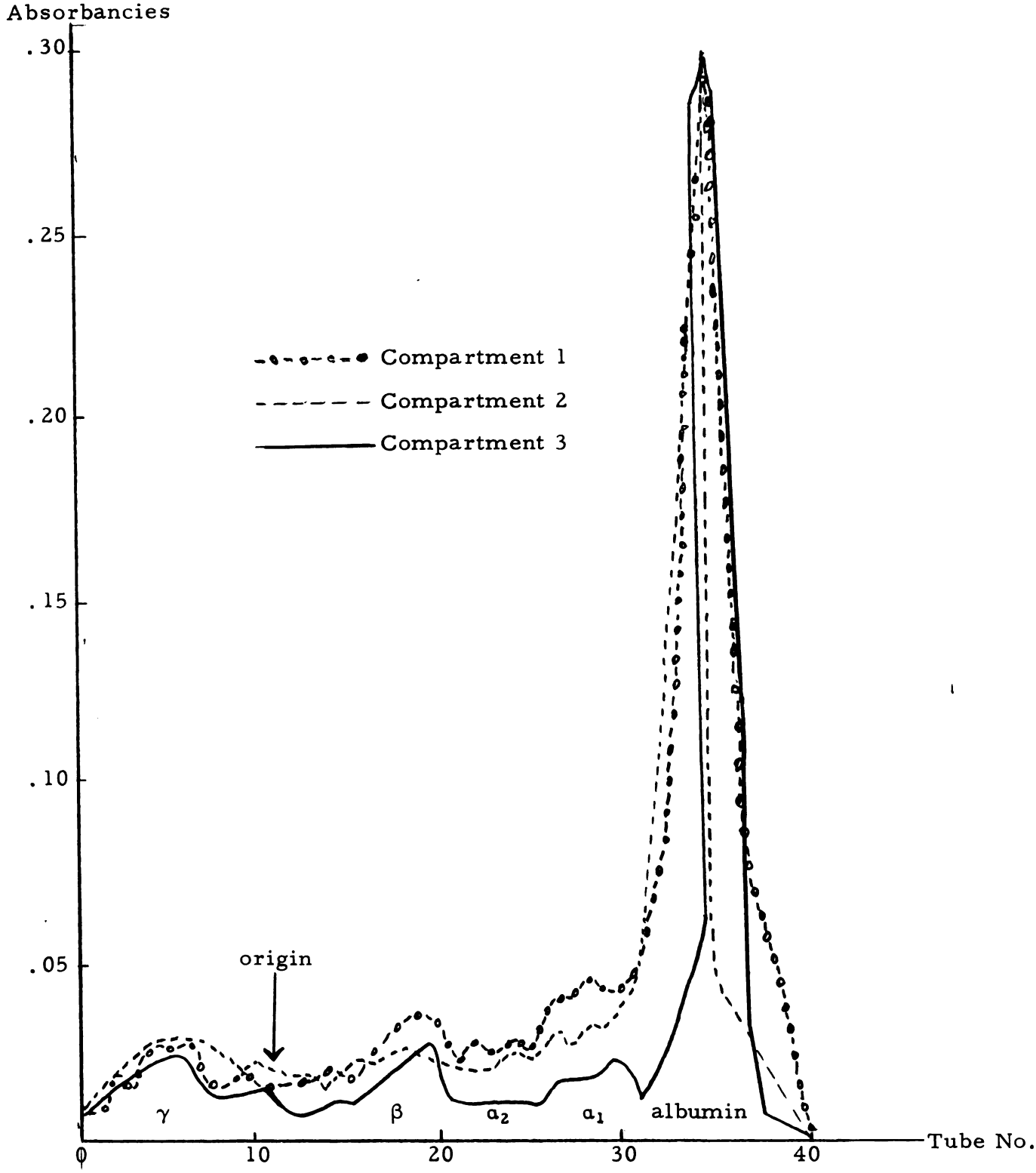

Figure 5. Biuret protein analyses of the eluates of starch block sections when all three compartments of the E-C Apparatus are used. The absorbancies of the eluates of each compartment are graphed separately. Three ml. of serum were separated in one starch block electrophoresis.

and two while loading strip three etc. The data from the Biuret determinations made separately on the three strips are similar but not precisely alike. In other words, superimposable curves are not obtained because of the time factor. Also, it was observed that the protein fractions obtained by electrophoresis of human blood serum specimens and after concentration by gentle heating and refrigeration produced crystals. This demonstrates that the starch block is a reliable means of preparative electrophoresis which produces protein fractions of considerable purity and reproducibility.

C. Sponge Rubber as a Supporting Medium

It was found in this investigation that the use of sponge rubber as a supporting medium for electrophoresis was less satisfactory than the starch block. The starch block could be eluted of protein by one extraction with physiological saline while the sponges necessitated three extractions. The protein distribution curves obtained by Biuret analysis of the saline eluates from the starch block sections were more uniform than those from the sponges. See Figure 6. Mitchell and Herzenberg (25) and Davidson (10) cite success with the use of sponges and point out the ease of recovery of the protein fractions. Nevertheless the observations of this study indicated starch to be more easily and readily extractable for its protein content.

D. Metal Ion Distribution in Serum Protein Fractions

To definitely establish the presence of a metallic element in a protein fraction, one should observe at least three lines of the element's spectra in the resulting photographic plates of the protein fraction (6). On this basis and from the data compiled in Table VIII and photographs

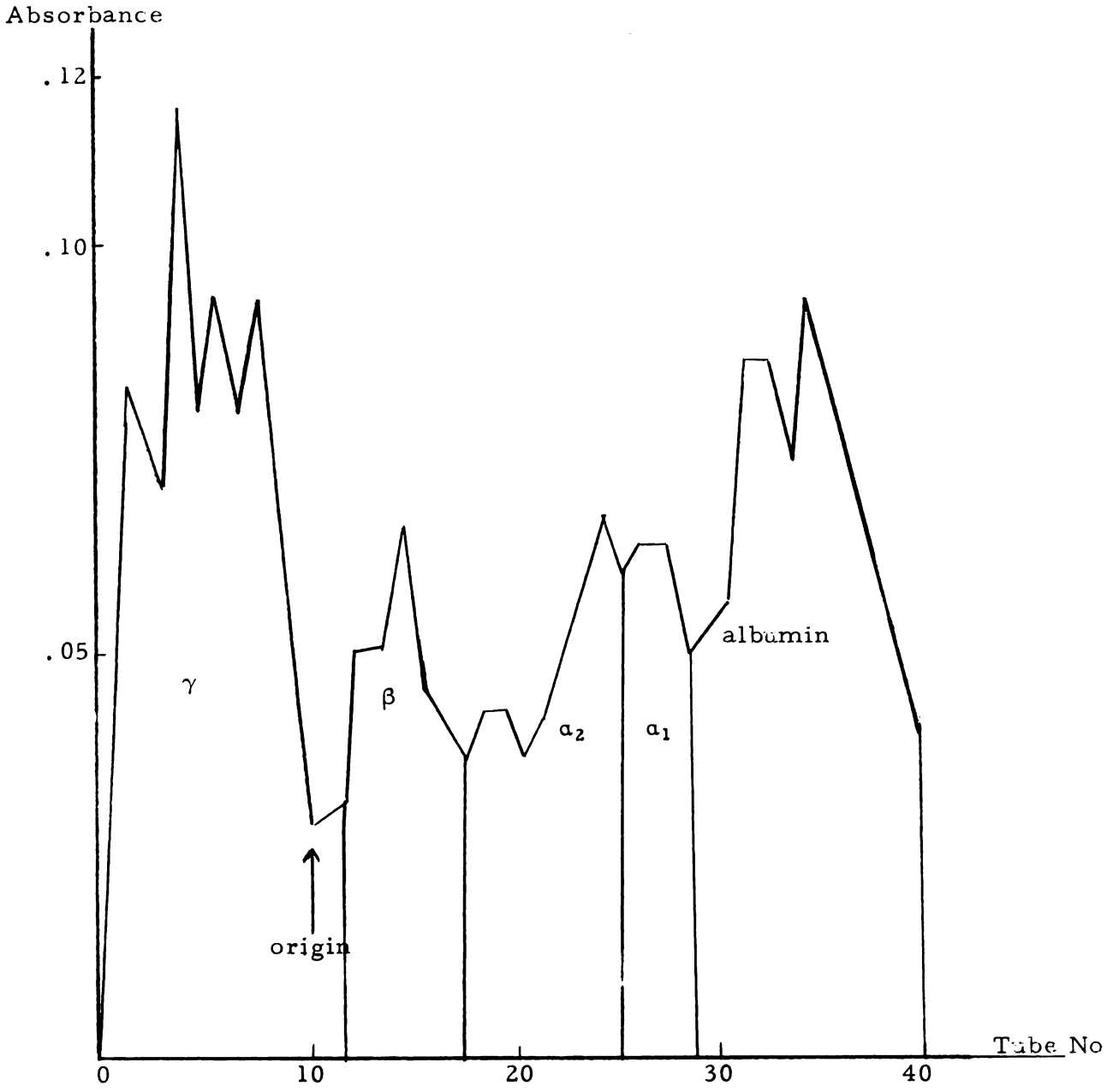

Figure 6. Absorbance vs. tube number of eluates of the foam rubber sponges after electrophoresis of a human serum sample.

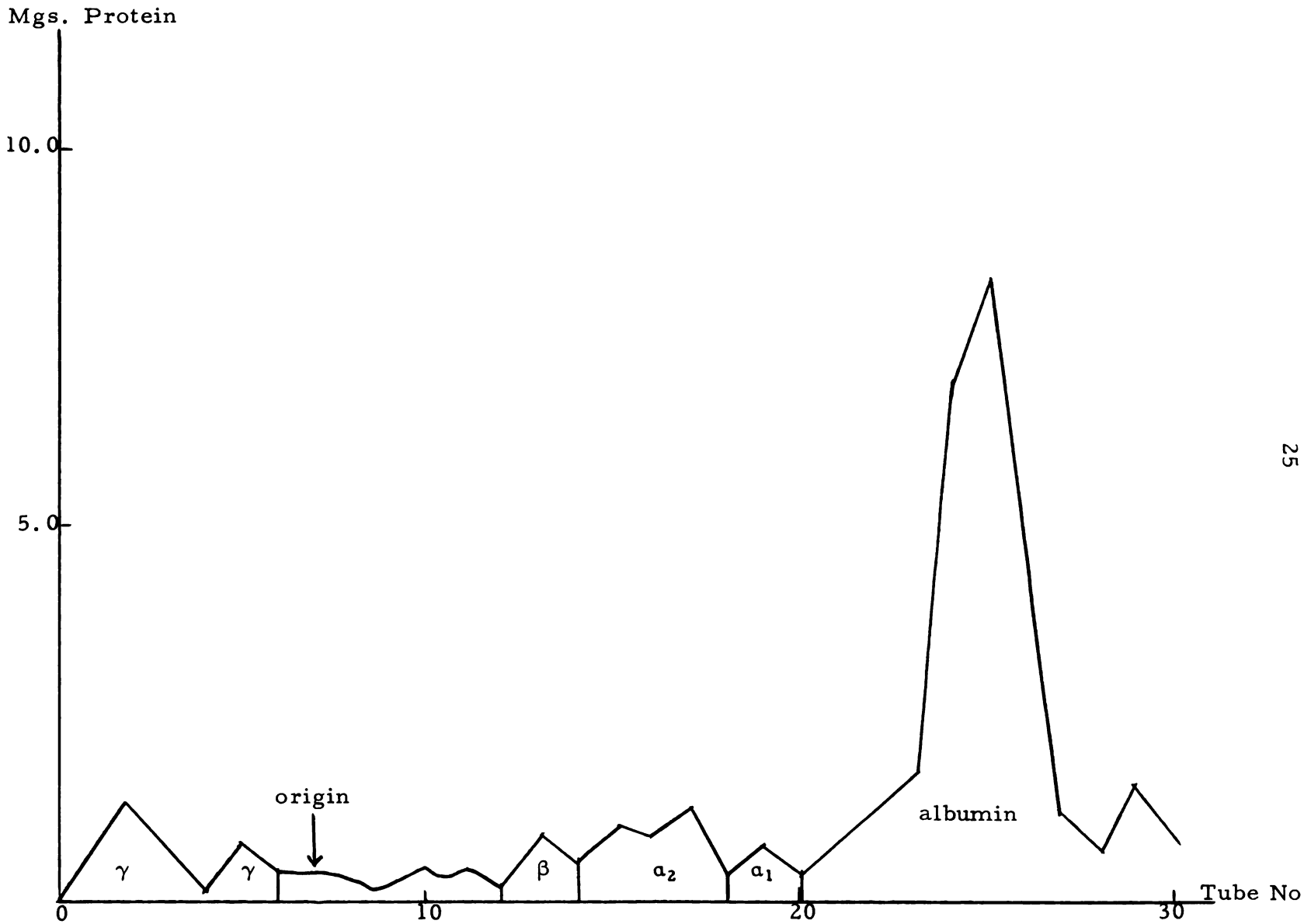

Figure 7. Biuret protein analysis of the eluates of starch block sections vs. tube number.

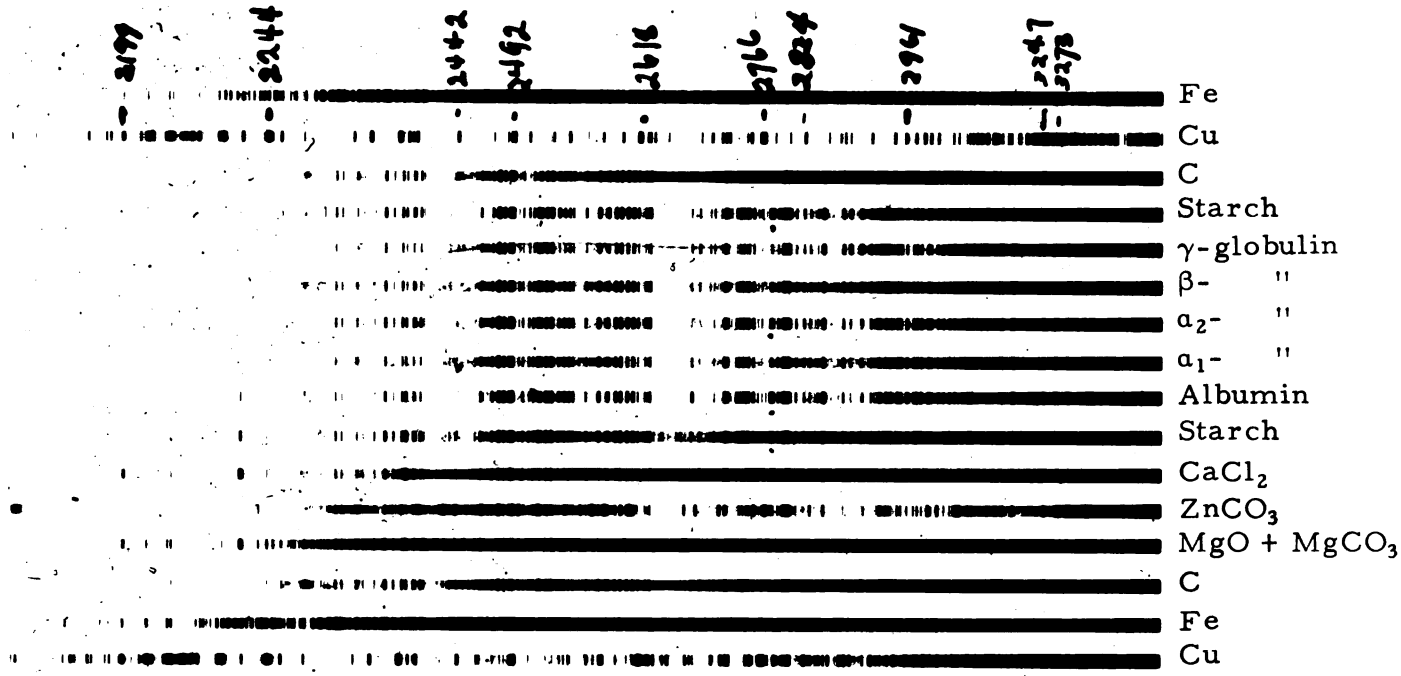

PLATE I

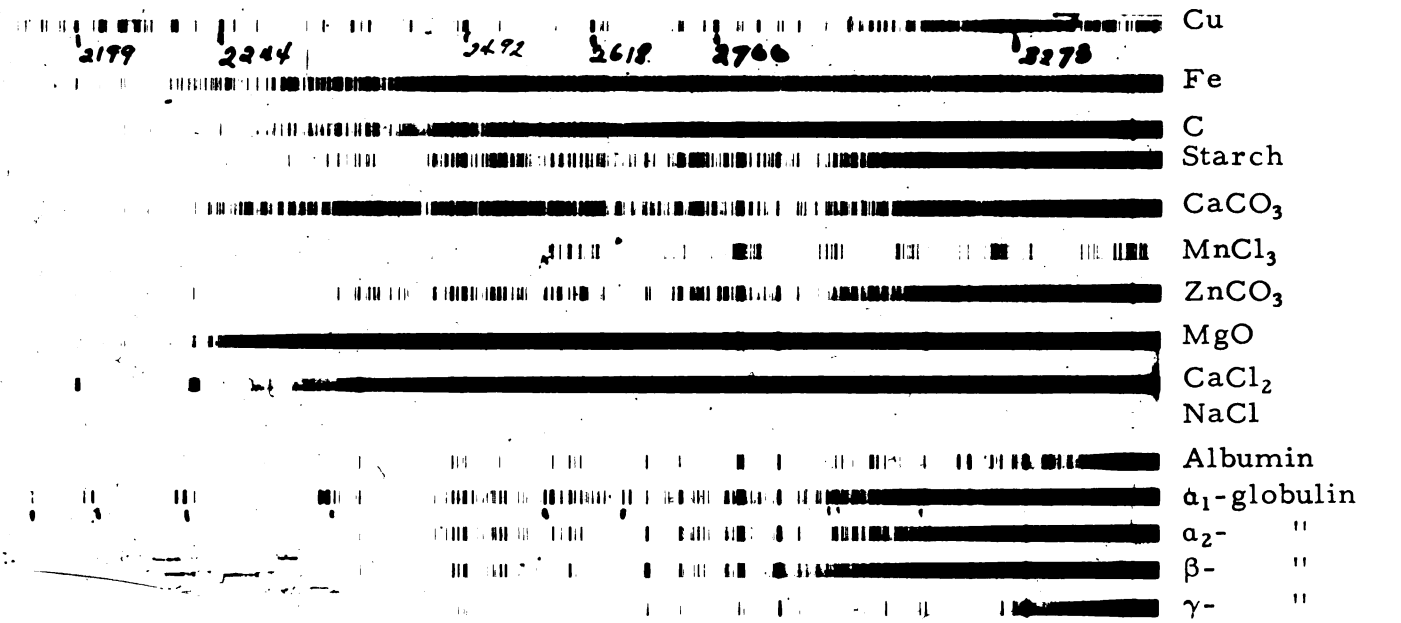

PLATE II

Figure 8. Spectrographic Plates

of Figure 8, it may be concluded:

1. The albumin fraction contains iron, copper and manganese.
2. The beta globulin fraction appears chiefly associated with iron.
3. The alpha one and alpha two globulin fractions have iron, copper, magnesium, cobalt, zinc and calcium present.
4. The gamma globulin fraction contains iron, manganese and cobalt.

Le Duc found calcium and magnesium present in all fractions, iron in the beta globulin and copper and zinc in the gamma globulin (23).

Foy (16) in his research on metal proteins of normal and pathological human sera found in all cases copper, iron, magnesium, calcium and zinc in all five fractions.

The results of this work when compared to the preceding show agreement in the finding of iron in all fractions. A difference is that Le Duc and Foy both observed calcium to be present in all fractions whereas the method of identifying the metal ions present by emission spectroscopy demonstrated calcium in the alpha globulins and albumin only. Foy reported copper present in all serum fractions, Le Duc found it appearing only in the gamma globulin fraction. This work demonstrated copper as occurring in the alpha globulins and albumin. They both also found magnesium and zinc in all fractions. Whereas in this work the presence of these two metal ions was seen only in the alpha globulins.

Experimental research along these lines should be further extended to a quantitative basis. This can be accomplished by using an internal standard as Vallee (38) did in his work with zinc metallo complexes of enzymes. It is also recommended that extending the list of metal ions to include chromium, beryllium, nickel and strontium (11, 27, 1) would be most revealing.

The possibilities of application of the emission spectroscopy method to metallo-protein study as developed in this investigation are

numerous. After adequate normal blood serum samples are run quantitatively to establish normal values for various metal ions in the different serum fractions, many sera from pathological cases could and ought to be similarly characterized. The results might lead to the possibility that a definite pattern or change of content of certain metal ions in certain protein fractions from various given pathological conditions could be established. Meanwhile this experimental technique may be regarded as a biochemical research tool to be utilized in such application as the localization of enzyme activating metal ions of blood, tissues or biological fluids.

V. SUMMARY

A technique for the investigation of metal ions present in human blood serum protein fractions has been developed. This was achieved by separating protein fractions by starch block electrophoresis and qualitatively identifying the metal ions present in each fraction by emission spectroscopy. The experimental technique has been described in detail and the results presented with suggested applications.

Specific developments were as follows:

1. The Biuret method of analysis is preferable to DU spectrophotometric analysis to obtain the protein content in saline eluates.

2. It is possible to run 3 ml. samples at one time, but for accurate results it is advantageous to run one ml. sample at a time.

3. The E-C spparatus is suitable for preparative electrophoresis of human serum protein and gives separation into fractions of exceptional purity and crystallizability.

4. The starch block proved to be a more satisfactory supporting medium than sponge rubber for electrophoretic separation of human serum samples.

5. Metal ion distribution by emission spectroscopy of the starch block electrophoretically prepared fractions proved to show that:

    a. Albumin contained iron, copper and manganese,
    b. Beta globulin contained iron,
    c. Alpha one and alpha two globulins contained iron, copper, magnesium, cobalt, zinc and calcium.
    d. Gamma globulin contained iron, manganese and cobalt.

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