วารสารเทคนิคการแพทย์ เชียงใหม่
BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY

Vol. 4 No. 1
January 1971

Serum Paper Electrophoresis Quantitative Study And By Elution and Scanning Method*

Nantaya Waiwattana, B.S. (M.T.)**
Chintana Kongton, B.S. (M.T.)***
Mnni Keoplung M.D.****

The first studies concerning the movement of substances under the influence of electric current were made many years ago. In 1809, Reuss[3] noticed that when he put sand in the bottom of two vertically held glass tubes and attached to the tubes the terminals of a battery he had constructed, the originally clear water in the tubes became turbid on the positive side because of the migration of the sand particles. This was perhaps the first clear description of the phenomenon of electrophoresis. Several years later, in 1816[12] the transport of water by galvanic current was also observed.

The traditional methods for determining the movement of particles under the influence of an electric field were improved with the passage of time until they reached a considerable variety of apparatus and techniques. These method can be roughly classified in three categories:

1) Transference method
2) Microscopic method
3) The moving boundary method

The transference methods for measuring the mobilities of particles are based on the work of Hittorf[12], published in 1853.

The microscopic methods are assigned for measuring the mobility of large particles by means of microscopes, such as immune reaction in liquid.

The moving boundary method includes techniques in which the registration of the movement of particles in an electric field is done optically, so that the movement of a zone bet-

*This work is a part of the Term paper for the degree B.S. (M.T.)

**Department of Medical Technology, Faculty of Medicine, Chiang Mai University
***Department of Pediatric, Chulalongkorn Hospital, Bangkok.
****Department of Medicine, Faculty of Medicine, Chiang Mai University.

ween the dispersed phase and the dispersing medium can be observed. The fundamental part of the apparatus is a U-tube in which a buffer solution can carefully layered over a dispersion of proteins or other substance in buffer. This method appears to have been introduced by Picton and Linder in 1892[12]. Various apparatus of this type appeared in the course of time until 1909, Michaelis, and others observed that the direction and rate of travel were a funtion of hydrogen ion concentration. A number of experimental difficulties and the prospective usefullness of the method were discussed by Tiselius in 1930.[5]

Because of the expense and time involed in the "classical" Tiselius technic,[4] simpler methods of electrophoresis were explored. The original idea of stabilizing the electrolytes at the place where electrophoretic migrations occur is of many years' studying. Lodge, in 1886,[12,3] appears to have been the first to attempt su h a procedure, using for their purpose a medium stabilized with gelatin in order to study the migration characterisrics of inorganic ions in an electric field: This work and that of Arrhenius, also published in 1886, were followed by that of Whetham, who in 1893 attempted to stabilize migration zones by msans of two solutions containing a common ion, and who showed in the following year that the addition of certain quantities of agar to solution of electolytes reduced the velocity of the ions he studied by about ten percent. A year later, Whetham published a new paper in which studies of the ionic migration using solution of electrolytes stabilized with agar were reported.

In 1937, Konig, in Brazil,[3,12] presented a paper on the electrophoresis fractionation on paper of the poison of the snake, **Bothrops jararaca.** This was probably the first time that the use of filter paper of a support was reported. However, this communication was forgotten until in 1939 Von Klobusitzky and Konig published with greater detail the results of their studies on the venom of the snake. In the same year, Strain described a method in which the combination of chromatography and electrophoresis in stabilized electrolytes with glass wool was attemptrd, and some time later, Kendall published a new paper on the separation of isotopes.

In 1948, Haugaard[12] and Kroner, studying the separation of amino acids by partition chromatography on paper, noted that by applying a potential difference to the end of the paper excellent separations were obtained. And in the same year, Wieland and

Fisher published papers on the separation of amino acids under the heading of electrophoresis. A year later, Wieland et al published a new paper on the application of this method to the study of inorganic ions and also substances of biological interest.

Finally in 1950, several papers were published dealing with the application of the method to the study of inorganic ions and in considerable detail with the separation of amino acids and proteins, especially of human blood serum.[13] Most of these papers seem to have been published independently by various authors working in different part of the world. Outstanding among them are those of Durrum and of McDonald et al in the U.S.A.; those of Cremer and Tiselius in Sweden, of Biserte in France; and finally those of Grassman and Hannig, Turba and Enenkel, Wieland and Wirth, Korver and of Kendel in Germany.

The term applied to the migration of particles through filter paper under the influence of an applied potential has not yet become standardized. Several terms have been suggested which would identify the various processes.[5] Because only the use of paper is to be consider here and the term has become increasingty entrenched in spite of any shortcoming, the term "paper electrophoresis" has been adopted.

Normally in paper serum electrophoresis as done in the clinical laboratory there appear only five fractions. They are designated according to the distance they travel in the electrical field. The fastest moving are the albumins, then alpha-1 globulins, alpha-2 globulins, beta-globulins and the gamma-globulins. In certain disease states and under running conditions of higher voltage, longer time for the run or higher ionic strength buffers more fractions may appear. Fractions appearing between the usual alpha-2 and beta are usually called "alpha-3" or if more than one fraction, successive subscripts as 3, 4 or 5 are used. For those fractions appearing between the usual beta and gamma fractions, the term beta is used with a corresponding subscript and the normal "beta" becomes then "beta-1".

Theory :

In general, an interfacial potential exists between two phases in contact. There are at least three ways this potential can arise : first, by ionization of surface groups, for example, protein molecules owe their charge principally to ionization of their amine and carboxyl groups; secondly, by the preferential adsorption of anions or

cations, and thirdly, by orientation of adsorbed polar molecules. Hemholtz had suggested in 1879 that an electrical double layer is formed at the interface, the first layer being the charged surface and the second layer consisting of a layer of oppositely charged ions. This has come to be known as the Hemholtz-double layer Modern theory, however, depicts the districbution of charges around the negatively charged surface.[2] The negatively charged surface is surrounded by an immobile layer of oppositely charged ions and this in turn is surrounded by a diffuse layer of ions of the same charge, which decreases in concentration and increases in mobility as distance from the charged surface is increased. If the surface is positively charged the surrounding ionic layers are negatively charged.

In electrophoresis, the mobility of a charged particle is a function of the magnitude of the charge, which in turn-varies with pH. For serum paper electrophoresis, the rate of movement on the strips under a given set of conditions is determined by the isoelectric point of the molecule and its molecular weight. Various factors of these conditions also influenc the movement.[5] The pH of the buffer in relation to the isoelectric point of the molecule affects the direction of the movement. At a pH 8.6 the albumin, alpha and beta globulins move toward the positive electrode. As the pH is near the isoelectric point of most of the gamma globulins, a portion of the gamma globulins will move very slightly toward the positive electrode, one portion will not have moved at all and a third fraction will have moved slightly toward the negative pole from the point of application. They appear as a single fraction in the area of the point of application

The ionic strength of the buffer affects the rate of migration.[2] If the ionic strength is too low, there is poor buffering power resulting in change in pH and slower movement of some fractions giving poor separation with indistinch boundaries. Too high and ionic strength could lead to some denaturation of proteins and some indistinctness in separation due to ionic interference of electron travel.

To prevent the migration of ions of the buffer solution from the electrode to the end of the paper strips where they would markedly alter the pH and interfere with electron flow, the chamber is fitted with a set of baffles to retard movement of the ions.[1,5] In addition, the ends of the strips do not dip into buffer solution, but are moistened by use of a heavy filter paper wick.

The support media, because of

adsorption of the protein, produces resistance to movement of the protein molecule and affects the sharpness of the separation. The older filter paper method, which used a relatively thick filter paper resulted in less distinct separation with more "trailing",[1,6] especially in the albumin fraction, than that of the thinner filter paper now in common use. Cellulose acetate paper, which is more resistant to wetting and adsorption, gives much clearer separations because it sets up less resistance to molecular movement. Also the electrophoresis time is 20-30 minutes compared to 16 hours for paper. But long cellulose acetate strips are difficult to handle because they easily become too soft or brittle during processing. The newer micromethods introduced by Beckman in their "Microzone" method and lated developped also by Gelman, in which eight samples are run on a single strip of cellulose polyacetate paper, have solved several problems. The papers are small and the technic has been improved so that the heets are handled on glass plates during the clearing process. The separations are very distinct with sharp boundaries making analysis of individual fractions easier and more accurate. The short electrophoresis run makes it easier to keep a constant power source during the run as well as making results available in a shorter time. Since this time various types of solid media have been developed.

Various kinds of apparatus have been designed and manufactured and methods for their use supplied by the manufacturer. The two common types used for paper electrophoresis are the horizontal type in which the strips are stretched across two horizontal supports, and the inverted-V or ridge-pole suspension type, used in this laboratory. According to the name, the paper strip is hung over a horizontal bar in a closed chamber. There is little question that good patterns can be obtained easily with this apparatus and that it is completely satisfactory for routine analysis,[5] but certain complication are introduced by the fact that the paper strips are not in the horizontal position. The enclosed space does not reach a uniform temperature or vapor saturation. Buffer continuously ascends the two limbs of the strip toward the apex and is concentrated by water evaporation, resulting in a gradient of increasing ionic strength and decreasing buffer volume in the paper toward the apex. There is therefore no linearity of movement of the components with

respect to time and there is an inconstancy of ratios of mobilities of components. In spite of attempts to compensate for these variables by analytical treatment of results this type of apparatus is generally regarded as unsuitable for mobility measurements or for many other investigational purposes. As a matter of fact, components frequently reach a point on the strip at which the factors operating to make it move down are exactly counteracted by the ascending steam of buffer, with the result that the component remains stationary. For some reason or other, however, there seem to be considerably less "edge effects" with the inverted-V type of suspension than with the horizontal suspension.[5]

The end of the filter paper strips dip into reservoirs of buffers across which the potential for migration is applied. It is essential that pH changes in these buffer during current flow be eliminated or minimized, especially when buffers of low ionic strenth are used.

Some methods of electrophoretic apparatus are equipped with a convenient tube connecting the two reservoirs for automatic leveling of the fluid therein. When measuring mobility it is important that this tube remains open to maintain equal levels and constant electroendosmosis flow through the paper. When open, a small current flow through this shunt. In routine analytical run it is not necessary to keep the tube open after equal levels have been obtain prior to beginning the run. In fact, a slight difference in levels has a negligible effect on resultant patterns.

The potential used for paper electrophoresis is usually in the range of 60 to 400 volts but may in some cases be as high as about 200 volts.[5] The source of this potential may be a series of dry cells but a power source working off 115 volt A.C. is generally used. For research purposes, it is necessary to be able to vary the voltage and to have a milliammeter and voltmeter (one meter may be used for both). For routine analyses these are convenient but not absolutely necessary. Mobility is proportional to the average potential gradient whereas the heat produced in the paper by the passage of current is proportional to the root mean square (RMS) or effective potential gradient.

For mobility or research studies it is essential that electronically control power supplied be used. Such power supplied frequently offer the choice of constant voltage or constant curent. In the type of electrophoretic

apparatus in which there is evaporation of solvent caused by the heat produced by current flow, the cycle of events leads to steadily decreasing resistant in the paper. With a constant voltage source, therefore, there is steadily increasing current flow. A constant current supply does not prevent the change in conductivity but does control the heat production at the expense of a steadily decreasing applied voltage and decreasing migration rates. It would seem, therefore, that a constant voltage source is required for mobility determination.

The general produce for an electrophoretic run in paper can be presented in 6 steps[5] :

1. placing the paper strip. The strip is wetted with buffer before or after setting it in place in the apparatus. This can be accomplished by letting buffer run freely on the paper from a pipet, distributing it as evenly as possible. The paper is then placed in taut suspension or between glass plate, depending on the type of apparatus.

2: Equilibrium period. A period about 15 minutes with the potential applied is allowed, therefore, for attaining equilibrim during which time the paper may gain or lose buffer.

3. Application of sample. After turning off the current, the sample may be applied to the paper as a spot by pipet or a stripe across the full width of filter paper. Protein sulutions must be applied to dry paper, or to wet paper with a brush, since denateration may occur in these instances. The sample should not contain particulate matter since this may foul the run. The quantity and concentration of sample to be applied is dependent on the width and thikness of paper strip, the nature of the migrants, and to a certain degree of personel preference. It is noted that the amounts greater than 100 microliters. (lambda is frequently used for microliter) applied to a single layer of paper strip tend to result in blurring and poor resolution In the inverted-V apparatus, the sample is usually applied in the center. In the horizontal apparatus the sample can be applied in the center or toward one end if the pattern is know to develop in the opposite direction.

4. Electrophoresis. The time for running depends on nature of substance, buffer pH, ionic strength, paper and the potential applied. This may be ranged from 1 to 24 hours.

5. Identification of components. Following removal of the paper from the apparatus, the strip must be dried quickly in the horizontal position, or

the zones will shift and and diffuse. The drying can be achieved in an oven at 100-130°C for 30 mins. If drying is not exactly uniform on both sides of the paper, components shift in the paper to the side where drying is more rapid. For colorless components any method developed for quantitative identification and subsequent quantitation of components on the paper strip may be used. These include dye-adsorption, chemical reaction resulting in a colored product. Ultraviolet light absorption, fluorescence and radiography.

6. Quantitation. When components are colored, quantition can be achieved by two methods. First, the spots or stripes may be cut out in their entirely and the color eluted by suitable means and read in a photometer or spectrophotometer.[14] In the second method the color is quantitated directly in the strip by a "densitometer" which is estentially a filter photometer or spectrophotometer constructed so it measures light transmission through a paper strip instead of a cuvet.[5,9] Many scanners are available commercially. In mannually operated instruments, after setting the instrument to 100% T on an area of the paper where one is sure that there is no component.[15] The paper is fed through the scanner and absorbances read through a slit of about 1 mm. width. Absorbance values are plotted vs. strip distance. The area under the curve is proportional to concentration, whereas the area under the curve of a paper electrophoretic pattern are proportional to absolute quantities present. In practice, however, the relationship between area and absolute quantities is a complex one. Certainly the basic laws of spectrophotometry apply[7,8,10,15] but there are application resulting from the fact that light transmission is taking place through a heterogeneous system of cellulose fibers. That the presence of "Stray radient energy" is undoubtedly at least partially responsible for observed deviations from Beer's Law. It is rather interestting that of the densitometers aviable as complete units, all employ filters. In most applications this restricts correction to be made for deviations from Beer's Law. In any event, it is absolutely prerequisite to quantitation that the apparatus be standardized for each component tobe scanned. If densitometer values are to be used in quantitation the values muat obey Beer's Law or first be converted to some function that does obey it. For example, the analytrol, a recoeding densitometer marked by Beckman (Palo Alto), converts readings to a linear

function mechanicall by specially designed cam, ' alled light balancing cam.

After the curve is constructed, lines are drawn perpendicular to the base line at the lowest points between peaks: The areas under the peaks between the construted vertical lines correstpond to the various components. This method of delineation of components is not the most accurate, but certainly is simplest and for routine purposes undoubtedly is adequate. The curve can be quantitated in variuos way: 1) Planimetry can be used but this is slow, tedious, and requires experience. 2) The segments can be cut out and weighed on an analytical balance. 3) The squares on the graph paper included in each area can be counted. If each absorbance reading is written down at the time of reading, the sums of the absorbances between minima are directly proportional to total numbers of squares. If the instrument does not automatically integrate the aereas this is probably the fastest method of all. 4) Some of the commercially available scanners automatically integrate the areas under the curve. This type of scanners is widely used in most laboratories.

Statement of Problem

The pupose of this experiment was to compare the results obtained between dye-elution and scanning methods after the papers are stained with Bromphenolblue dye.

Material and Method

In this experiment we used the Beckman Model R Paper Electrophoresis system with includes the following equipments:

- -Durrum type paper electrophoresis cell
- -Model RD-2 Duostat requlated power supply
- -Model RB Analytrol using B-5 cam and 500 millimicron interference filter.

For materials and specific proceedure see Beckman Model R paper Electrophoresis System Instruction Manual, Rim 5, November 1957.

Result

By the method of paper Electrophresis, using 70 serum samples from various conditions. Results obtained by the two methode, elution and scaning, and the differrencs in percent total protein are already shonw in Table I, II and III. Plus signs in the tables means the values from elution are greater than that from scanning, and minus signs mean the values from scanning are greater than the other.

In normal serum, as shown in Table I. The difference for albumin fraction is + 3.5, + 0.3 for L_1- globulin

- 0.3, - 2.5 and - 4.0 for L_2 -, B - and G - globulin respectively.

The standard deviations are ± 4.9 (Elution), ± 6.8 (Scanning) for albumin fraction, ± 0.67 (E) an ± - 0.99 (S) for L_1 globulin, ±1.4 (E) and ±1,3 (S) for L_2-globulin, ±1.2 (E) and ± 1.9 (S) for B-globulin, ± 4 1 (E) and ± 4.5 (S) for G-globulin fraction.

In miscellaneous cases as can be seem in Table II. The differences between the two technics are +4.6 for albumin, no differences for L_1 - globulin -0.4, - 2.7 and - 2.0 for L_2 -, B- and G globulin respectively.

As shown in Table III, in abnormal cases. The differences are +3.8 for albumin, - 0.2 for L_1 - globulin, - 0.9 for L_2 - globulin, - 2.5 for B - globulin, and finally - 7.1 for G - globulin

Discussion

The electrophoretic diagrams of Serum are not to be taken as specific for a particular disease but rather as an index to the physiological condition of the patient that cause certain changes in albumin and globulin.[11] The method of paper electrophoresis, is more advantagous than the chemical methods that fractionate only the globulins from albumin. On paper, albumin and globulins are separated into 5 fractions, albumin and alpha$_1$ - globulin separate well, whereas alpha$_2$ - Beta and Gamma-globulins tend to be compressed. However, we can locate all of the fractions easily by the naked eye after staining with bromphenol blue dye. In this experiment, two methods have been used for quantitation of serum protein separated by paper electrophoresis; dye elution and scanning.

Experiments showed that the color of bromphenol blue varied with the protein to which the dye was bound: this meant that scanning of the bound dye could give the results which differed from those obtained by eluting and measuring the free dye. However, it is also found that, when scanning paper strip at one paticular wavelength the effect of color differences in the dye bound to various proteins very nearly disappeared. The wavelength at which the absorption spectra were found to coincide was 495 Mili Micron. This fact meant that, if scanning were done at around this critical wavelength, agreement would be approached between results obtained by scanning and those obtained by elution. Consequently, a 500 Mili Micron interforence filter was introduced.

A difficulty in elution technique is the arbitrary method in which strips

must be cut up to define the separated zones, especially between Beta- and alpha- parts of globulin. The albumin trailing is still shown by this type of paper strip. This can be one source of error in both methods, the scanning method seems to have more error than the elution.

The advantages of the scanning method are of those the scanner can be operated easily by clinical and research personnel who have not received any special training. The system is provided with safety devices. It has a light balancing cam which corrects for scanner light energy distribution and the inhomogenecity of some filter paper. The B-5 cam may be used to produce records, which are essentially linear with dye concentration for many materials. The second pen is operated simultaneously with the densitometer, this can accurately integrate the area under the densitometer curve. The last thing is the rapidity in action. It is possible to scan 12 papers strips in an hour.

The results obtained in this experiment, which are given in Table I, II and III using normal, miscellaneous or abnormal serum, are closely similar. That is the scanning method showed the value to agree well with the results by the elution method, eventhough the scanning method tends to have a little lower value of albumin and a higher alpha-globulin than the elution method.

Conclusion

For this experiment we used 70 specimens of serum which came from normal 17 cases, various types of disease 18 cases and miscellaneous 35 speciments. The results which we obtained by elution and scanning are very close together. We can say that the scanning method is as good as eluton method. But, if we think about economy, the scanning method is much more expensive than the elution method. If we work in a small hospital, it is not necessary to spend so much money to buy a scanner even through the scanning method is much quicker than the elution. Scanning does not seem more accurate than the latter.

Summary

From the results of this work we find that quantition of serum paper electrophoretic strips of serum protein obtained with Bromphenol blue give equally satisfactory results by elution technic or by scanning on the integrating densitometer "Analytrol" of Beckman Instrument Co.

Variations between the two technics ever no greater than those within the technics themselves as can be seen in Table I, II and III.

Table I Comparison of results and differences in terms of total protein in Normal person, determined by dye elution and scanning methods.

No.	albumin %		alpha$_1$-globulin %		alpha$_2$-globulin %		Beta-globulin %		Gamma-globulin %	
	E	S	E	S	E	S	E	S	E	S
1	60 6	57.6	3.8	5.0	7.9	8.7	7.9	8.4	20.1	20.2
2	61.5	58.5	5.0	3.6	8.3	8.7	6.4	11.0	18.5	18.5
3	55.6	58.1	5.6	3.5	7.8	7.2	7.5	9.8	23.4	21.4
4	60.0	50.4	4.4	4.7	7.7	7.8	8.2	12.7	20.0	24.5
5	66.0	66.4	5.4	4.7	6.5	8.0	7.6	8.8	12.0	12.2
6	69.0	58.5	3.7	4.6	6.6	8.3	8.0	11.6	12.8	17.0
7	72.0	59.8	3.8	5.4	3.8	7.4	6.5	9.4	14.1	18.4
8	66.8	60.0	4.1	5.4	6.8	6.8	7.1	10.8	15.7	17.1
9	61.0	60.9	4.6	2.9	6 7	8.8	8.8	10.4	18.8	17.0
10	59.6	42.9	3.7	5.1	7.0	7.5	8.6	14.2	21.0	30.3
11	75.0	66.1	3.3	5.3	4.0	4.3	4.3	9.7	11.8	14.4
12	63.8	66.7	3.5	2.5	7.6	6.7	8.3	7.5	16.7	16.6
13	67.0	72.5	3.7	2.3	7.3	6.0	8.5	6.8	13.5	12.5
14	62.5	60.0	4.7	4.0	8.2	9.4	8.6	10.2	16.0	16.4
15	64.9	65.0	4.0	3.6	6.7	6.6	8.0	10.4	16.5	14.0
16	65.6	69.2	3.8	2.4	7.6	6.9	6.3	7.4	17.2	14.2
17	60.5	58.5	4.5	3.5	7.7	7.1	8.9	11.5	18.5	19.4
Mean	64.2	60.7	4.3	4.0	7.1	7.4	7.5	10.0	14.5	18.5
S.D.	± 4.9	± 6.8	± 0.67	± 0.99	± 1.4	± 1.3	± 1.2	± 1.9	± 4.1	± 4.5
Diff.	+3.5		+0.3		−0.3		−2.5		−4.0	

Table II **Comparison of results and differences, expressed in terms of percent of total protein, in Miscellaneous cases.**

No.	albumin %		$alpha_1$-globulin %		$alpha_2$-globulin%		Beta-globulin %		Gamma-globulin %	
	E	S	E	S	E	S	E	S	E	S
1	60.0	58.5	3.7	3.9	8.9	6.5	9.2	13.6	18.3	17.4
2	60.6	50.1	3.8	3.4	4.3	12.3	10.9	14.9	15.4	19.3
3	63.0	58.0	3.6	4.5	10.2	9.7	8.2	11.9	15.4	15.9
4	66.2	57.6	3.6	3.7	4.5	12.1	6.6	10.7	14.0	16.0
5	55.8	61.8	4.8	2.6	7.9	5.5	9.4	8.9	22.3	21.2
6	58.2	59.2	3.7	2.5	11.0	10.3	10.4	11.9	16.7	16.1
7	58.0	51.1	3.9	5.0	10.3	11.8	9.6	13.1	18.6	19.0
8	51.1	45.8	4.2	6.4	17.0	19.0	8.9	9.7	18.9	19.0
6	51.1	44.5	4.4	6.0	11.5	10.5	12.2	14.7	20.4	24.3
10	52.9	47.7	6.4	4.3	10.5	11.1	11.7	14.1	18.9	22.8
11	59.9	52.6	4.5	3.9	6.6	7.7	7.6	8.4	21.4	27.6
12	46.9	39.9	5.9	5.8	16.3	15.0	11.7	18.9	19.4	20.4
13	53.2	53.0	5.5	4.5	10.5	11.4	12.1	13.1	18.8	18.0
14	47.5	45.0	5.9	4.2	12.9	14.6	10.5	13.3	23.1	23.0
15	51.1	45.5	5.1	6.9	12.9	12.8	11.1	11.6	20.2	22.8
16	56.8	53.5	4.0	3.9	9.6	9.7	8.8	11.9	20.4	21.6
17	50.9	45.0	4.8	5.9	15.4	14.4	12.0	18.0	16.8	16.7

Table II (continue)

No.	albumin %		alpha$_1$-globulin %		alpha$_2$-globulin %		Beta-globulin %		Gamma-globulin %	
	E	S	E	S	E	S	E	S	E	S
18	66.5	62.8	3.0	4.1	9.4	9.2	8.1	9.5	13.0	14.5
19	52.1	48.2	4.6	4.9	14.1	14.0	10.2	16.6	19.0	16.3
20	57.5	53.1	3.0	3.0	9.7	10.4	11.8	14.0	18.1	19.5
21	66.1	63.2	2.7	3.8	7.8	6.1	10.5	13.0	12.8	13.6
22	60.5	61.0	3.8	4.1	9.5	8.5	10.7	8.5	15.5	18.0
23	61.5	57.0	3.6	4.8	6.3	7.0	12.6	14.8	16.2	16.4
24	57.5	53.0	4.5	4.5	7.8	9.2	12.2	15.0	18.0	18.3
25	56.1	47.5	3.8	3.1	8.6	8.5	11.8	12.1	19.8	29.0
26	52.0	45.5	5.1	3.7	13.1	11.7	13.6	19.8	16.2	19.3
27	60.5	54.5	3.7	3.2	9.7	10.1	10.8	17.7	15.3	14.5
28	53.1	47.0	4.1	5.3	9.9	10.1	13.3	13.0	19.6	24.6
29	56.3	43.8	3.7	3.9	10.3	12.4	9.2	14.7	20.2	25.3
30	57.2	51.7	3.6	3.7	10.2	11.9	10.0	14.3	19.1	18.5
31	36.5	31.0	8.2	7.9	9.4	6.9	5.5	8.0	41.0	46.3
32	8.5	9.0	4.7	2.8	37.2	38.0	12.8	14.4	36.8	35.9
33	60.0	54.5	6.0	6.4	12.0	10 2	5.2	9.5	16.8	19.7
34	41.9	39.6	6.9	6.7	12.7	12.9	14.3	15.3	24.1	25.4
35	45.8	40.3	7.0	8.5	9.7	10.1	10.5	8.1	26.8	33.0
Mean	54.1	49.5	4.6	4.6	11.1	11.5	10.4	13.1	19.4	21.4
Difference	+ 4.6		0		− 0.4		− 2.7		− 2.0	

Table III Comparison of results and differences, expressed in term of percent total protein, in abnormal cases.

No.	Diagnosis	albumin %		$alpha_1$-globulin%		$alpha_2$-globulin%		Beta-globulin%		Gamma-globulin %	
		E	S	E	S	E	S	E	S	E	S
1	ulcerative-colitis	47.0	51.9	7.2	7.2	14.0	6.4	11.8	14.7	19.9	19.7
2	Beriberi	48.5	46.9	6.4	7.1	8.9	11.3	4.5	11.4	21.8	23.3
3	Sclerodema	30.4	23.6	4.5	5.0	8.2	10.0	8.6	6.2	48.3	55.2
4	AIHA	50.1	50.5	7.1	7.8	8.8	7.5	7.0	12.0	25.8	22.2
5	N.S.	13.3	14.6	10.8	10.7	28.9	27.6	18.6	18.7	28.4	28.6
6	N.S.	24.9	24.0	9.6	9.5	18.8	18.0	14.7	21.2	32.0	27.3
7	N.S.	15.0	13.1	5.4	5.6	41.5	40.7	12.9	14.4	25.2	26.4
8	Obstr. Jaundice	45.5	36.4	6.1	10.3	9.9	7.6	8.1	17.2	30.4	28.6
9	Obstr. Jaundice	27.4	17.1	11.3	8.1	14.4	12.1	10.9	15.5	36.1	47.0
10	Hepatitis	53.2	51.5	6.1	4.8	6.7	8.5	11.3	13.3	22.6	21.8
11	ITP	56.3	54.0	3.5	4.5	9.4	6.4	8.7	15.3	22.2	19.8
12	Rheumatiod-arthritis	45.3	40.4	6.8	5.2	13.7	13.8	10.4	11.0	24.1	29.7
13	,, ,,	41.9	38.4	4.3	5.8	11.6	11.0	12.4	11.3	29.0	31.6
14	,, ,,	33.4	24.8	3.4	3.9	6.6	6.3	8.2	7.2	48.5	47.7
15	,, ,,	38.8	25.8	8.8	8.4	17.7	14.2	14.1	12.1	27.6	39.5
16	Multiple myeloma	31.2	30.0	3.9	4.2	7.6	7.5	52.0	54.5	4.3	3.9
17	Myocardial Infarction	48.6	44.5	5.7	7.4	7.9	9.8	14.3	16.7	23.4	21.6
18	Thalassemia	22.3	16.8	6.8	6.6	6.6	5.8	8.6	12.7	55.7	58.2
	Mean	37.4	33.6	6.5	6.7	13.4	12.5	13.4	15.9	28.6	30.7
	Difference	+ 3.8		- 0.2		+ 0.9		- 2.5		- 2.1	

REFERENCES

1. Beckman Model R: Paper Electrophoresis System Instruction Manual, Rim 5, November 1957.
2. Block, R.J., Durrum, E.L., and Zweig. G; A mannual of paper chromatography and paper electrophoresis, 2nd, 1958, 554
3. Gradwohl's clinical laboratory methods and diagnosis, Vol. 1;6th, Saint Louis, The C.V. Mosby Company, 1963 p 50
4. Griffiths, L.L: Electrophoresis of Body Fluids, J. Lab. Clin. Med., 41; 188, 1953
5. Henry, R.J. Clinical Chemistry. Principles and Technics. New York, Hoeber, 1964,93-109.
6. Henry, R.J., Orville J. Golub and Charles Sobel, Clin. Chem., 3: 49, 1957.
7. Joossens, J.V. and J. Cleaes. Quantitative Study of dyeprotein relationship by scanning and elution methods in paper electophoresis, J. Lab. Clin. Chem., 2: 386, 1956.
8. Latner, A.L., L. Molyneux, and J. Dudfield Roes. Semiautomatic recording densitometer for use after paper stip electrophoresis, J. Lab. Clin. Med., 43: 157, 1954.
9. Mackay I.R., Volwiler W., and Goldworthy P. D., Photometric quantitation and comparison with free electrophoresis. J. Clin. Invest., 33: 855, 1954.
10. Marie A. Andersch, Frances Barbusca. A graphic method for the conversion of transmission curves to a corrected density curve for the calculation of protein separated by paper electrophoresis, Am. I. Clin. Med. 45: 958, 1955.
11. Oosterhuis H.K. Studies on paper electrophoresis: A compari-on with the chemical method as an aid in clinical diagnosis, Am. J. Clin. Med. 44: 280, 1954.
12. Ribero, L.P., E. Mitidieri, and O.R. Affonso, Paper electrophoresis: A review of methods and results, 1961, 59-61.
13. Sunderman, Jr. and Sunderman: Clinical applications of the fractions of the fractionation of serum proteins by paper electrophoresis, Am. J. of Clin. Path., 27: 125 1957.
14. Sunderman Jr., and Sunderman: Studies on the serum protein; The dye-binding of purified serum proteins separated by continuous flow electrophoresis, J. Clin. Chem., 5: 171, 1959.
15. Wurm, M., and F.H. Epstein, Quantitative electrophoresis of serum proteins on paper, J. Clin. Chem., 2: 303, 1956.

Serum Paper Eiectrophoresis Quautitative Study by Elution and Scanning Method.

นันทยา วัยวัฒนะ, วท.บ. (เทคนิคการแพทย์)*
จินตนา คงทน, วท.บ. (เทคนิคการแพทย์)**
น.พ. มุนี แก้วปลั่ง พ.บ.***

ย่อจากต้นฉบับ

การแยก serum protein ด้วยกระดาษกรอง และเครื่องมือของ Beckman-Spinco ย้อมสีด้วย Bromphenol blue ใน absolute methanol เมื่อทำ Duplicate strips แล้วนำมาอ่านค่า protein เป็น percent ของ total protein ด้วยวิธี Elution ด้วย 5% $Na_2 CO_3$ และวิธี scan ด้วย "Analytrol" spinco Densitometer อีกวิธีหนึ่ง

ผลที่ได้จากการใช้ serum ทั่วไป 70 ราย ปรากฏว่าอ่านค่า protein ที่ได้มีค่าใกล้เคียงกัน วิธี scanning จะให้ค่า albumin ต่ำกว่า และค่า G-globulin สูงกว่าวิธี elution.

* ภาควิชาเทคนิคการแพทย์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
** ภาควิชากุมารเวช คณะแพทยศาสตร์ จุฬาลงกรณ์ มหาวิทยาลัย
*** ภาควิชาอายุศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่