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Some Practical Applications of Paper Electrophoresis.

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SOME PRACTICAL APPLICATIONS OF PAPER ELECTROPHORESIS

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INTRODUCTION

Due to the increased interest in electrophoresis as a clinical tool for diagnosis, it seems evident that an attempt should be made to obtain equipment for use in the small hospital.

The cost of commercially produced equipment prohibits the use of the electrophoretic procedure in the small hospital unless some method employing the basic theories can be found at lower cost. This paper attempts to show how a small hospital will have the essentials for this procedure at a cost of one-tenth of the commercially produced equipment. The completed electrophoretic patterns may then be sent to a larger hospital which may have an "Analytrol" or some other densitometer for obtaining the percentage of the serum albumin and globulins. The graphs can then be interpreted and sent back to the small hospital for diagnosis.

During the summer of 1956 the writer observed the equipment in use at the Naval Medical Hospital, Bethesda, Maryland. The results of the equipment there were compared to the results of this present investigation. The separation obtained at Hays corresponded so closely to that at the Naval Hospital, that the results of this investigation may be considered valid.

The present investigation was conducted at the St. Anthony Hospital at Hays, Kansas. It was designed to check the relative accuracy of an improvised instrument with an expensive commercial model, and to verify the use of such an instrument as a diagnostic tool.

HISTORY OF ELECTROPHORESIS

The movement of particles in an electric field, known as electrophoresis, is today one of the most important analytical tools available to the clinical laboratory. It may surprise many, in view of the fact that electrophoresis has only recently become popular, that this phenomenon of particle mobility in an electric field was observed as early as the beginning of the nineteenth century. The initial observation which eventually led to the development of electrophoresis as we know it today was made in 1800 by Nicholson and Carlisle when they noted that water decomposed under electrical stimulation. Shortly after this, in 1802, Cruickshank observed that metallic chlorides decomposed with the deposition of metals on the cathode. A similar experiment carried out by Hisinger and Berzelius in 1804, showed that neutral salts decomposed, liberating acids at one electrode and metals at the other. Davy, in 1807, used this technic for isolating sodium and potassium. Reuss, in 1808, the acknowledged founder of electrophoresis, found that water became positively charged when in contact with powdered quartz and clay, and, therefore, that a direct electric current could forcibly transport the charged water molecules through a porous quartz or clay diaphragm toward the negative pole.

However, of the various methods available for the study of the electrophoresis of proteins, the moving-boundary method has been most successful until the most recent "zone" and "continuous" electrophoresis. In 1886, Lodge used the moving-boundary method for the study of transference numbers, a technic brought to a high degree of perfection in 1932 by Longsworth and MacInnes. The first moving-boundary studies with proteins by Picton and Linder, in 1892, stimulated further work on proteins, in

the course of which Hardy, 1899, Pauli and Landsteiner, 1908, Michaelis, 1909, and others observed that the direction and rate of travel were a function of the hydrogen ion concentration. A number of experimental difficulties were encountered by the early workers, and these difficulties and the prospective usefulness of the method were discussed by Tiselius in 1930. Thorell, in 1934, succeeded in isolating the yellow respiratory enzyme of Warburg and Chrisian by use of an electrophoresis cell which could be divided into a number of sections by sliding ebony plates. In 1937, Tiselius described the apparatus that he had developed at the University of Uppsala which made it possible to make precise measurements on purified proteins and naturally occuring mixtures such as plasma.

The first report on the use of paper electrophoresis appears to be that of Konig, which appeared in Portugese in 1937. It is interesting to note that this report antedates the modern work on paper chromatography, which can be considered to have started with the work of Martin and Synge in 1945. In 1939 another paper appeared by Konig in collaboration with von Klobusitsky, in which paper electrophoresis was used for separation of a yellow pigment from a snake venom, its first use for protein mixtures. This work attracted little attention, however, perhaps because Konig's observations were temporarily eclipsed by the work of Tiselius with the moving-boundary method, which also appeared in 1937. In any case, it seems certain that the subsequent rediscovery of the method was influenced to a large extent by experience with paper

chromatography, as well as the silica gel ionophoresis of Consden, Gordon, and Martin. From 1947 to 1949 a number of laboratories independently developed methods of electrophoresis on paper which are widely employed without significant modification today.

Frankel (45) in 1955 reports there have appeared over 300 papers involving paper electrophoresis. Of these about fifty deal with various forms of electrophoresis. Fortunately, all of the types of apparatus which have been described fall into three basic categories as follows: (1) closed strip (evaporation prevented); (2) semi-closed (evaporation permitted); (3) open strip which includes the horizontal and hanging strip variations.

GENERAL PRINCIPLES OF PAPER ELECTROPHORESIS

Electrophoresis is the migration of charged particles in an electric field. The rate of migration depends upon the magnitude of the charge upon the particles, the viscosity of the medium, the voltage of the electric field and other factors. The particle charge is affected by pH and by the electrolytes and their concentration in the suspending medium.

All proteins are amphoteric: that is, they contain radicals which dissociate to give positive and negative ions (e.g. $-COO^-$ and NH_3^+). The relative and absolute numbers of positive and negative ions determine the sign and magnitude of the charge on the surface of the molecule or protein-containing particle. The charge is negative when negative ions predominate. A negatively charged particle migrates toward the positive electrode in an electric field. If such a suspension is made more acid, additional basic radicals dissociate and their positive charges reduce the net negative charge on the protein. At a certain reaction known as the isoelectric point the protein is maximally ionized, although negative charges are equal. The particle does not migrate in either direction in an electric field, but may precipitate out of solution. The isoelectric point of one protein usually differs from that of another.

Below the isoelectric point the particles possess a net positive charge and migrate toward the negative pole of its electrophoretic cell. Table I illustrates the electrophoretic mobility and isoelectric points of the four principal protein fractions of a normal serum. A comparison of distribution of serum proteins in normal and pathogenic conditions is shown in Figure 1.

The "classical" apparatus for electrophoresis was devised by Tiselius and consists of a glass U-tube composed of cells with parallel walls of optical glass connected through columns of liquid to the negative and positive electrodes. The ability to get a picture of the separated protein fractions is based on the fact that there is a refractive index difference between the buffer solvent and the protein solution. Gronwall (47), in order to remove the convection currents and stabilize the moving boundary separation, has performed the electrophoresis of protein mixtures and amino acids in packed cotton, glass wool, asbestos, silica gel and agar.

The principle of paper electrophoresis is essentially the same except that the migration occurs along a filter paper which has been moistened with a buffer solution and placed between two electrodes. This filter paper is placed in a wet chamber to eliminate evaporation from the paper.

The actual methods used vary somewhat but are all basically the same. For example, the sample to be tested is applied to a strip of filter paper which has been moistened with the electrolyte buffer. The ends of this paper are connected through reservoirs of electrolyte, usually also covered by heavy paper wicks, to the electrodes which consist mainly of platinum wire. In most methods these strips are supported in an inverted V. The sample is applied to the vertex of the V and allowed a short while to be absorbed. In some methods the sample is applied before moistening the filter paper, in others the paper is

dipped into the electrolyte first. The circuit is then completed and voltage is applied for 6 to 16 hours depending upon the magnitude of the current or applied voltage.

The buffer may consist of varying amounts of diethyl barbituric acid and sodium diethyl barbiturate or veronal. After electrophoresis the prescribed length of time the papers are dried in ovens varying from 120-140 degrees Centigrade. They should not receive the direct radiation and should be out of drafts. The papers are dried for about 30 minutes to insure the coagulation of the protein, then placed in a stain for about six hours. The dye is made up of bromophenol blue, zinc sulphate, and 5% acetic acid. The papers are then rinsed twice in 5% acetic acid, placed in a fixative consisting of sodium acetate and 5% acetic acid, and finally dried. The amount of dye which is bound is an index of the amount of protein in different parts of the paper. By cutting the paper and removing the dye and then colorimetrically determining the dye, a curve similar to the curve plotted in the "classical" Tiselius method is obtained by plotting the dye concentration against the distance traveled. The dye can also be photometrically determined without elution.

Gronwall (47) worked with the processes which occur during the electrophoresis when ions and charged protein molecules migrate. Diffusion occurs, heat is generated which increases evaporation from the surface, and a hydrodynamic equilibrium is formed between the capillary force and the force of gravity in the minute pores of the paper. For best results a constant migration must take place thus

necessitating a constant concentration and composition of the electrolyte which is altered by evaporation and the reaction at the electrodes. In order to keep all the deterrent forces at a minimum, a barbital buffer of pH 8.6 to 8.8, of ionic strength of 0.05, using a 35 cm. strip of filter paper and a potential gradient of about 7-10 volts long cm. must be used to get a complete separation of 0.005 to 0.03 ml of serum in about 5 hours. Gronwall also recommends not only bromphenol blue, but also Azocarmine S and Amidoblack 10B. In most methods the dye is eluted with precisely measured amounts of dilute NaOH. The methods vary depending upon whether the paper is cut in 5 mm strips, or whether the entire segment of a single protein is cut.

Much work has been done checking the accuracy of this method against the "classical" method of Tiselius. Levin and Oberholzer (63) found that after delineating the protein fractions on paper, a micro-Kjeldahl method of determining the protein nitrogen gave better comparisons than the dye eluting method used by most. This is due to the fact that albumin has a greater binding capacity for the bromphenol blue than for the gamma globulin. They found that 1.3 was a sufficient correction factor for the gamma portion. This would not apply to all fractions and is also specific to the time and concentration of the dye because of differential rates of adsorption. They also feel that the curve made by the dye elution method is difficult and not very accurate because of the job of measuring the 5 mm strips of paper, the number of solutions on which the dye must be determined, and the extreme difficulty

of analyzing the curve once it is made. Levin and Oberholzer ran a test to find out if the fractions were being correctly defined at the lowest point of concentration between the fractions. In one set the dye was eluted in 5 mm strips and micro-Kjeldahls were done on another set. The latter was discovered to be as accurate in the method of compiling and analyzing the curve as the Tiselius method. Comparison of the two methods showed that the albumin fraction agrees quite well, but the gamma-globulin, showed several differences over 6%. The greatest difference (over 10%) was shown on a patient with multiple myeloma. However, the gamma-globulin, being an end fraction like albumin, should be expected to agree. The discrepancy may come because of extra absorption at the point of application. Methods done on nephrotic serum showed difficulty in separating the alpha-2 and beta-globulin fractions partly due to the large amount of lipids. They also do not agree too well on the determination of hyperglobulinemia, possibly because of the dye elution method giving a measure of the protein part of the protein-lipid-carbohydrate complex. In comparing salt fractionation and paper methods, it was found that the two methods correlated well except in the case of nephrosis and in the alpha-2 and beta fractions. In salt fractionation most of the globulin appears in the beta fraction, in the paper the globulin is mainly in the alpha-2 fraction.

Harwicke (48), in a paper covering different determinations made to check the accuracy of paper electrophoresis against the "classical" Tiselius, says that the quantitative accuracy is found to be plus or

minus 6% of the total protein present. He feels that this method may be quantitatively more accurate in the analysis of pathological sera containing high proportions of lipid or carbohydrate than any processs used up to now.

Paper electrophoresis can also be used for spinal fluid. Before use, it is concentrated by dialysis in a cellophane bag against a concentrated solution of dextran. About nine ml. of spinal fluid or normal protein content can be concentrated to about 0.2 ml. after approximately 24 hours of dialysis in a cold room. After being concentrated spinal fluid will show a paper pattern similar to normal serum. Gronwall (47) worked out a method for the staining of serum-bound fat after the regular period of electrophoresis. After staining with a half saturated Sudan black in 50% ethyl alcohol, the fat will appear as blue-black spots situated in the globulin fractions. Glyco- and mucoprotein may be stained by a modification of the Hotchkiss method, using periodic acid for the oxidation of polysaccharides to polyaldehydes. The protein polysaccharides will appear on the paper with a red violet color.

Paper electrophoresis is also very useful in separating and analyzing abnormal hemoglobins. Apset (82) states that Pauling and his associates discovered that the sickling of red cells is associated with an abnormal hemoglobin of altered electrophoretic mobility. Later Itano and others found a second type having an electrophoretic mobility different from normal and sickle hemoglobins. When this second type is combined with normal blood, target cells are seen in peripheral blood smears although there is no clinical evidence of disease. This is

designated as Hemoglobin C. Itano also found another hemoglobin which has mobility of the same magnitude as sickle, but is more soluble and is not capable of causing the erythrocyte to sickle. It is also true that adult and fetal hemoglobins differ in their electrophoretic mobility.

In order to prepare bloods for electrophoresis the cells must first be hemolysed. Spaet (82) used oxalated blood, washed the cells three times with normal saline, hemolysed the packed cells by freezing, then thawing. He then diluted the resulting solution with five volumes of veronal buffer (pH 8.6) and finally centrifuged an hour at 20,000 rpm. Another method washes the cells with 0.9% NaCl, adds an equal volume of distilled water to the packed cells, using normal hemoglobin as a control. Spaet showed that normal hemoglobin travels farthest, sickle intermediate, and hemoglobin C the least. Motulsky, Milton and Durrum (66) showed this to be true and in addition they showed that fetal hemoglobin is between normal and sickle types.

Motulsky, Milton and Durrum (66) state that paper electrophoresis may be used for life span determinations of the erythrocytes in some of the hereditary hemoglobinopathies. The decay of normal adult hemoglobin from transfused normal cells may be followed in patients with homozygous C red cells by following the disappearance on paper. Conversely, homozygous hemoglobin S or homozygous hemoglobin C could be given to a recipient with homozygous hemoglobin A cells and the disappearance curve of the abnormal fractions followed.

APPLICATIONS OF PAPER ELECTROPHORESIS

Clinical Applications

At the present time the best complete work regarding paper electrophoresis and its clinical applications is the book A Manual of Paper Chromatography and Electrophoresis by Richard J. Block et. al. (1) which contains considerable theory and includes the different methods in current use. Instructions for making the apparatus for various types of separation are in detail. He includes problems in mobilities, diffusion, separations and their changes due to temperature, concentrations of the buffer, and changes in current. A good comparison with different buffers is shown in Figure 8. When the paper strips are made translucent, the comparison to the dry strip regarding transmitted light is given in Figure 9. A problem in ion migration regarding the 4% trailing error due to albumin tail is shown graphically in Figure 10. A thinner paper will decrease the error.

The theory of ionography is considered extensively by Hugh J. McDonald (3), who also includes general methods and types of apparatus. The location and identification of separated fractions by using different buffers is stressed, indicating how this can be used in industry.

The statement is proposed by William B. Wartman (4), that electrophoresis promises to become a useful means of differentiating ascites due to tumors and that from other causes. Another contribution of electrophoresis is the positive differentiation of obstructive jaundice from familial nonhemolytic jaundice with kernicterus is indicated by Wartman (4).

W. S. Adams (7) gives a good procedure on plasma cell tumors (multiple myeloma) using Tisileus technic showing that beta-globulin predominantly increases. The electrophoretic pattern may increase through all of the globulins.

Comparing the results of paper electrophoresis to Tisileus' method in multiple myeloma, H. O. Conn (8), indicates that results compare satisfactorily and are clinically more practical using paper strips.

Abnormal serum and urine proteins in a number of cases of multiple myeloma are compared by Osserman (9), and display identical mobility.

Serum lipoproteins studies by Ackerman (10), show definite patterns as illustrated in Figure 11. The lipoprotein pattern of normal values is compared to hypertensive cardiovascular disease, and hyperthyroidism. Both cholesterol and phospholipid values are given.

Serum electrophoretic studies on patients with familial primary systemic amyloidosis were made by Block (11). Serum findings on 5 related patients shows the presence of a typical peak between the alpha-2 and beta globulins which has been labeled alpha-2 prime.

Random fluctuations in the levels of alpha-1, alpha-2 and beta-globulins were seen in the serum of 3 patients with Rheumatoid arthritis during treatment with cortisone. The favorable clinical response to cortisone were not accompanied by a return to normal values for the alpha of beta glogulins as reported by Hess (12).

Macroglobulinemia and other paraproteins appear to be produced by a variety of disease states. Primary macroglobulinemia (waldenstrom) appears to be a distinct entity with multiple hematologic and related clinic abnormalities. The basic defect of primary macroglobulinemia appears to be a disturbance of the reticuloendothelial system with abnormal production of high molecular weight globulin. The condition resembles myeloma but Jim (13) reports that it is probably a separate disease.

Serum electrophoretic analysis in hemochromatosis and diacetes resistant to insulin is studied by Koeni (14). Comparisons are made between cirrhosis of liver, acute hepatitis, liver impairment and other liver conditions. Koeni (14) observes that both hemochromatosis and diabetes return to normal after treatment with ACTH.

Quantitative analysis of dyed filter paper electrophoresis of human serum has been facilitated by recent advances in instrumentation states Mackay (15). Reproducibility of commercial instruments have been compared and evaluated. The stain Amido-black for 10 minutes, then decolorized with methanol-acetic acid washes is compared to the bromphenol blue technic.

The electrophoretic study of the blood serum from lympho-granulomatous patients by Rottino (16) indicates that Hodgkin's disease does have a pattern different from other diseases.

The study of virus hepatitis indicates a drop of albumin without laboratory or clinical evidence of jaundice. In addition, there is a

severe drop of alpha-1 and alpha-2 in cases with severe icterus. A marked rise of gamma-globulin (less than in partial cirrhosis) appeared. This elevation did not occur in cases without jaundice, states Satoskai (17).

The simultaneous fractionin' of serum and urine proteins, proves useful in the differential diagnosis of proteinuria in cases of multiple myelomas with Bence-Jones urinary protein. Slater (18) further indicates the relative concentrations of urinary proteins depend on their molecular size and was a parent in various conditions. The high molecular weight serum lipoproteins were not detectable in the urine.

Plasma transfusions and treatment with corticotropin is discussed by Stickler (20), with resultant evidence of increase in the clearance of albumin and alpha-1-globulin during adenotous phase after this induced diuresis. Therefore the nephrotic syndrome might be explained on the basis of increased glomerular permeability to proteins of lower molecular weight. After administration of corticotropin, the decreased clearance values seem to suggest a decreased glomerular permeability.

Comparative studies in quantitative filter paper electrophoresis by walsh (21) agreed closely. Three differentsera were used and three different stains for each serum was carried out. Types of dyes were compared and the pH of the wash solution at 3 or less was explained. The elution of paper strips in sections and subsequent determination by means of a spectrophotometer is the method preferred by Walsh for greater accuracy. However, he states the results should be read as per cent of dye distribution.

Whitman (22), lauds the method of Tisileus as the method of choice in his study of portal cirrhosis. He compares the plasma, sera and ascitic fluid of patients with acute conditions. The serum albumin is decreased with the beta- and gamma-globulins increased in cirrhosis.

A good technic by Durrum (23), is included in his article "Lipid Detection in Paper Electrophoresis". The stains are new and show improvement.

The concept of protein purity using electrophoresis as a criterion is discussed by Hess (24). As more sensitive tests are applied as available the concept of protein purity will be revised. A protein may be pure for one operation and impure for another.

Using the pH gradient, Koch (21) shows the sharpness of the electrophoretic bands can be increased at the expense of the extent of the electrophoretic pattern. This increase of pH gradient is useful for analysis of very dilute solutions.

F. Larson (26), uses a new approach to the study of the nature of the circulating thyroid hormone as well as other tracer substances in "Localization of Protein-bound Radioactive Iodine by Filter Paper Electrophoresis."

By adding 5 to 15 percent glycerol or ethylene glycol or other low molecular weight substances to the buffer, the vapor pressure of the buffer is lowered with respect to water and thus can maintain a higher temperature for a longer period with minimum loss of solution. McDonald (27) also states the evaporation from the paper of the buffer solution

is caused by the heat generated by the electrical current.

An inexpensive stain for paper electrophoresis is given by Rideout (28). This stain is Light Green S. E., which is cheaper as well as adequate, and has less trailing than the commonly used Bromophenol Blue. The technic included was by W. Grassman.

The horizontal strip method described by Antonini (32) is a typical method, but uses the stain Naphthalene Black. The paper was then made transparent by dipping in paraffin and alpha-bromonaphthalene. The readings were made in a colorimeter with an attachment for strips.

A review of electrophoretic patterns of various diseases was made by Antonini (33), which included liver diseases, nephrosis, inflammatory diseases, tuberculosis, malignant lymphogranulomas, blood diseases, myelomas, and endocrine, kidney, and cardiovascular diseases.

The correlation between the cholesterol content and the material extractable from beta-globulin in normal and diabetic serum is discussed by Bowen (38). Lipid extraction of serum before electrophoretic analysis caused a reduction of the area of the beta-globulin peak and an increase in the alpha-2.

Baker (35) states that hydrolytic enzymes can be identified and separated. The four hydrolytic enzymes normally found in human serum are: Ali-esterase, pseudo-cholesterinase, alkaline phosphatase, and amylase.

Benhamou (36) comments on staining of the protein-bound polysaccharide of serum by oxidation with periodic acid following use of toluidine blue. In this study further oxidation with bromine water gives clear bands for the aldehyde groups.

Pulmonary silicosis is the problem of Boselli (37). The alpha-globulins were normal in almost all of simple silicosis, but above normal in cases of silicosis with infection. The gamma-globulin was above normal in almost all cases and definitely pathological in 75% of the cases.

The pathological sera of known cancer patients were considered by Boyland (39) and were shown to have significant increases in alpha-1 and alpha-2-globulins. The albumin content and the A/G ratio were lowered.

Investigation of the serum as a means of evaluating the so-called colloidal lability test is reported by Gaspani (42). An increase in the alpha-globulin fraction is accompanied by an increase in the sedimentation rate, a positive Wunderly reaction according to Weltmann a shift of the coagulation band to the left. A characteristic of an increase in beta-globulin is a positive McLagan reaction and a shortening of the Weltman reaction; the Takata and Hanger tests have some significance. Characteristics of an increase of gamma-globulin is a positive mercuric chloride test and Wunderly reaction and a prolongation of the Weltmann test. Fisher (44) discusses the clinical application and limitations of paper chromatography and paper electrophoresis.

Electrophoresis and its application to clinical chemistry is discussed by Frankel (45). Frankel gives a good report on the history of electrophoresis. Conditions indicating use of this new clinic tool and differential staining technics are detailed.

A simple method for continuous electrophoresis is given by Hobart (49) which differs from the elaborate plastic and hanging strip apparatus. One foot of 2 mm. silver wire is coiled into the required shape and first cleaned for a few seconds in concentrated nitric acid and washed with distilled water. Silver wire is then placed as the anode in an electrolytic cell with the platinum cathode in 6% hydrochloric acid and a current of 20 milliamperes is passed for 4 hours.

A comparison of electrophoretic patterns and clinical findings in myocardial infarct, by H. Kaufman (52) compare to previous findings. An increase in the alpha-globulin fraction was always seen, due to an increase of the alpha-2 fraction lasting for about a month, a transitory increase of the alpha-1-globulin, and the appearance in some cases of a globulin with a mobility between alpha-2-and beta-globulin. The rise in alpha-globulins was due to an increase in the glycoproteins which seemed to parallel the observed glycemia. The changes in the globulins may be an expression of the generalized stress response.

Blood donor electrophoretic patterns return to normal in about sixty days states Kellner (53). The serum protein concentration are higher in the more frequent blood donors. The protein composition shows no consistant change.

A short time zone-electrophoresis has been developed by Kiess (54). The time is 3.5 hours (350V) and the quantitative determinations by elution of dye and reading intensity of different portions at 595 u. results compare well with the moving boundary and are more favorable than chemical fractioning.

Clinical use of a new method of electrophoresis in liver diseases is reported by Knedel (56). He uses Amidoschwarz 10B as the dye, and uses a mixture of liquid petrolatum and alphabromnaphthalene to make the strips transparent. This strip is now placed between two glass slides and the optical density is determined for the different fractions.

Abnormal protein patterns in disease is discussed by Kuhns (57). He does not consider zone electrophoresis as being sensitive enough for a quantitative determination for serum protein fractionation.

A practical apparatus can be made for continuous electrophoresis, and is shown by Kulick (58). The preparation of the apparatus and tanks using ¼ inch plexiglass for the hanging paper assembly is shown.

Blood proteins and antibiotics travel independently in paper electrophoresis and is studied by Kutzin (59).

Lenke (62) reports that ACTH improves the serum electrophoretic pattern after this induced diuresis for nephrosis patients. Data are given for 3 infants with lipid nephrosis and one woman with chronic glomerulonephritis.

Paper electrophoresis as a quantitative method, measuring the alpha- and beta-lipoprotein cholesterol, is reported by Lanman (61). See Figures 11 and 12 for protein and phospholipid patterns and changes.

Electrophoresis and ionophoresis on paper is studied by Loos (64). This is a comprehensive summary and discussion of literature on the principle of ion migration. He studies blood serums, amino-acids, sugars, etc.

Peterman (68) shows the electrophoretic pattern returns to normal after surgically removing neoplastic conditions. Patients with gastric ulcer exhibit hypoprotein due to decrease of albumin, other constituents being normal. Patients with gastric cancer show a marked decrease in albumin; alpha-globulin and fibrinogen are above normal while beta and gamma-globulins are normal.

An acid protein present in the plasma is reported by Peterman (69). Electrophoretic analysis of human plasma and serum is acetic acid-chloride buffer at 4.0 reveals the presence of an acid protein component with a mobility of minus 2.7 x 10^{-5} cm^2/volt/second. The plasma of patients with gastric cancer or cancer of the lung contain this material in greater amounts than found in normal patients or non-neoplastic patients. High values have also been found in a few cases of lympathic leukemia and 2 cases of Hodgkin's disease.

Another study by Peterman (70) of Hodgkins disease, chronic myelogenous leukemia, and lymphosarcoma, revealed no characteristic pattern.

Instrumental requirements for the photometry of serum electrophorograms are discussed by Petzold (72), using the transparency method for the stained filter paper strips.

Electrophoresis of human blood serum by Portillo (73) was carried out to obtain standard values to which pathological serums could be compared. Anweiler microelectrophoresis apparatus was used. Little or no difference was observed between male and female sera, results were albumin 62%, alpha-1 4.5%, alpha-2 6.5%, beta 10.5% and gamma globulin 15.2%.

Results obtained with a compact and simple electrophoresis apparatus was discussed by Heiner (75).

Comparative studies of the beta disturbance of electrophoretic patterns in disease is reported by Routh (77). Results of 1600 determinations have been compared and tabulated.

Schneider (79) uses elution of paper strips to obtain the protein fractions and does so on the assumption that these fractions may be analyzed further for research value. Principles and methods are described for a 3.5 hour procedure.

The results of electrophoresis in serum protein changes in the condition of Trichinosis parallel the results of the Takata reaction reports Schonzen (80). The gamma-globulin increases at the expense of albumin and other globulins.

The Seminar by Sharpe and Dohme (81) includes a simple inexpensive electrophoretic apparatus. Technic is given and theory is simplified. This also includes a rapid staining technic. A satisfactory separation of protein can be made in as little as 3 hours, depending on the voltage applied. Visual observation of the serum migration is provided by adding a small amount of bromophenol blue to the serum prior to the determination.

A method for determining esterified fatty acid with zone electrophoresis is described by Thomsett (83). Serum lipids consist chiefly of the triglycerides (neutral fat), cholesterol (free and esterified), and phosphatides. The greater part of the lipid material seems to be combined with serum protein, forming substances referred to as alpha and beta-lipoproteins.

A simple method of analyzing paper strips in paper electrophoresis is discussed by Van Os (88). Valuable tips are given regarding technic of handling the electrophorograms.

Adaptation of paper electrophoresis for general clinical work by Verschure (90) gives the method for hanging strip electrophoresis. Reading on the strips is done on the Beckman spectrophotometer at wave length 480 mu.

Microelectrophoresis study on paper by Vitte (91) reports that except for the sharp increase in gamma-globulins, the proteins of cancer patients are not notably modified. In acute trachoma patients and in cicatricial states of trachoma, the albumin was decreased and the gamma-globulin was increased in the serum.

Control of the staining procedure after electrophoresis but before stains by using 0.02 cc. of 0.05% of aqueous polyethylenimine or "polymine" is applied to the paper strips at the side, prior to Sudan Black or Naphthalene Black 12B for lipids and lipoproteins. This polymine cannot be eluted from cellulose strips and therefore must be read directly. This technic, reports Wunderly (92), corrects the error of removing an excess of stain in the washes as other technics use.

Yamanaka (93) reports a study of protein fractions in the serum of trachoma patients. In the acute and cicatricial stages of trachoma, albumin was decreased and the gamma-globulin was increased.

IMMUNOLOGICAL APPLICATION

Chemical analysis reveals no uniform difference between antibody globulins and normal globulin. The association of particular radicals with the antibody function has been suggested in specific cases, but has not been confirmed for all antibodies. The distribution bases of amino acids in normal and immune globulin is essentially the same, so the alkaline property of the immune globulin may be associated with free amino groups. These, if properly oriented, can react with carboxyl radicals of antigens or haptenes.

Molecules of immune globulin possess at least two kinds of serologic specificity. One kind of specificity is peculiar to immune globulin and is shown by the reaction with homologous antigen. It depends upon antibody groups or radicals, which are probably few in number. The other type of specificity is common to both immune and normal globulins as species proteins.

Antibody globulin molecules seem to possess specific reactive groups present in normal globulin molecules of the same species as well as the radicals upon which their antibody function (antitoxin) depends. An antibody may be pictured as a globulin slightly modified in the process of manufacture to possess a few sites capable of combining especifically with certain characteristic chemical configuration of the antigen (1).

A method of absorbing and drying antibodies on paper disks was developed by Adams (30) for serological work. It eliminated many of the problems, such as bacterological spoilage, chemical denaturation and breaking of glass vials, associated with handling serum samples.

The antibody is eluted when the disk is immersed in a dilution of virus and could be detected by inoculation of the mixture into embryonated egg.

Electrophoretic examination by Anderson (31) shows that horse diphtheria-antitoxic plasma have different proportions of alpha, beta, and gamma-globulins and different distribution of antitoxin. These changes of composition are at least partly due to conversion of one component into another.

A method for dividing the electrophoretic pattern of serum into 17 components is described by Berry (34). The procedure is based on the assumption that each component is symmetrical about its axis and has a fixed relative mobility value.

The basis of virulence in Pasturella pestis has been described by Burrows (40). Virulent strains of P. pestis can be differentiated from protective avirulent strains of P. pestis by phagocytic tests in vitro.

Electrophoretic pattern of serum infected with Salmonella typhosa is compared to normal serum by Cagli (41). Total protein values are unchanged, but diminution of albumin and parallel increase of gramma-globulin is evident. The modification disappears in about 60 days.

Antigenic identity of hyperglobulinemic serum components with proteins of normal serum has been developed by Deutsch (43). A series of hyperglobulinemic components of human sera were reported and characterized in terms of their electrophoretic mobility.

Transport and binding in serum, as investigated by paper electrophoresis and radioactive indicators is discussed by Horst (50). Radioactive indicators seem to be selective in their binding with different fractions of serum. This may prove to be another method of positive identification and separation.

An investigation on antibodies by the use of partition chromatography was done by Humphrey (51). The antibodies of gamma-globulin were taken from rabbits at different stages of immunization with various antigens. The findings suggest that different cells, capable of producing slightly different globulins, may predominate in antibody production according to the route of injection and duration of the antigenic stimulus.

Electrophoretic studies in experimental rabbit syphilis was described by Kiimmel (55). Isolation of the specific reagen from the gamma-globulin was not complete, even though the rise and fall of serum tites was exactly parallel to increase and decrease of gamma globulin.

Detection of reactions of the type of antigen-antibody combination was discussed by Lang (60). Interactions of proteins can be qualitatively analyzed by zone electrophoresis. For example, a radioactively labeled antigen may be allowed to migrate past the antibody.

Immuno-electrophoretic analysis of blood serum is explained by Martin (65). Immuno-electrophoresis combines electrophoresis and an immuno-chemical precipitate in a jellified medium. The mixture of antigens and antibodies after diffusion shows a number of lines of

precipitation which indicates the albumin is not homogeneous, and the gamma-globulins are a group of proteins of different mobility although antigenically similar.

Mueller-Eberhard (67) discusses the carbohydrates of gamma-globulin and myeloma proteins. Gamma-globulin by zone electrophoresis revealed multiple components of different mean mobilities, but containing similar amounts of carbohydrates. Gamma-globulin isolated directly from normal serum by zone electrophoresis showed a heavy component in addition to the usual 7-S material. The heavy component concentrated by preparative ultra centrifugation was found to be considerably richer in carbohydrate than the rest of the gamma-globulin.

Quantitative complement fixation for estimation of antigens was validated by comparison with electrophoretic determination of serum albumin by Peterokofsky (71). The comparison was applied to the determination of heat labile alpha-2-glycoprotein (HLGP) in normal and pathogenic sera and in plasma fractions obtained by cold ethanol techniques. HLGP is markedly elevated in nephrosis.

Comparison of electrophoretic mobility of human and dog hemoglobins by Reich (74) was the same as the mobility of beta-globulin of their respective sera. The combination of hemoglobin with alpha-2-globulin in human and dog serum showed a linear relationship to the alpha-2-globulin level of the serum.

Schmidt (78) compared serological examinations of electrophoretically separated serum components with cardiolipin antigen and an "incomplete"

cardiolipin antigen. The question of possibly more than one antipoidal antibody or "region" with which the two antigens may react was discussed.

Observation of a fast-moving protein in avian malarial serum was made by Schinazi (29). These findings clearly depict the elaboration of a lipoprotein in the serum of pigeons infected with Plasmodium relictum whose electrophoretic mobility is greater than that of albumin. The substance seems to be correlated with the erythroblastosis produced by the infection. The similar observations reported for phenylhydrazine poisoning in pigeons would indicate that this phenomenon is a host reaction to pathological disturbances, erythropoietic or hepatic in nature, or both, which may be occasioned by either parasitic invasion or drug administration. Additional studies are in progress.

A study of paper electrophoresis of a purified specific antibody has been made by Telcmen (82). Rabbit antiserum against azo-globin of beef serum was prepared and the antibody precipitated from the antiserum with a homologous antigen. The antibody was separated from the complex with sodium chloride solution and 0.1 normal hydrochloric acid. Paper electrophoresis was applied at pH 8.8 and Ph 4.0. The strips were stained in a bromophenol blue solution. Pure antibody showed a single homologous peak at both acid and alkaline pH, corresponding at alkaline pH to the peak of serum gamma-globulin.

Thompson (84) showed the serum pattern of African infants in Uganda. The serum protein patterns of African babies change after the first month which may be due to malaria infections or to low protein

diets. American and European infants begin to change after one year to the dissimiliarity which is marked in adults.

Antibody and gamma-globulin formation in vitro in hemopoietic organs has been discussed by Thornbecke (85). Gamma-globulin regeneration is slower than that of other serum proteins. Antigens seem to be a better stimulant to gamma-globulin production than blood plasma. Plasma cells and gamma-globulin are absent prior to birth. Production of plasma cells and gamma-globulin are normal in response to antigens. There is no evidence of so-called "normal" gamma-globulin, therefore, all gamma-globulins may be antibodies.

The distribution of polio antibodies in serum proteins is discussed by Timaskeff (86). A monkey and a human polie-immune serum were fractionated by electrophoretic-convection method. In the case of the monkey serum, the activity was concentrated principally in the slow-migratory beta-globulin component. In the human, the activities against two strains of polio were present in different components of the gamma-globulin. See (Figures 6 and 7) for consecutive comparative gamma-globulin changes of polio patient.

Vannier (87) showed separation of antibodies in syphilitic rabbit sera by electrophoretic convection. The results show progressive increase in the mobility of the gamma-globulin isolated at successively lower pH's. No detectable differences in the mobilities of the beta-globulins were found. A comparison of the serological and the electrophoretic data indicated that the Wasserman reagen was concentrated in the slow gamma-globulin component and Treponema pallidum immobilizing

antibody was concentrated in the fast gamma-globulin component. No evidence was found for the concentration of Treponema pallidum agglutinating activity in any of the fractions, which may be a reflection of the importance of more than one antibody in the agglutination reaction.

Paper electrophoresis and its application to the study of the proteinogram in various pathological states is discussed by Van Os (89). Studies were made on blood serum, urine, ascitic, pleural and cerebrospinal fluids using the method of Grassman and Hannig (C.A. 45, 6680g). For thirty-four normal serums, the protein fractions were compared to serum from fifteen Congo Negroes. Serum from the Negroes had low albumin (45%) and high gamma-globulin (32%) levels as compared with the white subjects. Specific changes are bbserved in the serum protein distribution were observed in recurrent fever, laeshmaniasis infarct of the heart, cirrhosis of the liver, lipoid nephrosis and myelomas.

EXPERIMENTAL

Equipment and Materials

Two sets of electrophoretic equipment were used in making the separations. A commercial apparatus, and a home-made device were each used and a comparison of the effectiveness of resolution of the fractions and the relative cost of the process were made. The commercial apparatus is referred to as 1, while the home-made equipment is 1a.

1. Spinco Model R.

   Durrum-type electrophoresis cell.

   Duostat power supply.

   Analytrol Scanner and Integrator.

1a. "Impro".

   Durrum-type electrophoresis cell (plastic bread box).

   Heath-kit power supply.

   Analytrol Scanner and Integrator.

Buffers

Buffers for Proteins

Barbital Buffer, pH 8.6, ionic strength - 0.05 (short run experiments 3 to 7 hours).

1.84 gram barbital (diethylbarbituric acid)

10.30 gram sodium barbital

per liter 1000.

Barbital Buffer, pH 8.6, ionic strength 0.075 (longer run experiments 10 to 18 hour).

2.76 grams barbital (diethylbarbituric acid)

15.45 grams sodium barbital

per liter 1000.

Borate buffer, pH 8.6

8.8 grams sodium borate

4.65 grams boric acid

per liter 1000.

Borate buffer, pH 9.0

7.63 grams sodium borate

0.62 grams boric acid.

per liter 1000.

Phosphate buffer, pH 7.4

0.6 grams monosodium phosphate monohydrate

2.2 gram disodium phosphate, anhydrous

per liter 1000.

Buffer for fibrinogen

Phosphate buffer at pH 9.2

Buffer for Amino acids

Pthalate buffer, pH 5.9

5.10 gram potassium acid phthalate

0.86 gram sodium hydroxide

per liter 1000.

This buffer is useful in amino acid work because it permits separation of aspartic acid, glutamic acid, monoaminocarbocylic acid as a group, histidine, arginine and lysine.

Buffer for Peptides

Acetic acid solution from N/4 to 5N was used.

Buffers for Sugar Derivatives

Borate buffer, pH 10 may be used, however using the Barbital-Borate combination for simultaneous separation of proteins, sugar derivatives and lipoproteins is more practical in clinical use.

Buffer for Lipoproteins

Barbital-Borate combination, pH 8.6, ionic strength 0.075

Buffer for Hemoglobins

Barbital buffer, pH 8.6, ionic strength 0.05.

Stains for Proteins

Bromophenol blue (16 hours)

0.1 gram bromophenol blue

50 grams zinc sulphate

50 ml. glacial acetic acid

per liter 1000.

Amidoschwartz 10B (Naphthalene Blue Black 12B, Pontacyl Blue Black SX and Buffalo Black NBR or equivalent).

Saturated solution in methyl alcohol with 1% acetic acid

Dye 10 minutes,

Wash in methyl alcohol with 10% acetic acid until background is pale blue. Air dry. Elute if desired with 3-5% phenol in 10% acetic acid.

Azocarmine G (dye 10 minutes to 3 hours)

Saturated solution in 50% methyl alcohol with 10% acetic acid

Dye 10 minutes

Wash 5 minutes in methyl alcohol, 5 minutes in 10% acetic acid.

Stains for Lipid

Oil Red O (national Aniline)

60% alcohol saturated with dye for 16 hours at room temperature and filtered.

Stain overnight; rinse briefly in water to remove some background, and air dry.

Oil Blue N can be used similarly, but with a 4 hour staining period.

Sudan Black.

Wash solutions for proteins, 2% acetic acid; 3 rinses of 5 minute duration.

Fixing solution for proteins, 2% sodium acetate in 10% acetic acid; 2 minutes.

Dyeing and washing trays.

Paper wicks, for use in Durrum type cell to obviate dependent drops of electrolyte.

Paper strips (Whatman #3 MM).

Staining racks.

Procedure

A characteristic separation for paper electrophoretic analysis of serum proteins is as follows:

1. Obtain serum and store in refrigerator until ready to use.
2. Place labeled strips in the Durrum type cell and allow to moisten with the electrolyte, by capillary action. To save time, the strips may be moistened with a pipette.
3. Turn on current to 8 milliamperes for 15 minutes after strips have become moistened.
4. Using striper (turn off current) place 10 lambda of serum on strips at the apex.
5. Allow 15 minutes to reach equilibrium.
6. Turn on current to the desired milliamperage and note the time

   22.5 milliamperes for 6 to 7 hours

   6 to 8 milliamperes for 16 hours
7. After desired time turn off current and place rack of strips on top shelf of oven (which has been preheated). NOTE. Do not allow more than 60 seconds from time current is turned off until strips are placed in the oven at 110 to 120 degrees Centigrade. Migration of the protein fractions will result; another reason for migration of the protein fractions is the amount of time the strips remain in the inverted V position after the current is turned off, especially if no wick has been used at the reservoir end of the strips.

8. Dry strips for approximately 30 minutes.
9. Stain strips.
10. Partially dry, bring out blue color with ammonium hydroxide vapor.
11. Dry completely.

The strips obtained were evaluated for optical density by the use of the "Analytrol", an instrument produced by the Spinco division of Beckman Instruments, Inc. The "Analytrol" is a double cell photometer in which light transmitted through the stained strip via a narrow slit is registered by a photo cell; the same light passes through another aperture to fall upon the second balancing photocell, which is partially occluded by a rotating cam. A Servo motor drives the cam so as to vary the second photocell aperture and bring the light intensity difference on the two photocells to zero. Movements of the cam are transmitted to a recording pen, which traces the pattern. Optical densities of dyed protein on a paper strip as measured by light absorption photometers obey Beer's Law only within a narrow range of protein concentration; however, this source of error has been obviated by incorporating optical density corrections directly into the "Analytrol" cam.

Potential gradient, ionic strength of buffers and period of separation were experimentally varied in order to become familiar with the results. Optimum separation of serum samples with the Spinco Model R

was found to be 17.5 milliampers, a buffer of 0.05 ionic strength, separation for a period of 7 hours. Optimum separation of serum samples with the "Impro" Durrum type cell and Heath-kit power supply was found to be at 14.5 milliamperes, buffer of 0.05 ionic strength, separation for a period of 7 hours.

The influence of pH of the buffer on mobility of the migrant is very marked. At pH values of the buffer above the isoelectric point the movement is toward the positive end of the paper strip. This will bear a net negative charge. At pH values of the buffer below the isoelectric point of the ampholyte, the movement is toward the negative end of the peper strip; the net charge is positive

If all other factors are fixed - time, current or voltage, the velocity of the electromigration of a migrant can be altered greatly by varying the ionic strength of the buffer solution. The mobility increases with lower ionic strength buffers.

Temperature influences the mobility of the migrant. The mobility increases with higher temperatures. The temperature is greater with higher current. The original experiments with zone electrophoresis were with closed strip, sandwich type apparatus, in which temperature had to be regulated carefully. More recent experiments allow some evaporation of the electrolyte, but use a sealed type cell to maintain a relatively constant vapor pressure.

A series of experiments was performed on 149 different serum samples, over a period of 10 months. After a battery of

chemical determinations had been requested on a seriously ill patient, results were tabulated in an endeavor to identify an illness with a definite electrophoretic pattern. A table of illnesses showing changes in globulin fractions of the serum protein has been compiled and are shown in Table II.

A comparison of the results from the two sets of equipments is shown in Figures 2, 3, and 4, showing varied periods and magnitude of applied voltage.

Experimental results on a patient with thrombocytic thrombocytopenic pupura run at different periods during reatment is shown in Figure 5.

An undiagnosed case of myeloma was discovered that did not give characteristic bone changes in pelvis or skull X-rays. Several months later this patient expired and an autopsy revealed a large myeloma on the spine that did not delineate well on X-rays. The difference in this pattern is a very high increase of gamma-globulin, whereas in multiple myeloma the increase seems to be in the beta-1 or beta-2-globulin fraction. See Figure 5.

An electrophoretic study was requested on a patient with a condition suspected of being poliomyelitis of unknown virus type. Serum samples were examined at 3 periods during the first part of the illness as shown in Figures 6 and 7. These serum samples show a rise in the total globulins, but no outstanding features. Both sets of equipment were used.

Table I. Electrophoretic Mobility and Isoelectric Points of the Four Principal Protein Fractions of a Normal Serum

pH	Mobility in $cm^2/volt/sec \times 10^5$			
	Albumin	Alpha-Globulin	Beta-Globulin	Gamma-Globulin
6.02 8.03	-4.60 -7.15	-3.34 -6.16	-2.55 -4.20	0.01 -1.51
Isoelectric Point pH	4.64	5.06	5.12	6.0

TABLE II. GLOBULIN FRACTIONS

	ALPHA 1 Normal 3.1		ALPHA 2 9.1		BETA 13.9		Gamma 15.9
	Increase	Decrease	Increase	Decrease -	Increase	Decrease	Increase
1	----	----	----	Portal Cirrhosis	----	----	± Portal Cirrhosis
2	----	----	----		Obstructive Jaundice	----	----
3	Biliary Cirrhosis	----	----	----	----	----	Biliary Cirrhosis
4	Nephrotic (1) Syndrome		Nephrotic (1) Syndrome		Nephrotic (1) Syndrome		Nephotic (1) Syndrome
5	----(2)		Nephrotic (2) Syndrome		Nephrotic (2) Syndrome		---(2)
6	Multiple Myeloma (1)		Multiple Myeloma (1)		Multiple Myeloma (1)		
7	Staphylo-coccus Infection		----		----		----
8	----	----	----	Lymphoma	----	Lymphoma	Lymphoma (+)
9	----	----	----	----	----	----	Pulmonary Abscess
10	----	----	Recurrent Pulmonary Infection	----	----	----	----
11	----	-----	-----	----	----	----	Tuberculosis Adenitis
12	----	-----	----	-----	----	----	Bronchial Asthma
13	----	----	----	----	Rheumatic Pericarditis	----	Rheumatic Pericarditis (+)
14	----	-----	----	----	Rheumatic Pericarditis	----	Rheumatic Pericarditis
15	Scarlet Fever	----	Scarlet Fever	----	----	----	----

(CONTINUED ON THE NEXT PAGE.)

TABLE II. GLOBULIN FRACTIONS (Continued)

	ALPHA 1 Normal 3.1		ALPHA 2 9.2		BETA 13.9		GAMMA 15.9	
	Increase	decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease
16	----	Infectious Hepatitis (Severe case)	----	Infectious Hepatitus (Severe Case)	----	----	Infectious Hepatitus (severe case) not occur in cases without jaundice	
17	(1) L.E.Lupus Erythematosis	----	----	----	----	----	----	
18	(2) ----	Polyarteritis Nodosa (0)	----	----	----	Polyarteritis Nodosa	Polyarteritis Nodosa (78%)	
19					Accumulation of Lipoprotein			
20	----	-----	----	-----	-----	----	Multiple Myeloma(2)	----
21	Increase in Malignancy, Acute Febrile Diseases Inflammatory & Infectious (a) Rheumatic Fever		a.					
22	----	----	----	----	----	----	Waldenstroms Type-of Purpura	
23	(b)Rheumatoid Arthritis		b.					
24	Reaction to Immunization Procedure		c					

TABLE II. GLOBULIN FRACTIONS (Continued)

	ALPHA 1 Normal 3.1		ALPHA 2 9.1		BETA 13.9		GAMMA 15.9	
	Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease
25					Infection - Inflammatory Disease. (a) Idiopathic Hyperlipemia			
26					(b) Xanthomatosis			
27					(c) Lipoid Nephrosis			
28						Active Hepatic Necrosis		
29							Most Diseases of Infectious & Inflammatory Nature	
30							Any condition Associated with Production of Immune Bodies	
31								Lipoic Nephrosis
32								Severe Malnutrition
33								Diseases Associated with marked Wasting
34							Malignancies	
35			Metastatic Carcinoma of Liver					

(Continued on next Page)

	ALPHA 1 Normal 3.1		ALPHA 2 9.1		BETA 13.9		GAMMA 15.9		REMARKS
	Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease	
36A	-----	----	----	-----	----	----	Infectious Mononucleosis	---	
36B	----	----	----	Infectious Mononucleosis (Infrequent)	----	---	----	---	
37	---	----	----	----	Cardiac Failure	---	Cardiac Failure	---	
38	---	----	----	----	----	---	----	Hypo Protein-emia (in children)	
39	Pneumonia 1 or 2 (Confined)		Pneumonia 1 or 2 (confined						
40									Malignant Hypertension Decreased Albumin
41	Tuberculosis		Tuberculosis ALPHA 1 or 2		----	----	----	----	
42	----	----	----	----	Tuberculosis (Terminal		Tuberculosis (Terminal)		
43	Hyperthyrodism		Hyperthyrodism Alpha 1 or 2						
44	----	Hypothyroid (1 or 2)	----	Hypothyroid (1 or 2)	Hypothyroid	---	----	---	
45	Lobar Pneumonia	----	Lobar Pneumonia	----	Lobar Pneumonia	---	Lobar Pneumonia	---	
46	Lympho Gran-uloma (1) (Hodgkins)		Lympho Gran-uloma (1) (Hodgkins)		Lympho Gran-uloma (1) (Hodgkins)		Lympho Gran-uloma (1) (Hodgkins)		Decrease Albumin
47							Hodgkins (2)		

(Continued on next page)

TABLE II. GLOBULIN FRACTIONS (Continued)

	ALPHA 1 Normal 3.1		ALPHA 2 9.1		BETA 13.9		GAMMA 15.9
	Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase
48	----	----	----	----	Diabetes Acidosis (+)	----	----
49	Sarcoidosis (Lung Disease		Sarcoidosi (Lung Disease)				

Fig. I Graphic Representation of some typical Electrophoresis Patterns

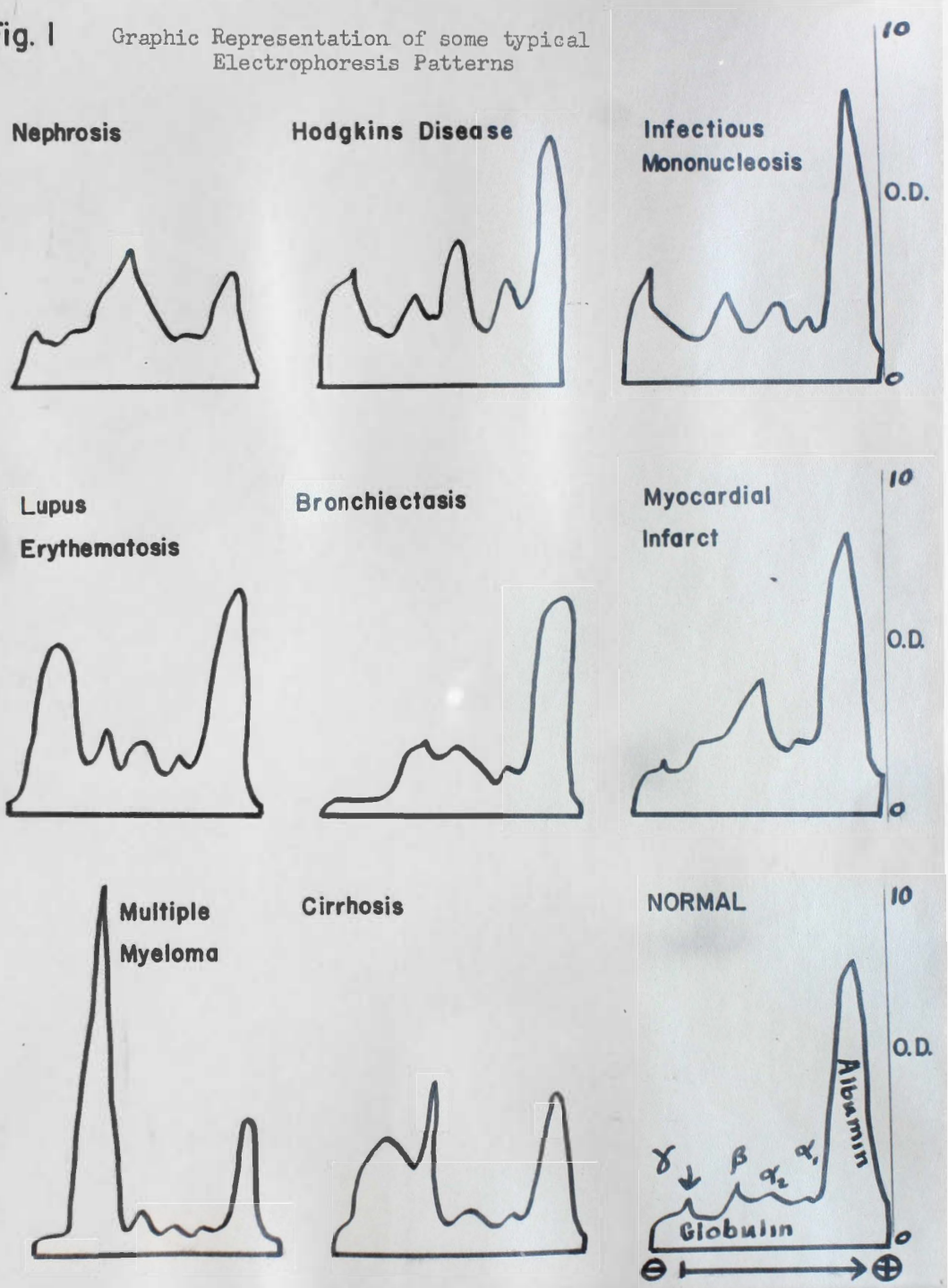
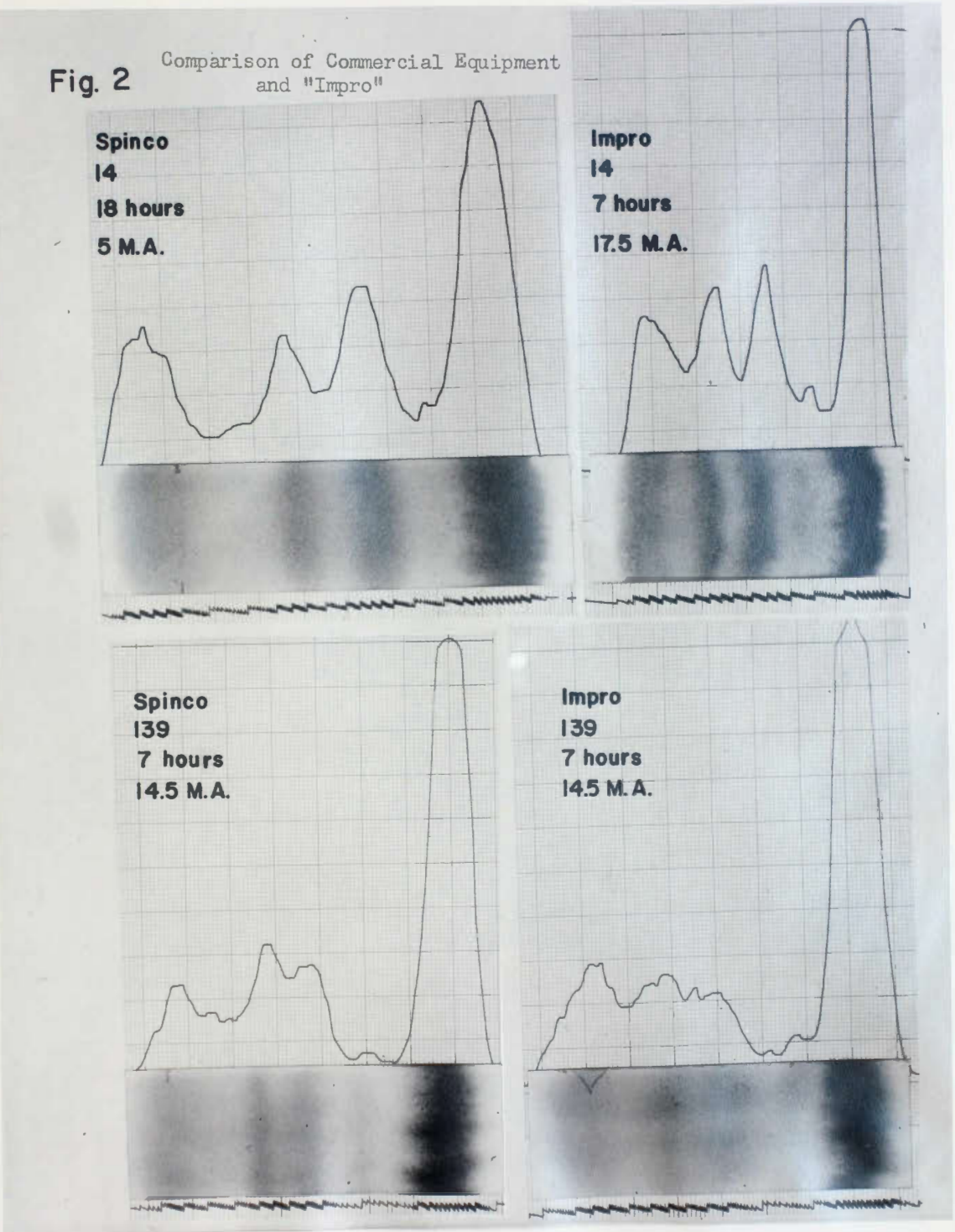
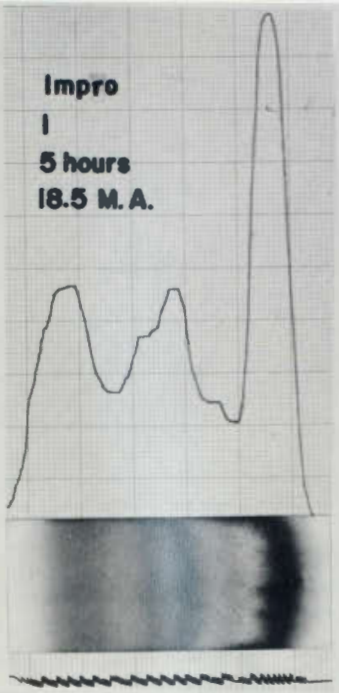


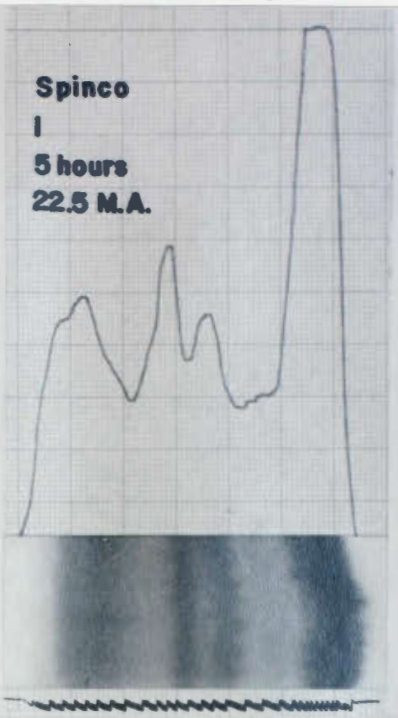
Fig. 2 Comparison of Commercial Equipment and "Impro"

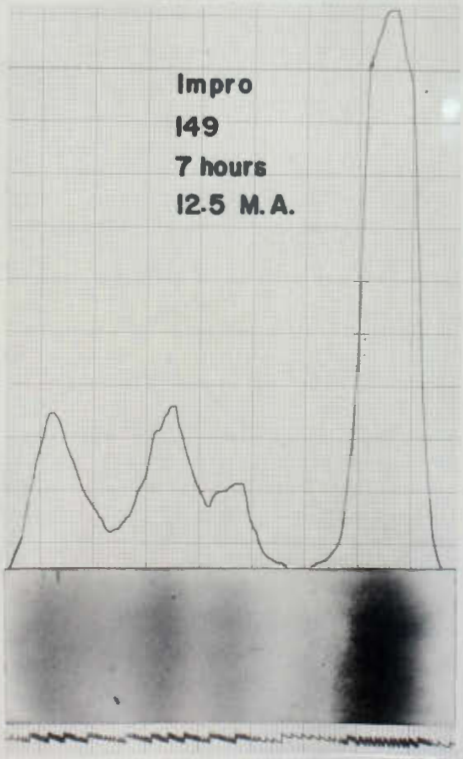


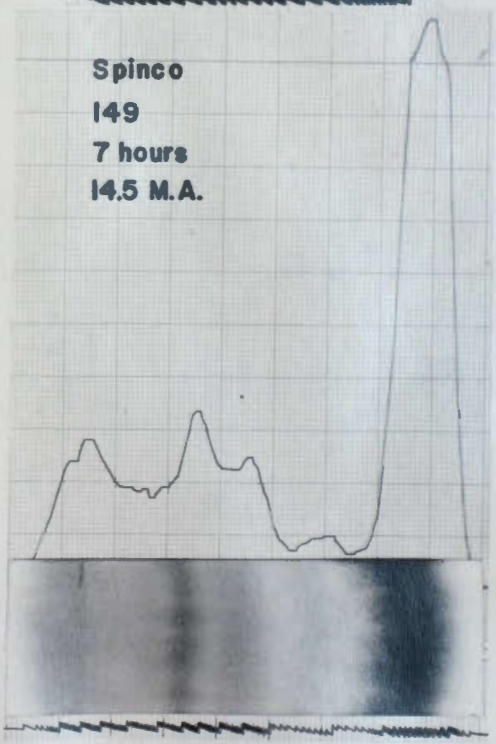
Comparison of Commercial Equipment and "Impro"

Fig. 3









Comparison of Commercial Equipment and "Impro"

Fig. 4

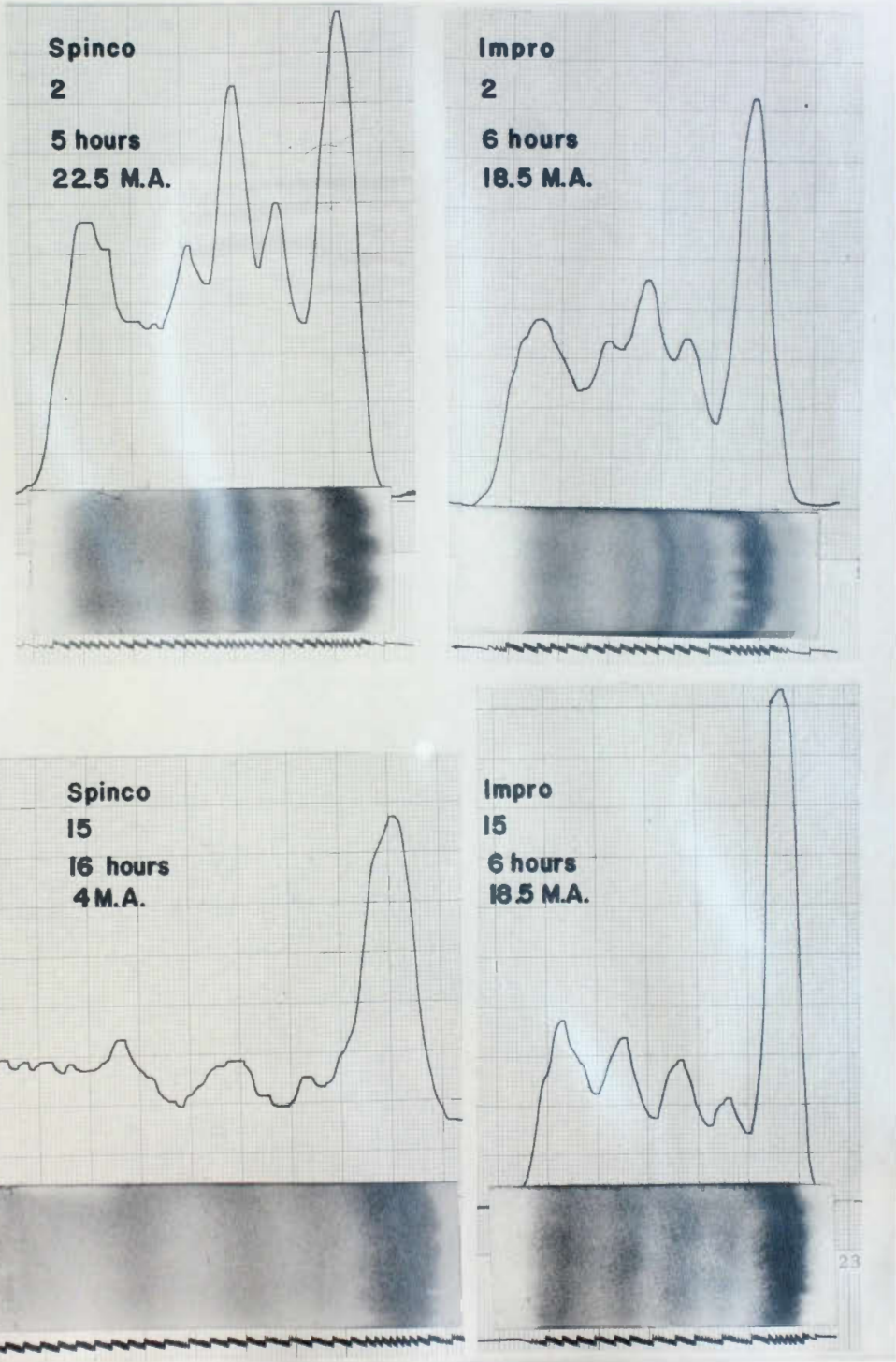
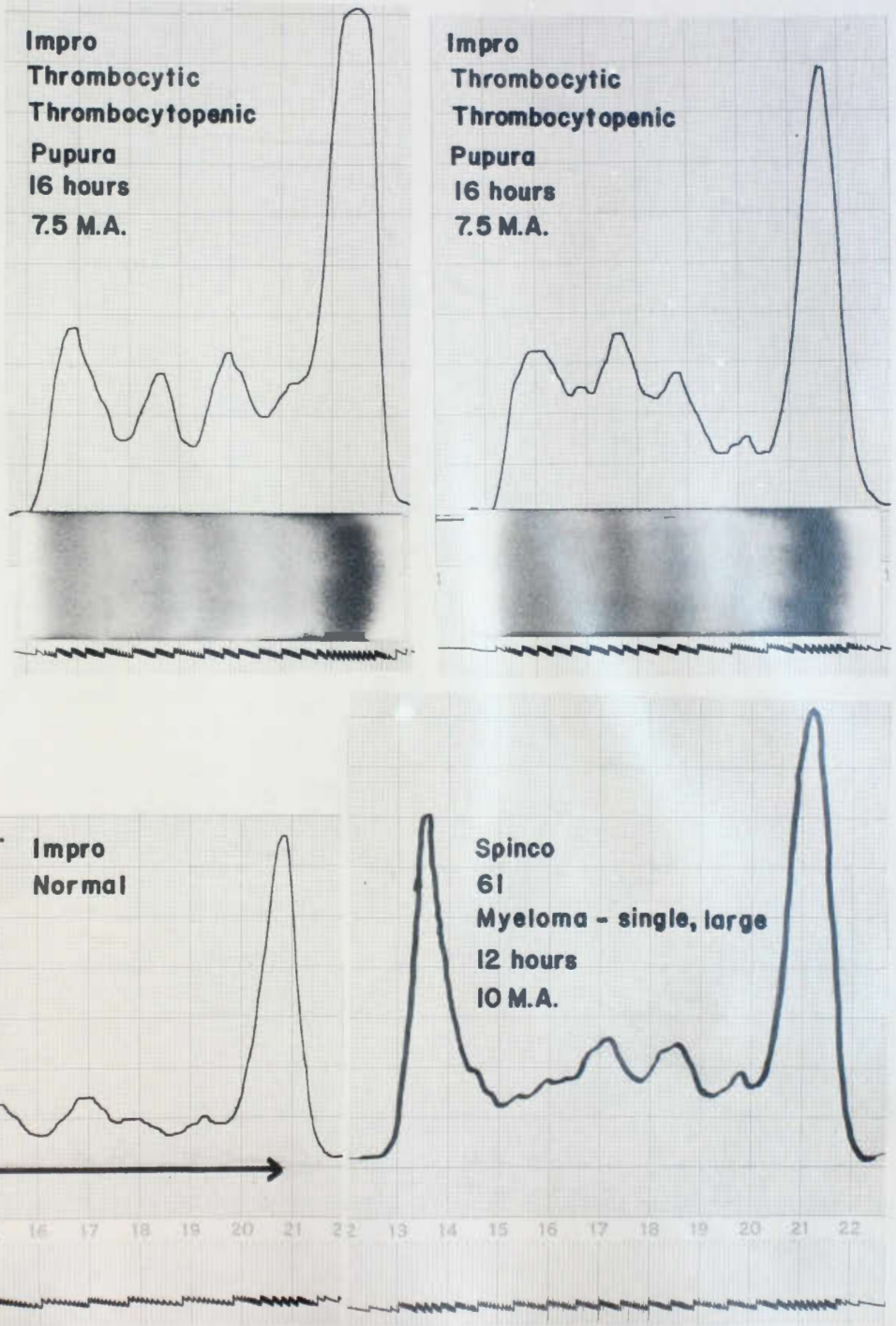


Fig. 5


Impro
Thrombocytic
Thrombocytopenic
Pupura
16 hours
7.5 M.A.
Impro
Thrombocytic
Thrombocytopenic
Pupura
16 hours
7.5 M.A.
Impro
Normal
Spinco
61
Myeloma - single, large
12 hours
10 M.A.
15 16 17 18 19 20 21 22
13 14 15 16 17 18 19 20 21 22

Clinical Changes During Treatment on "Impro"

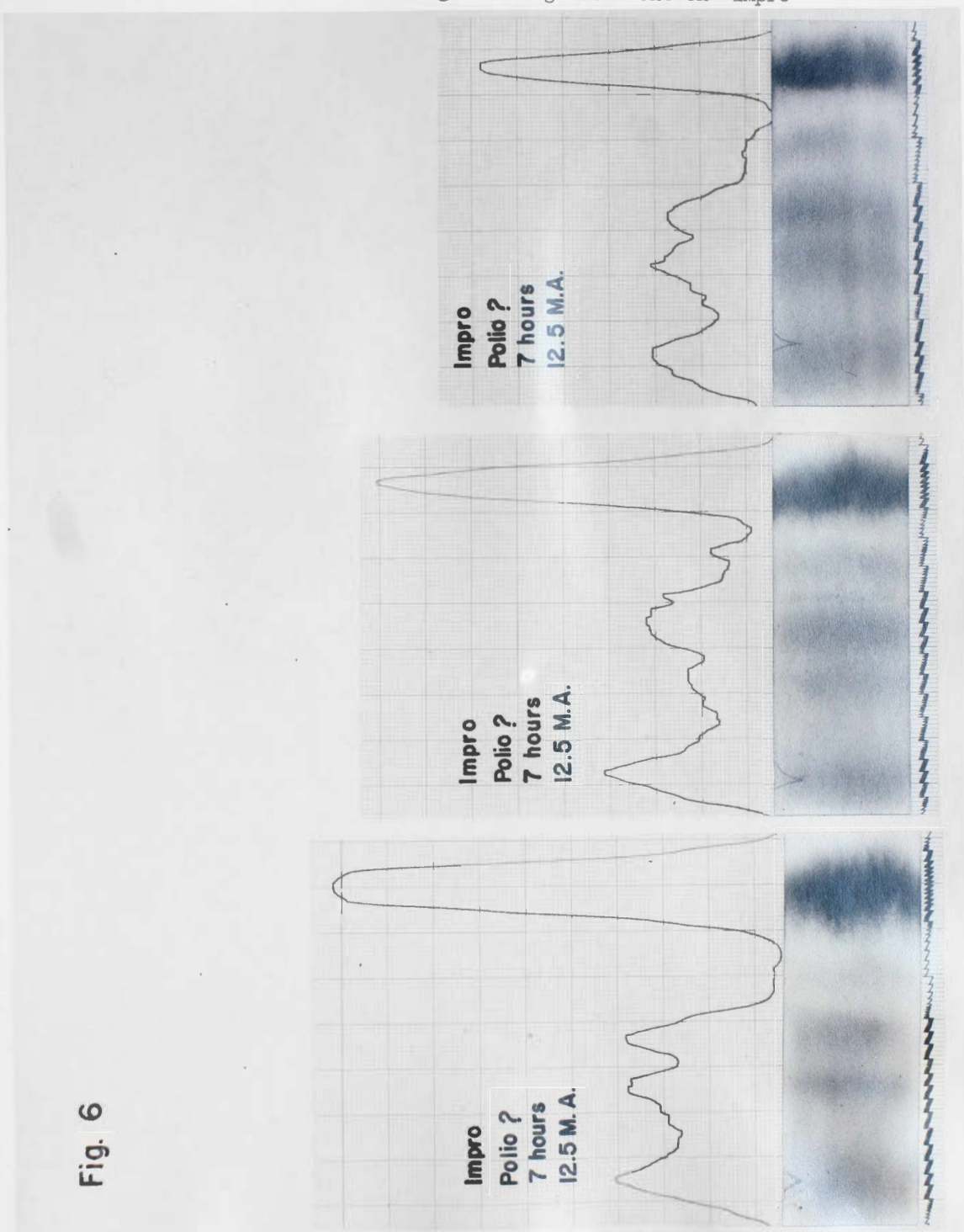


Fig. 6

Clinical Changes During Treatment on Commercial Equipment

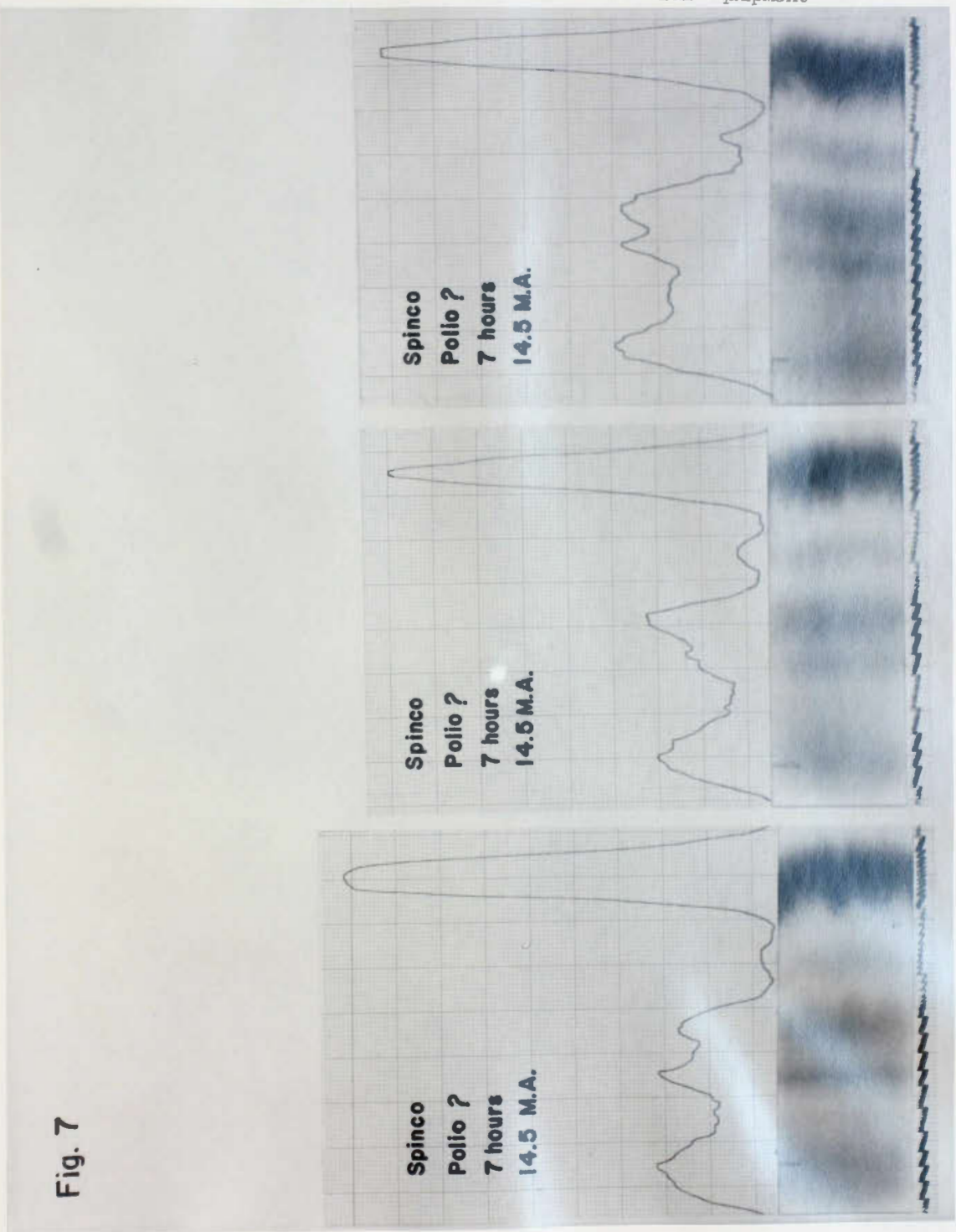
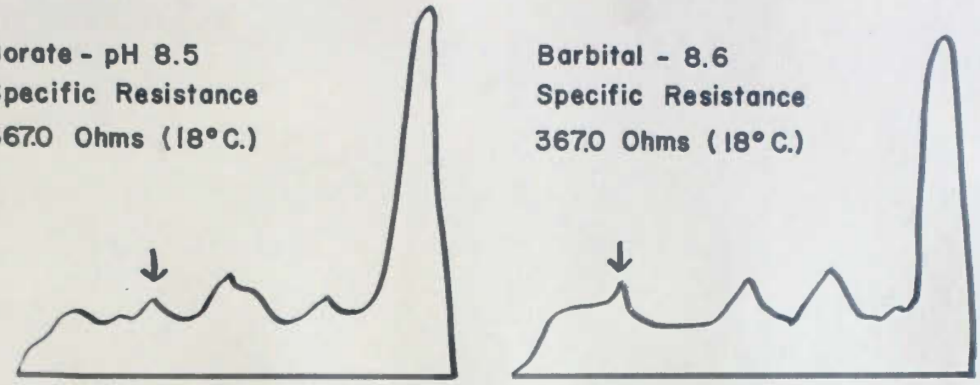


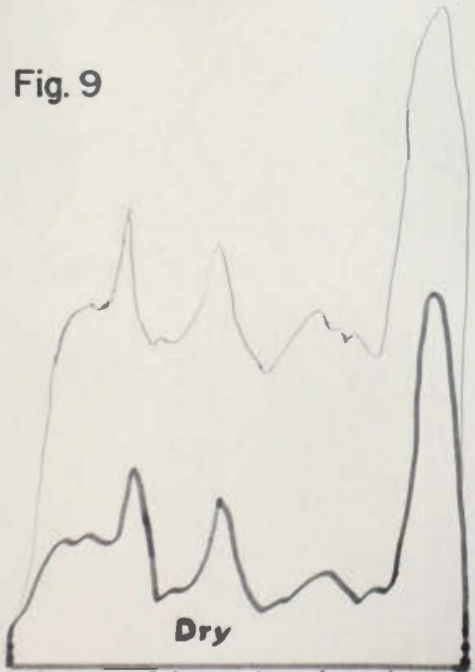
Fig. 7

Fig. 8



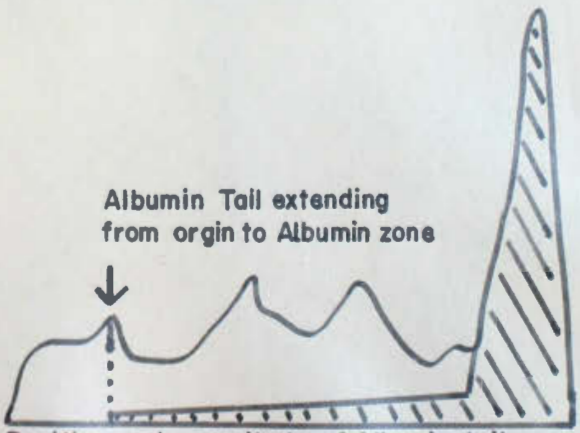
Comparison of Borate and Barbiturate buffers for normal human serum

Fig. 9



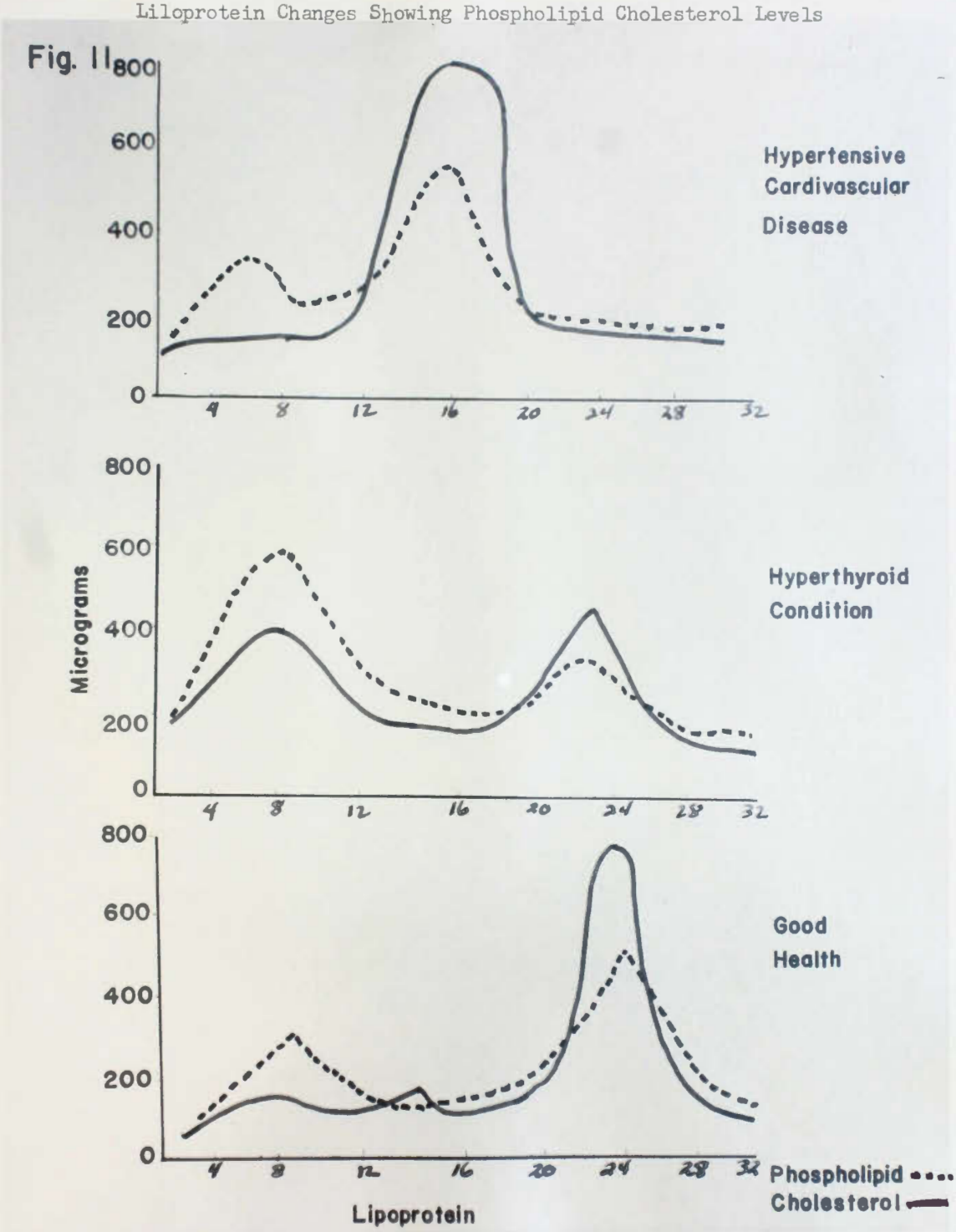
Influence of dry vs. translucent scans on relative protein % for bromphenol blue - stained strip

Fig. 10



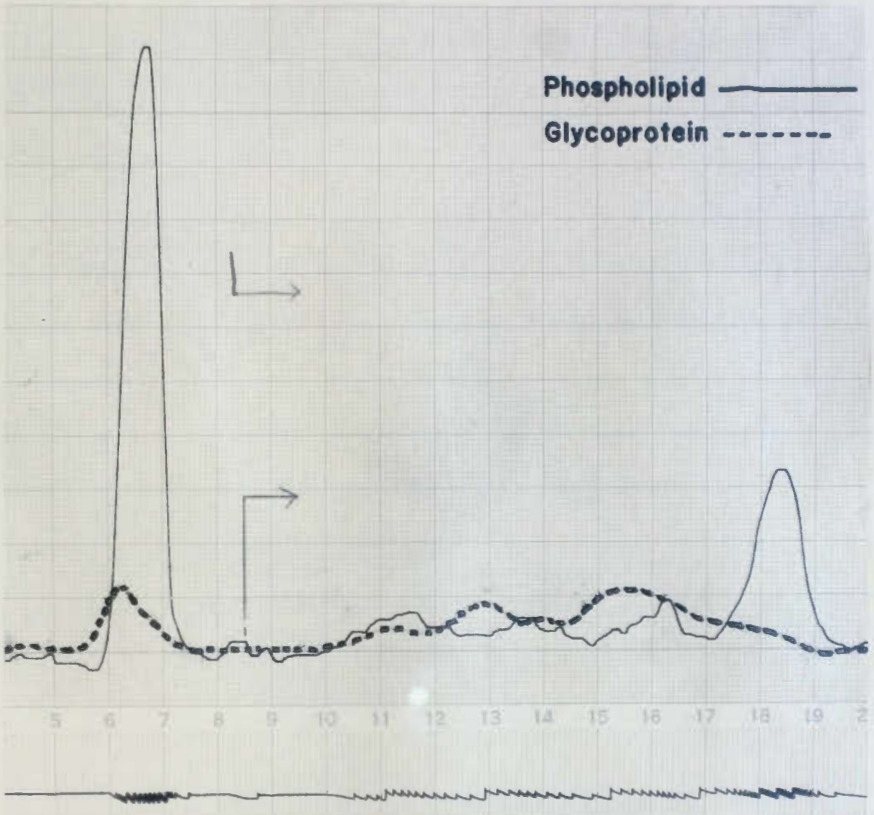
Position and magnitude of Albumin tail

Liloprotein Changes Showing Phospholipid Cholesterol Levels



Phospholipid and Glycoprotein Level for
Multiple Myeloma Patient

Fig. 12



Multiple Myeloma Patient
.075 ionic strength
16 hours
5 M.A.
Phospholipid stain - Hematoxin
from: B.A. Sachs, Scan. Jour. Lab. and Clin. Invest.,
Vol. 7, 3, 277, 1955

CONCLUSION

In the past, paper electrophoresis has been mainly a tool of research. Now it is becoming a very popular diagnostic tool. It is a practical method because of the many determinations which can be made, the small amount of specimen needed, the many specimens which can be run simultaneously, the simplicity of procedure, its ready availability, and its promise to make the analysis of colored and turbid solutions easier.

Of special interest is the application of electrophoresis with various equipment to increase the sensitivity of separations. Continuous flow electrophoresis seems to be the answer to purification of many substances which include enzymes, hormones, and many pharmaceutical preparations for medicine and industry.

Specific protein changes demonstrated by electrophoresis include

Albumin alterations --	Hypoalbuminemia in nephrosis, cirrhosis, malnutrition, malignancy, hemorrhage, burns, and acute malarial paroxysm.
Globulin alterations-	Gamma-globulin increase in malignancy, and acute febrile diseases, inflammatory and infectious conditions. Beta-globulin increase in infectious and inflammatory diseases and in association with accumulation of liproproteins in blood.

The results of homemade equipment which consisted of a plastic bread box and ridge-pole type of Durrum cell with current applied from

a Heath-kit power supply compared favorably in every way to a commercially produced equipment. The commercial equipment was more convenient but more than ten times as expensive. One consideration of a small hospital is the cost of equipment and whether this equipment will be productive. Another consideration of the newer hospitals and the younger physicans is to have the best equipment which science has to offer in order to give the best service of modern medicine. Due to this consideration for better medicine, the small hospital can now have the facilities for electrophoretic determinations of human serum and plasma at a reasonable cost. The actual expense for equipment used for the apparatus was less than $60. Buffer solutions, wash solutions, pipettes and applicators increase the initial cost to approximately $100.

In this investigation experimental evidence was obtained that paper electrophoresis is a valuable tool for diagnosis and that equipment can be devised which is within the budget of a small hospital.

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