IN THIS ISSUE

Electromigration in Stabilized Electrolytes Part III:
Applications Of The Techniques
H.J. McDonald, R.J. Loppe, E.P. Marbach, R.H. Spitzer, and
M.C. Ulbin ............................................................. page 51

A Standardized Plasma Preparation Suitable For Use As A
Control In The Determination Of Prothrombin Time
M.E. Hodes ............................................................. page 59

Fifth Annual Meeting Program, Chicago, III.
September 9-11 ......................................................... page 48
THE SECRETARY REPORTS

The fifth annual convention of the AACC is scheduled to take place in Chicago on September 9-11, 1953 during the 124th ACS National Meetings. It may be pointed out that each of the former national conventions was held during an ACS national meeting.

A short summary of the previous annual meetings of the AACC might be in order at this time. On September 15-17, 1949 the Association, then a toddler of less than one year of age, held its first national meetings in Atlantic City. This was attended by clinical chemists from all parts of the country, and a thrilling one for those of us fortunate to be there. The program consisted of a symposium, general session, dinner, and business meetings at which a great deal was accomplished.

The second annual convention was held in Philadelphia on April 11-12, 1950. In addition to the full program of the previous year, the AACC inducted Professor Walter R. Bloor as the first honorary member.

The third annual convention was held in Boston on April 2-3, 1951. At that time Dr. Donald D. Van Slyke was inducted as honorary member. Also a symposium on instrumentation in clinical chemistry was held with the collaboration of the Division of Analytical Chemistry of the ACS. This is of interest, since all the other scientific programs of the Association have been held with the Division of Analytical Chemistry.

Although not an official annual convention, the AACC took an active part in the Diamond Jubilee of the ACS during September, 1951. The international aspect of those meetings presented the opportunity to lay the foundation for the International Association of Clinical Biochemistry. At that time also honorary memberships were awarded to Dr. Norman R. Blethen, Professor E. J. King of Great Britain, and Professor E. C. Noyons of the Netherlands.

The fourth annual convention of the AACC was held in Milwaukee on April 1-2, 1952. At that time Professor P. A. Shaffer was inducted as an honorary member.

The fifth annual convention promises to be as fruitful as the previous ones. The AACC, in the relatively short period of its existence, has already written some exciting footnotes. Yet no historical review would be complete without noting the cordial collaboration given to us at all times by the American Chemical Society.

Max M. Friedman, National Secretary

FIFTH ANNUAL MEETING PROGRAM: CHICAGO, ILL., SEPT. 9-11

SCIENTIFIC SESSIONS

THURSDAY MORNING — Sept. 10

Symposium on Electromigration in Stabilized Electrolytes

In Participation with the Division of Biological Chemistry, American Chemical Society

Hugh J. McDonald, Presiding
9:00 — Hugh J. McDonald. Introductory Remarks.
10:05 — E. L. Dunham. The Separation of Serum Proteins by Zone Electrophoresis.
11:05 — Henry G. Kunkel. Zone Electrophoresis in a Starch Supporting Medium.

THURSDAY AFTERNOON

Symposium on Electromigration in Stabilized Electrolytes

In Participation with the Division of Biological Chemistry, American Chemical Society

H. H. Strain, Presiding

2:00 — H. H. Strain. Introductory Remarks.
4:30 — STATED ANNUAL MEETING

-46-
NARROW MOUTH, SCREW CAP

POLYETHYLENE BOTTLES

Flexible and unbreakable in normal use, and highly resistant to chemical attack.

2205-A.

BOTTLES, POLYETHYLENE, Narrow Mouth, Screw Cap. Polyethylene is a tough, lightweight, thermoplastic, paraffinic material with wax-like appearance and feel, possessing the following characteristics:

- Not attacked significantly at room temperature by mercury, concentrated alkali, concentrated hydrochloric, phosphoric, sulfuric, hydrofluoric or chromic acids, dilute nitric acid, or hydrogen peroxide to 90%.
- Can be used at temperatures up to approximately 70°C.
- Can be marked with usual glass marking waxes.
- Specific gravity is 0.92.

However, they are not suitable for use with bromine, carbon bisulfide and concentrated nitric acid, or for prolonged storage of acetone, ether, xylene and other volatile organic substances. Polyethylene caps do not require liners and provide a seal which does not tend to "freeze" or slip.

2205-B. Bottles, Polyethylene, Narrow Mouth, Screw Cap, as above described, complete with polyethylene caps.

Capacity.......... 2 oz. 4 oz. 8 oz. 16 oz. 32 oz. ½ gal. 1 gal.

Each.................. .22 .27 .41 .57 1.16 5.10 7.34
Per Dozen........... 2.36 2.93 4.44 6.16 12.65 55.20 79.39

NOTE—Also available, Polyethylene Caps only, for use with glass bottles; Bottles only, for use with either Polyethylene or moulded phenolic resin screw caps; and fittings for conversion of above Bottles into Wash Bottles.

POLYETHYLENE FUNNELS

FUNNELS, POLYETHYLENE, flexible, lightweight, and with reinforced rim. Unbreakable in ordinary use, with outside ridges on stem and base of cone to permit free air passage when funnel is reversed, and four inside ribs to improve filtering efficiency. Made from the same pure polyethylene as above Bottles and possessing the same characteristics.

5587-D. Funnel, Polyethylene, as above described.

Diameter, inches......... 4 7/16
Capacity, approximately, oz..... 6 1/22

Each.................... 1.20 2.26
10% discount in lots of 12, one size only.

ARTHUR H. THOMAS COMPANY
Laboratory Apparatus and Reagents
WEST WASHINGTON SQUARE
PHILADELPHIA 8, PA.
Teletype Services: Western Union WUX and Bell System 247-72
CANDIDATES CERTIFIED BY THE AMERICAN BOARD OF CLINICAL CHEMISTRY, INC.

The American Board of Clinical, Inc., since its formal organization in April 1950, has received requests for applications from 569 individuals. Of these 409 were completed and filed with the necessary data and supporting information. The Board during the past three years has met on several occasions at which time 378 of the filed applications were considered, were granted certification.

Applications were received from nearly every state as well as Canada, Hawaii, the Philippines, Puerto Rico and an occasional one from countries where there was a stationed United States Army or Navy personnel. The greater number of applications originated from areas of concentrated population and centers of scientific or medical training: New York, Pennsylvania, Illinois, California and Louisiana being outstanding. Many of those applying conduct private laboratories, the majority however, being connected with institutional work.

The Board carefully weighed the facts submitted in many instances found it desirable to seek additional information of those in the same field and areas as the applicant.

Those certified to date include the names of many who will be recognized by their numerous contributions toward the advancement of the profession, others who are younger will, it is expected, advance themselves to the same position of respect as the years pass.

The names of those certified up to and including the Annual 1953 Spring Meeting of the Board, have been published in the CHEMICAL AND ENGINEERING NEWS, Vol. 31, No. 31, page 3203, August 3, 1953.

NEW UNIVERAL BALANCE

A manual explaining the new Universal Balance has recently been published by the manufacturer, Voland & Sons, Inc., 32 Belaya Place, New Rochelle, N. Y.

This new instrument, which the manufacturer claims is a completely new concept in balances, is fully illustrated in the manual. Comprehensive diagrams and photographs describe the many unusual features of the Universal Balance, including the beam and projection systems, weight operation, compensating strip and other situations leading to identification and measurement of components in a mixture and assignment of structural formulas; the publication includes further specific data on the spectrometer and suitable magnet system. Data is included on basic operating characteristics; general arrangement of sub-units encompassing the probe, the radio-frequency transmitter and receiver, sweep device, power supply, and oscillograph. The folder is identified as publication No. 40. Varian Associates — 522 Hansen Way, Palo Alto, Calif.

RESEARCH PAPERS

The Editorial and Advisory Boards of THE CLINICAL CHEMIST invites for publication papers on original research. Authors should submit two copies of their manuscript and the format should follow that suggested in “Instructions To Authors” published in the JOURNAL OF BIOLOGICAL CHEMISTRY.

THE CLINICAL CHEMIST is now being distributed to the major libraries and papers will be abstracted in CHEMICAL ABSTRACTS.

IONOGRAPH

FOR ELECTROPHORETIC SEPARATIONS ON PAPER AND OTHER MEDIA

- SEPARATES Proteins, Amino Acids, and other mixtures of organic and inorganic compounds
- DETERMINES: Isoelectric points of Amino Acids and Proteins
- the presence of complex-ion formation in solution
- and some electro-kinetic properties

Double-walled insulated cabinet for wide temperature operation, holds 7 strips or large sheet. Use is not limited to water soluble substances.

Constant Temperature Media can be circulated through body and COVER. Voltage range from 0-2,000 volts. Inlet for inert gas atmospheres provides for water saturation, prevents drying of paper surfaces, aids in heat removal.

ALL SIMILAR TECHNIQUES ARE WITHIN THE SCOPE OF THE IONOGRAPH. WRITE FOR REPRINT PAPER AND BULLETIN 11329.

PRECISION SCIENTIFIC COMPANY
3737 WEST CORTLAND STREET — CHICAGO 47, ILLINOIS
The general fields of application of electromigration in mechanically stabilized electrolytes may be listed as: (1) simple fractionation and empirical resolution of mixtures of compounds, including ionic and colloidal substances as well as materials which are normally thought of as being relatively unchanged in solution, such as carbohydrates. (2) mobility determinations and related quantities, or derived relationships such as isoelectric point determinations, "pH versus mobility" curves for amphoteric ionic strength effects, studies of factors influencing electromigration, the relationship between mobility of a migrant and its molecular weight and volume, chemical kinetic quantites such as the energy of activation, and order of a reaction, etc., (3) reaction site studies, which include investigations of the effect of ionic strength on the dissociation constants of complex ions, the determination of equilibrium constants and such related thermodynamic quantities as the change in free energy, in heat content and entropy, etc.

For the simple fractionation of complex mixtures, and this constitutes the largest fraction of the papers published on ionography, variations in the design of the apparatus employed and the conditions under which the experiments are carried out seem not to be very significant, when used by experienced workers. In fact, while for mobility determinations the buffer ionic strengths are generally 0.05 or less, for higher buffer ionic strengths buffers may be used for empirical separations of mixtures and in some instances seem to yield sharper separations of the individual zones of the ionograms. In simple fractionation procedures, it is also unnecessary to go to any great length to eliminate the role of chromatography in the separation process, and indeed, the judicious simultaneous or consecutive combination of electromigration and chromatography may yield better results than when either technique is used alone. From the standpoint of simplicity of operation and the chances of getting usable separations (where mobilities are not involved) with the minimum of experience, it is evident from work in the authors' laboratory that any variation of the apparatus other than the compressed-sandwich type is preferable.

When mobility determinations are to be made, the many factors discussed in Part II of this series must be given consideration. The ridgepole suspension or hanging-strip technique (17) would seem to lend itself readily to work on mobilities than the horizontal suspension technique (52, 53, 59) or the sandwich technique (42, 153), although Maschebaouf and co-workers (173, 175) have suggested an analytical treatment of results obtained by means of the hanging strip method (17). Although both the sandwich technique and the horizontal suspension technique present certain difficulties when used in mobility studies, they both have been used successfully for such purposes (42, 52, 59, 171). The better control of evaporation in the compressed sandwich technique is offset to a large extent by factors as electromigration between the surfaces of the glass sheets and the paper filer, the interfering surface tension and capillary effects, by the difficulty of preventing a smear of the zone on the paper during the covering plates, and perhaps most importantly, by the difficulty of maintaining a uniform and reproducible ratio of "buffer solution" to "paper". If the pressure exerted on the glass plates varies from point to point throughout its length, and also will the potential gradient and the weight ratio of "buffer" to "paper", and consequently so will the thermodynamic activity of the migrant. Such factors have been pointed out in Part II of this series of articles under the section dealing with the determination of so-called free solution mobilities from those obtained in paper-stabilized electrolytes. To reiterate, at points where the covering plates are pressed together with greater force, there will be less water per given weight of paper, and a lower mobility of a migrant, than in a zone where the pressure is less and the water content of the paper higher. This same factor makes it difficult to attain complete agreement of results, as regards mobility, from one laboratory to another or between successive workers in the same laboratory using an identical sandwich-type apparatus. With such equipment it is not sufficient to state the usual conditions under which the experiments were run, such as buffer-type, ionic strength, pH, potential gradient, temperature, etc., but the pressure exerted on the paper filer by the covering plates should also be given, or in lieu of this information, the weight ratio of "buffer solution" to "paper filer".

It is of interest to note that much of the early work on electromigration in stabilized media was aimed toward applications of a biochemical or clinical chemical nature. For example, the studies of Field and Teague in 1907, concerned with electromigration characteristics of diphtheria toxin and antitoxin (122) as well as tetanolysin and antibiotolysin (123) in agar-stabilized electrolytes, and the work of Teague and Burton (204), also in 1907, on the electromigration behavior of hemolytic hemoglobin, hemoglobin in complement in agar jelly, may be mentioned. The earliest investigations on electromigration in paper-electrophoresis wetted with electrolytes dealt with biochemical studies on snake venom (153).

In Part I of this series, the article by Westland and Fischer, (132) published in 1948, was mentioned as the earliest known publication on this particular technique. Since then the paper by von Kredny and König (153), published in 1959, has come to the author's attention. These investigators employed a strip of medium-hard filter paper, 1 x 5 cm, the ends of which were connected to a 110 volt direct current source in a series with an adjustable resistance and a millimetre. The paper was moistened with a dilute solution of an electrolyte and one drop of the solution in water, containing yellow-colored snake venom (Brodura torquata) was placed on the moistened paper at a point midway between the ends. During some experiments, the paper strip was opened to the air while during others, it was placed between two glass microscope slides in order to retard evaporation. On allowing the current to flow for a period of 20 minutes at a pH of 6.0, the spot of colored snake venom was found to migrate toward the positive electrode. Starting at the center, the paper strip was cut transversely into several narrow sections, from each of which the material was extracted and suitably tested in various ways. The authors refer to an even earlier paper by König (154), in which the apparatus itself is described in more detail.

The material in the present paper will deal with the ionographic separation and some of the electromigration characteristics of the following substances: proteins, amino acids, carbohydrates, lipids, and lipopolysaccharides, enzymes and hormones, vitamins, inorganic ions, and some miscellaneous applications to less familiar compounds. No attempt will be made to cover all the minor variations of the technique, such as automatic scanning devices, apparatus for continuous separations, etc., since papers covering these aspects of the subject are listed in a separate bibliography (169).
Electromigration in Stabilized Electrolytes - Part III

describe the use of proteins as migrants. Davis et al. (15) and Gordon et al. (26) have investigated these compounds by using agar to stabilize the buffer solutions. The former group of workers carried out the electromigration in agar-filled glass capillaries, while the Gordon group employed a rectangular slab of agar jelly as the stabilizer in their separations of hemoglobin from ferritin, of the resolution of the proteins of egg white, and the investigation of a peptide preparation. For a more thorough discussion of Gordon's apparatus and the pertinent factors affecting the resolution of mixtures of proteins, an earlier paper (11) should be consulted.

By far the majority of workers employ filter paper to stabilize the electrolyte solution during protein studies. The references that appear to have definite value in describing and elaborating on the general apparatus and procedure accompanying protein investigations include: (13, 14, 15, 17, 21, 28, 29, 30, 31, 32, 34, 40, 41, 42, 75, 76, 77, 80, 93, 95, 96, 98, 104, 106, 115, 120, 133, 134, 139, 141, 159, 162, 166, 167, 179, 197, 202, 204).

Previous to the introduction of these methods, the separation and the quantitative estimation of proteins by free solution electrophoresis was not very practical. This traditional technique of electrophoresis required access to an expensive instrument which demanded the use of large volumes containing high concentrations of protein solutions, followed by difficult manipulations for the estimation and for the isolation of the protein constituents after their electrophoretic separation.

Two papers which illustrate the advantages of the new technique of ionophoresis in protein studies are those by Levin and Oosterhuis (163, 164). These workers estimated the absolute amounts of plasma proteins in blood by both micro-Kjeldahl and dye-solution analyses, and compared the results of their separations obtained by free solution electrophoresis and salt fractionation with those obtained via electromigration in paper-stabilized electrolytes. They concluded that the absolute amounts of plasma proteins in a blood sample measured by means of nitrogen analyses of the protein on the paper were in agreement with those obtained from free solution electrophoresis using optical methods of analyses. They also maintained that the method of separation is preferable to that of salt fractionation.

I. Techniques and Experimental Conditions Applicable to Studies of Proteins

In the discussion of the experimental conditions utilized during protein experiments it will be found that there is a variance of opinion as to the optimum choice of buffer, to the optimum choice of pH, ionic strength, temperature, filter paper to be used, etc. In this section an attempt will be made to evaluate the argument for or against a particular variable, but rather a presentation of the conditions maintained in certain successful experiments will be made.

(a) Pretreatment of the Proteins: Concentration and Dialysis

Flynn and de Mayo (21) state that with their procedure a concentration of migrant solution containing 2-3 gms. of protein per 100 ml. was satisfactory. In more dilute solutions a preliminary concentrating was necessary by some suitable means, as for example, by ultrafiltration. Further, for routine blood analyses serum was preferable to plasma since the extra fibrinogen band made the interpretation more difficult. These workers indicated that the serum specimens should be fresh and unhemolyzed.

When working with urinary proteins, Slater and Kunkel (195) took 1-5 ml. of urine and concentrated it to 0.2-0.3 ml. by dialysis in 25% aqueous polyvinylpyrrolidone. Earlier, Scheider and Wallenius (78) performed a similar manipulation but used dialysis as the colloid. Aside from the need, in some cases, to obtain an adequate protein concentration, the question arises as to the necessity of using the protein solution at the identical pH of the buffer solution. A number of sources indicate that preliminary dialysis is unnecessary when quantitative fractionations are desired (21, 42, 167); however, it would appear from work in the authors' laboratory that for clear-cut separations and mobility determinations, the protein solutions and the buffer solutions should be at the same pH values.

(b) Buffer solution: system, pH, ionic strength

Obviously the choosing of an appropriate buffer system is dependent on the characteristics of the proteins being studied. It would seem that the universal buffer system at pH 8.5 and ionic strength 0.05-0.10 is applicable to the separation of the serum proteins. The universal buffer at pH 8.5 may be formulated by using 0.05 M sodium diethylbarbiturate (1.0 gms./liter) and 0.01 M sodium diethyl barbiturate acid (1.8 gms./liter) (21, 73, 187). When the protein is to be subsequently determined by the nitrogen analysis via a micro-Kjeldahl technique, a reagent blank is necessary, since nitrogen is a constituent of the barbiturate molecule (187). The Michaelis' vernos-buffer system is convenient for varying the buffer strength and/or pH (180).

(c) Filter paper

The choice of an appropriate filter paper is also a function of the protein characteristics being investigated. Apparently the selection must also be based on the type of apparatus used. For example, the compressed sandwich type demands a thick hard paper.

(d) Identification and Quantitative Analysis of Protein Fractions

The proteins have been generally stained with the bromphenol blue reagent (1% bromphenol blue in 10% alcohol saturated with mercuric chloride) (21, 42, 167). Addition of an acid, such as acetic acid to this solution or to the washing solution, will enhance the separation of the proteins on the filter paper and minimize their loss on developing. The blue color may be intensified by passing the ionograms over ammonia vapor. Flynn and de Mayo (21) used a saturated solution of Naphthalene black 111,200 in methyl alcohol containing 3% acetic acid to stain the serum proteins. The strips are then washed with methyl alcohol alone. This represented a qualitative technique, but they described a quantitative technique also, in which bromphenol blue was used. A large number of dyes have been examined and discussed by Griffiths (134). He preferred to use the brom phenol blue reagent for general use but instead of using methanol as a wash suggests a different technique. The strip is washed with water until the background is almost white and then soaked in Dioxan (commercial grade dioxan) and then ether. Dioxan has the property of bleaching undesired dye while the other removes the Dioxan and hastens drying. Quastel and Von Straten (73) stained the proteins with a hot aqueous solution of Solvay purple (0.05%) containing 0.3% sulfuric acid. After five minutes in the solution the strips are washed with warm water to remove the excess dye and are dried. A saturated solution of the dye, anilinochrome 15B in methanol, containing 10% glacial acetic acid was used by Knedel (55). The above color tests for the proteins are not only able to qualitatively localize a protein fraction but in most cases may be extended to give a quantitative indication of the concentration. There are at least three general methods by which quantitative evaluation may be made. The first may be called the "dye elution method." This involves staining the proteins on the filter paper, cutting the paper up into smaller segments, eluting the dye from the segments and reading the color in a spectrophotometer (21, 42, 89). A variation of this technique involves the omission of the staining procedure, but nevertheless cutting the paper into sections and ultimately determining the proteins by nitrogen analyses (106, 116, 167). The third method of quantitative protein analysis renders the ionograms (in this case the stained proteins on filter paper) transparent by means of paraffin oil, etc., and then passing this filter paper through a light beam, thus obtaining a direct photometric estimation of the adsorbed dye (111, 134, 160). For further discussion the appropriate sections should be consulted. In other interesting methods to identify the proteins Stember (159) rendered them fluorescent by coupling with a few drops of riboflavin solution. Thus no fixing and staining is required. Homolka (140) after separating the proteins made a photographic print from the ionograms of the fractions.
II. Protein Studies of Special Clinical Interest

Fischer (124) in a recent review article lists many of the contributions of electrophoresis to clinical pathology. The work on free solution electrophoresis and electrophoresis in paper and solidified electrolytes as included in this survey of the literature from 1949 to 1952. The technique of electrophoresis has been found by Slater and Kinzel (195) to be applicable to the differential diagnosis of proteins especially in cases of multiple myeloma with Henoch-Jones urinary protein. In the process they simultaneously analyzed under identical conditions specimen of serum and urine from the same patient. The clinical utility of electrophoresis as applied to liver disease is described by Kneidel (151). Knauf et al. (140), Gross (122) and Kusnezow (145) have determined clinical changes in the serum protein components. The separation of methalbumin and hemoalbumin has been demonstrated by Rensi and Lettenhov (197). These compounds are identified with the hematinic reagent and subsequently a quantitative determination is attained. Bemis et al. (111) achieved protein fractionation correlated the results with other laboratory findings to diagnose diseases of the liver and biliary tract. In their studies on the hepatic and extrahepatic regulation of serum protein metabolism, Roberts and Bruns (189) injected C14-labeled albumin acids into normal and hypercholesterolemic rats. The serum proteins from the respective animals were separated and the radioactivity of the compounds determined. Using alamine-2414 they concluded that the liver is directly involved in the formation of albumin and alpha globulin, and that the gamma globulin is not immediately dependent on liver activities for its formation. Kliew et al. (155) find that the value for protein concentration obtained when using paper as the stabilizer compares well with those from the Tiselius apparatus and are suitable for clinical purposes.

Application of the technique to the separation of the various hemoglobins has been attempted by Spoto (198). He separated the adult normal hemoglobin, sickle cell hemoglobin and gamma globulin but not type F hemoglobin. Maibach et al. (174) use the method to obtain information from hemoglobin sera, especially from cases of liver nephritis, multiple myeloma, and Lassen's cirrhosis. A discussion on the separation of protein fractions, lipids, and carbohydrates bound to the proteins was undertaken by Grunwell (135). Methods for the visualization of protein-bound lipids and carbohydrates, and radiographic detection of isoelectric points, have been described. The determination of albumin-alpha globulin ratio has been put on a routine basis by Nation (67). Sternberg (199) has described a technique that he has found applicable to clinical work.

III. Cerebrospinal Fluid Proteins

A logical consequence of the many papers devoted to the investigation of the serum proteins is the extension of the studies to other protein solutions, as for example, the cerebrospinal fluid proteins. And there we, in this case, several reports on this topic (6, 79, 114, 119, 121, 312, 205, 181).

Schneider and Wallenda (78) have described a procedure for the diazotization and immunologic separation of this fluid. They concluded that the albumin-alpha globulin ratio is similar to that of blood serum and it possesses corresponding components with similar electrophoretic behavior.

Preliminary concentration of the cerebrospinal fluid in a desiccator while dialyzing against a buffer solution previous to the electrophoretic separation was performed by Hess et al. (121). Eiser and Heinzel (119) utilized a ultracentrifugation method to concentrate the protein.

Sicher et al. (8), however, concentrated the fluid by precipitation with acetone at low temperatures. In contrast to Schneider and Wallenda, they found that on fractionation, there appeared to be two extra bands, in addition to the usual zones observed in serum protein patterns.

A number of conclusions were made by Wellensius in his studies (205). He found the cerebrospinal fluid proteins are normally identical to the serum proteins and possess an albumin-alpha globulin quotient near that of serum.

IV. Iodine-Protein Relationships

The versatility of the immunologic technique was further expressed in a series of papers dealing with the localization of protein-bound radioactive iodine after the administration of 131I to the circulatory system (3, 17, 25, 43, 75).

In 1950 Durman (17) injected 131I intraperitoneally into rats. An extract of the thyroid was obtained and utilized as the antigen. Autoradiographs were then made of the tissue to indicate the distribution of radioactive material.

Gordon et al. (25) using the usual buffer system at pH 5.5, fractionated the serum collected from patients who had been given doses of iodine-131. They found the maximum radioactivity in a band immediately following the albumin and a smaller amount associated with the albumin fraction. In vitro experiments showed that radioiodine has a mobility near that of the alpha globulin. In similar work Lumen et al. (42) pursued the serum of euthyroid patients with a Huthte cell carcinoma of the thyroid with metastases. Iodine-131 had been previously administered to the patient. After protein fractionation they found that the main concentration of radioactivity was near the alpha globulin area with an indication of some in the albumin.

Fobbeins and Fiall (75) likewise were in close agreement with the distribution of radioiodine in the serum.

V. Immunological and Related Considerations

Contained in this section are six papers which are unusual due to the substances the authors chose to investigate by the technique of electrophoresis in stabilized electrolytes. The substances included, among others, antibodies, antigens, hemolytic, agglutinating, toxic, and antitoxin. Several decades ago, in the year 1907, three papers appeared in the literature utilizing the technique of electrophoresis in agar-gel (125, 133, 235). The authors, Field, Teigue, and Burton expressed an awareness of certain difficulties that have concerned contemporary workers. For example, they were cognizant of the fact that the antigen may be adsorbed by the stabilizer and that the products of electrophoresis could affect the direction of electrophoresis and that due to the heat generated in the agar as the current passes through it, the antigens may be modified or deviated from their native state. They studied the electrical change of antibodies and toxins in the presence of certain agglutinins, and hemolytins.

In 1953 Cober and Williams (131) described a method permitting the simultaneous study of electrophoretic and immunologic properties of a protein mixture using agar to stabilize the electrolyte.

Gros et al. (30) used paper as the stabilizer in their work on the speed of renewal of antibodies and other protein fractions of blood serum through the medium of marking with C14 in the isopropyl group.

In 1954 (45) in his studies on the allergenic fractions of Alternaria sp. has utilized ionophoresy to indicate association of allergenic activity with a carbohydrate fraction of an antigen and antitoxic activity with the protein portion.

Purified diphtheria toxin was separated by Polak (137) into four fractions, one of which showed fucocytolytic activity.

VI. Miscellaneous Ionomophoretic Studies of Proteins

Included in this section are topics which in some cases could have been described elsewhere in this paper but due to the specificity of their subject nature were included here. All papers pertain to protein studies even though in certain cases only indirectly.

In the first group are a series of investigations concerned with peptides and the hydrolitic products of the proteins. Conant and Gordon (12) in their studies of the peptide of cystine in pure hydrolytic fractions of wool, separated the acidic peptides into groups with similar eluting mobilities using silica gel as the stabilizer. Roullinger and Risette (110) in their review paper of chromatographic techniques for separating small and large peptides and proteins discussed electrophoresis of water soluble systems. A crude polyglutamyl peptide was chemically fractionated by Strange and Harkema (201) from B. anthracis capsule and the components of the peptide mixture were separated by ionophoresy. An agent to identify the peptides is described.
ELECTROEMIGRATION IN STABILIZED ELECTROLYTES – PART III

Kurtzian (157, 158) in his study of blood proteins has indicated generally that the sulfamides move independently of the proteins.

In an interesting experiment Chalmers et al. (11) have separated the protein components of beef and human lens. Two components are isolated from the beef lens while a single constituent was present in the human lens extract.

Zweig and Block (215) have resolved the α-, β- and γ-gamma-casomes using paper as the stabilizer. It is interesting to note that they found the relative mobilities of the caseins of the same order as those determined by the free solution electrophoretic technique.

A comparison of the serum proteins of man, monkey, horse, rabbit, guinea pig, and rat has been made by Gomori et al. (225). It was demonstrated that the patterns differed in the spacing of the globulin spots.

Lauer and Unger (163) separated human gastric juice on filter paper. Several protein fractions were found. Besides all possessing vitamin B12-binding activity one peak contained either a mucoprotein or mucopolysaccharide.

Wunderly (108) separated the serum proteins on filter paper, segmented the strips eluted the proteins with physiological saline and studied the lyophilic properties of the fractions.

AMINO ACIDS

Wieland and Fischer (202) utilized electromigration in filter paper to study the complexing of amino acids with cupric ion. In further work Wieland et al. (103) incorporated radioactive copper into the complexes to attempt a quantitative determination of the amino acids. Wieland and Bauer (101) made separations of amino acids, and putrim and pyridine derivatives. Fogg and Kramer (31) used paper chromatography and electromigration simultaneously to achieve good separations of amino acids.

As described in Part I of this series of papers, Durum (17) studied among other compounds, the separation of amino acids and peptides. He found the ridge-pole technique to be satisfactory for simple separations of amino acids and peptides. He found the ridge-pole technique to be satisfactory for simple separations of amino acids and peptides.

CARBOHYDRATES AND RELATED COMPOUNDS

The immunographic method is divided into two sections. In the first part there is considered the separation of carbohydrates which are usually thought of as being electronically neutral substances, but which are made loads by methods involving the formation of complexes. In this group may be included carbohydrates and nucleic acids. In the second part, there is considered the separation of carbohydrates-containing materials by the methods not requiring "ion complex formation". This group includes the nucleic acids, polyaminoacids, and protein containing carbohydrates.

Carbohydrates and Nucleosides

Immunography has been used for the separation of carbohydrates as well as for the identification of the carbohydrate constituents of a mixture. In general, the procedure employed is to saturate the mixture with a borate, a borate-NaOH, or a borate-EDTA solution of requisite ionic strength and pH, and then to apply the mixture to the paper. Under the influence of a potential gradient applied for the proper period of time, the carbohydrates will separate one from the other according to the amount of borate with which they have complexed.

The amount of borate that carbohydrates will bind depends upon (1) the static configuration of the macromolecules, (2) the ratio of borate with which they have complexed.

The amount of borate that carbohydrates will bind depends upon (1) the static configuration of the macromolecules, (2) the ratio of borate with which they have complexed.

The amount of borate that carbohydrates will bind depends upon (1) the static configuration of the macromolecules, (2) the ratio of borate with which they have complexed. An excellent review concerned with the configurational capacities of the binding of borate with carbohydrates has been provided by Ebran (109), and (2) upon the number and type of carbohydrate units constituting carbohydrate polymers.

In the separation of polysaccharides the usefulness of the particular apparatus used to effect separation than in the procedure used to stain and locate the carbohydrate materials on the paper strips or sheets which themselves are composed of carbohydrates. In general, the staining techniques which are employed in chromatography are utilized in this method.

After a period of electromigration of 6-9 hours, Jancokov and Vollbrechtshausen (144) separated the ribonucleic acid from ribonucleic acid and found the successive groups of amino acids.
cylidine with quinazoline and uridine. In 0.1 M KOH, the ribo-
sides do not migrate. In acetic buffer at pH 3.2, quinazoline and uridine can be sep-
parated not because their constituent sugars are complexed with borate ion, but
instead because of the ionic charge of the carbonyl and amino groups of the quinaz-
oline and uracil. That is to say, under the con-
ditions employed for the electrophoretic fractionation of these two substances, the
separation depends on their inherent ionic charge. They detected the ribosides by
ultraviolet photography or by spraying the paper with a fluorescent dye, adenamine-1’
-phosphoric acid and adenine-5’
-phosphoric acid can be separated by ad-
dition of boracic acid or borate buffer to the
solution.

Michael and van de Kamp (182) combined electrospray in filter paper with chro-
matography for the separation of sugars into mixtures. The mixtures were used as
borac acid complexes and the electrophore-
etic separation was carried out simultane-
ously with the downward flow of the solution through the filter paper. Fractions were
collected and then chromatographed separa-
tely.

By choosing conditions of pH and time such that mobilities differ by 30% or
more, Casid and Stammer (12a) were able to separate fructose, sorbose, glucose,
galactose, mannose, arabinose, ribose, rhamnose, raffinose, and cellobiose using borate buffer. The same
sheet of paper which they used for the electrophoretic separation was subse-
sequently employed for a further chromatographic resolution. By this procedure a
newly described庶 by ionophoresis employing a
10% borax or borax acid solution as the
electrolyte. The flavonoids migrate to the
cathode, the distance being related to
the number of adjacent --hydroxyl groups of the sugar and the number of
hydroxyl groups of the aglycone. The
flavonoid spots were made visible with
etheral LiA1H4 and subsequent treatment with water or dilute HCl. The
spots were developed with orindine hy-
drogen phosphate (186).

Foster (125) found that 2,3, 2,4, and
3,4-diimethyl-1-hydropyrones chromatog-
raphically are separable, the latter two
only difficulty. Of the three dimethyl-
hydropyrones only the 3,4-diimethyl
derivative had a structure suitable for
complex formation with boracic acid since it
has cis-hydroxyl groups at carbon atom 1
and carbon atom 2 in the beta-form. In
his work Foster (126) employed the tech-
niques in which a sheet of filter paper is
sandwiched between two glass plates. The
lower plate was cooled by means of a
cooler coil through which ice water
flowed.

In borate buffers at pH 10 and from
the migration of numerous methyl sugars via ionography, Foster (127) ascertained
that the evidence suggests that the
aldheydo-forms, in addition to ring
structures of the derivatives, interact
with borate ion.

Foster and Stacey (128) attempted to
derive a value, "M" relating to the
movement of sugars in an electric field, analogous to the "R" values used in
chromatography. They defined this factor as

\[ M = \frac{d}{v} \]

By the so-called "true distances of migation" they determined values of migration (mobilities) that have been cor-
rected for electrosmotic flow by the use of a non-borate-complexing deriva-
tive (2,3,4,6-tetramethyl-D-glucose).

The choice of this electrosmotic indicator was arbitrary because several other "in-
ert" indicators did not "migrate" at the same velocity.

These authors discuss complexing in relation to sugar structures and point out
that carbon atom one and carbon atom two are
paramount in complex formation.

Nucleosides, Nucleic Acids, Mucopolysac-
charides and Proteins

Carbohydrates of blood serum were studied by Kli and Geval (154) in connection with clinical altera-
tions of blood-serum carbohydrate values.

After suitable electromigration in non-
complexing buffers, the proteins on the
tongue were separated, and their carbohydrates content as a dye specific for
carbohydrate but not for the paper (cellu-
lose) which was used as the stabilizing medium. The dye was a modified tuchsin-
 sulfide solution.

Since no stains for acid-mucopolysac-
charides are known which will not also
stain the paper, Ritter (188) after suit-
able ionography in a non-complexing
buffer, e.e., phosphate or acetate, cut the
spots, eluted the polysaccharides, and analyzed the eluates for hex-
oseamine or hexosuronic acid. Employing
a phosphate buffer, he studied the elec-
trophoretic migration of hyaluronic acid,
chondrosine sulfate, and heparin. The
positions of these on the ionography were
detected either by staining with toluid-
dine blue or more satisfactorily by elution
followed by a suitable spectrophotometric test.

By incubation with trypsin Woolf (211)
was able to prepare cornal acid mucop-
ysaccharide free of protein. Using
ionophoresis on filter paper as one of
several analytical tools including occa-
sional prior chromatography of the mucop-
ysaccharides, he was able to fractionate and to evaluate the chemical composition of the compound.

Although Kondaljens (32) also did not use a non-carbohydrate-complexing buf-
er like borate, he was able to separate
nucleic acids in 4-6 hours by electropho-
resis on filter paper at pH 5-5.3. The nucleic acids migrate on an electric field due to the
charges on the carboxylic acid and
amino residues of the constituent purines and pyridines. He stained the
ionograms for non-cellulose carbohydrates
using fuchsin-acetic acid solution and
nucleic acids by the method of Zinnozade (214).

A limited attempt at elucidating the
structure of nucleotide anhydrides by cor-
relating mobilities of the nucleotides with their possible structures, Markham
and Smith (178) employed a 0.05 M ammo-
inum formate buffer at pH 3.5. According
to these workers the charge on the am-
hydride derivatives of the nucleotides in
entirely due to that on the -OH groups of
the phosphoric acid radicals and the
amino groups of the bases.

In means of a moderately successful electrophoretic isolation using agar gels as the
supporting matrix for ionographic separation, followed by the more auspic-
ious use of filter paper instead of the gel, Deverdine and Smollett (116) were able to
separate, analyze for, and recover in high
percentages the nucleotides in a hydro-
lyzate of ribonucleic acid. These authors
used a 0.02 M citrate buffer at pH 3.5
found that adequate separation required 16 hours at a potential gradient of 11
volts/m. It was interesting to note that
they dried their ionograms not in an oven
but by means of a heat lamp, and recovered
all the known ribonucleotides can be sep-
parated by ionophoresis on filter paper,
that the method is most convenient to
employ, and that it is suitable for small
quantities.

In a study of the nucleoproteins of cyto-
plasm and the nuclei of the cells of rat
liver and other tissues, Irving and Irving
(142) found the average mobility of yeast
nuclear acid to be 13.5 x 10^-5 cm/sec per
volt/cm at pH 6.5 (no buffer skited) and
ionic strength 0.22. No electrosmotic
connection is given. The bands of nucleo-
proteins or nucleic acids were located in
the dried paper strips by direct measure-
ment of optical densities at 260 milli-
microns in a Beckman spectrophotometer
equipped with a device which permitted
continuous passage of the rula strip past
the exit slit of the monochromator.

Employing both ionophoresis on filter
paper and chromatography, Gordon and
Reichard (130) investigated the mixture
of oligonucleotides formed by the action
of pancreatic ribonuclease on deoxyribonucleic acid. The elec-
trophoretic separation was carried out in
ELECTROMIGRATION IN STABILIZED ELECTROLYTES—PART III

aqueous jelly first at a potential gradient of 3.2 volts/cm for two hours followed by an increase to 3.7 volts/cm and an additional 15 hours.

In order to separate chondroitin sulphate from hyaluronic acid, Gandel, et al. (129) utilized Hyflo Super-Cel (a commercial kieselguhr) as the stabilizing agent for the electrophoretic separation of these two substances. After suitable electromigration the slab was cut up into strips which were extracted with water in order to elute the polysaccharides, and these were assayed by a suitable carbohydrate test. Three different buffers (citrate - 0.1 M and pH 3.7, acetate - 0.1 M and pH 4.7, and phosphate - 0.1 M and pH 6.7) were each individually used in the electrophoretic separation at a potential gradient of 3 volts/cm. The time for which the electrophoresis was conducted was estimated from the visible movement of the dye which these authors had incorporated into the agar, and amounted to a distance of 30-35 cm. from the agar inlay containing the migrating which traveled toward the anode.

LIPIDS AND LIPOPROTEINS

Several analyses of lipids and lipoproteins by the method of electromigration have been reported. The chief difficulties encountered in the study of these substances are in the detection of the bands or zones on the long strip, in the adsorption of the lipids or lipoproteins on the surface of the paper, and in the retention of the lipid stain by the paper itself. The usual technique is to run at least two strips, one of which is stained for lipids and lipoproteins while the other is stained for proteins. In this manner the positions of the lipoprotein bands can be given relative to the protein bands.

Swahn (91) used a semi-saturated solution of Sudan black B in 50% ethanol for the detection of lipids and lipoproteins. Since Sudan black B is not a dye in the technical sense, it does not stain, but colors lipids black by dissolving in them. The coefficient of distribution for Sudan black B between lipids and 50% ethanol is very favorable to the former. The coloring appears to be quite specific in that at present the list of substances known to be colorable with Sudan black B includes nothing but lipids and lipid-complexes such as lipoproteins. Swahn stained the paper strips for 30-45 minutes and then rinsed them with 3-4 changes of 50% ethanol until only a faint blue tone remained on those parts of the paper where there were no lipids. He also reported that with Munkholt’s No. 20 paper there was no adsorption of the Sudan black B than with Whatman’s No. 1 paper. At room temperature the separation of the lipid and beta-lipoproteins was obtained using a 0.05 molar barbital buffer solution at pH 8.5.

Employing an apparatus similar to that which was used by Gandel, Wolanska and Goldwall (38), he studied the distribution and relative amounts of the serum lipids from normal and from pathological patients. Fossli (20a) obtained rather sharply differentiated bands of the alpha and of the beta-lipoprotein fractions using an instrument similar to that employed by Flynn and de Mayo (21). He stained the lipids with a saturated solution of Sudan III in 50% ethanol for 30 minutes at 45°C, then washed them thoroughly in 50% ethanol and subsequently in distilled water.

The conditions for carrying out his separations were: a buffered solution, pH 7.3, ionic strength 0.05, S., and S. No. 540 paper; a potential gradient of about 3 volts/cm for a duration of 4 hours. Rosenberg (190) in his studies of human serum has confirmed the observations of Swahn (91) and Fossli (20a).

Duxon, et al. (10) separated the alpha and beta-lipoproteins using Whatman 3MM filter paper employing a veronal buffer of pH 8.4 and ionic strength 0.05. The lipids and lipoproteins were stained for 18 hours in a bath comprising a saturated solution of oil red 0 in 60% ethanol. The strips were then rinsed with tap water, blotted, and dried. The resulting strips show a red pattern against a pink background. The use of oil red 0 as a lipid or lipoprotein stain is not too practical because the beta-lipoproteins do not show up clearly. Lerner, Smith, Crawford, Jetten and Duxon (190) state that studies by paper electrophoresis of top fractions of serum prepared by the Goldman technique have proved unsatisfactory due to adsorption of the material by the paper. It has been shown by Munkholt (38) that if the lipoprotein fraction is deoxygenated or partially denatured, the adsorption on the paper is greatly increased. Kunkel and Slater (40) used both Whatman 3MM paper and potato starch to obtain lipoprotein patterns of serum with a buffer of pH 8.6 and ionic strength 0.10.

Merkbach and McDonald (51) determined the electrophoretic properties of bovine serum lipoprotein using the technique of isoelectric focusing. They determined the electrophoretic mobility of bovine serum lipoprotein over the pH range from 2.7 to 8.8. The pH versus mobility plot they obtained is similar to that of a protein, except that the beta-lipoprotein is a much slower moving substance; its isoelectric point was determined to be approximately 5.2. The conditions for the experiment were: a veronal buffer pH 2.7 to 8.8; ionic strength 0.015; potential gradient 10 volts/cm; temperature 10°C; time 5-4 hours; atmosphere: helium saturated with water vapor.

Niklitsch (182) using Munkholt’s No. 20 filter paper, fractionated 0.25 ml of serum in 4 hours. He analyzed 10 normal and 2 other-eleutherian male sera and one hypercholesterolemic rabbit serum show that all beta-lipoprotein is richest in lipid material. A high gamma-lipoprotein lipid value is found but it is believed to be caused by some technical difficulty. In patients with active arteriosclerosis the conditions of the shift of lipids from alpha-lipoprotein to slower fractions.

ENZYMES, HORMONES AND VITAMINS

The technique of electromigration on wet surfaces has been applied to the fractionation of several enzymes, hormones, and vitamins. Schaper (28b) was able to distinguish between a purified and a partially purified preparation of Schardinger dextrinase using this technique. Falesch (185) using a 100 mg sample of lyophilized cytochrome c from beef or chicken hearts, in 1 ml of pH 9.8 glycine buffer (and similarly using a phosphate buffer at pH 7) was able to distinguish between ferric and ferrous cytochrome. Niklitsch (184) showed that the partially purified pancreatic amylase contained 3 components of which the middle fraction had amylase activity, with a mobility of 2.9 x 10^-5 cm/sec per volt/cm. at pH 8.6 and ionic strength 0.05. He also showed that under similar conditions cytochrome c appeared to contain 2 fractions; the larger and more active fraction had zero mobility (isolectric point) under the conditions of the experiment, while the other fraction exhibited a mobility of 7.9 x 10^-5 cm/sec per volt/cm.

Mills and Smith (66) used a slight modification of the apparatus of Cramer and Thiebus (14) but without any special cooling arrangement, to study certain amylase preparations. The filter paper used was Whatman 3 MM in strips 3 or 4 cm wide, the other conditions being similar to those employed by Cramer and Thiebus. Enzyme activity was localized by means of the chromogenic substrates, phenolphthalein g-hemostrene for glucosidase, phenolphthalein phosphate for phosphatase and p-nitro phenyl butyrate for esterase. A strip of filter paper soaked in substrate and buffer was placed in contact with the amylase, the two were clamped between glass plates, incubated, and the color developed after a suitable time. Wallenfels and Von Pechmann (99) separated amylase, protease, lipase, and phosphatase from mushroom culture.

Simonsen et al. (194) showed the presence of pepsin in commercial rennet by three methods, including the technique of electromigration on filter paper using a modification of the apparatus described by Munkholt and Smith (176). Munkholt et al. (52) separated two components from a chorioic gonadotropin preparation, by means of electromigration in paper-stabilized electrolytes. McDonald and Merkbach (170) fractionated isoelectrically a low-potency ACTH preparation from pig pituitaries, with the result that the active material was separated out, largely into one well-
of motion of the boundary could be followed. The addition of agar did not appear to affect the results, so it was used to stabilize the boundaries. Since the potential gradient was constant through the tube, the mobility of each could be calculated from the measured current and the motion of the boundaries. Archemedes (108) studied the relationship between the fluidity of electrophoresis stabilized with various amounts of jelly and their conductivity.

Kendall and co-workers (93,147,148, 149,150,151) from 1923-38, applied the method of electrophoresis in agar gels to the separation of isoproteins of cereals, of human serum, and of human plasma. Using this medium, they were able to separate various proteins in a single gel.

Several authors have used the technique of electrophoresis to study the fractionation of vitamins. Ericson, et al. (118) have demonstrated that vitamin B12 in the fermenting liquor of Streptomyces griseus.

Schweizer and Witten (82) used the technique of electrophoresis to study the ratio of the activity of vitamin B12 to the activity of vitamin B12 in the fermenting liquor of Streptomyces griseus.

INORGANIC IONS

The earliest known investigations on electromigration in stabilized media dealt with inorganic ions. Lodge (168) in 1886 filled a tube with solid methylene blue, which had been dyed with red by a drop of alcohol. On being in contact with a dilute acid, and on passing a current through the tube, the color was progressively bleached. From the rate of motion of the boundary the velocity of hydrogen gas, which was less than 0.002 cm./sec. per volt/cm. Lodge’s method was improved by Westrum (207,208,209) who used two solutions with a common ion, with the same concentrations and nearly the same specific conductance, but different in color and density and stratified in a vertical tube. In one case, 0.1 N potassium dichromate and potassium carbonate were used. The colored ion moved and the rate of electromigration was determined.

MISCELLANEOUS APPLICATIONS

King and Doeye (152) employed the technique of electromigration to determine whether unknown antibodies in culture fluids were active; thus, they could separate proteins and to approximate their isoelectric points. It is interesting to note that they used chloromycetin, which shows a monoclonal movement in filter paper close to that of dextran, as an electromotive indicator. They found that the conductance of the whole paper sheet, on drying, was a factor to be considered when comparing electrophoretic measurements made in "non-stabilized" and in "paper-stabilized" buffer solutions.

Schild and Mauer (192) utilized P32 to make an in vivo study of the phosphorus metabolism of red blood cells. The phosphorus turnover of the erythrocytes, as organic phosphorus, was found to be 3% per hour, of the total phosphorus content. The phosphorus esters were then extracted with triethylacetic acid, separated electrophoretically on paper strips, and their concentration determined by their radioactivity. Three maxima were identified as representing adenosine triphosphate, creatine phosphate, and inorganic phosphate.

McDonald and Spitzer (171) have determined the electromigration characteristics of the plasma expander polyvinylpyrrolidone (PVP) on paper. They describe a color test for locating the substance on paper and outline the necessary conditions for separation of PVP from the albumin, and a and B-globulin in fractions of blood plasma. Weber (206) has described the separation of organic anilies on filter paper by migration under the influence of an electric field. The sensitivity was found to be about 10-6 mole. Human urine was shown to contain methylamine, dimethylamine, and another amine not yet identified.

Welsh and Field (210) have described the isoproteic separation and determination of hydroxyxycarboxylic acids. Wundley (212) has described the separation of 4-dimethylaminoazobenzene (butter yellow) in serum.

Penniston, Agar, and McCarthy (71) utilized the technique of electromigration in agar-stabilized electrolytes to fractionate sulfonated aromatic substances comprising dializable sulfonic acids and phenolic substances obtained by the alkaline oxidative degradation of lignin. The agar was contained in Vycor glass tubing, which permitted analysis after migration by direct scanning of the tube for absorption of ultraviolet radiation by use of a spectrophotometer. In some cases up to 10 micrograms of a component was found to suffice for analysis. Ion mobilities for vanillin, ferulic acid and vanillic acid were determined. Acetone and benzene were used as electroscopic indicators. Isoproteic separations were made of known mixtures of four phenols, guaiacol, catechin, vanillin, and vanillic acid, in a phosphate buffer system, made up to a pH of 7.

ELECTROMIGRATION IN PAPER-STABILIZED NON-AQUEOUS SOLVENTS

The technique of electromigration is not limited to compounds which are water-soluble; it is only necessary that they be dispersed or solvable in the solvent, that it acquire a net charge and that the medium conduct an electric current. Paul and Duran (70) separated dye mixtures on filter paper using non-aqueous solvents such as dimethyl ether, glacial acetic acid, and ethyl alcohol. In the authors' laboratory aqueous solutions containing up to 50% dioxane have been used successfully by Marko and McDonald in measuring the effect of dielectric constant on the mobility of isoproteic blue.

ACKNOWLEDGMENT

That part of the work reported in this series of three papers which was carried out in the authors' laboratory was aided by financial assistance from a number of sources. The authors are pleased to acknowledge the support given the project by grants from the American Heart Association (153-S2) and the Chicago Heart Association (155-S4), and by...
ELLEMIGRATION IN STABILIZED ELECTROLYTES – PART III

a research grant (51-1341) from the National Heart Institute of the National Institutes of Health, Public Health Service (1955-6). In addition, E. F. Marbach held the Feilmeier Myron Strong Fellowship during 1955-56, and the Royal E. Coillon Fellowship during 1955-56. M. C. Utz was awarded a Graduate Research Fellowship, Standard Oil Company (Indiana), during 1956-61. Acknowledgment is also made of a grant-in-aid during 1952-53 from the Committee on Research, of the Council on Pharmacy and Chemistry, of the American Medical Association, for the purchase of some special equipment needed to expedite the research program. R. J. Lappe and R. H. Spitzer held research assistantships in the Department of Biochemistry of Loyola University during the periods (1950-53) and (1951-53) respectively.

BIBLIOGRAPHY

References for Part III Not Cited in the Bibliography Appeared to Parts I or II.

[133] Hennemann, W., and Hamag, K., Naturwissenschaften 37, 397 (1950).

An ionograph in compact self-contained form with line-operated rectifier, water-jacketed chamber, levelling screws in base, adjustable frame shown with filter paper striplets in place, window in cover through which migrant may be applied and anti-condensation pads on each side of window. At left, in front, shown separately, feeder vessels for use in certain experiments where special precautions are necessary to maintain constant the water content of the strip (S2).
A STANDORIZED PLASMA PREPARATION SUITABLE FOR USE AS A CONTROL IN THE DETERMINATION OF PROTHROMBIN TIME

by

M. E. Hodes*

From the Department of Physiological Chemistry, U.S. Naval Medical School, National Naval Medical Center, Bethesda, Maryland.

To the chemist who has to do an occasional prothrombin time, as well as to him who has many determinations each day, the problem of securing a satisfactory control plasma is well known. There is always uncertainty as to whether a random specimen is really normal, and a technician often finds himself the unwilling donor of a control sample. To overcome these objections, various attempts have been made to provide stable control preparations of plasma. The present paper describes our results with a preparation known as Diagnostic Plasma Warner-Chilcott®, which we tested under the designation CH-90, and an essentially similar product known as CH-70.

METHODS AND MATERIALS

Thromboplastin: Either Simplastin® or Multime Thromboplastin® was used.

Lyophilized Control Plasmas, CH-70 and CH-90: These were reconstituted by addition of distilled water to volume. To produce the 12.5% cont plasma, 0.5 cc of the reconstituted material was diluted to 4.0 cc with normal saline.

Some of the stability experiments required pooling of several ampules of the reconstituted material, so that there was not sufficient plasma in a single ampule for all the determinations required. Pooled material was stored in the refrigerator at about 4°C, except for the room-temperature experiments. Several ampules were stored at room temperature for stability studies on the dry material.

Determination of Prothrombin Time: The method used was the Link-Engine modification of the Quick one-stage test, as described by Sudath (1). Tests were done in the routine laboratory by one technician. Questionable determinations were repeated. 

RESULTS

Comparison of CH-70 and laboratory normals.

A condensation of the results of a two-month comparison of the laboratory normals with CH-70 is presented in Table I. Most values for CH-90 are 13 and 14 seconds for whole plasma, and between 30 and 40 seconds for the dilute plasma. These figures are for plasmas run using Simplastin®. When Multime Thromboplastin® was used, the prothrombin times were somewhat shorter.

* Present address: Cell Chemistry Laboratory, Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York.
A STANDARDIZED PLASMA PREPARATION

TABLE I

Comparison of CH-70 and Laboratory Normals

<table>
<thead>
<tr>
<th>Thromboplastin*</th>
<th>Range of Laboratory Normals</th>
<th>Range of CH-70 Determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>whole plasma</td>
<td>dilute plasma</td>
</tr>
<tr>
<td>Simplastin (16)</td>
<td>13-15</td>
<td>33-37</td>
</tr>
<tr>
<td>Thromboplastin (9)</td>
<td>12-15</td>
<td>36-35</td>
</tr>
<tr>
<td>Simplastin (6)</td>
<td>13-16</td>
<td>32-40</td>
</tr>
</tbody>
</table>

* The number in parentheses indicates the number of individual determinations in each series.

Comparison of CH-70 and CH-90

CH-90 prothrombin time values were compared to CH-70 values. Results were essentially the same with both preparations.

Stability of CH-70 and CH-90

A. Before reconstitution

[1] Under refrigeration: The material appeared to be stable indefinitely in the refrigerator.

[2] At room temperature: The material was stable for about 3 months during a hot spring and summer. A sample of CH-70 58 days at room temperature, and one of CH-90 28 days away from refrigeration, gave essentially the same results as identical samples kept under refrigeration.

B. After reconstitution

[1] Under refrigeration: Experiments on several batches of CH-70 indicate that the material is stable for at least 4 days if kept in the refrigerator after preparation. During this period there is a slight increase in the dilute prothrombin time. The results of several experiments are summarized in Table II.

CH-90 also showed essentially no deterioration over a 4-day period.

[2] At room temperature: The material was stable 58 hours when kept at room temperature of about 25°C. At 60°C, it deteriorates in less than 2 hours, as does normal plasma.

Reproducibility of results

A dilution error of ± 10 per cent in the 1 cc. size ampoule produced no significant change in the whole or dilute prothrombin times. Quadruplicate determinations on CH-70 showed the results to be reproducible within a few tenths of a second in both the whole and dilute ranges (Table III). The results were very similar even with different batches of Simplastin, of the same or different lot numbers, and with different batches of CH-90. Experiments were performed on CH-90 left unrefrigerated for 20 days.

SUMMARY AND CONCLUSIONS

Comparisons have been made between the prothrombin time values determined using lyophilized normal plasma preparations (CH-90 and CH-70) and routine laboratory (patent) normal plasma. These indicate that the lyophilized preparations, when used in conjunction with Simplastin, give constantly reproducible prothrombin time values in both the whole and dilute (12.5 per cent) plasma ranges.

TABLE II

The Stability of CH-70 Under Continued Refrigeration After Reconstitution

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Hours Old</th>
<th>Patient Normal*</th>
<th>CH-70</th>
<th>Lot No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>whole</td>
<td>dilute</td>
<td>whole</td>
<td>dilute</td>
</tr>
<tr>
<td></td>
<td>minutes</td>
<td>minutes</td>
<td>minutes</td>
<td>minutes</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>13</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>14</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>13</td>
<td>35</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>13</td>
<td>37</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>13</td>
<td>33</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>13</td>
<td>34</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>190</td>
<td>15</td>
<td>40</td>
<td>19</td>
</tr>
</tbody>
</table>


* Refers to a fresh normal drawn the day of the determination.

TABLE III

The Reproducibility of Results With CH-90

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>whole</td>
<td>dilute</td>
<td>whole</td>
</tr>
<tr>
<td>I</td>
<td>15.0-15.5</td>
<td>47.2-47.5</td>
<td>94421</td>
</tr>
<tr>
<td>II</td>
<td>14.8-15.3</td>
<td>42.0-42.4</td>
<td>94421</td>
</tr>
<tr>
<td>III</td>
<td>14.4-14.6</td>
<td>43.1-44.9</td>
<td>94421</td>
</tr>
<tr>
<td>IV</td>
<td>15.0-15.2</td>
<td>45.0-45.3</td>
<td>94421</td>
</tr>
<tr>
<td>V</td>
<td>15.1-15.5</td>
<td>43.8-45.0</td>
<td>93821</td>
</tr>
</tbody>
</table>

* This experiment was performed on CH-90 stored 28 days at room temperature.

ACKNOWLEDGEMENT

The author gratefully acknowledges the technical assistance in laboratory determinations by E. Bites, Chief Hospital Corpsman, United States Navy.

Bibliography

ONE PAN
BERANGER TYPE BALANCE
For General Laboratory and Animal Weighing

No weights to attach—or to loss, no complicated reading device: compact, simple to operate, it is ideal for rough laboratory work, animal weighing, general autopsy work, and general batch weighing. Employed a uniquely convenient method of reading, a built-in sliding weight with a micrometer adjustment. Included in the equipment is a simple zero-ing device and a beam arrangement. Finished in stove-white enamel, with removable chrome-finished pan.

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>MASS CAPACITY</th>
<th>CUBIC CAPACITY</th>
<th>SENSITIVITY</th>
<th>READABILITY</th>
<th>PRICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>18460</td>
<td>6K</td>
<td>1650cc</td>
<td>2 g</td>
<td>5 g ¼ &quot; between Divisions</td>
<td>$39.50</td>
</tr>
<tr>
<td>18461</td>
<td>6K</td>
<td>1650cc</td>
<td>1 g</td>
<td>2 g ¼ &quot; between Divisions</td>
<td>$42.50</td>
</tr>
<tr>
<td>18462</td>
<td>10K</td>
<td>2500cc</td>
<td>3 g</td>
<td>10 g ¼ &quot; between Divisions</td>
<td>$46.50</td>
</tr>
<tr>
<td>18463</td>
<td>15K</td>
<td>2500cc</td>
<td>3 g</td>
<td>10 g ¼ &quot; between Divisions</td>
<td>$49.50</td>
</tr>
<tr>
<td>18464</td>
<td>15K (Flat Plate)</td>
<td>2500cc</td>
<td>3 g</td>
<td>10 g ¼ &quot; between Divisions</td>
<td>$46.50</td>
</tr>
</tbody>
</table>

For Thorough Mercury Cleaning

The Bethlehem Oxifier and gold-adhesion filter constitutes a radical change in methods of mercury cleaning. The Oxifier reduces all base metals to oxide precipitates. The filter removes these precipitates, as well as all floating impurities—all water, oil, grease, dust of every character.

Oxified and filtered mercury possesses a high degree of purity and contains no base metals. It show no skin on the surface and retains its brightness after long storage. It is bone dry and entirely free of acid or oxidized element. The Oxifier-filter process renders low cost prime virgin mercury equal, and in some respects superior to, expensive triple distilled. It meets the U.S.P., the A.C.S., and the A.P.H.A. specifications and is widely used in instruments such as Thermo metric Manometers, McLeod Gauges, Mercury Switches, Flow Meters, Office Meters, Rectifiers, Polarographs and instruments of precision.

No. 57165 — 5-LB. OXIFIER $77.50
Height 7", table space 9" x 5"; weight 8 lbs.; shipping, 15 lbs. Water, direct-connected 1/10 HP. 115 V., 60 Cy., A.C., with line switch. Cast iron frame, finished in green and black enamel.

Cleans 20 lbs. a day in 5-LB. BATCHES OXIDES DISOLED BASE METALS

No. 57165—TYPE ‘Q’ FILTER $240.00
Height 11", table space 3½ x 8", weight 1 lb. 2 oz.; shipping, 2 lbs. No reservoir or shut-off valve; mercury can be poured through at rate of 5 lbs. in two minutes. Plastic and stainless steel.

Eliminates:
- Floating Impurities: Water, Oil, Oxide-Dust: But Not Dissolved Metals

HAEMOSCOPE
COMBINES SPEED WITH ACCURACY!
The Automatic Phonometrically Operated Instrument that Achieves a Blood Count in Approximately 10 Seconds!! Requires no special equipment other than accessories included with the Haemoscope as standard equipment.
Is the fastest known method of determining the erythrocyte count. Has remarkable precision.
Gives direct reading on dial, in millions of RBC per cubic millimeter.
Compensates automatically for cell size.

Write for Brochure with Complete Information
No. 52083 — Complete with Cassette Holder, cord set for use on 115 volts A.C. and numerous accessories...

$585.00

Make Your Labels Washable with "VARNITON" LABEL VARNISH
When coated twice with "Varnite" Label Varnish, labels on bottles, flasks, caps, beakers, storage bins and reference files become resistant to oils, greases, gasoline, DRIES FAST!
8 oz. Can with 2 oz. dispensing bottle, containing a “Cello-Lume”...

$1

STANDARD SCIENTIFIC SUPPLY CORP.
34 West 4th Street • New York 12, N.Y.
LABORATORY APPARATUS—REAGENTS AND CHEMICALS