VOL. 5
NO. 2

April 1953

# The CLINICAL Chemist

* * * * * * * IN THIS ISSUE * * * * * * *



*Official Publication*
*of*
*AMERICAN ASSOCIATION*
*of*
*CLINICAL CHEMISTS,*
*INC.*

**THE STANDARD METHODS OF CLINICAL CHEMISTRY, VOL. I TO BE PUBLISHED JUNE, 1953**

The CLINICAL Chemist

P.O. Box 123
Lenox Hill Station New York 21, N.Y.



VOL. 5, NO. 2 APRIL 1953

## THE SECRETARY REPORTS

It becomes necessary for us periodically to bring up the unpleasant subject of finances. Our income is derived primarily from dues, and these assessments are kept at a minimum, even in view of our relatively small membership. By the specialized nature of our Association, the membership of necessity can expand only as the specialty expands. The Association is fortunate in that so many of its members are giving freely of their time and efforts. However, there still remain minimal expenses that must be borne by any national organization.

There are some other sources of income that could help to partly defray some of the expenses, as well as add to the treasury surplus so painstakingly being built by the National Treasurer. The first of these is the membership directory of the AACC. Although this is not a very large list when compared with some other scientific and professional societies, yet it is the only acceptable list of clinical chemists in the United States, and as such is a valuable asset of the Association. This list is available to appropriate advertisers at a standard fee, and with written permission for its use.

Another source of income comes from advertisements in THE CLINICAL CHEMIST. The membership could contribute by encouraging dealers and manufacturers to use the facilities of the AACC. This could easiest be done by advising the advertiser that you saw the product being ordered advertised in THE CLINICAL CHEMIST, as well as calling this publication to the attention of prospective advertisers. We could readily increase the income from this source without relaxing the present standards of accepting only ethical advertising pertaining directly to clinical chemistry.

And finally there is the matter of membership certificates available to members on payment of four dollars. The initial costs of engraving and printing have already been written off, so that certificates ordered in the future will allow for a small profit to the national treasury. This membership cer- is artistically designed and suitable for framing.

*Max M. Friedman, National Secretary*

## NOTE From The Editor-in-Chief of the *STANDARD METHODS OF CLINICAL CHEMISTRY – VOL. I.*

*THE STANDARD METHODS OF CLINICAL CHEMISTRY* by the American Association of Clinical Chemists, Volume I will be published by the Academic Press Inc. New York, June, 1953. The list of chapters is as follows:

### VOLUME 1, June 1953

Editor-in-Chief, Miriam Reiner, Gallinger Municipal Hospital, Washington, D.C.

CONTENTS*

**Introduction**
Marion Edward Hodes

**Amylase**
**Margaret Kaser,** Nelson F. Young

**Bilirubin**
**George R. Kinsley, G. Getchell and R.R. Schaffert**
David Seligson and Marjorie Knowlton
Albert E. Sobel

**Calcium**
**Otto Schales**
Mariam Reiner

**Carbon Dioxide Content** (Titrimetric)
**Marion E. Hodes**
Miriam Reiner

**Carbon Dioxide Determination**
(Van Slyke Volumetric and Manometric Apparatus)
**Miriam Reiner**
Julius J. Carr

**Chloride**
**Otto Schales**
Marschelle H. Power
Nelson F. Young
Joseph Benotti

**Total and Free Cholesterol**
**Margaret M. Kaser**
Louis B. Dotti

**Creatinine I. Alkaline Picrate Method II. Dinitrobenzoate Method**
**George R. Kingsley and R.R. Schaffert**
Miriam Reiner

**Glucose** (Folin-Wu)
**Nelson F. Young**
Albert E. Sobel

**Glucose** (Nelson-Somogyi)
**John G. Reinhold**
Margaret Vanderau and P.E. Halpern

**Lipase**
**Carl Alper**
Marion E. Hodes and B. Garland

**Phosphatase** (Alkaline and Acid)
**Julius J. Carr**
Miriam Reiner

**Inorganic Phosphate**
**Marschelle H. Power**
Nelson F. Young

**Total Protein, Albumin and Globulin**
**John G. Reinhold**
David Seligson, G.E. Schreiner, L.V. Riddle
Margaret Vanderau

**Prothrombin**
**H.C. Sudduth**
Miriam Reiner

**Sodium and Potassium**
**Joseph Benotti**

**Thymol Turbidity**
**George R. Kingsley and G. Getchell**
Jos Kahn, Martin Rubin, David Seligson and M. Knowlton

**Urea Nitrogen**
**Otto Schales**
Albert E. Sobel, Joseph Benotti

**Uric Acid**
**Samuel Natelson**
Margaret Kaser

* The original *Submitters* of the method appear in bold type, followed by the name of the person that checked the method.

Some of the members of the Association of Clinical Chemists have become impatient at the seeming delay in its publication, but they do not realize the trials and tribulations in setting up such a project. There were so many things to be decided in the first volume: the scope of the book, method of organization, the presentation of a variety of methods in a fairly uniform manner (allowing for individual differences in the literary style of the authors). Most of the difficulties and delays were due to the fact that the chemists taking part were from all parts of the country and all communication and discussions were by mail and not in person; since there are about 25 coauthors there was a prodigious amount of correspondence.

Our book of STANDARD METHODS is not just another book of methods recopied from the literature. It is a practi-

**STANDARD METHODS – VOL. I** *(Cont.)*

cal and reliable collection of procedures that has been tested 1) in the laboratory of the *Submitter* on normal and pathological sera (including directions for setting up standard curves); and 2) retested in at least one other laboratory by the *Checker.* Sometimes the method did not work as well in one laboratory as another; this led to further work, sometimes far afield into the recrystallization of compounds or rechecking of buffers, etc. until what seemed like a simple task turned into a long and arduous one. Even though this was tedious, I think that all coauthors will agree that they learned quite a lot during this testing and they have a new respect for the methods we carry out daily in our laboratories and take so much for granted.

We would like to take this opportunity to thank all the members of the American Association of Clinical Chemists who have helped in the publication of the *STANDARD METHODS;* first the *Submitters* and *Checkers* who have done such a thorough job and who have made our plan a reality. To the Executive Committees, Officers of the Association, and others too numerable to mention who have helped with advice, assistance, and moral support, we offer our grateful thanks.

To all members of the American Association of Clinical Chemists we dedicate VOLUME I of the STANDARD METHODS. We hope you will find it useful. Please let us know how you like it by comments, criticisms and suggestions, so that we may improve it, and increase its scope and usefulness through the years.

*Miriam Reiner, Editor-in-Chief, Vol. I*
*Washington, D.C.*

## QUID NUNCS

Captain **David Seligson** reverted to inactive status in the Army in January. On March 1, he took up his new position as Chief of Chemistry at the Graduate Hospital of the University of Pennsylvania and Assistant Professor of Internal Medicine at the Graduate School of Medicine.

Dr. **Ralph E. Peterson** has returned to Washington, D.C. after having spent a year at the Peter Bent Brigham Hospital in Boston, Massachusetts. His new position at the National Institutes of Health, Clinical Center, is that of a Medical Research Investigator.

# ABSTRACTS OF PAPERS ON CLINICAL CHEMISTRY

*Presented in Cooperation with The Division of Biological Chemistry ACS as part of the 123rd National Meeting*

REVIEW OF INTERLABORATORY ACCURACY SURVEYS OF CLINICAL CHEMICAL ANALYSES. Richard J. Henry, Bio-Science Laboratories, Beverly Hills, Calif.

A review of all available interlaboratory accuracy surveys conducted in the field of clinical chemistry was made with the idea that from such a compilation might come indications for better design of future surveys.

Among the factors discussed are the method of reporting results, type of check specimens, accepted "true" result, permissible tolerances, design of surveys, and purposes of surveys.

The conclusion is reached that such surveys at the present stage of clinical chemistry should not be run with the purpose of "policing" laboratories, but rather with the intent of analyzing the faults of tests and laboratories, their causes, and possible means of rectifying them where they occur.

AN EXPERIMENTAL PROPOSAL OF NEW METHODS FOR THE DETERMINATION OF BARBITURATES IN BIOLOGICAL FLUIDS. Earl M. Bilger, Leonora Neuffer Bilger, and Edward Izawa, Department of Chemistry, University of Hawaii, Honolulu, Hawaii.

The paper presents a review, in brief, of the steadily increasing seriousness of the problem of barbiturate consumption and the consequent importance of clinical laboratories being provided with a reliable method for the determination of barbiturates in biological fluids, in particular, urine and blood. The unfavorable status of the two procedures at present utilized, gravimetric determination following extraction and the colorimetric gold chloride method, based upon appraisals as set forth in the literature is emphasized.

Experimental work is described in which a mercuric chloride titration procedure, adapted from the La Motte urea determination, and a volumetric silver nitrate method, already used for determining large amounts barbiturates, were applied to the analysis of urine and blood for barbiturates. These procedures were compared experimentally with the widely used cobalt method. Four different drugs were studied in each of three states: (1) as pure drugs extracted from commercial tablets, (2) in urine, (3) and in blood. About 80 analyses were made, and data are set forth in some twenty tables.

The mercury and silver procedures were found to be significantly more reliable than the cobalt method and the silver titration was superior to the mercury for pure samples of drugs and for urine.

The cobalt method was investigated by substituting six other metals for cobalt, but results were unreliable and erratic.

DETERMINATION OF BARBITURATES IN BLOOD. Bernard F. McKenzie and Marschelle H. Power, Mayo Foundation, University of Minnesota, and Mayo Clinic, Rochester, Minn.

Measurement of the increase of optical density in the ultraviolet range of the spectrum when solutions of barbiturates are made alkaline has been utilized in recently reported methods for the quantitative determination of barbiturates. In our technique 2 ml. of oxalated blood is extracted with peroxide-free ether for 45 minutes in an all-glass apparatus. The extract is evaporated to dryness and the residue is taken up in 3 ml. of 95% ethyl alcohol containing 0.2 ml. of acetate buffer at pH 4.65. The optical density of this solution in comparison with that of the alcohol-buffer mixture as a blank is measured over the range 220 to 330 mu by means of the Beckman spectrophotometer. A 0.2-ml. portion of borate buffer of strength sufficient to increase the pH to 9.5 is then added to each absorption cell and the readings are repeated over the same range. The increase in optical density at 240 mu is proportional to the content of barbiturate. Individual barbiturates cannot be determined, since the absorption curves of the various barbiturates are similar. For pentobarbital sodium (nembutal), an increase in density of 0.262 unit corresponds to 1 mg. per 100 ml. In our experience recovery of barbiturate added to blood has been satisfactory and the alcohol-buffer solutions have invariably remained clear.

Certain drugs that might be present in blood were studied for possible interference. The presence of aureomycin and metabolites of caffeine introduced small positive errors, equivalent at most to not more than about 0.2 mg. of barbiturate per 100 ml.

SERUM CHANGES IN DISEASES AS FOUND BY PAPER CHROMATOGRAPHY. Henry Tauber, Wilton E. Vannier, Edward L. Petit, and Harold J. Magnuson, Venereal Disease Experimental Laboratory, U.S. Public Health Service, School of Public Health, University of North Carolina, Chapel Hill, N.C.

We have recently presented improvements concerning the two-dimensional paper chromatography of proteins and developed a new staining reagent for locating the movement of proteins Tauber and Petit, J. Am. Chem. Soc., *74*, 2865 (1952) . When the plasma albumins (Fraction V) were mixed with the $\gamma$-globulins (Fraction II) there occurred a characteristic separation of a portion of each plasma fraction (Tauber and Petit, Proc. Soc. Expti. Biol. Med., *80*, 143 (1952)).

We found by using our technique and 10 microliters of diluted serum (to contain about 120 micrograms of total protein) that typical patterns are obtained with serums from patients suffering from diseases which are accompanied by an abnormal albumin-globulin ratio. Under the conditions of our technique the serum albumins fraction moves only slightly in the lower region of the second dimension. With normal serum a short streak is obtained. When the globulins fraction increases in proportion to the albumins fraction, there is a proportional lengthening of the "globulins streak." In such cases an occasional decrease in the albumin portion of the pattern may also be noted. Sera which had an abnormal chromatogram showed also an abnormal electrophoretic pattern. Chromatograms and electrophoretic patterns with quantitative data are presented.

DETERMINATION OF URINARY ESTRONE AND ESTRADIOL. Albert L. Chaney and Wm. E. McKee.

The urinary estrogen conjugates are hydrolyzed by autoclaving in the presence of 1.2 *N* sulfuric acid. An extract which is free of most of the usual red and purple pigments is obtained by adjusting the urine to pH 9 before extraction with benzene. The solvent layer is purified by sodium carbonate and sulfuric acid washes. The phenolic fraction is extracted with 1 *N* sodium hydroxide, and then the pH is reduced to 10.5 with sodium bicarbonate. Shaking the bicarbonate layer with benzene re-extracts the estrogens. Final purification is accomplished by chromatography on 1 gram of alumina. Estimation of the combined estrone and estradiol is made by fluorimetry in 60 volume % sulfuric acid.

NEW AND RAPID METHOD FOR THE DETERMINATION OF FREE SERUM CHOLESTEROL. Albert Zlatkis, Bennie Zak, Harold H. Brown, and Albert J. Boyle, Departments of Chemistry, Pathology and Medicine, Wayne University, Detroit, Mich.

Precipitation of the digitonide of cholesterol is effected by the use of the polyvalent aluminum ion which is added to an alcohol-acetone extract of serum. The precipitation of the digitonide in these circumstances is complete and almost immediate. The spun down precipitate is then reacted with a sulfuric-acetic acid iron reagent, which yields a purple color reaction that is used for the estimation of the free cholesterol.

The advantage of the acid iron reagent is its sensitivity, which is several times that achieved in current methods. In addition, the color development is complete within 3 minutes and requires no temperature control. The resultant color is stable for several hours.

The combination of rapid precipitation of the cholesterol with digitonin and the use of the new color reagent make possible a continuous processing of serum for the estimation of free cholesterol in a relatively short time.

DIRECT MICROTITRATION OF SERUM CALCIUM. Albert L. Chaney and Kenneth D. Johnson.

The direct titration of solutions of calcium oxalate with sodium Versenate fails to yield satisfactory end points because of the competition of the oxalate and Versenate ions for the calcium. This type of interference has been overcome by the destruction of the oxalate by microdigestion with perchloric acid.

The calcium is precipitated in a micro centrifuge cone from an aliquot of serum with a buffered solution of ammonium oxalate. The precipitate is collected by centrifugation and the supernatant removed as completely as possible. Twenty to 50 microliters of 72% perchloric acid is then added to the unwashed precipitate and the mixture is digested at about 200°C. The oxalate is rapidly and completely destroyed along with traces of organic matter. The digest is taken up in a few milliliters of water and ammonia-cyanide buffer, and is titrated with standard Versenate solution. Eric-chrome black T is used as indicator. The end point is sharp and permanent.

This technique has been used on serum samples as small as 50 microliters, and work is continuing on the adaptation of the method to still smaller aliquots. The precision and accuracy of the method compare favorably with those of the conventional macrotechniques of clinical chemistry.

DETERMINATION OF SERUM AND URINE CALCIUM BY FLAME SPECTROPHOTOMETRY USING A PHOTOMULTIPLIER TUBE. Alfred D. Winer and Dwight M. Kuhns, Laboratory Service, Walter Reed Army Hospital, Walter Reed Army Medical Center, Washington, D.C.

The applicability of flame spectrophotometry employing a 1P28 photo-multiplier tube attachment for the Beckman DU spectrophotometer has been studied for the determination of calcium in 0.5 ml. of serum or urine and a simple routine clinical method developed. The radiation intensity of the atomic spectral line of calcium at 422.7 mu was measured on diluted serum or urine (1/10) which was atomized directly into the hydrogen-acetylene flame. The increase of radiation intensity of the Beckman instrument using the photomultiplier tube was approximately 10 times that of the wave length employed. The spread obtained for calcium was in the order of 1 transmission division per 0.06 meq. per liter of calcium.

The time required for a single calcium determination was less than 5 minutes, with the average laboratory technician capable of performing 25 determinations in 1 hour.



Comparison determinations of calcium were evaluated and agreed well with those obtained by the Roe-Kahn, Clark and Collip, and gasometric determination of calcium oxalate methods.

EFFECT OF ORGANIC SOLVENTS ON THE EMISSION SPECTRA OF CATIONS IN SERUM AND AQUEOUS SOLUTIONS. George R. Kingsley and Roscoe R. Schaffert, Clinical Biochemistry Laboratory, Laboratory Service, Veterans Administration Center, Los Angeles, Calif.

An investigation of the enchancing effect of organic solvents on the emission spectra of sodium and potassium has been extended to other solvents and cations. The emission spectra of calcium in serum and aqueous solutions are greatly increased by the addition of acetone and acetic acid. The use of this solvent mixture with a photomultiplier attachment — Beckman spectrophotometer acetylene — oxygen burner assembly, produces a very strong calcium spectrum at 422.7 mu light transmission of sufficient intensity to make spectral measurements with slit widths of 0.01 mm. This light transmission and slit width permit direct calcium determinations on 0.2 ml. of serum in 10 ml. of solvent without interference from sodium, potassium, magnesium, or proteins which are present in the serum. The effects of other solvents such as dioxane, methyl Cellosolve, butyl Cellusolve, methanol, ethyl alcohol, propyl alcohol, isopropyl alcohol, etc., on the emission spectra of calcium or other cations have been investigated.

*(continued on page 16)*

## ABSTRACTS OF PAPERS

QUANTITATIVE ESTIMATION OF SERUM BILIRUBIN WITH THE ACID IRON REAGENT. Bennie Zak, Albert Zlatkis, and Albert J. Boyle, Departments of Pathology, Chemistry and Medicine, Wayne University, Detroit, Mich.

The use of a sulfuric acid-iron reagent added to serum diluted with phosphoric acid converts bilirubin to a green substance, presumably biliverdin. The resultant color has a linear relationship to bilirubin concentration and may be used for its quantitative estimation. The presence of $\Delta^5$ steroids does not interfere with the color production nor do they lend any measurable color to a mixture of acid iron reagent, phosphoric acid, and serum. This is due in part to the phosphoric acid and to the relatively large amount of water present. The sensitivity is comparable to the diazo reaction for total bilirubin and may be done directly on the serum sample. Color development is complete within 3 minutes.

RAPID TITRIMETRIC METHOD OF THE ESTIMATION OF SERUM LIPASE. Kenneth D. Johnson and Herbert I. Harder.

A technique for the estimation of serum lipase, based on the continuous titration of the acids liberated from a fatty substrate, is described. The combination of glass electrode pH meter, magnetic stirring, and a syringe-type microbureto yields a compact unit suitable for routine use in the clinical chemistry laboratory. This method permits the estimation of se.um lipase levels within a period of less than half an hour.

The unbuffered substrate is adjusted to the desired pH and temperature, and the rate of alkali addition required to compensate for nonenzymatic hydrolysis of the substrate and the absorption of atmospheric carbon-dioxide is determined. This rate constitutes the blank. The serum is then added to this system, alkali added to readjust the pH, and the rate of alkali addition required to maintain the pH constant redetermined. The excess of this rate over that of the blank is a measure of the lipase concentration in the serum.

Data are presented on the influence of substrate composition, pH, and temperature on the enzyme activity as determined by this technique. Normal values and data on the stability of lipase in serum are also given.

## CORRECTION PLEASE

We wish to correct two errors that appeared in our book review column in our last issue, February, 1953. The author of CONDENSED REVIEW OF PHARMACY, published by John Wiley and Sons, Inc., is George W. Furo. The editor of the volume, SYMPOSIUM ON RADIOBIOLOGY, published by John Wiley and Sons, Inc., is James J. Nickson.

## SOUTHERN CALIFORNIA SECTION PARTICIPATES IN ACS MEETING

The Southern California Section of the AACC participated in the 123rd National Meeting of the American Chemical Society, Los Angeles. On March 16 the local section, along with visiting AACC members, met for lunch with the ACS Division of Biological Chemistry. Dr. R. H. Barnes and Dr. Otto Schales, chairman and secretary-treasurer respectively of the Division, and Dr. R. J. Henry, local AACC chairman, each spoke briefly, all joining in the sentiment that the get-together between the two groups established a worthwhile precedent that should be carried through in all future national ACS meetings. That afternoon five local members delivered papers before the symposium on clinical chemistry, sponsored by the ACS Division of Biological Chemistry.

## NATIONAL ASSOCIATION OF CLINICAL LABORATORIES CONVENTION – MAY 15-17

The National Association of Clinical Laboratories will hold its Fifth Annual Convention at the Hotel Traymore, Atlantic City, N.J., May 15-17.

The three day meeting will feature an all day scientific session under the general heading "Ways and Means of Improving Performance of Routine Laboratory Tests." This symposium will be held on May 16. The morning session speakers will discuss topics concerned with the management and supervision of laboratories. The afternoon session will be devoted to scientific topics dealing with clinical chemistry, haematology, bacteriology and parasitology.

### NEW MEMBERS ELECTED BY THE EXECUTIVE COMMITTEE

| | |
|---|---|
| Richard A. Bonofiglio | Worchester, Mass. |
| Alice L. Kittinger | Long Beach, Calif. |
| Louise Wells | Kansas City, Mo. |
| Marjorie Knowlton | Washington, D.C. |
| Richard S. Wayne | Brooklyn, N.Y. |
| Olive E. McElroy | Silver Spring, Md. |
| Albert F. Lingle, Jr. | Takoma Park, Md. |
| Daniel J. Zeloyle, Jr. | New Kensington, Pa. |
| Roy L. Mundy, | Silver Spring, Md. |
| Cecilia W. Spearing | Washington, D.C. |
| Jerry Thompson | Washington, D.C. |
| Chiadao Chen | Chicago, Ill. |

## NEW BOOKS

**STATISTICAL METHODS FOR CHEMICAL EXPERIMENTATION.. W.L. Gore, xi + 210 pages. Interscience Publishers, Inc. 250 Fifth Avenue, New York 1, N.Y. $3.50.**

This is the first of a series of volumes designed as reliable tools of laboratory procedures. The emphasis is placed upon techniques rather than theory and are geared to provide sound guidance for those not conversant with the full scope of the scientific development.

W.L. Gore has presented a collection of statistical methods most commonly employed in a well run analytical laboratory. No attempt has been made to show the origin of mathematical relationships of the formulae used. The basic assumptions and approximations are given and the assay of the utility and limitations of the various techniques are shown.

The chapters are concise and each concept is illustrated with data from a typical experiment. Of interest is the chapter, Design Of Experiments, in which the importance of the statistical aspect of experimental design is shown to yield to the experimentor the most constructive information.

Certain statistical tables are included in the appendix as well as a list of formulae used in the volume. The bibliography is something new. The author enumerates the modern work in the field with his comments so that the reader caring to go further into the field is given an idea of the scope of the work.

**NON-AQUEOUS SOLVENTS (Application As Media For Chemical Reactions) Ludwig F. Audrieth and Jacob Kleinberg xii + 284 pages. John Wiley and Sons, Inc., 440 Fourth Avenue, N.Y. 16, N.Y. $6.75**

The authors present the use of non-aqueous solvents for carrying out both inorganic and organic reactions. They discuss the physical characteristics of the various solvents and show what properties determine their usefulness.

Liquid ammonia chemistry is covered in four chapters. Other solvent groups discussed are the nitrogen-containing solvents, acetic acid, sulfuric acid, hydrogen fluoride, liquid sulfur dioxide, acid chlorides, halogens and interhalogens, and high temperature systems. Modern concepts of acids and bases are presented in the light of the new systems.

## NEW MAILING ADDRESS FOR NATIONAL SECRETARY

Dr. Max M. Friedman, National Secretary of the AACC, announces that after May 1, 1953 the new address of the National Secretary will be

**DR. MAX M. FRIEDMAN**
**LEBANON HOSPITAL**
**NEW YORK 57, N.Y.**

# ELECTROMIGRATION IN STABILIZED ELECTROLYTES

## PART 1; THE DEVELOPMENT OF THE TECHNIQUE

*by*

**H. J. McDonald, E. P. Marbach, and M. C. Urbin,**

*The Graduate School and Stritch School of Medicine,*
*Loyola University, Chicago.*

The effectiveness of the relatively new technique, whose development is to be described here, depends on the electromigration of charged particles, on wet surfaces of filter paper or similar materials. The extent of movement of a single migrant, or the separation of a mixture of migrants, is determined by such procedures as developing colored derivatives, using light-absorptive characteristics or incorporating radioactive tracers. It lends itself equally well to the study of electromigration of ions and colloidal materials, and requires smaller amounts of material and at lower concentrations than can be investigated by other comparable methods. It has been especially helpful in the fractionation, absolute separation, and identification of a wide range of complex systems, including proteins, peptides, amino acids, enzymes, hormones, inorganic ions, etc.

Despite the development of the moving-boundary type of electrophoresis apparatus to the point where it became a practical tool for studying high molecular weight charged substances, and their naturally occuring mixtures such as blood plasma proteins, (1,7) it failed to satisfy all needs. The absolute separation of the constituents of a mixture, and the handling of small ions, for instance, were not very practicable on the moving-boundary apparatus. The microscopic method for studying the electrophoretic behavior of charged particles in a liquid is even more limited in application (7). Because of this situation, various workers have investigated electromigration through electrolytes stabilized with substances such as agar (15, 26, 33, 47, 71), ground glass wool (13), cotton (86), cotton gauze (100), asbestos fiber (9), silica gel (11, 12, 26), starch (40, 41), and cation exchange resins (84). In these techniques, (except for the last named, which is a rather specialized one) the preparation of the stabilized column is usually time consuming, the removal of separated fractions of such materials as protein hydrolysates is not easy, and a large element of chromatography is inseparably bound-up with the electrophoresis. Ingenious combinations of chromatography and electrophoresis have, in fact, been developed (6, 16, 88, 90), and are often very useful in the resolution of complex mixtures, but are, of course, of little value in determining such properties of substances as, for example, the isoelectric point of proteins or amino acids (55, 59, 60, 81) which are determined from mobility data. In fact it might be said that if electromigration through stabilized electrolytes is to be utilized for any purpose involving mobilities of the migrant, every effort should be made to reduce chromatographic interference to a minimum. How this objective is accomplished, using paper and similar materials in sheet or strip form, to stabilize the electrolyte, will be made evident later in this article. The use of paper strips or sheets, saturated with buffer solutions, as a medium for electrophoretic separations, was developed independently and almost contemporaneously by a number of investigators in different countries. Wieland and Fischer, in Germany, seem to have published the first account in 1948 (102). In 1950, reports on the subject of electrophoresis on paper were also published by Cremer and Tiselius (14) in Sweden; by Durrum (17), by Kraus and Smith (39), and by McDonald, Urbin, and Williamson (57) in the United States; by Biserte (4), in France; by Grassmann and Hannig (28), and by Turba and Enenkel (95) in Germany. In 1951, an even larger number of papers on the technique appeared (16, 18, 21, 24, 29, 32, 37, 42, 46, 48, 49, 55, 58, 59, 60, 61, 62, 63, 66, 72, 76, 77, 78, 79, 81, 83, 88, 89, 94, 99) and the number of contributions reporting on various applications, modifications and limitations of the technique, have shown a steady growth since that time.

### Types of Apparatus

As mentioned above many variations in the technique of carrying out separation processes due to differences in electromigration rates have been developed, in attempts to control variables inherent in the process. The separation of charged particles has been carried out in tubes (15, 33, 71) or columns (9, 13, 86) filled or packed with various substances, in slabs of gel (12, 26, 27, 47), in sheets or strips of filter paper and similar materials (4, 14, 16, 17, 28, 39, 57, 95, 102). Where paper has been used, it has been suspended between solutions in a closed vessel (61), hung over a rod between the solutions (17, 18, 20a, 21, 25), stretched horizontally in a closed vessel (24, 52, 59, 75) or compressed between plates of glass (42, 38, 88) or plastic (88). In order to control the temperature, the moist paper has been immersed in a vessel containing chlorobenzene (14), it has been sandwiched between glass plates and laid on a metal block (39), or placed in a cold room (42), and it has been suspended in a water saturated hydrogen or helium atmosphere in a closed double-walled vessel through which liquid from a thermostat was circulated (52, 59). All these variations in technique might be included as separate items in a scheme of classification, but it was decided, for the sake of simplicity, to base the classification (where a single potential gradient served as the driving force) on the method by which the paper strips were held in place in the apparatus, and to discuss mainly the earlier known papers in each case.

### Single Potential Gradient Serves as the Driving Force

#### Simple Catenary Suspension

The apparatus of Wieland and Fischer (102) consisted of a strip of filter paper, 1 cm X 20 cm, which was suspended between two glass plates 5 cm X 20 cm so as to close off the strip from the atmosphere and retard evaporation. The paper strip was soaked in buffer solution and the two ends allowed to dip into Petri dishes, containing more of the same buffer solution, and the electrodes. To begin operations, a drop of amino acid solution was applied to the paper, and the electrodes connected to a 110 volt potential source. Using an acetate buffer solution (0.1M) at pH 3.7, aspartic acid could be separated from glutamic acid; alanine, glutamic acid and histidine were separated in 1 hour's time. At a pH of 7.0 histidine could be separated from lysine. The strips were dried at 110°C, treated with acetone containing 10% formamide, dried again and then dipped into a solution of copper acetylacetonate in chloroform. On rising through the strip, this solution converted the amino acids into copper acetylacetone complexes. Excess of the reagent was removed by washing with chloroform. The amino acid spots were then made visible by spraying with an acetone solution of rubeanic acid which converted the areas with copper present into dark spots. While suspension of the paper strip or sheet as a simple catenary is satisfactory for some empirical separations of mixtures, it does not lead to linear movement of the migrant with time (52, 58, 59) and hence does not permit of mobility determinations.

#### Ridgepole Suspension

Durrum (17) constructed an apparatus consisting of two 150 ml tumblers, covered with lucite plates which sealed their tops and supported an inverted L-shaped glass

rod. The horizontal portion of the glass rod served to support the apex of the filter paper strips, which were draped symmetrically over it, with the ends of the strip passing through slots in the Lucite covers and extending into the buffer solution in the tumblers below. A third tumbler was inverted to cover the paper draped over the glass rod and a spot of test solution was applied to the paper at the apex. A potential of 300 to 600 volts was applied across the carbon electrodes inserted directly into the tumblers containing the buffer solution. After about 3 hours, the components of the test solution were located on the dried strip. Amino acids and peptides were detected by spraying with a ninhydrin solution, proteins by spraying with a solution of bromphenol blue, and radioactive iodine by means of a radioautographic technique. Using this apparatus, Durrum analyzed mixtures of amino acids, separated peptides from one another, and fractionated serum proteins. When the the height of the rod was kept constant, reproducible fractionations could be obtained. However, Durrum found that he was not able to obtain linearity of movement with time for a test substance, and hence this design of apparatus is not suitable for mobility determinations.

Flynn and de Mayo (21) have described a modification of Durrum's apparatus, with which they have been able to obtain some very good blood plasma protein separations. Gordon and co-workers have described (25) another modification of the apparatus.

### Horizontal Paper Strips in a Closed System

McDonald and co-workers have described an apparatus (52, 53, 59, 60), in which several paper strips or a paper sheet are held, in a taut horizontal position, in a removable frame, which in turn is inclosed in a double-walled chamber. Liquid can be circulated through the walls of the chamber to maintain constant temperature in the interior. The platinum electrodes and electrode vessels are separated by means of agar salt-bridges, from the buffer vessels into which the ends of the paper strip dip, thus protecting the migrating material from unfavorable pH changes due to accumulation of electrode products. To be suitable for mobility studies the section of the filter paper through which the migrant moves must be reasonably uniform with respect to water sheath, ionic composition, potential gradient and temperature. A criterion of sufficient uniformity, as regards these factors, for the purpose in mind, is afforded by the constancy of the reading on the milliammeter in series with the paper strips, and by the fact that the migrant moves linearly with respect to time. The use of helium to help dissipate the heat developed in the paper strips was found helpful in achieving these objectives.

Experience has shown that it is of prime importance in mobility work to have the region of the paper through which electromigration rates are being measured, held horizontally, and that the level of the liquid in all the vessels within the chamber be identical. When the paper strips were allowed to sag, nonlinearity of movement with time was observed (52). Durrum (17) has reported that he was not able to obtain linearity of movement with time, in experiments where the center portion of the paper strip was elevated above the level of the ends of the paper. The reason for establishing a uniform and constant liquid level in all the inside vessels is to prevent capillary siphoning from one vessel to another through the paper strips, with its concomitant chromatographic interference. Uniformity of liquid level was achieved by maintaining throughout the course of a run a small-bore siphon in place between the buffer vessels into which the ends of the paper strips dipped.

Goa (24) used an arrangement which in many ways resembles that described above, wherein the paper strip lies flat on a horizontal glass plate. A second glass plate, which is placed over, but not touching the paper, is separated from the base plate by long narrow glass strips. An apparatus very much like that used by Goa, has been employed by Robbins and Rall (75). Michl (65) has developed an apparatus with many features in common with that used in the author's laboratory, and Schulz and Wegner (80) have described a modified version of the apparatus.

### Paper Strips Clamped Between Glass Plates

Cremer and Tiselius (14) described an arrangement in which the paper strip, immersed in buffer and containing the specimen to be analyzed, was placed between glass plates, immersed in a solution of chlorobenzene and connected to electrode vessels. The chlorobenzene retarded evaporation from the paper and acted as a partial cooling agent. While reasonably good separations could be made, the apparatus was certainly not convenient to use.

Kunkel and Tiselius (42) have reported on a modification of the apparatus, in which the use of chlorobenzene was discontinued. The paper sheet or sheets were clamped between glass plates and the edges sealed with silicone grease, thus retarding evaporation. The two ends of the filter paper dipped into buffer solution contained in the electrode vessels, on which the glass plates rested. To reduce pH changes at the ends of the filter paper due to the products of the electrode reactions, the principal measure taken was simply to enlarge the electrode vessels themselves until they held sufficient buffer solution to offset pH changes in a normal run. A rubber tube connecting the electrode vessels could be opened to balance the liquid levels. The polysaccharide, dextran, which has a very low electromigration rate in free-solution electrophoresis, was used in an effort to approximate the extent of electroosmotic flow. The authors also apply another correction factor to their observed electromigration rates to correct them for "increased migration path length". Experimental evidence will be given in Part II of this series which makes questionable the validity of these correction factors. The apparatus was used to fractionate several artificial mixtures of purified proteins, and serum proteins. Curves were obtained showing the same five major peaks for normal serum as found by the classical moving-boundary method. With proper precautions, the apparatus can be used both for separation process (38,42) and mobility determinations (42).

While the technique of clamping the paper sheets between glass plates would seem to offer certain advantages over the previously described methods, as far as evaporation from the paper is concerned, other complicating factors are introduced which in many cases more than offset the gains. For example, the factor of electroosmosis between the glass and paper surfaces, the interfering surface tension and capillary effects, and the difficulty of preventing a loss of the migrant on the glass surface may all be mentioned. Flynn and de Mayo (21) tried, then discarded, the technique of holding the paper between glass sheets. They found that suspending the strips in a moist atmosphere was preferable from the standpoint of experimental results.

More recently, Robbins and Rall (75) described a modified form of the Kunkel and Tiselius apparatus. They raised the upper glass plate about 0.5 cm. above the paper, and sealed the sides of the glass plates with masking tape. The paper sheet itself was pressed flat on the lower glass with a roller.

## Driving Force: Potential Gradient Combined with Gravitational and Magnetic Fields or with Another Potential Gradient

### Combinations of Electromigration and Chromatography

A number of workers have reported on combinations of electromigration and chromatography. Discontinuous separations on paper were described by Haugaard and Kroner (31). Svensson and Brattsten (90) utilized a cell filled with small glass beads, with solvent flowing continuously, for the separation of dyes.

Strain and Sullivan (88) described an arrangement for the separation of inorganic ions on paper, held between vertically-arranged glass sheets, with simultaneous downward flow of solvent, horizontal electrophoresis, and continuous sample collection. Durrum (17) has described a somewhat similar system, using free hanging paper rather than paper between glass plates. Brattsten and Nilsson (6) placed the sheets of filter paper between glass plates, and cooled the apparatus with circulating water.

They separated a mixture of glycine, alanine, and glycylglycine in an acid medium, and a mixture of serum albumin and gamma globulin in neutral solution, as a continuous process.

**Simultaneous Crossed-Currents**

The technique of electromigration in stabilized electrolytes has heretofore been used exclusively by imposing a potential in one direction at a time. Recently, McDonald and Urbin have described an arrangement (56) in which simultaneous crossed-currents are used. Two glass plates (20 cm X 20 cm) are fitted about the edges with a rubber gasket, and then they are brought together so as to form an enclosure. Within the inclosure is suspended horizontally, a sheet of (E. & D. 613) filter paper (20 cm X 20 Cm) which has been immersed in a suitable buffer and allowed to drain on an absorbent paper. On each of the four sides of the glass there is placed a buffer vessel which is connected to the filter paper sheet by means of filter paper tabs (2.5 cm X 7.5 cm) which overlap the square by about 1.5 cm. The four vessels are then connected to individual electrode vessels by means of agar salt-bridges. An equal potential is imposed in both directions at 90 degrees to each other, so that the migrants move at a 45 degree angle, with respect to the sides of the paper square.

Twelve amino acids and five dinitrophenyl amino acids were studied in this manner. The amino acids were chosen because of the closeness of their isoelectric properties, their difference in molecular weight and difference in structure. They were alanine, glycine, hydroxyproline, isoleucine, leucine, methionine, phenylalanine, proline, serine, tryptophane, tyrosine, and valine. The dinitrophenyl amino acids were derivatives of alanine, glycine, methionine, proline, and tryptophane. For the amino acids, 0.01 N HCl, pH 2.2 was used as the buffer, with a potential of 200 volts for 3 hours. The dinitrophenyl derivatives were run in veronal buffer, pH 8.3, at 200 volts for two hours. When the movements of the amino acids or their derivatives were plotted against their respective molecular weights a linear relationship was noted. Such was not the case when the same substances were run under the influence of a single potential gradient. It would appear from these results that the method offers, with certain restrictions, a new way of determining molecular weights at least for amino acids and some of their derivatives.

**Superimposed Electric and Magnetic Fields**

In the authors' laboratory attempts have been made to utilize combined electric and magnetic fields for the separation of ions. A magnetron magnet (4869 gauss), was fitted with pole face plates, 4.4 cm in diameter, in such a way that the gap between the pole face plates was about 0.64 cm. Two standard 3X4" lantern slide glasses were fitted with a thin rubber gasket around their edges, and a paper strip moistened with a suitable buffer solution was suspended horizontally between them. The ends of the paper strip dipped into suitable buffer vessels which in turn, were connected by small agar salt-bridges to electrode vessels and electrodes. The magnet was mounted in such a way that its pole faces were parallel to the plane of the paper strip. The behavior of several migrants, including amino acids, dinitrophenyl derivatives of amino acids, and proteins was investigated in the combined electric and magnetic field.

It might be expected from *a priori* considerations that an ion undergoing electromigration would swerve from its normal linear course under the influence of the magnetic field. For a given ion, the direction of the swerve, whether to the right or the left, should be found to reverse on reversing the position of the pole faces. Actually, no definite influence of the magnetic field could be observed in the case of the amino acids and proteins which were studied, — a result which is in agreement with the thoughts expressed by English (20). However, in the case of the dinitrophenyl derivatives of amino acids, for example, N — (3,5 - dinitrophenyl) — glycine, which attain unusually high electromigration velocities as compared to most other substances under similar conditions, a definite influence of the magnetic field was observed. The dinitrophenyl compounds, due to their intense yellow color, could be observed visually. Due, apparently, to an insufficiently powerful magnetic field, the direction and amount of swerve of the migrants' electromigration path was not sufficiently large and reproducible enough to justify publication of the results. The method, however, merits further study with magnetic fields of much greater power and with horizontal pole faces of much greater area. As these seem to lie beyond the financial resources of this laboratory, the problem remains to be investigated elsewhere. Kolin (36a) has recently described an arrangement in which electrically neutral particles migrate in a magnetic field traversed by an electric current.

**Terminology**

As mentioned earlier, the technique of resolving mixtures of compounds based on the relative differences in their electromigrations rates on wet surfaces of filter paper and similar materials was developed independently in several laboratories. It is only natural, then, that several different terms should have been suggested to describe the operation. In their first paper describing the technique, which to their knowledge at the time had not been described before, McDonald, Urbin, and Williamson suggested the term "ionography" (meaning, literally, "the record of a wanderer") as a convenient and useful one (57). The term is a convenient one, especially when used in conjunction with the terms "ionograph" to describe the apparatus,* and "ionogram" to describe the paper ribbon or sheet on which the migrant has been fractionated into discrete zones or areas.

Other terms which have been used to describe essentially the same process, include (micro) paper electrophoresis (17, 21, 42), (micro) paper ionophoresis (17) electrochromatography (88), zone electrophoresis (94), electrochromatophoresis (100) electromigration (52, 84). The term "paper electrophoresis" can hardly be applied to cover the use of woven glass—fiber ribbon, nylon, and other similar materials which have been used in the authors' laboratory as supports for the buffer column. It has been suggested (50) that the term "ionophoresis" be used to describe the movement in an electric field of ions, as opposed to the term "electrophoresis" for the movement of particles of colloid size. On this basis, the use of any term incorporating the word "electrophoresis" to describe the technique of electromigration in stabilized electrolytes would seem to place an undue restriction on the general applicability of the method, since *both* ions and particles of colloid size can be handled with equal ease.

The terms "zone electrophoresis" or "zone ionophoresis" would seem relatively satisfactory as general terms if in the light of the remarks made above, due regard is given to the ionic or colloidal nature of a migrant in a given instance, but they would again seem to present a conflict with the established usage of the terms "electrophoresis" and "ionophoresis" (50). They also fail to distinguish between electromigration from a specific wide zone of solution, as in the moving boundary method, and electromigration from a narrow zone of solution in another fixed solution, as exists in stabilized electrolytes.

Electrochromatography probably describes fairly well much of the work which has been reported in the literature, where no special attention was paid to eliminating chromatographic interference. It also is an apt term to describe arrangements where electromigration and chromatography are deliberately combined to give discontinuous or continuous separations of mixtures (6, 16, 88, 90). However, it seems hardly a descriptive term for those arrangements where every effort has been made to reduce chromatographic interference to a minimum, and where the separation of components results almost solely from a difference in electromigration rates.

Electromigration in stabilized electrolytes is a perfectly general way of describing processes in which the chromatographic element has been largely eliminated. However, no convenient related derived terms for the apparatus or the developed paper strip are apparent, in contrast to ionography, ionograph and ionogram.

**Available from Precision Scientific Co., 3737 Cortland St., Chicago 47, Illinois.*

# ELECTROMIGRATION IN STABILIZED ELECTROLYTES

Despite the superficial resemblance of ionography to paper chromatography, a careful distinction should be made between the two separation processes (52, 57, 59). In chromatography, separation of solution constituents depends on a distribution between a mobile and a less-mobile or non-mobile phase. In ionography, separation results from the migration of ions or charged particles at different rates, in an electrical field, rather than from distribution equilibria, adsorption-desorption effects or counter-current exchange.

### Some Details of Ionography

Schematic diagrams, representing the development of the ionographic apparatus as used in the authors' laboratory, have been published elsewhere (52, 53, 57, 59). A few aspects of the apparatus and experimental details will bear re-emphasis. The apparatus is easily modified to make possible the carrying out of separations by most of the various electromigration methods so far suggested in the literature, thus permitting a comparison of results. For example, glass plates on one or both sides of the paper strips or sheets may be supported conveniently on the removable frame, if a need for carrying out the experiments in this fashion should arise. Two dimensional experiments are easily carried out, since the end portions of the paper-holding frame can be moved freely along the side bars so as to accommodate, for instance, a square sheet of paper. A small quantity of the migrant mixture is then applied at one corner of the sheet. It may then be partially separated by one-way electromigration using a buffer of low ionic strength and definite pH. After some definite time, the paper is rotated through 90 degrees, dried quickly in an oven, and set back in the ionograph, using a buffer of the same (or different) kind, but which is usually made up to a greater ionic strength, and either the same or different pH.

In the authors' laboratory, the solution (5-10 microliters, usually) of the migrant (for amino acids and proteins, 3-5 mg per ml, usually) is applied to the paper strip with a calibrated micropipet as a thin streak (0.5 mm or less) across the full width of the paper ribbon. A potential of 10 volts per cm is ordinarily used. Eaton and Dikeman paper, No. 613, 8 mm in width which was chosen for its reasonably good uniformity and high wet-strength, has been used more than any other, although papers similar to Munktell 20 and Whatman No. 3 have been found particularly good for the simple separation of proteins. The runs are ordinarily made at either 1-4° C., or at room temperature. For mobility work, the buffer ionic strengths are generally 0.05 or less, while for empirical separations of mixtures much higher ionic strength buffers may be used, and indeed, seem to give sharper separation of the individual zones.

### Location and Identification of Separated Fractions

To determine the location of the various zones occupied by the individual components of a mixture after electromigration for a suitable time, all the specialized techniques developed in the field of paper chromatography may be employed (2, 5, 87). For example, the presence of amino acids on the ionogram may be detected by spraying with a solution of 0.1 percent ninhydrin in butanol (52), proteins may be detected by one of the modifications of the bromophenol reagent (14, 17, 52, 53, 96) or by the use of amido black 10B, (29, 74), zones containing lipid may be colored by means of a semi-saturated solution of Sudan Black in 50 percent alcohol (91), by oil red O (19) or by Sudan III (20a), etc. Radioactive elements, or compounds containing them, may be located by the usual radio-autographic techniques (2, 17, 43, 103).

To estimate the relative amounts of a given substance in a particular zone, at least two methods are available. (1) The strip may be cut into sections, the colored material eluted with suitable solvents, and its intensity determined in a standard spectrophotometer fitted with small cuvettes (14, 21, 24, 38, 91). On the other hand, a key element in the various fractions for example nitrogen, may be determined by microkjeldahl and the concentrations of the original components computed. Similar methods rely on the absorption of ultraviolet light, determination of a particular amino acid, etc. (b) The distribution pattern of various substances on a strip can be determined directly by a transmission densitometer, (29, 64, 69, 91, 93, 97). In this procedure, the paper is usually treated with some material to render it translucent, for example, bromonaphthaline dissolved in paraffin oil to give a solution having a refractive index of 1.51 (29), or one of the patented transparentizers, e.g. Keuffel & Esser's "Translux". It is then passed through the densitometer by some geared device which moves the strip forward a mm or so at a time. The impulses from the photoelectric cell then go to a sensitive galvanometer whose deflexions are read.

More recently, in the authors' laboratory, a variable wave-length densitometer, (ultraviolet up through the visible) with a motor-driven feed for the paper strip, was hooked up with a Brown strip-chart recorder. This arrangement permits an automatic recording of the zones on an ionogram. In many cases it has been found unnecessary to transparentize the strip first, and if the material in the zones absorbs in the ultraviolet, no prior dyeing of the strip is necessary.

When a staining process is used for blood plasma proteins, the proteins will continue to take up dye for some time and the rates of dye adsorption are not the same for the various protein fractions (21, 38, 91). Strips stained for 15 minutes may adsorb up to twice as much dye as those stained for 5, and the increase in uptake of dye affects the albumin fraction to a greater extent proportionately than the globulins (21). All these factors must be given due consideration.

### Comparison of Schlieren Patterns from the Moving Boundary Apparatus and Densitometer-Based Curves from Paper-Strips

The patterns obtained by means of the schlieren method when used in conjunction with the moving boundary apparatus, and those derived from transmission densitometer readings on paper or other similar materials, differ not only in degree but in kind. Due to this intrinsic difference the two patterns cannot be compared directly.

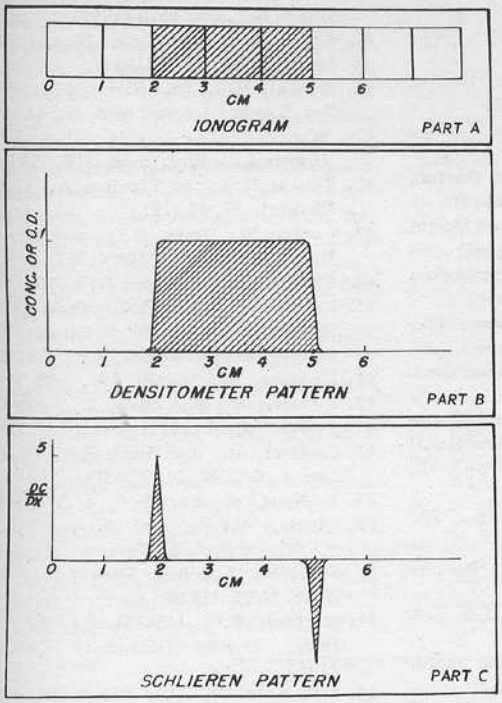
Differentiation of the densitometer curve yields the schlieren pattern. This can be shown as follows; assume the following hypothetical case, a strip of homogeneous paper 1 cm wide to which is applied evenly 1 mg of dye per 1 $cm^2$ over 3 cm of paper length. Figure 1, part A, is a diagram of these conditions. If light of proper wave length passes through a slit 1 $cm^2$, then is allowed to pass through the paper, and finally is picked up by a photoelectric cell, a densitometer curve can readily be obtained. A curve as shown in Fig. 1, part B, will be obtained if optical density is plotted against distance, x, moved along the paper length. Assuming Beer's law to hold optical density can be replaced by concentration. As the light passes through the paper containing no dye, that is, between the 0 and 1 cm mark, the optical density is set to read zero. Now, if the paper is moved 1 cm, so that the light passes through the paper between the 1 and 2 cm mark, the optical density reading will still be zero because no dye is present. However, when the paper is moved forward another distance of 1 cm, that is, so light passes through it between the 2 and 3 cm mark, the dye will absorb a quantity of light. Assume, for convenience's sake, that the constant in Beer's law is 1, when the units of concentration for the dye are mg per cm length of paper. Then the optical density reading will be 1. The same reading will be obtained over the next two cm because the same amount of dye is present. However, when the light passes through the paper between the 5 and 6 cm mark, the optical density reading will fall back to zero because no dye is present.

If the curve shown in Fig. 1, part B, is integrated, the area under the curve will be obtained, and it can be shown, as follows, to be proportional to the quantity of dye applied.

$$\text{area} = \int_{2cm}^{5cm} C\,dx = C\,(3cm)$$

$$\text{but } C = \text{mg/cm}$$

$$\text{area} = 3\ \text{mg}$$




**LEGEND TO FIGURES**

**PART A;** A schematic representation of a developed ionogram, which is 1 cm wide, containing dye at a concentration of 1 mg per cm length, over a length of 3 cm of a paper ribbon. The total dye present is therefore 3 mg.

**PART B;** Schematic representation of a densitometer pattern based on the ionogram shown in Part A. The area under the curve, namely 3 $cm^2$, represents the *total amount* of dye applied.

**PART C;** Schematic diagram of a schlieren pattern based on the ionogram in Part A. The area under each separate section of the curve, namely 1 $cm^2$, represents the *concentration* of dye present.

**FIGURE 1**

However, in practice, concentration changes with respect to x, therefore the area cannot be determined practically by intergration but rather it must be obtained either by means of a planimeter or by direct weighing on a analytical balance. In the densitometer curve it can be seen that concentration, C, is plotted against distance. However, if one differentiates the curve, that is, plots $\frac{dC}{dx}$ against the distance, x, (this can be looked upon as plotting the slope of the curve against the distance, x) the classical schlieren pattern is obtained.

Here the area is proportional to concentration. This can be shown as follows:

$$\text{area} = \int \frac{dC}{dx}\,dx = \int dC \cdot \frac{dx}{dx} = \int dC = C$$

Since C usually changes with respect to distance x this expression is very difficult to intergrate. Here again, evaluation of the area is therefore accomplished either by a planimeter, by direct weighing on an analytical balance, or by other practical methods.

From the following considerations one can readily see that areas from the densitometer patterns are proportional to the absolute quantity of material present, while the areas from schlieren patterns are proportional to the concentration of the material. Although the curves from a complex mixture such as blood plasma proteins may, therefore, show a certain correspondence in shape and height, no exact correspondence is to be expected because of the intrinsic, fundamental difference in the nature of the two somewhat similar patterns. This conclusion holds whether the measurements are made directly on the paper strip by means of a transmission densitometer, or are based on cutting the strip into narrow slices, elution of the material, and making use of a spectrophotometer or other device to determine the concentration present originally in the individual slices.

**General Fields of Application**

The general fields of application of the ionographic method may be listed as: (1) Simple fractionation and resolution of mixtures (2) mobility determinations and related quantities (3) reaction-site studies.

The great bulk of the papers so far published on ionography may be included under the first class. For the simple empirical fractionation of complex mixtures it would seem from the reports in the literature, and results in the authors' laboratory, that variations in apparatus design may be almost insignificant. However, even in the case of the simple resolution of the components of a mixture, some standardization of apparatus design is in order, if results from one laboratory are to be compared with those from another.

Where mobility determinations are among the goals of the investigator, certain designs of the apparatus to be used have clear-cut advantages over others. Mobility measurements are of particular significance in the determination of such factors as: (a) the effect of ionic strength of a given electrolyte on the migration rate of the migrant (52, 59) (b) the construction of pH versus mobility curves, for ampholytes such as proteins and amino acids, etc. (42, 54, 55, 59, 81) (c) the effect of temperature on electromigration rates, and the determination of the energy of activation of such processes (52). (d) the determination of isoelectric points of various substances (52, 59, 81). (e) the study of electroosmosis (42, 52, 60, 65) (f) the effect of molecular volume of the migrant versus average pore size of the paper, etc. (85).

Whenever measurement of electromigration mobilities are involved, every effort must be made to reduce the chromatographic factor to a minimum, and to cancel out the effect of the geometry of the apparatus used in making the measurements. A very helpful criterion as to whether the data obtained in any given set of experiments can be utilized for mobility purposes, is to determine whether the movement of the migrant with respect to time and potential, respectively, is linear or not (52, 58, 59). If, at a given potential gradient along the paper strip, of say, 10 volts per cm., a given migrant moves the same distance in the first, second, third, fourth, etc. hours (all other conditions such as ionic strength and pH of the buffer, temperature, etc. being constant) then it must be admitted that the possible influence of the many factors which could theoretically affect the mobility (evaporation from the strip, unevenness of electrolyte distribution, etc.) are reduced to a point where they actually do not influence it.

A similar argument holds for the potential gradient. It should be demonstrated for any given set of experiments, that (with all other conditions being constant), the migrant moves linearly with increase in potential gradient (59). For example, suppose a migrant moves 1 cm. in 1 hour at a potential gradient of 5 volts per cm; then it should be demonstrated that it moves 2 cm in 1 hr. at 10 volt per cm and 3 cm in 1 hr. at 15 volts per cm.

When the two criteria discussed above, are fully met, the particular design or geometry of the apparatus fades out of the picture as far as having any influence on electromigration rate is concerned, and it becomes legitimate to measure electromigration rates in terms of mobilities, that is, in cm/sec per volt/cm. Under the conditions outlined above, it can be said that, if there are non-uniformities in the path or other possible interfering factors, they do not affect the migration velocity of the migrant. Any desired voltage, as well as any convenient length of paper, may also be used, as long as the results are expressed in terms of mobility.

It should also be emphasized that whether or not the electromigration mobilities obtained in stabilized electrolytes agree with those obtained by the more classical so-called free-solution electrophoresis, is not really too important. What is important is that the data obtained from ionographic measurements be reproducible. At the rate at which the technique is being forwarded in various laboratories, it can soon stand

on its own merit as a distinct discipline, without need of reference, at least for corroborative purposes, to other methods of electrophoresis.

A newer aspect of electromigration studies in stabilized media, is to utilize the paper strip as a site on which chemical reactions can be carried out under controlled conditions, and the products of the reaction later separated. Early applications of this idea are found in the work of Kraus and Smith (39), who utilized electromigration on paper to estimate the stability constants of complex ions in solution, in particular, mercuric and iodide ions in chloride solutions. Michl (65) has investigated the reaction of certain carbohydrates in a borax, and, or boric acid solutions, using electromigration on paper strips.

Spitzer (85) has investigated the changed electromigration behavior of PVP under various conditions. In the authors' laboratory, the combining power of various proteins for several metal ions, (for example bovine serum albumin and nickel ion) have been studied.

In the usual case, microquantities of the reactants are placed on the paper strips under pH conditions which will cause the protein, for example, and ionogenic material to migrate in opposite directions, simultaneously. Thus, at same time during the course of their migration the two materials will pass counter-currently through each other's zones, thus making it possible for a reaction to take place. By varying the concentration of the buffer solution and extrapolating to zero ionic strength, it is possible under certain conditions to calculate the amount of binding at zero ionic strength, which in this case, is simply the amount of reaction of protein and reactant. By suitably varying the temperature of the environment in which the reaction is taking place (and this is readily accomplished using the ionograph), it is possible to determine certain chemical kinetic and thermodynamic properties of the system.

**Bibliography**


1. Alberty, R.A., J. Chem. Education *25*, 426, (1948) J. Chem. Education *25*, 619,(1948)
2. Balston, J.N., and Talbot, B.E., "A Guide to Filter Paper and Cellulose Powder Chromatography"; Edited by Jones, T.S.G.. H. Reeve Angel and Co. Ltd., London, and W. and R. Balston Ltd., Maidstone (1952)
3. Bennhold, H., Kallee, E., and Roth, E., Z. Naturforsch. 7b, 324 (1952)
4. Biserte, G., Biochim. et Biophys. Acta *4*, 416 (1950)
5. Block, R.J., LeStrange, R., and Zweig, G., "Paper Chromatography; A Laboratory Manual", Academic Press, Inc., New York, (1952)
6. Brattsten, I., and Nilsson, I., Arkiv. Kemi. *3*, 337 (1951)
7. Briggs, D.R., "Biophysical Research Methods", edited by F.M. Uber, Interscience Publishers, Inc., New York, N.Y., pp. 271-300 (1950)
8. Bucher, T., Matzelt, D., and Pette, D., Klin. Wochschr. *30*, 325 (1952)
9. Butler, J.A.V., and Stephen, J.M.L., Nature *160*, 469 (1947)
10. Choisy, A., Derrien, Y., and Gaetan, J., Compt. Rend. *234*, 1918 (1952)
11. Consden, R., Gordon, A.H., and Martin, A.J.P., Biochem. J. *40*, 33 (1946)
12. Consden, R., Gordon, A.H., and Martin, A.J.P., Biochem. J. *46*, 8 (1950)
13. Coolidge, T.B., J. Biol. Chem., *127*, 551 (1939)
14. Cremer, H., and Tiselius, A., Biochem. Z. *320*, 273 (1950)
15. Davis, W.W., Parke, T.V., and Gamewell, B., Abstracts, *XIIth* International Cong. Pure and App. Chem. New York, Sept., 1951, p. 65
16. Durrum, E.L., J. Am. Chem. Soc., *73*, 4875 (1951)
17. Durrum, E.L., J. Am. Chem. Soc. *72*, 2943 (1950)
18. Durrum, E.L., J. Colloid Sci. *6*, 274 (1951)
19. Durrum, E.L., Paul, M.H., and Smith, E.R.B., Science *116*, 428 (1952)
20. English, A.C., Science *113*, 367 (1951)

20a. Fasoli, A., Lancet *1*, 106 (1952)

21. Flynn, F.V. and de Mayo, P., Lancet *261*, 235 (1951)
22. Ganzin, M., Macheboeuf, M., and Rebeyrotte, P., Bull. Soc. Chim. Biol. XXXIV, 26 (1952)
23. Ganzin, M., and Macheboeuf, M., Bull. Soc. Chim. Biol. XXXIV, 32 (1952)
24. Goa, J., Scand. J. Clin. and Lab. Investigation, *3*, 236 (1951)
25. Gordon, A.H., Gross, J., O'Connor, D., and Pitt-Rivers, R., Nature, *169*, 19, (1952)
26. Gordon, A.H., Keil, B., and Sebesta, K., Nature *164*, 499 (1949)
27. Gordon, A.A., and Reichard, P., Biochem. J. *48*, 569 (1951)
28. Grassmann, W., and Hannig, K., Naturwissenschaften *37*, 496 (1950)
29. Grassmann, W., Hannig, K., and Knedel, M., Deut. Med. Wochschr. *76*, 333 (1951)
30. Gros., P., Dubert, J.M., Sung, S.S., Coursaget, J., and Macheboeuf, M., Bull. Soc. Chim. Biol. XXXIV, 37 (1952)
31. Haugaard, G., and Kroner, T.D., J. Am. Chem. Soc. *70*, 2135 (1948)
32. Kanngiesser, W., Naturwissenschaften, *38*, 503 (1951)
33. Kendall, J., Science *67*, 163 (1928)
34. Kirk, P.L., and Duggan, E.L., Anal. Chem. *24*, 124 (1952)
35. Knedel, M., Med. Monatschr. *5*, 707 (1951)
36. Knedel, M., Arzneimittel-Forsch. *2*, 182 (1952)

36a. Kolin, A., Science *117*, 134 (1953)

37. Koiw, E., Wallenius, G., and Gronwall, A., Bull. Soc. Chim. Biol. *33*, 1940 (1951)
38. Koiw, E. Wallenius, G., and Gronwall, A., Scand. J. Clin. Lab. Invest. *4*, 47 (1952)
39. Kraus, K.A., and Smith, G.W., J. Am. Chem. Soc. *72*, 4329 (1950)
40. Kunkel, H.G., and Slater, R.J., J. Clin. Invest. XXXI, 677 (1952)
41. Kunkel, H.G., and Slater, R.J., Proc. Soc. Exper. Biol. and Med. *80*, 42 (1952)

41a. Kunkel, H.G., Taylor, S.P., and du Vigneaud, J. Biol. Chem. *200*, 559 (1953)

42. Kunkel, H.G., and Tiselius, A., J. Gen. Physiol. *35*, 89 (1951)
43. Larson, F., Deiss, W.P., and Albright, E.C., Science *115*, 626 (1952)
44. Latner, A.L., Biochem. J., *51*, XII (1952)
45. Le Beau, L.J., Ph.D. Thesis, Bacteriology; University of Illinois, Chicago Professional Colleges, (1952)
46. Lederer, M., Nature *167*, 864 (1951)
47. Lederer, M., and Cook, I., Australian J. Sci. *14*, 56 (1951)
48. Lederer, M., and Ward, F.L., Australian J. Sci. *13*, 114 (1951)
49. Lederer, M., Research, *4*, 371 (1951)
50. Martin, A.J.P., and Synge, R.L.M., "Adv. In Protein Chemistry", Vol II, Page 32, Academic Press, Inc., New York, N.Y., (1945)
51. Marbach, E.P., M.S. Thesis, Biochemistry, Loyola University Chicago, (1952)
52. McDonald, H.J., J. Chem. Education *29*, 428 (1952)
53. McDonald, H.J., and Marbach, E.P., J. Biochem. *40*, No. 2, March (1953); in press
54. McDonald, H.J., and Marbach, E.P., J. Am. Chem. Soc. *74*, 1619 (1952)
55. McDonald, H.J., Marbach, E.P., Urbin, M.C., and Williamson, M.B., Fed. Proc. *10*, 218 (1951)
56. McDonald, H.J., and Urbin, M.C., Fed. Proc. *12*, (1953); in press
57. McDonald, H.J., Urbin, M.C., and Williamson, M.B., Science *112*, 227 (1950)
58. McDonald, H.J., Urbin, M.C., and Williamson, M.B., J. Am. Chem. Soc., *73*, 1893 (1951)
59. McDonald, H.J., Urbin, M.C., and Williamson, M.B., J. Colloid Sci. *6*, 236 (1951)
60. McDonald, H.J., Urbin, M.C., and Williamson, M.B., Abstracts, *XIIth* International Cong. Pure and App. Chem., New York, N.Y., Sept., 1951, p. 67
61. Michl, H., Monatsh. *82*, 489 (1951)
62. Michl, H., Riedl, K., and Wessely, F., Monatsh., *82*, 539 (1951)
63. Michl, H., Monatsh., *82*, 944 (1951)
64. Michl, H., Monatsh., *83*, 210 (1952)
65. Michl, H., Monatsh., *83*, 737 (1952)
66. Mills, G.T., and Smith, E.E.B., Biochem. J., Proc. *49*, iv (1951)
67. Natelson, S., Rockford Memorial Hospital, Rockford, Ill., Private Communication, (1952)
68. Ott, H., Huber, H., and Korver, G., Klin. Wochschr. *30*, 34 (1952)
69. Paladini, A.C., and Leloir, L.F., Anal. Chem. *24*, 1024 (1952)
70. Paul, M.H., and Durrum, E.L., J. Am. Chem. Soc., *74*, 4721 (1952)
71. Peniston, Q.P., Agar, H.D., and McCarthy, J.L., Anal. Chem. *23*, 994 (1951)

72. Pluckthun, H., and Gotting, H., Klin. Wochsch., *29*, 415 (1951)
73. Quastel, J.H., and Van Straten, S.F., Proc. Soc. Exper. Biol. and Med., *81*, 6 (1952)
74. Reinhold, J.G., and von Frijtag Drabbe, C.A.J., University of Pennsylvania, Philadelphia, Pa., Private Communication, (1953)
75. Robbins, J., and Hall, J.E., Proc. Soc. Exper. Biol. and Med., *81*, 530 (1952)
75a. Sato, T.R., Diamond, H., Norris, W.P., and Strain, H.H., J. Am. Chem. Soc. *74*, 6154 (1952)
75b. Sato, T.R., Norris, W.P., and Strain, H.H., Anal. Chem. *24*, 776 (1952)
76. Schmid, L., Michl, H., and Zwettler, G., Monatsh. *82*, 526 (1951)
77. Schneider, G., Acta Chem. Scand., *5*, 1020 (1951)
78. Schneider, G., and Wallenius, G., Scand. J. Clin. Lab. Invest. *3*, 145 (1951)
79. Schraub, F., and Alder, A., Schweiz. Med. Wochschr. *81*, 483 (1951)
80. Schulz, D.M., and Wegner, C.J., Lab. Invest. 1, 186 (1952)
81. Schwarz, V., Nature, *167*, 404 (1951)
82. Schweitzer, D.H., and Wittern, A., Arzneimittel-Forsch. *2*, 185 (1952)
83. Smellie, R.M.S., and Davidson, J.N., Biochem. J., Proc. *49*, xv (1951)
84. Spiegler, K.S., and Coryell, C.D., Science *113*, 546 (1951)
85. Spitzer, R.H., M.S. Thesis, Biochemistry, Loyola University, Chicago, 1953
86. Strain, H.H., J. Am. Chem. Soc. *61*, 1291 (1939)
86a. Strain, H.H., Anal. Chem. *24*, 356 (1952)
87. Strain, H.H., and Murphy, G.W., Anal. Chem. *24*, 50 (1952)
88. Strain, H.H., and Sullivan, J.C., Anal. Chem. *23*, 816 (1951)
89. Suzuki, T., and Maekowa, Y., J. Pharm. Soc., Japan, *71*, 1298 (1951)
90. Svensson, H., and Brattsten, I., Arkiv. Kemi Mineral. Geol. 1, 401 (1949)
91. Swahn, B., Scand. J. Clin. Lab. Invest. *4*, 98 (1952)
92. Takeda, K., Otsuka, H., and Kimura, T., J. Pharm. Soc. Japan 72, 1055 (1952)
93. Tennent, D.M., Florey, K., and Whitla, J.B., Anal. Chem. *23*, 1748 (1951)
94. Tisellus, A., Abstracts, *XIIth* Int. Cong. Pure and App. Chem., New York, N.Y., Sept., 1951, p. 67
95. Turba, F., and Enenkel, H.G., Naturwissenschaften, *37*, 93 (1950)
96. Urbin, M.C., M.S. Thesis, Biochemistry, Loyola University, Chicago, 1952
97. Van Os. G.A.J., Biochim. et Biophys. Acto 9, 111 (1952)
98. Vignato, L., Minerva Med., *43*, II, 43 (1952)
99. Wallenfels, T., and Von Piehmann, E., Angew. Chem. *63*, 44 (1951)
100. Weller, G.L., Chromatography Bulletin *1*, 5 (1950)
101. Wieland, T., and Bauer, L., Angew. Chem. *63*, 511 (1951)
102. Wieland, T., and Fischer, E., Naturwissenschaften, *35*, 29 (1948)
103. Wieland, T., Schmeiser, K., Fischer, E., and Maier-Leibnitz. H., Naturwissenschaften, *36*, 280 (1949)
104. Wieland, T., and Wirth, L., Angew. Chem. *62*, 473 (1950)
105. Wunderly, C., Nature *169*, 932 (1952)

## CLINICAL EVALUATION OF HYPERGLOBULINEMIAS

by

**Pietro de Nicola,** *University of Pavia, Department of Internal Medicine, Pavia, Italy*

*The following is a summary of Dr. de Nicola's lecture, delivered before the Southern California Section of the AACC, Hollywood, California, October 7, 1952. The data presented is from original research carried out in association with Prof. Wuhrmann and Dr. Wunderly at the Department of Internal Medicine, University of Zurich, Switzerland.*

During recent years it has been possible to characterize the physiopathological variations of the plasma proteins by means of relatively exact and reliable methods. Electrophoresis can be considered today as one of the most useful methods for clinical evaluation of plasma proteins. By means of electrophoresis, the results of two simple serum reactions, the cadmium and the Weltmann reactions, have been correlated with the levels of the various protein fractions.

The *Weltmann reaction* is based on the heat and calcium sensitivity of diluted plasma. 0.1 ml. aliquots of plasma are placed in a series of 12 or more tubes and diluted to 6.0 ml. with calcium chloride solutions of increasing concentration: (0.03, 0.06, 0.09, 0.12, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, etc. %). The tubes are boiled for 15 minutes and at the end of that time a more or less extended flocculation takes place. For normal plasma the flocculation occurs with calcium chloride concentrations of 0.25-0.30% and above, while lower concentrations do not show any flocculation. In other cases flocculation occurs with minimal amounts of calcium, and this is called a prolonged Weltmann reaction. In other cases it occurs only with calcium chloride concentrations of 0.35% or more, and this is called a shortened Weltmann reaction.

A further evaluation of the Weltmann reaction may be obtained by measuring the turbidity of flocculated samples after filtration and 1:10 dilution with distilled water, by means of a nephelometric procedure. The curves obtained by plotting turbidity against calcium chloride concentration are called *nephelograms* and may vary in the different physiopathological conditions.

The *cadmium reaction* is based on the flocculability of the serum in presence of cadmium sulphate. By adding 0.2 ml. of a 0.4% cadmium sulphate solution to 0.4 ml. of serum, we observe the persistence of a fair transparence in normal serum. In pathological conditions, a more or less pronounced turbidity may take place.

By evaluating these two reactions and the electrophoretic patterns together, a series of *combinations* is obtained:

1) Shortened Weltmann reaction and positive cadmium reaction: increase of the alpha globulins;
2) Shortened Weltmann reaction and negative cadmium reaction: increase of the beta-1 globulins;
3) Normal Weltmann reaction and positive cadmium reaction: increase of the alpha plus gamma globulins or of the beta-2 globulins;
4) Normal Weltmann reaction and negative cadmium reaction: normal globulin values or increase of the beta plus gamma globulins;
5) Prolonged Weltmann reaction and positive cadmium reaction: increase of the gamma globulins;
6) Prolonged Weltmann reaction and negative cadmium reaction: particular protein alterations, generally increase of the the gamma globulins.

This scheme is not exact, but it gives valuable results in a great number of cases. If many protein fractions are increased, the results of the reactions may be determined by predominance of only one of the fractions.

On the basis of the combination of the cadmium and Weltmann reactions and electrophoresis, it is possible to take into consideration the various groups of diseases, in which there are pathological modifications of the plasma proteins.

A group of diseases which present the most impressive alterations of the plasma proteins includes the *myelomata.* These diseases are characterized by an increase of plasma globulins. Wuhrmann and Wunderly subdivided the myelomata into four forms: alpha, beta-1, beta-2 and gamma myelomata. The most typical patterns are represented by the beta and gamma myelomata, in which very sharp and high electrophoretic waves are observed. Such a subdivision not only corresponds to a chemical differentiation, but also to clinical observations. It has

been pointed out that the alpha myelomata correspond to a more acute and severe course of disease, while the gamma myelomata are usually less severe, and the survival is usually longer. Also in the bone marrow the differences are marked: in the myelomata the plasma cells or so-called myeloma cells are much more immature than the gamma myelomata. Conversely in the gamma myelomata the plasma cells present characteristics of maturity.

A number of experiments support the concept that the proteins of the myelomata are different from the normal proteins. First of all, by injecting myeloma globulins in a rabbit, we obtain a serum which contains antibodies against such proteins, but not against the normal proteins nor against proteins of other physiopathological conditions. The ultraviolet absorption curve of the myeloma globulins is quite different from that of normal globulins, probably because of the higher tyrosine and tryptophane content of the myeloma globulins, as compared with the normal globulins.

The form of the electrophoretic wave may be interpreted in connection with the homogeneity of the concerned protein. In our researches the ratio, height-base, of the electrophoretic wave has been evaluated. The higher the ratio, the more homogeneous is the concerned protein. Analysis of a great number of electrophoretic diagrams shows that the behaviour of the myelomata, and especially of the gamma myelomata, is very typical, as compared with other pathological conditions, and shows the presence of a considerably high height-base ratio of the concerned electrophoretic wave. On the other hand, we may have hyperglobulinemias in which the protein content is about the same as in the myelomata, but the wave is very flat and spread out. This happens especially in cases of cirrhosis and agranulocytosis.

The uroproteins in the myelomata have the characteristics of the globulins, at least from the electrophoretic point of view, while the molecular size and the ultraviolet absorption are typical for the albumins.

The *hyperglobulinemias*, which are currently observed, usually belongs to other forms. Generally the various physiopathological conditions produce more or less pronounced modifications of the total proteins of the plasma. The variations of the protein fractions, always or almost always, result in a relative increase of the globulins and in a relative diminution of the albumins, within certain limits, regardless of the total protein amount. As an increase of the albumin fraction is never or almost never observed, practically all physiopathological conditions result in hyperglobulinemias, and for this reason the A/G ratio cannot be considered any longer as a significant expression of the protein alterations. A definite characterization is obtained only if the ratio between albumins and the single globulinsfractions is evaluated.

Some typical disease states were evaluated by means of the combination of reactions. They may be summarized as follows: The usual finding in *carcinoma* is represented by an involvement of the alpha and beta globulins. The liver often causes an increase of the beta fraction, while the presence of necrosis usually shows an increase of the alpha fraction. In *sarcomata* and other connective tissue growths an increase of the gamma globulins is usually observed. In *liver cirrhosis* gamma hyperglobulinemia is quite typical. In *hepatitis* the increase of the beta and gamma globulins is a very usual finding. *Nephrosis* is characterized by a marked increase of the alpha and beta globulins and the decrease of the gamma globulins, in agreement with the increase of the lipid fractions, which migrate together with the alpha and beta globulins, and probably with the decrease of antibody activity in nephrosis. In the *infectious diseases* gamma hyperglobulinemia is generally correlated with the increase of the antibody activity, In some forms, as for instance in tuberculosis, alpha and beta hyperglobulinemias are usually correlated with more or less activity, while gamma hyperglobulinemias are quite typical of the chronic forms.

In nephrosis, in contradistinction to myelomata, proteinuria is chiefly represented by high amounts of albumins. Such data are worth noting as in nephrosis the serum albumin values are usually low.

The hyperglobulinemias associated with *macroglobulinemias* are of pertinent interest, as they are often accompanied by hemorrhagic tendency, as happened in a personal case, documented by ultracentrifugal examinstion. It seems that the increased globulin fraction had, in this and in other similar cases, an inhibiting effect on the thrombin-fibrinogen reaction.

The association of alterations of the bone marrow with hyperglobulinemias, particularly of the gamma type, as happens in many cases of *pancytopenia* and also in other blood diseases, gives another opportunity of studying the formation of the blood proteins in the bone marrow.

To conclude this brief presentation of our investigations, it should be pointed out that the significance of these clinical chemical data must not be overestimated for diagnostic purposes. They must always be used in conjunction with a full clinical evaluation of the patient.

---

# REVIEW OF CURRENT LITERATURE

**ELLENMAE VIERGIVER – EDITOR**
**CECILIA RIEGEL, C. VON FRIJTAG DRABBE, HARRY G. ANRODE**

WATER AND ELECTROLYTE BALANCE IN SURGERY. H.T. Randall (Cornell Univ. Med. Coll., New York, N.Y.). Surg. Clin. No. Amer. 1952,445.

A review with references, of requirements of surgical patients for water, glucose and electrolytes. H.G.A.

MICROMETHOD FOR THE DETERMINATION OF TOTAL LIPIDES IN SERUM. Bengt Swahn (Univ. Lund, Sweden). Scand. J. Clinc. & Lab. Invest. 4:247, 1952

0.02 ml. serum is dried on filter paper, treated with Sudan black and washed with 50% EtOH. The dye is eluted with 25% AcOH in EtOH and the extinction coefficient read at 590 mu. Results compare well with gravimetric procedures and the nature of the lipid constituents do not affect the results. H.G.A.

PAPER ELECTROPHORESIS OF SERUM PROTEINS. B. Levin and V.G. Oberholzer (Queen Elizabeth Hosp. for Children, London). Nature 170:123, 1952.

Kjeldahl N analysis of the bands is substituted for dye elution. Comparison of data obtained in this manner and that obtained by the classical Tiselius technique indicates that the paper method is accurate. H.G.A.

AN IMPORTANT CAUSE OF ERROR IN THE MICRODETERMINATION OF POTASSIUM BY THE COBALTINITRITE METHOD. P. Cristol and M. Caron (Faculte med., Montpellier, France). Bull. soc. chim. biol. 34:228, 1952.

The reactions should be carried out at 5° C. to prevent excessive solubility and decomposition of the precipitate.

PREPARATION AND PROPERTIES OF SERUM AND PLASMA PROTEINS. XXXIV. AN X-RAY STUDY OF CRYSTALLINE HUMAN SERUM ALBUMIN PREPARATIONS. B. A. Low (Harvard Univ.). J. Am. Chem. Soc. 74:4830, 1952

An x-ray study is made of several human serum albumins and some metalloalbumin derivatives. Estimated mol. wts. are of the order of 66,000. Miller indices are given and mol. size, shape and orientation are discussed. H.G.A.

SOURCE OF ERROR IN THE URIC ACID DETERMINATION IN SERUM ACCORDING TO FOLIN. K. Dirr and E. Dietz (Univ. Munich, Germany). Munch. med. Wochschr. 93:2605, 1951

Vitamin C also reduces phosphotungstic acid.

ELECTROLYTES AND CONGESTIVE HEART FAILURE. T.S. Donowski (Univ. of Pittsburgh, Pittsburgh, Pa.). Ann. Internal Med. 37:453, 1952.

A review with 110 references. H.G.A.

PLASMA CHOLINESTERASE ACTIVITY IN LIVER DISEASE: its value as a diagnostic test of liver function compared with flocculation tests and plasma protein determinations. A. Wilson, R.J. Calvert, and H. Geoghegan (Univ. Coll. Hosp. Med. School, London). J. Clin. Invest. 31:815, 1952.

Patients with liver disease show low cholinesterase activity. Normal values may occur in attacks of cholangiohepatitis and in malignant disease. H.G.A.

ELECTROLYTES IN SURGERY. W.H. Cole and J. Laws (Univ. of Ill. Coll. of Med., Chicago). Surg. Clin. No. Amer. 1952,3.

A review of electrolyte deficiencies and replacement therapy in surgical patients. H.G.A.

THE DIFFERENTIAL DIAGNOSIS OF JAUNDICE. H.J. Tumen and E.M. Cohn (Univ. of Pennsylvania, Philadelphia, Pa.) Surg. Clin. No. Amer. 1952, 1637.

A review of clinical and laboratory procedures. H.G.A.

THE CATALYTIC EFFECT OF MOLYBDATE ON THE HYDROLYSIS OF ORGANIC PHOSPHATES. L. Lutwak and J. Sacks (Brookhaven National Laboratory, Upton, N.Y.). J. Biol. Chem. 200:565, 1953

The catalysis of the hydrolysis of organic phosphates by molybdate is accompanied by the formation of molybdate-organic phosphate complexes. The absorption spectra of these complexes are the same as those of reduced phosphomolybdate. Calculations of the kinetics based on the color formation by the addition of Fiske-Subbarow reagents lack significance because part of the color is due to the molybdate-organic phosphate complex. C.vF.D

INTERACTION OF EGG ALBUMIN AND PEPSIN. D.S. Yasnoff and H.B. Bull (North-western Medical School, Chicago, Ill.). J. Biol. Chem. 200:619, 1953

Egg albumin and pepsin when mixed in the pH range 3.1 – 4.2 form a precipitate which is not denatured protein. The optimal ratio of the proteins is 1:1. The amount of precipitate also depends on the salt concentration. The precipitate dissolves at higher salt concentrations. C.vF.D

METHOD FOR THE DETERMINATION OF GASTRIC ACIDITY WITHOUT STOMACH TUBE BY EXAMINATION OF URINE SPECIMENS. W. Maurer and A. Zimmer (Univ. Cologne, Germany). Munch. med. Wochschr. 94:1072, 1952

After oral administration of $Ca^{45}CO_3$, urinary $Ca^{45}$ is determined at intervals. The results correspond to those obtained by intubation, except where psychic factors produce errors in the intubation method.

THE REPRODUCIBILITY AND CONSTANCY OF THE PLATELET COUNT. George Brecher, Marvin Schneiderman and Eugene P. Cronkite. (National Inst. of Health). Am. J. Clin. Path. 23:15, 1953

The error of single direct platelet counts on venous blood collected in siliconed test tubes performed with the phase microscope was 11%, and could be reduced only by doing multiple counts. The error of platelet counts on capillary blood from finger puncture was 24%, and the counts were 2.5% lower. In 13 healthy males platelet levels varied greatly in individuals but was constant in single individuals over a five month period. The authors discuss the errors involved in counts with the phase microscope and in other method. C.R.

CLINICAL VALUE OF SERUM THYROXINE DETERMINATION. V.E. Chesky, W.C. Breese, B.O. Duboczky, W.H. Hall, and C.A. Hellwig. (Hertzler Clinic, Kansas). Am. J. Clin. Path. 23:41, 1953

Thyroxine was determined by a combination of Taurog and Chaikof's extraction method for thyroxine with Barker's ashing and distillation method for protein-bound iodine, the latter modified by the addition of glycocoll to the blank to furnish organic material. 134 patients with thyroid disease and 79 patients without endocrine disorders were studied. Av. values found were: Hyperthyroidism, 6.5; non-toxic goiter, 3:0; hypothroidism, 1.0; euthyroid, 3.0 μg%. There was overlapping between groups. C.R.

METHOD FOR THE MEASUREMENT OF THE ESTERASE ACTIVITY OF BLOOD SERUM. A. Mariani and P.B. Camponovo (National Inst. cardiol., Cordoba). Acta. argentina fisiol. y fisiopathol. 1:683, 1951

Na acetylsalicylate is substituted for Na acetylcholine. The Na salicylate formed is measured colorimetrically.

A SIMPLE, ACCURATE METHOD OF BLOOD pH DETERMINATIONS FOR CLINICAL USE. F.A. Graig, K. Lange, J. Oberman, and S. Carson (New York Med. Coll., New York, N.Y.). Arch. Biochem. Biophys. 38:357, 1952.

INFLUENCE OF SMALL DOSES OF HEPARIN ON FAT TOLERANCE CURVES IN VIVO AND IN VITRO. W.J. Messinger & Y. Porosowska. (Goldwater Memorial Hospital, Welfare Island, New York City). Proc. Exptl. Biol. & Med. 82:164, 1953

By use of the fat tolerance test, it has been demonstrated that small doses of heparin administered intravenously *in vivo* have a clearing effect on the serum of human subjects. The ability to develop this "clearing factor" varied from individual to individual and did not appear to be related to the amount of heparin injected. Ability to clear their own sera *in vitro* after incubation likewise varied from one subject to another. The data show that those subjects who clear well *in vivo* will probably clear well *in vitro*. E. V.

PLASMA LEVELS OF VIOMYCIN IN MAN AFTER SINGLE INTRAMUSCULAR INJECTION. William E. Dye and William F. Bull (Fitzsimmons Army Hosp. Colorado). Am. J. Clin. Path. 23:56, 1953

Of 20 patients with pulmonary tuberculosis 10 received 1.0 gm Viomycin sulfate intramuscularly and 10 received 0.5 gm. Analysis by an agar diffusion assay procedure of blood samples taken at intervals thereafter showed the peak plasma level was obtained 1-2 hours after the drug was given. 19 of 20 patients had a max. plasma level ranging from 22-118 μg per ml, well above the concentration required for in vitro inhibition of virulent strains of tubercle bacilli. C.R.

ORAL FOLIC ACID TOLERANCE TEST IN NORMAL HUMAN SUBJECTS AND PATIENTS WITH PERNICIOUS ANEMIA. S.L. Clark, Jr. (Vanderbilt University, School of Medicine, Nashville, Tenn.). Proc. Soc. Exptl. Biol. & Med. 82:25, 1953

The concentration of free folic acid in serum was determined microbiologically before and at various intervals following the ingestion of 1 mg of folic acid by normal subjects and by patients with pernicious anemia. No defect in gastrointestinal absorption of folic acid in patients with pernicious anemia could be demonstrated. It is suggested that this test may prove useful in the study of folic acid metabolism. E. V.

CHLORIDE EXCHANGE BETWEEN HUMAN ERYTHROCYTES AND PLASMA STUDIED WITH $Cl^{36}$. W.D. Love & G.E. Burch. (Tulane University, School of Medicine, New Orleans, La.). Proc. Soc. Exptl. Biol. & Med. 82:131, 1953

By *in vitro* studies with $Cl^{36}$ the authors have demonstrated that the exchange of chloride between plasma and erythrocytes is at least 600-1300 times greater than that of sodium and potassium respectively. Changes in temperature, addition of various enzyme inhibitors, and conversion of hemoglobin to methemoglobin had no effect on the rate of transfer. These experiments do not suggest the presence of any metabolic processes controlling the transfer of chloride, but they do not eliminate this possibility. E. V.

GENERAL PHYSIOPATHOLOGY OF PLASMA PROTEINS. J. Gras (Munic. hosp. infectious diseases, Barcelona). Rev. espan. fisiol. 6:275, 1950

A review-320 references.

AN EVALUATION OF THE IODOACETATE TEST FOR CANCER. Joseph Lebowich (Saratoga County Hospital, N.Y.). Am. J. Clin. Path. 23:45, 1953

The Huggins, Miller and Jensen iodoacetate test was performed on serum from 170 subjects (85 carcinoma, 85 non-cancerous). The results show 35% of the non-cancerous patients as falsely positive; 21% of the cancerous as falsely negative. The test has no value as a diagnostic test for cancer. C.R.

## REVIEW OF CURRENT LITERATURE

THE EFFECT OF LECITHINASE A ON THE SUCCINOXIDASE SYSTEM. A.P. Nygaard and J.B. Sumner (Cornell Univ., Ithaca, N.Y.). J. Biol. Chem. 200:723, 1953

Lecithinase inactivates the succinoxidase system. Component parts of the system are affected less. Lecithin may be a part of a component linking succinic dehydrogenase and cytochrome c. C.vF.D

THE INHIBITION OF THE ACTION OF PANCREATIC LIPASE BY ESTERS OF POLYOXYETHYLENE SORBITAN. F.N. Minard (Abbott Laboratories, Chicago, Ill.). J. Biol. Chem. 200:65., 1953

Tweens are currently used to promote fat absorption. Under certain conditions Tweens have an inhibitory effect on pancreatic lipase. Tween 80 (oleic acid ester) is the most inhibitory, followed by 60 (stearic acid ester) and 20 (lauric acid ester). The experiments were carried out with corn oil emulsion and pork pancreatic lipase (Pangestin). The inhibition can be reversed by bile salts. The Tweens are themselves hydrolysed by pancreatic lipase. Apparently the less reactive substrate inhibits the enzyme action upon the more reactive substrate. C.vF.D

THE DIRECT PREPARATION OF CRYSTALLINE UROBILIN FROM BILURIBIN. C.J. Watson (Univ. of Minnesota, Minneapolis, Minn.). J. Biol. Chem. 200:691, 1953

50 mg. bilirubin are mixed with 0.2 ml. of 0.1 n NaOH and 0.8 ml. of distilled water in a 5 ml. bottle. 5 gm. of 4% sodium amalgam are added, the bottle corked tightly and shaken for one hour. Dilute with distilled water and extract with 300 ml. petroleum ether in a separatory funnel. Acidify to pH 5-6 with buffered acetic acid. Wash with petroleum ether, three times. Extract the petroleum ether with distilled water containing an appropriate amount of a 1% ethanolic solution of iodine (0.45 mg of iodine per mg. of mesobilirubinogen) four times. Acidify to 1.0 N HCl with 7.5 N HCl. Extract the aqueous fraction with small amounts of chloroform. Concentrate under reduced pressure. Add hot acetone-crystallization usually commences at once. Yield: 30%. C.vF.D.

THE ROLE OF ADENOSINETRIPHOSPHATE IN THE ACTIVATION OF FATTY ACID OXIDATION IN VITRO. R.F. Witter, E.H. Newcomb and E. Stotz (School of Medicine and Dentistry, Univ. of Rochester, Rochester, N.Y.). J. Biol. Chem. 200:703, 1953

ATP level of rat liver could be maintained by phosphopyruvate or phosphocreatine (from rabbit muscle)-ADP transphosphorylation. The phosphopyruvate-ADP system is a more effective activator of sorbate oxidation than the phosphocreatine-ADP system. ATP is essential for the oxidation of sorbate (unsaturated 6-C fatty acid). C.vF.D.

ELECTROPHORESIS BY THE MICROSCOPE METHOD: A SIMPLE EXPERIMENTAL ASSEMBLY. R.S. Hartman, J.B. Bateman and M.A. Lauffer (Camp Detrick, Frederick, Md.). Arch. Biochem. Biophys. 39:56, 1952

PREPARATION OF SERUM LIPIDE EXTRACTS FREE OF INORGANIC PHOSPHATE. D.D. Van Slyke and J. Sacks (Brookhaven National Laboratory, Upton, N.Y.). J. Biol. Chem. 200:525, 1953

Lipide extracts, prepared with Bloor's reagent, directly from serum containing radio-active tracer, or from zinc and ferric hydroxide precipitates, contained less than 0.1% inorganic P. The extracts were prepared without heating. C.vF.D.

A REVIEW ON FLAME ANALYSIS IN THE CLINICAL LABORATORY. Bennie Zak, Robert E. Mosher and Albert J. Boyle. (Wayne Univ., Detroit). Am. J. Clin. Path. 23; 60-uu, 1953

66 references. Methods of the authors for Na, K, Ca and Mg are given in detail. Gas-oxygen or acetylene serves as fuel. Glucose, phosphate, gelatin, urea and the above electrolytes are contd. in the standards solns. in concns. approximating those of plasma. For Na and K plasma or urine is diluted suitably with water. For Ca and Mg the plasma is ashed with nitric acid-perchloric acid, and the ash dissolved in water to a suitable vol. C.R.

THE QUANTITATIVE RELATION BETWEEN INSULIN AND ITS BIOLOGICAL ACTIVITY. W.C. Stadie, N. Haugaard and M. Vaughan (Univ. of Pennsylvania, Philadelphia, Pa). J. Biol. Chem. 200:745, 1953

Glycogen synthesis is proportional to the amount of insulin bound by muscle. There is no difference in binding of insulin between normal and hypophysectomized rats under comparable conditions. Acceleration of glycogen synthesis per microgram of bound insulin is shown following removal of the pituitary. Evidently the pituitary hormone has a contra-insulin effect of the periphery. C.vF.D

THE EFFECT OF HYPERGLYCEMIC-GLYCOGENOLYTIC FACTOR AND EPINEPHRINE ON FATTY ACID SYNTHESIS. E.S. Haugaard and W.C. Stadie (Univ. of Pennsylvania, Philadelphia, Pa). J. Biol. Chem. 200:753, 1953

Crystalline insulin stimulates the incorporation of acetate into liver fatty acids. Hyperglycemic factor and epinephrine depressed the incorporation of acetate into fatty acids. C.vF.D.

EFFECT OF A LYSINE-POOR DIET ON THE COMPOSITION OF HUMAN PLASMA PROTEINS. A.A. Albanese (St. Luke's Hospital, New York, N.Y.). J. Biol. Chem. 200:787, 1953

Six babies were studied, who were put on a wheat gluten diet. This diet caused an increase in arginine of the plasma protein. No difference in A/G ratio was observed by the Howe method. C.vF.D.

THE NATURE OF PREECLAMPSIA. L.C. Chesley. Bulletin Margaret Hague Maternity Hosp. 4:60, 1952.

A review of the physiol. and biochem. factors involved.

STUDIES ON VITAMIN B6. I. BIOCHEMICAL CHANGES IN VITAMIN B6 DEFICIENCY IN RATS. J.R. Beaton, J.B. Beare, J.M. White, and E.W. McHenry (Univ. of Toronto, Toronto, Canada). J. Biol. Chem. 200:715, 1953

B6 deficiency causes a fall in the fasting level of glutamine and a significant increase in the fasting level of blood urea. C.vF.D

ANTIDIURETIC ACTIVITY OF THE SERUM OF NORMAL AND DISEASED SUBJECTS. W.F. Perry & T.W. Fyles. University of Manitoba, Winnipeg, Can.). J. Clin. Endocrin. 13:64, 1953

The antidiuretic activity of the sera of 10 patients with congestive heart failure and 9 subjects with liver disease showed no significant difference from that of normal subjects. From these experiments it is concluded that the water retention associated with cardiac failure and liver disease is not due to alterations in serum antidiuretic activity. E. V.

THE PHYSIOLOGY OF THE ADRENAL CORTEX. J.H. Mulholland (New York Univ., New York, N.Y.). Surg. Clin. No. Amer. 1952, 347.

A review with bibliography. H.G.A.

A SIMPLE MICROHOMOGENIZER. FOR PREPARING SUSPENSIONS OF TUBERCLE BACILLI AND TISSUE HOMOGENATES. Maurice S. Tarshis (Kennedy Hosp., Memphis, Tenn.). Am. J. Clin. Path. 23:94, 1953

The apparatus consists of a Kolmer centrifuge tube, and a pestle made from a liquid plastic prepn. Directions for making the pestle are given. C.R.

CHANGES IN FREE AMINO ACIDS OF BRAIN AND MUSCLE AFTER TOTAL HEPATECTOMY. E.V. Flock, M.A. Block, J.H. Grindlay, F.C. Mann, and J.L. Bollman (Mayo Foundation, Rochester, Minn.). J. Biol. Chem. 200:529, 1953

The concentration doubles in 13 to 24 hrs. after total hepatectomy in the dog. This is mainly due to the 5-fold increase in glutamine. A similar but smaller change was observed in muscle, accompanied by a simultaneous decrease in glutamic acid. the glutamic acid content of the brain is unchanged. C.vF.D.

FURTHER MODIFICATIONS IN THE MEASUREMENT OF BLOOD GLUCOSE. F. William Sunderman (Jefferson Hosp., Phila. Pa.). Am. J. Clin. Path. 23:193, 1953

A micro adaptation of the Sunderman-Fuller procedure for estimation of blood glucose is described. A description of a new tube for use with the method, having graduations at 5, 10, and 20 ml and with a glass stopper, is given. C.R.

## SOUTHERN CALIFORNIA SECTION

Herbert O. Carne, Ph. D., Biochemist, Long Beach Veterans Administration Hospital, addressed the local section February 3 at the Hollywood Presbyterian Hospital. His discussion of "Turdidimetric Methods" will be summarized in a later issue.

The instrumentation and applications of paper chromatography were reviewed for the local section and guests last December 2 (Veterans Administration Center, Los Angeles) by William Werkhaiser, Ph.D., of the University of Southern California.

At its simplest, paper chromatography involves applying a small spot of sample to a piece of filter paper, then allowing an appropriate solvent mixture to advance over the spot, carrying the various components forward at different characteristic rates, thereby accomplishing fractionation. Dr. Werkhaiser led off with a brief discussion of proposed underlying phenomena: (1) adsorption (by the cellulose fiber) and (2) partition (distribution between two immiscible liquid phases, a stationary phase bound to the paper over which a mobile phase passes). He observed that it has been possible, upon the basis of the partition theory, to calculate distribution coefficients (within 5% error) from data obtainable from appropriate paper chromatograms.

Dr. Werkhaiser classified for discussion some of the many instrumental modifications that have been employed: ascending, descending and combined ascending-descending procedures (according to the direction in which the solvent front advances); concentric ring procedures (in which fractionation takes place radially from the point of sample application); the chromatopile (a vertical column formed by a pile of filter paper discs pressed together); paper electrophoresis and ionophoresis (with migration under control of an electric current); and the continuous fractionation apparatus in which horizontal ionophoresis is imposed on a normal descending chromatogram such that separated fractions may drip off simultaneously and continuously from different points along the lower edge of the paper sheet chromatogram. Other modifications include phase reversal (when a stationary organic phase replaces the usual aqueous phase on paper that is coated, for example, with latex), or adsorption may be made the controlling factor when the paper is impregnated with a strong adsorbent such as alumina.

For qualitative evaluation of chromatograms, Dr. Werkhaiser mentioned examination under ultraviolet light (causing certain colorless compounds to fluoresce), spotting with various color developing agents, elution and subsequent tests of eluate fractions, also the use of enzymatic and microbiological tests on chromatograms. For quantitative evaluation, he mentioned measurements of spot area, maximum density of spots, and total light absorbancy of spots.

In particular, Dr. Werkhaiser demonstrated and discussed the apparatus he has employed for the ionophoretic analyses of nucleotides, and showed representative graphs of absorbancy (260 $m\mu$) against displacement along the chromatographic strip. (Absorbancy measurements were performed with the aid of a strip moving attachment he made for his Beckman DU spectrophotometer). As a check on purity, he measured, for each position along the strip, absorbancies at two wave lengths, and plotted the resulting absorbancy ratios against displacement; a horizontal straight line curve (constant absorbancy ratio) evidences that a single pure compound is located over the corresponding region of the chromatogram.

As examples of the many applications to clinical chemistry, Dr. Werkhaiser cited the work of William Bergren on porphyrins, Robert and Kay Fink on betaaminoisobutyric acid excretion patterns, and R. J. Williams and associates on the comprehensive chemical excretion patterns of many individuals (evidencing the proposal that each individual has, within limits, a distinctive metabolic pattern of his own).

Always the discovery of spots on chromatograms, spots that cannot be identified with known compounds, is an interesting invitation to further investigation. All in all, Dr. Werkhaiser emphasized that the applications of paper chromatographic techniques know no bounds.

## PHILADELPHIA SECTION

The third meeting of the 1952-53 season of the Philadelphia Section, was held at 7:45 P.M. on Tuesday, January 27, 1953 in Alumni Hall of the Hospital of the University of Pennsylvania. Prior to the meeting, there was an informal dinner in honor of the speaker at the Lido Restaurant.

The president, Dr. Cecilia Riegel, introduced Dr. Harry Shay, Director of the Fels Research Institute of Temple University School of Medicine, who spoke on "Liver Function."

Dr. Shay discussed the clinical use and limitations of the most practical of the large number of tests that have been proposed for studying liver function. Since liver function in disease changes rapidly, and the value of some tests is limited to certain stages of liver damage, it is necessary to use serial determinations with several tests in establishing a diagnosis and in following the effects of treatment. Various groups of the practical tests were suggested for studying the different types of jaundice.

After the formal program, Dr. Shay answered numerous questions related to the subject of his lecture.

## WASHINGTON – BALTIMORE – RICHMOND SECTION

The first meeting of the new section of the AACC was held December 11, 1952 at Georgetown University. Lipoproteins were discussed by three speakers from the Section of Cellular Physiology, National Heart Institute, Bethesda, Maryland. The speakers were Dr. Ray K. Brown, Dr. Edwin Boyle, and Dr. Joseph Bragdon. Abstracts of their talks follow this news item.

Our second meeting was held January 22, 1953 at the Army Medical Center. Local By-laws were approved and have been submitted to the National Executive Committee. Following the business meeting, Captain David Seligson gave a discussion of micro methods as used by the Department of Hepatic and Metabolic Diseases at the Walter Reed Army Medical Center. He pointed out the misleading nature of the term "micro" and showed how some of the frequently used clinical procedures measure smaller quantities

*(continued on following page)*

## WASHINGTON – BALTIMORE – RICHMOND SECTION

of material than some of the most refined micro methods. He demonstrated methods and apparatus which measured small amounts of $CO_2$, ethanol, ammonia, urea, NPN, total N, Cl ion and other substances. Apparatus was shown for doing electrometric titrations on small samples for chloride or for doing titration curves on volumes down to 0.1 ml. Also, special microdiffusion technics and apparatus for $CO_2$, ethanol and ammonia were demonstrated. He emphasized throughout that micromethods often were more precise, accurate, and specific than the classical methods. The ones demonstrated were in use in his Department often because of convenience rather than necessity.

The March meeting was held on Friday, March 27 at the George Washington Medical School, 1335 H. Street, N.W., Hall A. Dr. Bernard Armbrecht and Dr. Harold Jeghers, both of Georgetown Medical School, spoke on "Porphyrinurea".

### ULTRACENTRIFUGE STUDIES ON SERUM LIPOPROTEINS

**by Edwin Boyle**

The serum lipids, cholesterol, phospholipids and neutral fats circulate in the blood as giant molecular complexes with serum proteins. These lipoproteins vary in size in a progressive fashion from the smallest or alpha-1 lipoprotein with a molecular weight of about 250,000 and a density of 1.12 containing about 75% protein, up through the so-called beta lipoproteins containing about 25% protein and 75% lipids, to the giant chylomicrons that have a diameter up to .2 of a micron and contain about 95% lipid. It is believed that the larger lipoproteins are progressively broken down to form smaller lipoproteins of the alpha class by an enzyme system. This enzyme system is greatly accelerated by the administration of heparin. By varying the density of serum samples by the addition of salts, the various lipoproteins may be separated and purified in the ultracentrifuge. Patients with coronary heart disease have been found to have a high concentration of a lipoprotein of the $S_f$ 10-100 class, which are slightly larger and less dense than the normal beta lipoprotein. Normal young females have most of their serum lipoproteins in the alpha-1 species and normal young males in the beta species. After the age of 55, both males and females have high concentrations of beta lipoproteins. The clinical significance and importance in this lipoprotein breakdown enzyme system is apparent as this heparin stimulated system transforms the so-called pathological molecules into what is considered a more normal distribution.

### SEPARATION OF SERUM LIPOPROTEINS

**by Joseph Bragdon**

The lipoproteins of the serum may be separated for analytical study by paper electrophoresis, by Cohn's alcohol precipitation, or by ultracentrifugation. The general chemical procedure of determining the ratio of total phospholipid to total cholesterol in the whole serum is not a reliable test for the presence of Gofman's pathological lipoproteins.

The usual method of determining total serum lipids by extraction, evaporation, and re-extraction is not satisfactory as some lipid is usually oxidized, reducing its solubility in the second solvent; and as certain non-lipid materials always carry through. A better procedure is to precipitate the lipids with the proteins with a protein precipitant, wash out water soluble substances, and then extract the lipids from the proteins. Of the protein precipitants tried, the zinc sulfate technique of Van Slyke appears the most promising.

The neutral fats must be determined as the difference between the total lipids and the combined cholesterol and phospholipids. Total lipid may be readily determined by digesting the sample with potassium dichromate and sulfuric acid and determining the reduced chrome ion colorimetrically.

### CONVERSION OF LIPOPROTEINS BY HEPARIN

**by Ray K. Brown**

The conversion of large serum lipoproteins is being investigated. *In vivo* heparin forms clearing factor which decreases serum turbidity as measured in a photoelectric colorimeter and causes a breakdown of large lipoproteins and formation of $\alpha$ lipoproteins as determined ultracentrifugally.

Clearing factor may be formed *in vitro* from a plasma precursor, heparin and a tissue substance found in heart, lung and pylorus. It causes changes similar to in vivo formed clearing factor. Washed lipoproteins are prepared free of other plasma constituents by layering lipemic plasma under physiological saline and floating them up in the ultracentrifuge. Washed lipoproteins are not cleared by purified clearing factor unless another plasma component, coprotein, is present. During clearing triglycerides are partially broken into fatty acids. Plasma esterases play a part in this breakdown.

The following scheme is proposed:

Precursor + heparin + tissue factor → clearing factor

Clearing factor + coprotein + large lipoprotein → smaller low density lipoproteins + alpha lipoproteins.

## BOSTON SECTION

The Boston Section held its sixth meeting of the current season at the New England Medical Center on March 12. The speaker of the evening was Dr. Fabian Lionetti, Assistant Professor of Biochemistry at Boston University Medical School. His talk was on the subject of "Isotope Analysis."

Dr. Lionetti showed how isotopes can be used as a valuable diagnostic tool. Since it is well established that certain tissues exhibit a selective affinity for a given element, detection of that element if radioactive, is quite simple with radio-counters. Considerable use has been made for some time of radioactive iodine in evaluating thyroid states. In cases of metastatic thyroid carcinoma, $I_{131}$ appears to concentrate in the metastases as well.

In the diagnosis of brain tumors, diiodofluorescein containing $I_{131}$ concentrates in the tumors and tumor areas can be visualized.

Sodium chloride, containing $Na_{24}$, is useful in detecting impaired circulation. The method is rapid, and permits precise localization of arterial constriction.

Radioactive chromium is being used in blood volume studies. As the cation ($Cr^{+++}$), it remains in the plasma, whereas, anionic chromium ($CrO_4^{=}$), is taken up by the red cells. Precise determinations of both plasma and cell volumes is thus possible. In the determination of whole blood volume, radioactive phosphorus is being used.

Radioisotopes are now well established in the field of therapy. Cobalt ($Co_{60}$), having a high gamma ray emmission, is used locally as implants in the form of "seeds". $P_{32}$, an inexpensive isotope, is being used in both polycythemia and leukemia.

### 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.