The CLINICAL Chemist

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Official Publication
of
AMERICAN ASSOCIATION
of
CLINICAL CHEMISTS,
INC.

STATED ANNUAL MEETING
September 10, 1953
Chicago, Ill.
THE SECRETARY REPORTS

The members of the AACC can well be proud of their official publication, THE CLINICAL CHEMIST. To those with editorial experience it becomes immediately obvious that a great amount of work is necessary to get out this bimonthly publication, under favorable circumstances. With insufficient funds for clerical and incidental expenses, this job becomes more difficult. Without adequate cooperation from the membership the task becomes even more difficult. And such has been the situation for some time.

The editorial board has been producing ever better issues, and the members thereof have applied themselves to this task with a great deal of devotion. But this still remains the publication of all the members, and as such they and their local sections must be expected to submit comments, criticism, and items of interest to other members.

About sixty per cent of our income at the current rate of costs is being expended for the maintenance of THE CLINICAL CHEMIST. This leaves very little funds for other important activities. Our main source of income is from dues, and there is no desire on the part of the Executive Committee to recommend any further increase at this time. However, it is certain that with some effort it should be possible to increase the membership of the Association without relaxation on standards for admission. The advantages of the Association and the burden of costs should be equally distributed amongst all the chemical clinics in the United States. Also the area of our activities can be expanded as the organization strengthens.

Max M. Friedman National Secretary
MICHAEL SÓMOGYI NOMINATED FOR ERNST BISCHOFF AWARD

Dr. Otto Schaes, Chairman of the AACC Award Committee, announced that Dr. Michael Somogyi of the Jewish Hospital of St. Louis, has been chosen as recipient for the 1953 Ernst Bischoff Award and Medal.

The Honorary members of the AACC, acting as Award Nomination Committee, designated Dr. Somogyi from a number of candidates submitted to them by Dr. Schaes' committee, Joseph H. Roe, and Fritz Bischoff.

The Ernst Bischoff Award, consisting of $500, a scroll and medal, will be awarded at the Stated Annual Meeting Dinner to be held Thursday evening, September 10th in Chicago. Dr. Somogyi will deliver the Second Ernst Bischoff Lecture.

Michael Somogyi is noted for his investigations of the metabolism of carbohydrates and ketone bodies and his clinical studies of diabetes and the physiology of the action of insulin. He has been associated with the Jewish Hospital of St. Louis since 1925, heading one of the first independent hospital laboratories of clinical chemistry.

INTERNATIONAL SURVEY

Dr. E. J. King, Professor of Biochemistry, University of London, Postgraduate Medical School, has invited twelve laboratories in the United States to collaborate with groups in Great Britain in testing the reliability of analytical results obtained in clinical chemistry laboratories. Identical samples will be sent to collaborators for the analysis of eleven blood constituents.

In a letter to Dr. Albert E. Sobel, President of the AACC, Dr. King asked Dr. Sobel to invite the laboratories in the U.S. to participate. The idea of international participation in this type of survey was suggested at the first General meeting of the International Association of Clinical Biochemistry which was held in Paris the summer of 1952.

STATED ANNUAL MEETING
CHICAGO, SEPTEMBER 10

The Fifth Annual Meeting of the American Association of Clinical Chemists, Inc., will be held on Thursday afternoon, September 10, in Chicago, Ill. The meeting will be held during the 124th National Meeting of the American Chemical Society.

Thursday, September 10th, in Chicago, will virtually be "clinical chemistry day." In participation with the Division of Biological Chemistry, the AACC scientific session will feature a symposium on "Electromigration in Stabilized Electrolites." A panel of noted scientists will discuss the various aspects of this new tool for protein separation and quantitation as well as its use with other biological material.

The Association Dinner will be held Thursday evening. At the dinner, the 2nd Ernst Bischoff Award will be presented and will feature the Ernst Bischoff Lecture by the recipient of the Award.

Registration for the meeting and tickets for the dinner will be available at the Conrad Hilton Hotel, 720 South Michigan Ave., beginning September 6.

Reservations for hotels may be made by writing to the Housing Bureau, American Chemical Society, 134 N. LaSalle Street, Chicago 2, Ill.

As the publishing schedule of THE CLINICAL CHEMIST will not allow adequate time for the presentation of the entire scientific program, place and time of the dinner, Annual Meeting, and other events scheduled, members are urged to consult the CHEMICAL AND ENGINEERING NEWS, August 3rd issue, which will carry the complete program of the meeting.

WASHINGTON - BALTIMORE - RICHMOND SECTION ELECT NEW OFFICERS

Officers for 1953-54 are: Chairman, Miss Miriam Reiner; Sec. Treas., Miss Pearl Anderson; Councilors, for 3 years, Dr. Martin Ruben, Georgetown University, for 2 years, Dr. Oliver J. Irish, Veterans Administration, for 1 year, Dr. Joseph Roe, George Washington University Medical School.

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1953 FEDERATION MEETING

Members of the AACC attending the Thirty-Seventh Annual meeting of the Federation of American Societies for Experimental Biology held in Chicago, met at a dinner meeting on April 8. The Chicago Section of the AACC was host, with Dr. Clarence Cohn, Chairman of the local section, presiding.

The meeting offered the opportunity for the exchange of views among the membership as nearly every section of the country was represented. Dr. Albert Sobel, President of the AACC, gave a brief review of the progress made by the AACC during the year. Positive accomplishments include the growth in scientific and professional strength of the various local sections; the unusual interest shown at the national meeting; the completion of the book on standardized clinical methods; plans for additional books, including one on toxicology; development of educational standards; growing interest on the part of the medical profession in qualified clinical chemists in hospitals; integration of our activities with other professional societies, particularly the group at the APHS meeting; and our good relations both with the American Chemical Society and the Federation group.

Among the speakers of the evening were Professor Walter R. Bloor, University of Rochester, Dr. Oliver H. Guebler, Edsel E. Ford Institute for Medical Research, Professor Kurt G. Stern, Brooklyn Polytechnic Institute and Chairman of the New York Section AACC, and Professor Hugh J. McDonald, President-elect of the Association.

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
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Letters From Members

ASSOCIATION OF CLINICAL BIOCHEMISTS
Royal Berkshire Hospital,
Reading,
England.
18, May 1953.

Dear Dr. Friedman,

An Association of Clinical Biochemists was formed at a meeting held on 28 March at the Postgraduate Medical School of London. I am now writing to you on behalf of the Council who thought that the American Association of Clinical Chemists would wish to know of the aims of the new Association and of the circumstances which prompted its formation.

While clinical biochemistry had for some time been developing into a more or less well defined branch of science, the first impetus for forming an association came from the professional side. When during the first months of the National Health Service conditions of employment in the Service were being negotiated, the Ministry of Health had to find an organization which represented clinical biochemists. The lack of any such organization led to the setting up of a temporary committee formed by the Biochemical Society, the Royal Institute of Chemistry, and the Association of Clinical Pathologists through which these societies jointly undertook this task.

For many years systematic schemes of research had been under way in the clinical biochemistry departments of several medical schools, and some meetings of the Biochemical Society had been organized into what were virtually clinical biochemical meetings. The possible number of such meetings was necessarily limited, and a growing need made for the formation of local societies (first in Birmingham and Glasgow) for informal talks, and later for the holding of formal meetings and the reading of papers.

While the various aspects of our needs were tackled piecemeal, national organizations of clinical biochemists were being formed in the U.S.A. and in several countries in Europe. The foundation last summer in Paris of the International Association of Clinical Chemists presupposed that a national organization in Great Britain would eventually be formed.

The Association now formed is primarily scientific in character, although it intends also to concern itself with professional matters as these arise. Membership is open to practising clinical biochemists who are University graduates in science or medicine, registered medical practitioners or members of the Royal Institute of Chemistry. The Association intends to hold meetings locally and on the national scale for reading papers and giving demonstrations and hopes to hold joint meetings with all organizations interested in similar aspects of science. The Council will be glad to join with others in all activities to which clinical biochemists in this country can usefully contribute, in particular they look forward to an active cooperation with the American Association of Clinical Chemists and the other member organizations of the International Association.

Yours sincerely,

A. L. Tidmack, Hon. Secretary.

June 4, 1953

Dr. A. L. Tidmack
Royal Berkshire Hospital
Reading, England

Thank you very much for your letter of May 18th, in which you advise us of the formation of the Association of Clinical Biochemists in Great Britain. You may be certain that the members of the American Association of Clinical Chemists will be happy at all times to cooperate fully with your group in Great Britain, as they have with other national groups that have already been formed.

I have submitted your communication to the editors of THE CLINICAL CHEMIST, our official publication. On behalf of the AACC, I wish to extend our best wishes to your society.

Sincerely yours,

Max M. Friedman, Ph.D., National Secretary
ORGANIZING VOLUME II OF "STANDARD METHODS"

Dr. Nelson F. Young of the Medical College of Virginia has been appointed by the Executive Committee to head the Editorial Board of Volume II. The methods will be compiled in much the same fashion as in Volume I, in order to assure critical evaluation of all procedures. Each method included is to be sponsored by at least two, and preferably more, members of the Association. The submitter and checker of the procedures to be evaluated will be selected by the Editor. Work will commence immediately on the formulation of a list of methods that will comprise the book.

All AACC members are urged to participate in building the second volume in the series on methodology by filling out completely the accompanying form and, wherever feasible, volunteering to assist by submitting methodology and in the checking of methods. Suggestions of tests which do not appear on the appended list, and recommendations of methods for inclusion in the book will be welcome. The completed form, together with recommendations and comments, is to be sent to:

Dr. Nelson F. Young
Medical College of Virginia
Richmond, Virginia.

The information supplied by the membership at large will be an invaluable aid in determining which methods are in the greatest demand, and in providing the manpower with which to work in writing another outstanding volume on "Standard Methods of Clinical Chemistry."

The Table of Contents of Volume I (C.C. 5 12 (1953)) shows that Volume II must be made available soon in order that even the common determinations be adequately covered. The Editorial Board believes that the methods selected in the first two or three volumes should be those which have a history of proven reliability under various laboratory conditions. For this reason, more complex and untried procedures will in general be left for later volumes.

A tentative Table of Contents for Volume II is as follows:

1. Serum Amylase (method based on disappearance of starch-iodine color)
2. Protein-bound I (2 procedures if possible - I with distillation and the Barker, Humphrey)
3. Hemoglobin (Oxyhemoglobin (spec.) and one other)
4. Bromide
5. Sulfhydryl
6. Thiosemicarbazide
7. Sulfa. (Includes factors for newer drugs)
8. Total Lipids
9. Lipid P
10. Phosphates (nitrite & phosphate)
11. Phosphorus
12. 17-Keto-steroids
13. Corticoids
14. Titrimetric calcium (chelating)
15. Total cholesterol

This list is by no means closed and the editor will welcome any suggestions for additions.

CHICAGO SECTION

The Chicago Section of the AACC held a wide variety of scientific lectures presented at the monthly meetings for the 1952-53 season. In November 1952 Dr. Hans Popper of the Heiseen Institute spoke on "CONDITIONED AMINO ACID DEFICIENCIES." On January 30, Dr. Israel Davidson, Professor and Chairman of the Department of Pathology, Chicago Medical School, and Pathologist and Director of Laboratories, Mount Sinai Hospital, spoke at the Professional Services and Research Laboratories Building of Mount Sinai Hospital and Chicago Medical School, on the subject: "BLOOD GROUPS AND RF FACTOR." Dr. Davidson traced the historical development of the various blood groups of the ABO system and discussed the various tribal and racial differences in reference to incidence, locality, etc. There is also a wide distribution of the "A" substance in animals. After discussing the various sub-groups such as "MN" etc., the major presentation centered around the Rh factor and its importance in erythroblastosis fetalis. The tissue changes were discussed and also various laboratory methods to make the proper diagnosis. Unfortunately, very little chemistry is known about this important disease entity.

On February 27, Dr. Henry S. Guterman, Director of the Department of Research in Human Reproduction, Medical Research Institute, Michael Reese Hospital, Chicago, spoke at Michael Reese Hospital on "PROGESTERONE METABOLISM." Dr. Guterman traced the development of the localization of the hormone of the corpus luteum and its excretion product, pregnanolone, in the urine. He also reviewed the various chemical methods, especially the ones in which he was instrumental in developing as an aid in diagnosis of pregnancy and as follow-up therapy in threatened abortion.

The March meeting featured a talk by Dr. Carl A. Moyers, Chairman, Department of Surgery, Washington University School of Medicine on the subject: "FLUID AND ELECTROLYTE BALANCE IN RELATION TO ACUTE TRAUMA."

On May 22, Dr. A. E. Sobel, President of the AACC and Chemist to the Jewish Hospital of Brooklyn, spoke on "THE CHEMISTRY OF BONE AND TOOTH FORMATION." A synopsis of Dr. Sobel's lecture is published in the scientific section of this issue.

WASHINGTON - BALTIMORE - RICHMOND SECTION

About a year ago, Dr. John Reinhold, then President of the American Association of Clinical Chemists, Inc., suggested the formation of a Washington section. Fourteen members of the Association were then residing in this area. Representatives from the medical laboratories of the local hospitals, medical schools, Surgeon General's Office, Army, Navy, Veterans Administration, and National Institute of Health, Washington, Baltimore, and Richmond, were invited to meet with Dr. Reinhold at Walter Reed Army Medical Center. It was agreed to proceed with formal organization.

The first meeting, May 1952, at Walter Reed Army Hospital included the formal organization to petition recognition by the National Association and a symposium on chemical and clinical aspects of flame photometry. Attendance of 100 was ample assurance of interest and support.

The most significant events of the first year, 1952-53, may be summarized as: four scientific programs on the biochemistry and clinical aspects of new micro-analytical techniques in clinical chemistry, the serum lipoproteins, the porphyrins, and the identification of intoxication, detection, and identification; the average attendance at these meetings was approximately 35; the local section was officially established by the National Association; membership was increased from 14 to 35; over 85% of the membership participated in the annual election of section officers.
ELECTROMIGRATION IN STABILIZED ELECTROLYTES

PART II: FACTORS INFLUENCING MOBILITY

by

H.J. McDonald, R.J. Lappe, E.P. Marbach, R.H. Spitzer, and M.C. Urbn

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

This article will deal with the character and range of some of the more important factors that influence the electromigration of a migrant in electrolytes stabilized with paper strips and sheets. Some of the fundamental aspects of the technique include the relationships that exist between the movement of the migrant and such factors as time, potential gradient, pH of the buffer solution and its ionic strength, temperature, adsorption, nature of the paper and of the migrant and the role of electrosorption. In addition, the influence on mobility of such factors as the method of supporting and wetting the paper strips, the separation of electrode products from the paper itself, capillary infiltration of liquid through the strip, the evaporation of the paper, the drying of the strip, the point of application of the migrant, the relative amounts of water held by various types of paper, and the development of the ionogram will be considered briefly.

Movement as a Function of Time and Potential

In experiments involving mobility, as opposed to simple empirical fractionation procedures, one criterion which must be met is that the motion of the migrant be a linear function of the time, under conditions of constant potential gradient and temperature. It should be emphasized at this point that a non-linear relationship can readily be obtained using any modification of the open- or closed-strip apparatus thus far proposed if precautions are not taken to minimize water shifts about the paper. That a linear relationship is an achievable goal, however, has been demonstrated in this laboratory, for a varied range of substances as amino acids (99), proteins (98), lipoproteins (51), PVP (88), carbohydrate-borate complexes, some inorganic ions, etc.

Some practical factors important in achieving linearity of movement of the migrant with time include: 1. The employment of siphon-filled salt bridges to separate the products of electrode reactions from the buffer vessels into which the ends of the paper strips dip. This precaution is especially important when buffer solutions of low ionic strength are used. 2. The establishment and maintenance of a uniform and constant liquid level in the buffer vessels. This can be achieved by allowing a siphon with a bore of small diameter to remain in place between the buffer vessels during the course of the experiment. 3. The maintenance, in a horizontal plane, of that portion of the paper strip, throughout which electromigration of the migrant under study takes place. In carefully controlled experiments it has been observed that non-linear movement of the migrant with respect to time is obtained when the center of the paper strip or sheet is elevated appreciably above, or lowered much below, the level of the ends of its suspended portion (52, 4). The use of a water-saturated atmosphere around the strips and the reduction of the free gas space to a minimum due to the low molecular weight of helium and the consequent high relative velocity of its molecules it exhibits a heart conductivity, or 0°C, approximately six times that of air. It serves, therefore, on an excellent conductor of heat and consequently aids in dissipating the electrical energy developed in the strip, and in minimizing the loss of water. When evaporation from the paper strip is reduced to a minimum and the liquid level in the buffer vessels is maintained constant, thus offsetting bulk water shifts or surge through the paper, chromatographic interference is largely eliminated.

When the electrical energy developed in a strip exceeds about 0.005 watt per square cm. of radiating surface at 25°C, the use of helium is recommended. However, on the temperature is reduced down from room temperature, the need for helium decreases rapidly, so that many experiments may be carried out in the neighborhood of 0°C with good results using a simple air atmosphere. When ionographic measurements are to be used for mobility studies the ionic strength of the buffer solution used is generally 0.05 or less.

If the various precautions outlined above are enforced, it is found that, with all other conditions being constant, the rate of electromigration of the migrant is directly proportional to the voltage impressed across the ends of the paper strip or to the potential gradient along the strip (50, 88). If the movement of the migrant is linear with respect to both time and potential, it is obvious that the size or design of a particular apparatus no longer exerts an influence on electromigration rate, and the latter quantity can legitimately be stated simply in terms of cm/sec per volt/cm.

The Wetting and Drying of the Paper Strips

The method of wetting the paper strips with the buffer solution can affect the mobility of the migrant. In one earliest work, the dry strips were placed in a horizontal position with the ends dipping into the buffer vessels, and the buffer solution was allowed to wet them by simple wick action. In this procedure there is the possibility of introducing some chromatographic separation of the buffer salts themselves along the strips, thus leading to non-uniform distribution of electrolyte and a concomitant non-uniform potential gradient along the strips. Although this non-uniformity can be smoothed out by allowing the wetted strips to equilibrate for 1.5–2 hours, with the current on, before applying the migrant, the trouble can be circumvented in the following way. The paper strips are wetted by allowing about 3 ml of buffer solution for a 50 cm. length of Eaton – Dinkelmann #613 paper, 8 mm. in width; the amount of buffer needed, of course, depends on the particular type of paper being used and its width, to run freely onto each strip from a pipet, care being taken to distribute the buffer as evenly as possible over the whole length of the filter paper. The paper strips are again pulled taut and allowed to equilibrate, with the current on, for 0.5–1.5 hours, before applying the migrant, in general the migrant is applied to the strips at a point midway between their ends, although for a 50 cm. length of paper, a central zone of about 35 cm., will usually be found to yield linear movement of migrant with time. This useful zone can be increased by careful adjustment of the experimental conditions.

When a run has been completed it is important in mobility work, that the strips be dried quickly. Even in the empirical fractionation of mixtures such as serum proteins this still holds, as has been noted by Peasehold (79), if shifts of the separated zones are to be avoided. This is usually done by taping them gently from the frame and drying them quickly in an oven set at about 105–110°C. In this way, chromatographic shifts of zones on the ionogram and diffusion of the edges of the individual zones can be kept to a minimum.

In replicate experiments, under identical conditions of buffer, ionic strength, pH, temperature, etc., the displacement of the forward edge of the initial zone of application (5–12 cm., usually) can be kept to within limits of ±1–2 mm. variation from the average of all such displacements.

The Influence of pH of the Buffer on the Mobility of the Migrant

For ampholytes such as amino acids, proteins and lipoproteins, the pH of the buffer solution saturating the paper strip exerts a profound influence on the mobility of the migrant. If the mobility of such substances is measured in several buffers of constant ionic strength but varying pH, it
ELECTROMIGRATION IN STABILIZED ELECTROLYTES – PART II

It is observed that at some definite pH (the isoelectric point, pI) the mobility of the migrant, and therefore its net charge, approaches zero. At pH values below the isoelectric point, of the ampholyte, the migrant bears a net positive charge and will move toward the negative end of the paper strip, while at pH values higher than the isoelectric point, the migrant bears a net negative charge and moves toward the positive end of the paper (96).

Fig. 1 represents the 'pH-mobility' curve of bovine beta lipoprotein, which acts as an example of an ampholyte. The isoelectric points of several substances have been determined by isoelectric focusing (51, 52, 59, 81) and are found, in general, not to differ very much from the values determined by more classical methods.

The Influence of Buffer Ionic Strength on the Mobility of the Migrant

If all other factors influencing the mobility are fixed, it is found that the velocity of electromigration of a migrant can be altered greatly by varying the ionic strength of the buffer solution. The mobility of the migrant increases with decreasing ionic strength in such a way that the rate of increase of migration velocity becomes greater as the ionic strength is lowered (59). Fig. 2 illustrates this relationship for the case where the migrant is polyvinylpyrrolidone (85). The observed behavior is the result expected on the basis of known effects of electrolyte concentration on the ion atmosphere of an ion in solution or on the so-called 'zone potential' of a colloidal particle. It is evident that the time required for separation of a mixture of components is less at lower than at higher ionic strengths of the buffer solution used to saturate the paper.

When the mobility of the migrant is plotted against the reciprocal of the square root of the ionic strength of the buffer saturating the paper strips, a straight line relationship is obtained over a considerable ionic strength range (59, 85). This may be interpreted to mean that when the migrant is actually undergoing electromigration, it behaves as if it were deep in the body of the liquid (52, 59).

The Influence of Temperature on the Mobility of a Migrant

As might be expected, the mobility of a migrant increases with increasing temperature. As before, consider the mobility of PVP, which was determined at five different temperatures over the range 2-29°C (85). As the electromigration of the migrant through the buffer solution saturating the paper strip is fundamentally a simple rate process, it would be expected that when the logarithm of the mobility is plotted as a function of the reciprocal of the absolute temperature, a straight line would result, as shown in Fig. 3. In a similar way, when the reciprocal of the viscosity of the buffer solution was plotted as a function of the reciprocal of the absolute temperature, a straight line was also obtained, as shown in the same figure.

The viscosity measurements were determined by means of a simple Ostwald viscometer, at four different temperatures. The slope and the energy of activation as determined from the two curves can serve as a basis of comparison for the two processes. The energies of activation as determined from the two curves, which have essentially identical slopes, are shown to be 3500 ± 500 calories. It would appear, therefore, that the two processes are fundamentally related, that is, the shear of the liquid layers that occurs during the movement of PVP is similar to the shear of the liquid that occurs when the viscosity of the buffer is measured.

Correction for 'Added Length of Migration Path', in Paper-Stabilized Electrolytes

In a paper by Kunkel and Tiselius (42) the electromigration path of an ion or particle is viewed as a sort of meandering funnel through the paper (Fig. 4, left side). The assumption is made that, for a given paper all migrants follow along similar paths, and that since the path is much longer than the actual length of the paper, the potential gradient actualizing the migrating particle will be less than the value given by simply dividing the potential difference impressed across the paper strips by their length. On this basis, then, and after applying a correction factor for electromigration (using dextran as an indicator of the amount of electromigration movement), they apply a second correction factor for 'added migration path length', which appears to bring the mobility of the migrant up to that observed in free solution. They state that this latter correction factor, for a given type of paper, can then be used for mobility calculations under widely varying conditions. In other words, the correction factor for 'added migration path length' is a function principally of the paper, and not of the migrant.

If the view of Kunkel and Tiselius is correct it should be found that if the mobilities of different migrants are determined on several varieties of paper, the percentage change in migration velocity, for any migrant X, from say, paper A to paper B, to paper C, should be the same as for a different migrant, Y. Experimental evidence, as shown in Table 1, does not support this conclusion. As a particular example, consider aspartic acid and poly-

Fig. 2 The mobility of polyvinylpyrrolidone as a function of the ionic strength of the buffer solution. Buffer, veronal-acetate, pH, 8,17; temp., 25°C; various potential gradients and periods of time; water saturated helium atmosphere.

Fig. 3 The effect of temperature on the mobility of polyvinylpyrrolidone and on the viscosity of the buffer solution. Upper curve, the logarithm of the mobility as a function of the reciprocal of absolute temperature; buffer, veronal-acetate; ionic strength, 0.0075; pH, 4,17; water saturated helium atmosphere. Lower curve, the logarithm of the reciprocal of the viscosity of the buffer solution as a function of the reciprocal of absolute temperature.
The mobility of aspartic acid in practically the same on the different papers, while that of PVP varies from 0.18, on Schleicher and Schuell No. 413 paper, to 0.30 on Cramer-Tissius Munkells paper, that in a variation of approximately 50 per cent. In the case of the aspartic acid, the average deviation of the mobility for a given paper, from the average of the measurements on all papers, was 74 per cent, while in the case of PVP, it was 22 per cent. This experimental data can be interpreted to mean that different migrants apparently follow quite different migration paths and that therefore no single correction for so-called "added migration path length" can be applied for different migrants on the same type of paper in used to stabilize the electrolyte.

The authors have suggested an alternative view of the mechanism of electromigration in buffer solutions stabilized with paper which is consistent with the experimental facts. For ease in discussing this view, it will be referred to as "the barrier theory". According to this latter view, the paper fibers act as barriers in the path of migration and their effect will differ for migrants of different molecular volumes (Fig. 4; right side). While the particle is undergoing its forward movement, however, it is doing so essentially in the buffer solution trapped in the interstices between the paper fibers, and the potential gradient which is actuating its movement is given directly by the potential difference impressed across the paper's length divided by the length of the paper (from the surface of the liquid in one buffer vessel to the surface of the liquid in the other). According to the barrier theory, then, the correction which is applied must be a function of both the paper and the migrant. The lower mobility observed on paper, as compared to that not to an increased length of migration path over that of the actual length of the paper itself, but to the fact that the individual molecules of the migrant suffer collision with the paper fibers and therefore are momentarily slowed down, that is, in essence, their thermodynamic activity is reduced. On the barrier theory, then, the movement of the migrant is pictured as a halting one, but nevertheless, when the migrant molecule does move, it does so under the impetus of the full potential gradient given directly by the voltage impressed across the paper divided by the paper's length (from the surface of the liquid in both buffer vessels, to that in the other). It will be shown in the next section of this paper, that to agree in favor of an "increased migration path length" with the concurrent implications regarding the lowered actuating potential gradient is tantamount to denying Ohm's law itself.

A Suggested Method For Determining Free-Solution Mobilities From These Obtained in Paper-Stabilized Electrolytes

The migration of a charged particle or ion under the influence of an electrical field may be expressed in terms of "mobility" and mobility may be defined as velocity (distance of migration in cm divided by time in seconds) per unit field strength or potential gradient. Field strength is expressed as voltage drop per cm (V/cm). Hence, mobility, \( \mu \), represents the distance a particle moves in unit time per unit field strength, that is:

\[
\mu = \frac{d}{V} \frac{1}{t}
\]

where:
- \( d \) = distance the particle moves in cm
- \( t \) = time of migration
- \( V \) = voltage applied over 1 cm of distance

Therefore, in mobility experiments, potential gradient (voltage/cm) must be kept constant. However, potential gradient can only be directly measured when the conductor is of uniform cross-sectional area (and hence of uniform resistance). In moving-boundary electrophoresis the U-tube across which the voltage is impressed is not of uniform cross-sectional area throughout and the field strength cannot be obtained simply by dividing the voltage applied by the length of the U-tube. To overcome this difficulty the section of the U-tube through which the particle actually migrates must be of uniform cross-sectional area. Since

\[
V = IR
\]

where \( I \) is current and \( R \) is resistance through the solution, and

\[
R = \frac{1}{K \cdot a}
\]

for a conductor of uniform, cross-sectional area, \( a \), and length, \( l \) through which the particle migrates; \( K \) equals the specific conductivity of the solution. It follows that if \( V \) is replaced by its equivalent, that is:

\[
\frac{V}{l} = \frac{1}{K \cdot a} \frac{1}{l} = \frac{1}{K \cdot l}
\]

Assuming \( K \), the specific conductivity of the solution does not change, current, \( I \), is kept constant to obtain a constant potential gradient. It is not necessary in determining potential gradient to make these substitutions, when the conductor is of uniform cross-sectional area throughout and it is possible to measure \( V/l \) directly, as in the technique of isochroscopy, where electrolytes are stabilized with paper or other similar materials.

According to Kunkel and Tissius (42) the expectations for field strength, that is, potential gradient, namely

\[
\frac{V}{l} \text{ and } \frac{1}{K \cdot a \cdot l}
\]

are not equal in paper electrophoresis. Here, \( V \) is the voltage applied across a length of paper \( l \), \( I \) is the current flowing through the paper with an effective cross-sectional area of \( a_0 \), saturated with a buffer whose specific conductivity is \( K \). The non-equality is stated to be due to the fact that \( l \) does not represent the true distance of voltage drop through the paper. There is no need to explain the inequality by using the term "tortuous channel". Instead, it should simply be stated that there is no justification for expecting an equality when using...
**ELECTROMIGRATION IN STABILIZED ELECTROLYTES – PART II**

K, the specific conductivity of buffer solution instead of \( k_p \), the specific conductivity of the system over which the potential gradient is to be determined, that is, the "buffer-paper system." This can readily be shown by combining the equation of Ohm's law,

\[
V = IR
\]

and an equation defining resistance,

\[
R = \frac{1}{k_p}
\]

to give the equation,

\[
\frac{V}{I} = \frac{1}{k_p} + \frac{x}{D_p}
\]

If this, in turn, is substituted into the expression for field strength, \( V/I \), the equation

\[
\frac{V}{I} = \frac{1}{k_p} + \frac{x}{D_p}
\]

is obtained. From this equality, it is evident that there is no justification for "\( \frac{1}{k_p} \)" to equal 'potential gradient' because \( \frac{1}{k_p} \) is not the proper expression to be used for a system containing both buffer and paper fiber. The correct expression for field strength for a system containing buffer and paper fiber is either \( V/I \) or \( 1/k_p \), both of which are equivalent. And since it is easier to measure the voltage drop across the strips and the length of paper strips from buffer surface to buffer surface, \( V/I \) is the most convenient expression to use for field strength.

A method for determining so-called free-solution mobilities from those obtained from paper-stabilized electrolytes, which does not require a denial of the validity of Ohm's law is outlined here, and will be represented in greater detail in a later publication from this laboratory. In a specially constructed conductivity cell employing platinum black electrodes which are embedded in opposite parallel wells so that only one side of each electrode is exposed to the inside of the cell, the conductivity of the buffer solution is determined. Varying amounts of paper, which are cut into pieces as to fit inside the cell, are added. A plot of "conductivity" versus "fraction of buffer displaced" may be obtained as shown in Fig. 5. Curve AC indicates the drop in conductivity due to the displacement of buffer by paper fiber. Another curve, AB, is drawn on this graph and indicates the drop in conductivity due solely to buffer displacement without paper. That is to say, when one-half of the buffer is displaced, the conductivity should then be one-half of the original. The difference between the two curves represents the decrease in conductivity of the buffer due to the presence of paper fiber. The decrease in conductivity of the buffer ions can be looked upon as a decrease in the thermodynamic activity of the buffer ions due to the presence of paper fibers which introduce a "Barrier effect" upon the buffer ions. The more paper fiber present, the greater the decrease in conductivity and the greater the decrease in mobility. The conversion factor is then determined from the graph; the point along the abscissa which corresponds to the particular degree of wetness when the mobility measurements is calculated readily from the ratio of "buffer weight" to "unit weight of paper as follows:

\[
\frac{\text{fraction of buffer displaced}}{\text{by paper}} = \frac{1/D_p}{1/D_p + x}
\]

where:

- \( D_p \) is the density of the paper fiber
- \( x \) is the ml. of buffer present in one gram of paper.

**Fig. 5** A plot of "conductivity" versus "fraction of buffer displaced";

Curve AB represents the decrease in conductivity due solely to the buffer displaced. Curve AC represents the decrease in conductivity when the buffer is replaced by the paper fiber. The difference between the two curves represents the effect the paper fiber has on the thermodynamic activity of the buffer. The vertical dotted line represents the fraction of buffer displaced when E&D No. 613 paper is used in the ionograph. The ratio of the value of the ordinate corresponding to the upper horizontal dotted line, to that represented by the lower horizontal dotted line, represents the correction factor for determining free-solution mobilities from those obtained ionographically.

The conversion factor is then the ratio of the ordinate of line AB to the ordinate of AC, where these two curves intersect with the dotted line drawn vertically from the point along the abscissa which corresponds to the experimental conditions existing at the time the mobility measurements were made. For example, bovine serum albumin (in a veronal buffer, pH 8.6, ionic strength 0.10, using E and D paper No. 613 at a temperature of 25°C) has a mobility of 3.00 ± 0.10. The ratio of "buffer weight" to "unit weight of paper" existing during the run is 2.09; hence, the fraction of buffer displaced by paper is 0.248. This is represented by the vertical dotted line in Fig. 5. At the point where this line crosses curve AC, the ordinate is 3.90, and at the point where it crosses curve AB, the ordinate is 2.42. The conversion factor is then 3.90/2.42 or 1.60. The free-solution mobility is then (3.00 ± 0.10) X 1.60 or 4.80 ± 0.16. Add to this figure a correction factor for "electroosmotic flow" (as indicated in this instance by the movement of dextran) bringing the value up to 5.73, which is in the range observed in free solution, under similar conditions.

In preliminary experiments, the conversion factor has been found to hold quite well; however, it is felt that perhaps the migrant itself, buffered at the pH under which the mobility is determined, is the more proper material to be placed in the conductivity cell when determining the decrease in thermodynamic activity, instead of the buffer alone, since it is the mobility of the migrant that is being determined. This latter conversion factor, then, would be dependent on the type and wetness of the paper used, on the nature of the buffer solution and on the characteristics of the migrant. It is felt that this conversion factor would then perhaps include the effects of adsorption, barrier and electrophoresis.

**Electroosmosis – Statement of the Problem**

On classical grounds it has been pointed out that in electromigration in stabilized electrolytes, the mobility of positively charged ions might be expected to be increased while that of negatively charged ions might be decreased, due to the phenomenon of electrophoresis (32, 37). In the case of electrolytes stabilized with paper, this conclusion is based on the observation that the paper surface generally appears to bear a negative charge, while the paper solution in contact with it, acts as if it were positively charged. Consequently, the resultant velocity vector of the positive ions would be expected to be the sum of the simple electromigration velocity and the electrophoretic velocity of the solution in which they are being buoyed along. Negative ions, on the other hand, have been pictured as breaching the electrophoretic tide. The resultant velocity vector in this latter case would then be the vector difference between the simple electromigration velocity and the electrophoretic velocity of the liquid.

**Attempts to Reduce Electroosmosis**

Michel (55) has described an apparatus that resembles in many respects the equipment used in the author's laboratory. There is one important difference, however: he isolates the horizontal portion of the paper strips, which have been saturated with buffer, from the end portions which dip into the buffer solution, by means of cellophane dialysis tubing, thus permitting the cellophane barrier in the manner described above. He reasons that water shifts due to electroosmosis are reduced to a negligible
For while at the same time electrical conductivity is essentially unimpaired, although the rationale of the procedure adopted by Möbius is not unsound, experiments with his technique have so far proved inconclusive as regards the efficacy of the cellulose barrier to overcome the problems associated with electroosmosis.

Attempts to Measure Electroosmosis

It would seem reasonable on first consideration that, if a substance which exhibited a negligible electronegative mobility in the traditional moving-boundary apparatus were placed as a thin streak across a paper strip, in the isocratic technique, its movement would serve as a measure of the amount of electroosmosis. In the authors' laboratory attempts have been made to use amino acids in the neighborhood of their isoelectric points as electroosmotic indicators. Creatinine has been used (12a) and tryptophan and alanine by the same investigators below pH 9.0 (12b). Xylose has also been used by Schwindt (82b). Starch, amylose, sucrose, and other polymers have also been used (42) but probably the most widely used substance is dextrans (42), a higher molecular weight polysaccharide.

In the light of the barrier theory of electrophoresis, in electrolytes stabilized with paper, it would seem that the use of an electroosmotic indicator is valid only if its molecular volume approaches closely that of the particular migrant under study. If the indicator and the migrant differ widely in molecular volume, the indicator, for example, having a much larger molecular volume than the migrant, it will suffer many more collisions with the paper fibers than will the migrant, as they are buoyed along by the electroosmotic tide (classical theory), and it should undergo considerably less electroosmotic displacement than the migrant. The application of a correction factor to the mobility of migrants of a wide variety of molecular volumes, based solely on the movement of dextran, is highly questionable, and the indiscriminate use of such correction factors should perhaps be discouraged. Unless an investigator has some evidence which indicates that the molecular volume of the indicator he proposes to use is not widely different from that of the migrant, it would seem preferable to report simply the experimental mobility data as obtained rather than attempting to apply a questionable correction factor. When enough indicators to cover a wide range of molecular volumes become available, correction factors of less questionable validity may then be applied.

In preliminary experiments in the authors' laboratory, run under identical conditions of buffer, ionic strength, pH, temperature, etc., the movement of creatinine, proline and dextran have been found to be different, the dextran showing the least movement. These results support the views expressed in earlier paragraphs relative to the effect of the molecular volume of an electroosmotic indicator on its movement. There is a tendency, nowadays, to discount the role played by the Helmholtz double layer in the phenomenon of electroosmosis. This school of thought may perhaps be exemplified by the work of Dumas (146) who explains the transport of water molecules associated with electroosmosis on the basis of transport of water molecules by ions. His work on ultraviolet absorption studies leads him to the conclusion that the number of water molecules electrically bound to a chloride ion is of the order of 1000, in a solution of 6.05% normal NaCl, and that the great difference in the hydration of cations and anions can explain the electroosmosis phenomena. In general, positive ions are more highly hydrated than negative ions, and hence there would be a tendency for a net flow of water toward the negative electrode. He derives an equation, in fact, purporting to relate in a quantitative way, the rise in solution at one electrode due to electroosmosis, the potential, the number of ions of one sign per milliliter, the viscosity of the media, the mobilities of the positive and negative ions and the number of moles of water-in-hydration associated with the positive and negative ions, respectively.

Whether the phenomenon of electroosmosis is viewed from the classical or modern viewpoint, however, does not invalidate the idea that there may be some effect on the mobility of a migrant, and from the standpoint of this paper, these theories can be shown to lead to essentially the same conclusion.

Influence of Electroosmosis, Absorption and Barrier Effect

In Fig. 6 an attempt is made to represent, in an idealized, schematic fashion, the possible influence on the mobility of an emulsol, under a wide range of pH, of such factors as electroosmosis, absorption and barrier effect. The curve DFJ represents the shift to be expected from electroosmosis alone, all other interfering effects being assumed to be absent; the effect, as indicated in this instance by the movement of dextran and PVP, is greater at higher than lower pH values. In the region, pH 6 - 9, the effect of electroosmosis is reduced but little as the pH is reduced. As the pH is reduced to values lower than 6, the effect of electroosmosis falls off until in the region of pH = 4.5, it is perhaps only 30% of the value between pH 6 - 9. In any case, the net effect of electroosmosis alone is to cause a shift to the right throughout the entire "pH-mobility" curve, and it would then follow that isoelectric points determined by a study of electromigration rates in stabilized electrolytes would be higher than those obtained by other techniques. The magnitude of the difference in the isoelectric points should be a measure of the amount of electroosmosis. The difference observed would be expected to be smaller, the lower the isoelectric point of the migrant. The effect of electroosmosis will also appear to be manifested in direct ratios as the migrants chosen for study exhibit lowered mobilities. That is to say, if the slope of the pH-mobility curve is steep, the effect of electroosmosis will appear to be less than if the slope is small in particular, the effect of electroosmosis in shifting the isoelectric points of a slower-moving migrant, such as a serum gamma globulin, to higher values than observed in free solution will appear to be greater than for a faster-moving migrant such as serum albumin. In Fig. 6, a given shift in the mobility of some particular migrant may be represented by the dotted line, EK. For a slower-moving migrant, such as gamma globulin, it is obvious that a correspondingly large shift, EP, in the isoelectric point, will be greater than for a faster-moving migrant.

The curve AEG illustrates the effect to be expected if there is any tendency for adsorption of the migrant on the paper surface. It might be expected that (in the absence of complex-ion formation) due to the slight negative charge on the paper surface, the electroosmotic velocity of positive ions would be decreased, while that of negative ions would be relatively unaffected. Thus the factor of adsorption by itself would not be expected to cause any appreciable shift in the isoelectric points of emulsol when determined by immunology. It would be expected, in general, that the lower the pH of the buffer solution below that of the isoelectric point of the migrant, the greater would be the adsorption of the latter. As the pH of the buffer solution approaches the pH of the migrant, however, the effect of adsorption would be expected to decrease to zero. As
ELECTROMIGRATION IN STABILIZED ELECTROLYTES – PART II

...a rule it would seem preferable to run isotropic determinations on ampholites in a buffer whose pH was greater than that of the pl. of the migrant. Under such conditions the factor of adsorption would be minimal. The same recommendation can generally be made with regard to eluting the dispersed zones of ampholytic substances from an isoelectric point.

The line BEH represents the effect on the mobility of a migrant, of stabilizing the electrolyte medium with a material such as paper. Due to the barriers in migration introduced by the presence of the cellulose fibers, the mobilities of both positively and negatively charged ions or particles would be reduced, but there should be no effect on the experimentally-determined isoelectric point of a migrant. In summary, then, it can be said that neither adsorption phenomena between the migrant and the paper surface, nor the "barrier effect", would be expected to alter the value of the isoelectric point of an ampholyte, when determined by isoelectrofocusing. They simply cause a counter-clockwise rotation of the pH-mobility curve about the pl of the migrant, as a pivot. Electromigration, on the other hand, might be expected to yield pl values, when determined in stabilized electrolytes, which were greater than these determined in non-stabilized electrolytes, the magnitude of the effect seeming to be magnified in the case of migrating lower mobilities and higher pl values.

Comparison of Isoelectric Points Obtained from Mobility Data in Stabilized and in Non-Stabilized Electrolytes

The isoelectric points of a number of ampholytes such as amino acids (5), proteins (51,52,81,96) and lipoproteins (51) have been determined by isoelectrofocusing methods, and it is perhaps surprising that, in general, the differences from those determined by more classical methods is not as great as might have been anticipated. The interfering effect of electromigration in isoelectrofocusing measurements would therefore also appear to be less than might have been expected from a priori considerations of the extent of the interfacial surface between the buffer solution and the material used to stabilize it. This observation may perhaps, be explained by the behavior of hydronium ions in an electric field. The idea has been put forward that when a potential is applied across a column of water some few hydronium ions travel through the solution in the ordinary diffusion manner. However, the principal mechanism responsible for the rapid ionic transport involves the transfer of a proton from a hydronium ion to an adjacent water molecule, as follows (25):

\[ \text{H}^+ + 
\begin{align*}
\text{H}_2\text{O}^-&+ \text{O}^- \rightarrow \text{H}^- + \text{H}_2\text{O}^- \\
\text{H}^- + \text{H}_2\text{O}^-&+ \text{H}_2\text{O}^- \\
&\rightarrow \text{H}_2\text{O}^- + \text{H}_2\text{O}^-
\end{align*}
\]

The newly formed hydronium ion, in turn, can now transfer a proton to still another water molecule, in a sort of Grothuss chain conduction. In this way, the hydronium ion acquires a high mobility. After the passage of the proton, the water molecules are oriented in a manner different from that which existed while the proton was being transferred. If the prototropic process of conduction is to continue, therefore, each water molecule must rotate after the proton has been passed on, so that the donor water molecule may again be in proper position to accept a proton. It has been computed that the prototropic contribution to the conductance of hydronium ions in water at 25°C, is approximately 85% (23). Thus the apparent movement of hydronium ions through the solution from anode to cathode, which, according to the modern school of thought, must play a contributing role to what looks like a bulk movement of water from the positive to the negative end of the paper strip, is actually largely a movement of protons. On this basis, the electromigration effects due to the bodily transfer of water associated with the movement of hydrogen ions would be expected to be relatively small as compared to the result to be expected if there were no proton transfer between hydronium ions.

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STUDIES WITH Labeled ERYTHROCYTES AND IODO-ALBUMIN

by

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Cr⁶¹, a soft X-ray emitter, with 26.5 day half-life has been employed for the measurement of circulating red cell volume (mcm). When added to red cells as sodium chromate, the isotope is firmly bound by hemoglobin. On reinjection into the subject, the labeled erythrocytes retain their radioactivity without appreciable loss through the course of a day.

Human serum albumin labeled with 2-3

iodine atoms per molecule remains tagged after intravenous injection in rabbits and behaves like native protein by immunological tests. On injection into human subjects, plasma radioactivity falls relatively rapidly during the first few days, signifying distribution of the labeled protein in the body's exchangeable albumin pool. Thereafter a gradual decline of plasma radioactivity occurs, due to slow metabolic degradation of the protein. The experimental curves have been resolved into two exponential decay rates representing distribution and degradation. Qualifications of these interpretations are discussed.

Abstract of lecture delivered before the New York Section AACC, April 18.
THE SPACES OF DISTRIBUTION OF TAGGED ERYTHROCYTES AND SERUM PROTEINS AND OF Na²⁴ AND Br²²* 

by 
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In man, the spaces of distribution of red blood cells tagged in vitro with K₂¹ and P₂² give essentially identical values for total circulating erythrocyte volume in simultaneous studies (1). Using labeled human serum albumin and gamma globulin also mix in identical volumes during the first 15 minutes after intravenous administration (2). However, simultaneous determinations with tagged erythrocytes and tagged albumin reveal an overall relative cell volume of the body approximately 75% lower than that indicated by the centrifuge hemocrit or the peripheral blood corrected for trapped plasma (3). This is presumed to result from the relatively hypovascular blood of minute vessels (4).

The intravascular biological half life of P₂² tagged erythrocytes averages 24 hours. In individual subjects, this rate of fall may be determined precisely over a period of several hours and extrapolated beyond, for the evaluation of blood volume changes in acute experiments, thus obviating reinfusion techniques (5).

Distribution of K₂¹ labeled human serum albumin results in a fall in plasma concentration at two major exponential rates equivalent to half times of about 3 and 24 hours. Distribution equilibrium is reached in 4-6 days in the non-obese subject but requires as long as 16 days in the patient with emaciated anemias. The apparent space of distribution of albumin averages about 2/3 times the plasma volume (6, 7, 8). Na²⁴ and Br²² eventually distribute into apparent spaces considerably greater than that occupied by extracellular fluid which is presumed to be accurately measured by insulin (9) and sucrose (10). However, in non-obese subjects, Na²⁴ and Br²² (corrected for red blood cell penetration) diffuse at a rapid rate within the first 15-30 minutes into almost identical and reproducible apparent spaces approximating that of extracellular fluid (10). Subsequent increases in spaces of distribution of Na and Br occur at a considerably slower rate and are presumed to be due chiefly to intracellular penetration. Extrapolation of this slow rate to zero time permits correction for cellular perversions during the "extracellular" diffusion period. However, analysis of leg edema, pleural fluid and ascitic fluid in subjects with heart failure, leukemia and cirrhosis of the liver respectively reveal that uniform distribution of Na²⁴ in extracellular fluid in far from complete at the time of the "slow" second phase of distribution. Since there is no assurance that extracellular diffusion is complete at this time in the normal subject as well, it is concluded that the early space of distribution cannot be presumed to be identical to that occupied by extracellular fluid. More likely, sodium and bromide, as well as other predominately extracellular electrolytes with an intracellular phase, have at the end of 15-30 minutes, diffused into a large fraction of the extracellular fluid and a small portion of the intracellular space. The sum of these two spaces incidentally approximates the true extracellular fluid volume in non-obese subjects. The determination of extracellular fluid volume by such methods is therefore considered to be unreliable.

Dr. Sidney S. Schreiber, Joseph Post and Lawrence H. Waishon participated in various phases of the studies reported here.

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*Abstract of lecture delivered before April meeting New York Section, AACC.

THE CHEMISTRY OF BONE AND TOOTH FORMATION

By 
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The inorganic composition of bone, enamel, and dentin may be represented in the formula shown below:

\[
Ca_{10}(PO_4)_2\cdot\text{CoCO}_3 
\]

where \( n = 2.1 \) to 9.3

About 5% of the calcium may be replaced by magnesium, sodium, potassium, lead, etc. Some of the carbonates is probably replaced by citrate, fluoride, hydroxyl ion, sulphates, and other anions.

Despite the wide variation in inorganic composition, the crystal structure as shown by X-ray diffraction studies is invariably that of an apatite. After ignition of 90% of this structure changes to \( \beta^{-}\text{Co}_4(PO_4)_2 \)

when the \( \text{Ca}_2\text{PO}_4 \) mole ratio is 1.5 or less, and continues to be apatite when this ratio is 1.6 or more.

The \( \text{CO}_3^{-}\text{PO}_4 \) ratio of growing enamel, dentin, and bone is related to serum \( \text{CO}_3^{-}\text{PO}_4 \) ratios. Serum \( \text{CO}_3^{-}\text{PO}_4 \) ratios are, in turn, related to dietary \( \text{CO}_3^{-}\text{PO}_4 \) ratios. Thus, the \( \text{CO}_3^{-}\text{PO}_4 \) ratio of enamel, dentin, and bone can be influenced in a predictable manner by regulating the diet.

*Abstract of lecture delivered on May 22, 1953 before joint meeting of Chicago Sections of AACC and ACS.
BONE AND TOOTH FORMATION

by

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Dr. Brodie (1) has discussed some features of the relationship between antipyrine and heavy water as agents for measuring the distribution of water. The chief argument for the use of heavy water rests upon the fact that it is tracer water, as it is water made with tritium or with oxygen-18. Any other agent has to be compared with a form of tracer water before its validity as an agent which measures the distribution of water in a given situation is assured. Agreement in the apparent volumes of distribution of deuterium oxides and another agent in one species or in normal subjects does not assure similar agreement in other species or under pathological conditions.

In kinetic studies the use of a tracer of water is essential. Unfortunately, the measurement of tracer water is more difficult than that of substitutable agents. The only radioactive form available, tritiated water, is difficult to count because the energy of the tritium beta particle is only 0.041 Mev, or about 1/8th the energy of carbon-14 beta particles. The stable isotopes, deuterium and oxygen-18, form varieties of heavy water which differ from ordinary water in a number of physical properties. The mass differences make analysis with a mass spectrometer possible. Other methods are based upon the differences which occur in the density, the vapor pressure, the refractive index, or other properties (2). Among the more satisfactory methods for measuring small differences in density has been the falling drop method. Briefly, in this method one observes the rate of fall of a drop of the sample being tested as it falls through a column of ethylfluorobenzene, an organic liquid which at 27°C has a density slightly below that of water. By carefully adjusting the temperature one can establish convenient falling times for the range of concentrations expected. This method is sensitive to small changes in temperature and a water bath regulated to 0.005°C is essential. The method is also especially sensitive to the presence of impurities and the samples must be purified by distillation to assure accuracy. Schlichter, Moore and associates (3) have used this method in many studies and have developed an elaborate distillation apparatus for processing several samples simultaneously (4).

I have been more interested in the compartmentation of the body water and in the kinetics of its exchange among these compartments than in equilibrium measurements of total body water. There are several approaches to the problem of compartmentation. One is to compare the volumes of distribution of other agents which have more restricted distributions, such as labeled red cells, dyes, or tagged albumin for the blood volume and sodium, chloride, bromide, tritium, sucrose or other agents for the extracellular fluid. Some of these are discussed in other papers on this panel. Another set of clues to the compartmentation is obtainable from the curve of the approach of the concentration of the tracer to equilibrium. In general this curve can be approximated by a few exponential components. Granted that there is some controversy as to how well justified one is in representing such a presumably complex set of processes by as few exponential components as the procedure does have rational theoretical support and seems to me to be well worth using. A greater hazard arises from the ways in which these exponential components can be interpreted. In a compartmental system in which there is exchange back and forth, the behavior of a tracer in one compartment is affected by the events in each of the other compartments. In particular, it is usually incorrect to assume that a given component, as obtained experimentally, represents any particular process. A key reference for the correct procedures and interpretations is the article by Shepard and Householder (5).

1 Based upon talk (Body Water Studies with Deuterium) presented at meeting of New York section of the American Association of Clinical Chemists April 14, 1953.
It turns out that the exchange of deuterium oxide among the body compartments is quite rapid. Moore and coworkers (3) found the rates of exchange in the arm to be so rapid that the fast components seen in the arterial plasma curve are effectively masked in the venous plasma curve. The two curves are widely separated at first and it takes about an hour before they appear to coincide. Furthermore, even the arterial curve is markedly affected by the exchange which occurs in the pulmonary circulation, so that the apparent volume of distribution as measured by the first obtainable arterial sample is much greater than the plasma volume. An implication of this is that from the kinetic viewpoint the boundary between the blood and some of the major divisions of the extravascular fluids is of little importance—these rapidly exchanging spaces are effectively part of the vascular system so far as water exchange is concerned. There are at least two ways of accounting for the slow components which are seen: the effect of slowly exchanging portions of the extracellular fluid or the effect of exchange with intracellular water. Edelman (6) has pursued this problem and concludes that transcapillary exchange of water is important only during the first minute following injection. He explains the slower components which are found on the basis of differing rates of exchange with the intracellular water of various tissues. 

A different picture is obtained in the edematous states. The approach to equilibrium is much slower. Prentice, Sli, and Joiner (7) have compared ascitic fluid and plasma equilibration curves in subjects with ascites due to cirrhosis or to metastatic carcinoma, and show that in these conditions the behavior of tritium-labeled water can be approximately described by the equations for a two-compartment system, with ascites as one compartment and the rest of the body water as another. The ascitic fluid turnover rate was found to be 40 to 80 per cent per hour. We have some similar studies made with deuterium in edematous nephrotic children but the analyses have not been completed and cannot be reported at this time. We do have the results on antipyrine studies which were made simultaneously and find that in edematous subjects antipyrine is not entirely satisfactory. When an agent is metabolized or excreted at a comparatively rapid rate, the mathematics which are applicable to closed systems no longer hold. The situation is closely analogous to the parent-daughter relationships which occur in some radioactive decay schemes. Figure (1) shows curves obtained with antipyrine in the venous plasma and in the ascitic fluid of a nephrotic child. The only instant that the concentration in the same in these two compartments occurs when the curves cross. Any other compartment, such as the peripheral edema fluid, will most probably have a concentration curve which crosses the plasma curve at a different time, so there is no time at which the entire system is in concentration equilibrium. Figure (1) also shows the curve obtained with sodium 24 in a simultaneous study.

Since this subject was not excreting much sodium, the closed system principle does apply. This and other studies made with sodium-24 support the hypothesis that the relatively slow rates of water exchange seen in the edematous nephrotic are extracellular phenomena.

In conclusion, the argument for the use of tracer water in body water studies has been reviewed and some applications of the methods of determining the compartmentation and kinetics of body water have been discussed.

Bibliography
1. Brodie (First paper on this panel)

REVIEW OF CURRENT LITERATURE
ELLENMAE VIERGIVER—EDITOR
CECILIA RIEDEL, C. VON FRITTAG DRABBE, HARRY G. ANRODE


In the method described, o-estradiolbenzoate, estrone, oestradiol, and ethyl estradiol were used for separation. A number of color reagents are described and the behavior with various compounds is tabulated. Quantitative measurements were made by means of a spectrophotometer at 280 to 252 mλ.


A modification of the method of Cook for the detection of sub-microgram quantities of urea.

H.G.A.


Quotis's micromethod is modified by using CHCl₃ instead of xylene as the solvent to obtain a more intense color. H.G.A.


The substrate contains acetylcholine which is buffered to pH 7.5 and phenol red. Cholinesterase activity is calculated from the decrease in intensity of the absorption band of the indicator at 550 mλ.

H.G.A.

MICROANALYTICAL METHODS FOR PROTEINS IN BLOOD PLASMA. H. B. Salt (Royal Infirmary, Worcester, England). Analyst 76, 4-14, 1951.

A review article with 112 references.

H.G.A.
REVIEW OF CURRENT LITERATURE


Intervenous injection of sodium cholate into normal fasting rats produced an experimental hyperlipemia. The lipemia responded to a comparable percentage increase in the concentration of each plasma lipid fraction, and not from preferential increase in any one particular plasma lipid. E.V.


Parathyroid extract produces an increase in blood phosphorus, but no change in the alkaline phosphatase activity of the kidney of male rats. E.V.


By a system of cross-dialysis, blood of a uremic subject was cross-dialyzed with that of a subject with normal kidneys. There would be no mixing of blood between the two circulations. In such a system, the PD is anticipated that the normal subject would remove through dialysis "toxic products" from the uremic subject and then utilize its own kidneys to excrete them. It is believed that the procedure is relatively uncomplicated, safe, and requires few laboratory checks during operation. Further study is required to determine whether or not it has any value as a clinical test. E.V.


The kinetic interrelationship of the amino acid and protein metabolism is assumed to be illustrated by the schema:

PROTEIN

The author's experiments indicate that the metabolic pool is small and that it turns over rapidly. The immediate impact of an injected amino acid appears to be largely disrupted within several hours; from theory and in vitro, amino acids are released from the protein and the picture. Previously reported measurements of the metabolic pool did not take into account the importance of urea nitrogen excretion as a rate determining step.

C.V.F.D.


A general discussion of methods and applications.

H.G.A.


A critical discussion.

H.G.A.


In two series of 12 and 13 consecutive analyses of the same serum variations of 2 to 10% of the individual fractions were observed.

H.G.A.


A.A.


0.4 to 0.5 mg. of aqueous amino acid solution (containing 0.05 to 0.5 percent of amino acid) in a volume of 1 ml. of KCN-pyridine solution and 1 ml. of 80% phenol reagent. After the mixture reaches the temperature of the waterbath, add 0.2 ml. of ninhydrin solution. Stopper the tube, allow to stand for 5-5 minutes, Cool, Dilute to 10 ml. with 50% ethanol. Read at 700 m. against a blank of 0.4-0.5 ml. of ammonia-free water, treated as the unknown. All amino acids, except tryptophan and alanine, react quantitatively.

0.1 ml. of urine or sample containing 1-15 mg. of hydroxyproline are placed in a Polyn-Wu tube. The solution of the urine is adjusted to pH 7.0 with solid dicyclohexyl potassium phosphate (to some solutions add 0.1 ml. of phosphate buffer pH 7.0, 0.1 M). Add 2.5 ml. of benzene. The tube is attached to a vibrator, the bulb dipping into a waterbath of 75°C. The vibrator is started and 0.2 ml. of ninhydrin solution is added. Allow to stand for 5 minutes. Remove the tube from the waterbath, Make up the benzene layer to 10 ml.

in a volumetric flask. Read the density of 550 mg. in a Beckman spectrophotometer. Normal value for urine: 0.5-2.5 mg. of hydroxyproline per day.

C.V.F.D.

A STUDY OF THE SYNTHESIS OF CREATINE BY LIVER PREPARATIONS. S. Cohen (Department of Pediatrics, University of Colorado School of Medicine, Denver, Colorado). J. Biol. Chem. 201, 93-102, 1953.

Homogenate of livers of adult guinea pigs synthesizes creatine in the presence of glutamic acid, L-methionine, and ATP. Some of embryonic guinea pigs require in addition a component of the tricarboxylic acid cycle, e.g. fumarate. The latter also enhances the formation of creatine by whole homogenates of the liver of adult rats when a pteridine derivative such as folic acid, aminoala, or 6-pteridinol-dehyde was added.

Soluble preparations capable of creating synthesis have been obtained from guinea pig and rat livers. But liver preparations require more ATP than those of guinea pigs.

Oxygen is required by whole homogenates. Methionine serves as a specific methyl donor in the reaction. Magnesium ions are required.

C.V.F.D.


The availability of a phosphate acceptor is believed to be an important requirement for oxidation in organized systems in which oxidation is coupled with phosphorylation. Adenosinediphosphate is in such a system present in mitochondria and its concentration in the mitochondrion rather than in the cell as a whole is believed to regulate the rate of which oxidation occurs in a system such as that involved in citric acid cycle from oribifolate, carbamylglutamate and NH₄Cl. In resting mitochondria a slow breakdown of ATP provides ADP in low concentration so that little oxidation occurs. When the citric acid cycle is in operation, an increase in O₂ uptake occurs as synthesis proceeds.

The concentrations of the nucleotides involved, ATP, ADP and AMP, appear to remain unchanged in the medium. It is thought that such changes are localized in the mitochondria.

Dinitrophenol, hexokinase and calcium ions which also convert ATP to ADP show the same general picture of increased oxidative rate with constant levels of nucleotides in the medium.

C.V.F.D.

COLORIMETRIC MICROESTIMATION OF HUMAN BLOOD CHOLINESTERASES AND ITS APPLICATION TO POISONING BY ORGANIC PHOSPHATE INSECTICIDES. F. L. Metcalfe (Univ. of California, Citrus Exp. Sta., Riverside). J. Econ. Entomol. 44, 802-90, 1951.


A review. C.R.


A review with 227 references. H.G.A.


Paper electrophoresis studies of the effect of heparin on serum patterns. H.G.A.


Mg++ is retained following surgery. Excretion of Mg++ is influenced by ACTH. H.G.A.


The amino acids and nitrogen bases of urine of normal males have been studied by means of a chromatographic separation on Dowex 50, supplemented by paper chromatography. Concentrations of amino acids were measured by means of ninhydrin.

Approximately 1 g. of amino acids was found to be excreted each day in unconjugated forms, 70% of which consists of threonine, glycine, histidine and histidine. Others identified are: threonine, serine, asparagine, alanine, aspartic acid, cystine, valine, isoleucine, leucine, tyrosine, phenylalanine, and lysine.

The following were either absent or present in concentrations of less than 15 mg. per day: aspartic acid, proline, methionine, citrulline, glucosamine, hydroxylysine, ornithine, and arginine. Glumatic acid appeared only after urine had stood several days.

Following hydrolysis of urine with HCI the amino acids are extracted each day in conjugated form. Glycine, glutamic and aspartic acids constitute the major portion of the conjugated amino acids. C.V.F.D.


The administration of ACTH to humans has been shown to be effective in the excretion of the excess of uric acid, ammonia, urea, and some undetermined nitrogenous constituents. All of the urinary nitrogenous constituents returned to normal levels shortly after cessation of treatment. The possible significance of some of the findings is indicated. E.V.


Following electrophoresis of blood serum on filter paper, the strips of paper are cut out the boundaries of the various protein fractions, and these cut pieces after extraction with method alcohol are analyzed for N by micro-Kjeldahl procedure. Protein fractions are isolated from 30 ml. of blood. This method was shown to give results in good agreement with free electrophoresis by the Tiselius method, and at least as good results as those reported for the dye edition method following paper electrophoresis described by Crema and Tiselius. The results by the procedure described herein are in better agreement with results the results obtained by salt fractionation methods. C.R.


Determination of the in vitro dehydrogenase activity of various human cancer tissues indicates 1) no correlation between in vitro dehydrogenase activity of the tumor tissue and clinical behavior of the tumor; 2) the degree of inhibition of in vitro dehydrogenase activity of tumor tissue by urethan and by triethylene melamine in variable but there appears to be correlation between the inhibition by these drugs and the radio sensitivly of the tumor tissue. C.R.


A statistical study of errors involved in pipetting and in various steps of the procedure in determination of blood glucose, serum inorganic phosphate and serum cholesterol according to the manual accompanying the Klett-Summerson photometer.

C.R.


The case reported is that of a 45 yr. old woodcutter engulfed in a gasoline fire in a gasoline arnest. Chemical analysis of the liver showed a concr. of 45 mg Dornson per 100 gm tissue. C.R.


1. The extent of the incorporation of the methyl group of methionine and of betaine into tissue choline and creatine is decreased in the folate acid-deficient rat.

2. Homocysteine or cystine increased incorporation of C14 into choline or creatine.

3. Added choline or betaine partly overcome the effects of folate acid deficiency.

4. Less creatine was synthesized from the methyl group of choline than from the methyl group of methionine, betaine, formate, the L-carbon of glycine, or the f-carbon of serine. C.V.F.D.


α-ketoglutamic acid + DPNH + C5A ~ α-succinyl C5A + DPNH + CO₂ + H₂. The reaction is catalyzed by α-ketoglutaric oxidase. Succinyl C5A is a thioseter. The reaction is specific for DPNH. At high concentrations pentonate can react C5A. The reaction equilibrium is far toward α-succinyl C5A production, and the DPNH formed in independent of pH.

C.V.F.D.
REVIEW OF CURRENT LITERATURE

CHOLESTEROL SYNTHESIS BY LIVER. III. ITS REGULATION BY INGESTED CHOLESTEROL. C. van T. Monty. H. Sheppard and L. H. Chiu (Depart. of Physiology of the University of California School of Medicine, Berkeley, Calif.). J. Biol. Chem. 201, 137-141, 1953.

Female rats were fed diets with and without added cholesterol. After 5 to 8 days the rats were sacrificed, the livers were excised, and 500 mg. of slices incubated for 3 hours at 37° C. with 1 μM of carboxyl-labeled acetate.

When a diet contained 5 per cent cholesterol, cholesterol synthesis from acetate practically stopped. Marked depression of synthesis occurred also in rats fed 0.5 per cent cholesterol diets for 7 days. Even a single feeding of cholesterol showed an effect. The results indicate that cholesterol synthesis in the liver is under homeostatic regulation by dietary cholesterol. The findings raise the question as to whether severe restriction of dietary cholesterol offers any real advantage in the control of atherosclerosis.

G. F. D.


After a one hour rest period the blood proteins of 30 pregnant women showed a marked drop from original values. The A/G ratios were not altered.

H. G. A.


The object of the study was to assess the value of endoderic creatinine clearance as a measure of glomerular filtration rate (GFR) in infants and young children. It is concluded that the method of Sennens and Tausky is not suited for measuring GFR in infants and young children.

H. G. A.


A review and discussion with 23 references.

H. G. A.


It is suggested that the distribution, rather than the total vol. of extracellular fluids has a regulatory action on Na+ excretion.

H. G. A.


Ca2+ binding by plasma protein does not impede transcapillary movement of Ca2+. The redistribution of Ca²⁺ between the bone and ionized states in a rapid process. The rates of transcapillary movement of plasma Ca²⁺ and Na⁺ are equal.

H. G. A.


Fractional salting out with phosphate salts shows human hemoglobins to consist of hemoglobin a in its relative proportions in fetal and adult blood.

H. G. A.

MODIFIED PROCEDURE FOR DETERMINATION OF PROTEIN-BOUND IODINE IN SERUM. H. Sobel and S. Sapin (Cedars of Lebanon Hosp., Los Angeles, Calif.). Arch. chem. 24, 1929-31 (1952)

A modification of the Chowney procedure to cut the running time in half is described.

H. G. A.


A biochemical and physical review with 216 references.

H. G. A.


A review with 113 references.

H. G. A.


H. G. A.


Acute leukemia may be accompanied by hypokalemia which is attributed to neoformation of tissue.

H. G. A.

SOUTHERN CALIFORNIA SECTION

Telfer B. Reynolds, M.D., Instructor in Medicine, University of Southern California Medical School, was guest speaker before the local section and guests April 7 at the Cedars of Lebanon Hospital, Los Angeles. Dr. Reynolds recently returned to this country after one year as research fellow at Hammersmith Hospital, London.

Dr. Reynolds listed the following determinations as of particular importance to "Diabetes Mellitus in Electrolyte Disturbances:" NPN, CO2, Cl, Na, K, P, blood pH, urine pH and specific gravity, and plasma specific gravity or hematocrit.

Dr. Reynolds expressed the opinion that the chemical clinician can render valuable service as a consultant to the physician who is not a specialist in electrolyte disturbances, particularly by indicating to him the possible causes for any abnormal levels found in the above determinations. Following up this suggestion, he illustrated his address with a series of charts, each of which concerned a particular abnormal level. (For example, high NPN) and concisely listed those pathological conditions that are most frequently found responsible.

John W. Mehl, Ph.D., Professor and Head of the Department of Biochemistry and Nutrition, University of Southern California Medical School, returned for a second engagement as guest speaker May 8 at the Los Angeles County Hospital. Dr. Mehl, a specialist in protein research, especially by electrophoretic techniques, spoke on "Lipoproteins in Human Plasma."

The season was concluded with the annual dinner meeting at the Carolina Pines restaurant, June 2. Merle L. Lewis, Ph.D., University of Southern California Medical School, automatically succeeded Dr. R. J. Henry as Chairman. Herbert O. Carne, Ph.D., Long Beach Veterans Administration Hospital, was elected to succeed Dr. Lewis as Program Chairman and Chairman-Elect. William McClesky, Chemist Chemical Laboratory, was elected to succeed Kenneth Johnson as Secretary-Treasurer.

During the past season, the local section has customarily met on the first Tuesday of each month for a scientific session followed, after a brief interval, by a business session (except in March when a luncheon meeting was held during the 123rd National Meeting of the American Chemical Society). During the season, the section and guests were addressed by five guest speakers and four member speakers. When the new season begins next September, the local session will again extend to all those interested in its customary welcome to attend all scientific sessions. (Individual announcements
PHILADELPHIA SECTION

The fourth meeting of the 1952-53 season of the Philadelphia Section, American Association of Clinical Chemists, was held on Tuesday, February 24, 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. Albert E. Sobel, President of the American Association of Clinical Chemists, Head of the Department of Biochemistry of the Jewish Hospital of Brooklyn, and Professor of Chemistry at the Polytechnic Institute of Brooklyn, who spoke on "Quantitative Ultramicro Analysis in Clinical Laboratories."

After considering the role of the chemistry department in a hospital, Dr. Sobel described the development of clinical chemical procedures, with emphasis on the considerable progress that has been made in recent years in refinements of techniques that have permitted the use of smaller and smaller quantities of unknown materials without sacrifice in the precision and accuracy of the methods. In pointing out the increasing demands that are made on the clinical chemist for more analyses on the same or smaller amounts of blood, Dr. Sobel cited specific cases to show the valuable role that some of the ultramicro chemical analyses have had in establishing diagnoses in critical cases.

Dr. Jaime Rizman of the University of San Paolo, Brazil, who has been doing graduate study in pediatrics at the Jewish Hospital of Brooklyn, assisted Dr. Sobel in presenting a most excellent demonstration of some of the quantitative ultramicro analyses that can be carried out on a few drops of blood from a finger puncture.

After the formal program, Dr. Sobel and Dr. Rizman answered numerous questions related to the subject of the lecture.

The fifth meeting of the 1952-53 season on Tuesday, March 24, 1953 in Alumni Hall of the University of Pennsylvania. There was an informal dinner in honor of the speaker at the Lido Restaurant preceding the meeting.

In the absence of the president, Dr. Cecilia Riegel, the vice-president, Mr. A.G. Keller, presided at the meeting. Mr. Keller announced that officers for the coming season would be elected at the next meeting on April 16, 1953 and that Dr. Riegel had appointed the following members of the section to serve on the nominating committee:

Dr. Ellinmae Viergiver, Chairman
Mr. Herman Stiplet
Mr. O.C. Beckord

Mr. Keller then introduced Mr. Robert O. Gorson, Physiologist in the Department of Radiology of the Hospital of the University of Pennsylvania, who presented a most interesting and informative lecture on "Clinical Use of Radioactive Isotopes."

With due consideration to their limitations as well as to their advantages, Mr. Gorson described the techniques used with the various isotopes that have been found of value in clinical diagnosis and treatment. He then showed a moving picture that clearly demonstrated the use of radioactive gold in clinical medicine.

The sixth and final seasonal meeting of the Philadelphia Section, American Association of Clinical Chemists, was held on Tuesday, April 28, 1953, in Alumni Hall, Hospital of the University of Pennsylvania. There was an informal dinner in honor of the speaker at the Lido Restaurant preceding the meeting.

The president, Dr. Cecilia Riegel, introduced Dr. John G. Reinhold, Associate in Charge of Chemistry, William Pepper Laboratory of Clinical Medicine of the Hospital of the University of Pennsylvania, who spoke on "Applications of Electrophoresis in Clinical Chemistry."

After giving a brief history of the development of electrophoretic methods of analysis, Dr. Reinhold considered the various types of instruments that have been developed for such analyses. He discussed the advantages and disadvantages of the different types of equipment and outlined the techniques that are used and the precautions that must be observed.

After outlining briefly his experience with a Tiselius apparatus constructed by Dr. Britton Chance in the Johnson Foundation of the University of Pennsylvania, an Amino-Stern apparatus, and a paper electrophoresis apparatus, Dr. Reinhold presented numerous lantern slides which indicated the variations in blood serum proteins that are encountered in various clinical conditions as determined by electrophoretic methods. The electrophoretic patterns depicted some serum protein variations that are encountered in lobar pneumonia, tuberculosis, sarcoidosis, cancer, lymphosarcoma, pregnancy, nephrosis, viral hepatitis, chronic hepatitis, cirrhosis, and multiple myeloma.

It was pointed out that in liver disease the protein alterations depend much on the nature of the lesion and on the extent of the disease.

Later Dr. Riegel presided at a business meeting for the members of the Philadelphia Section during which Dr. Ellinmae Viergiver, reporting for the nominating committee, proposed the following candidates for the respective offices for the coming year:

President: Mr. A.G. Keller
Vice-President: Dr. Robert H. Hamilton
Secretary-Treasurer: Mr. Harry G. Amode

There being no nominations from the floor, the nominations were declared closed, and the secretary was directed to cast a unanimous ballot for the above candidates.

The treasurer's report was approved as read.

NEW MEMBERS ELECTED BY THE EXECUTIVE COMMITTEE

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<td>Herbert J. Eicher</td>
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<td>Gertrude Salzberg</td>
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<td>Foster W. Burke</td>
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<td>Lynn Carbamace</td>
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<td>John N. McConnell</td>
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POSITION OPEN

CLINICAL CHEMIST, to head the laboratory of a 700 bed county hospital. Supervision of routine work with opportunity for a personal work. Salary: $330-412 per month depending on background. M.S. or equivalent desirable. Address: J.D. Rainwater, Personnel Director of Kern County, Bakersfield, California.
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