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# The CLINICAL Chemist

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*of*
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Sixth Annual Meeting
September 13 – 16
New York City

The CLINICAL Chemist

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VOL. 6, NO. 3 JUNE 1954

## THE SECRETARY REPORTS

On various occasions in the past we have pointed with pride to the accomplishments of the Association. At this time we refer to a very real advance, namely, a regular journal to be administered by the AACC. The publication, CLINICAL CHEMISTRY, is to replace the present CLINICAL CHEMIST and will be published for us by Paul B. Hoeber, Inc., Medical Book Department of Harper & Brothers. The first issue is to make its appearance in January-February, 1955 and for the start it is to be a bimonthly periodical. A summary of the terms is included in the Executive Committee Minutes of April 14, 1954 and may be found elsewhere in this issue. There will still be three numbers of THE CLINICAL CHEMIST for the remainder of the year and progress in the preparation of CLINICAL CHEMISTRY will be detailed as it develops.

This new publication will be in regular journal format and all the features of the present publication will be continued. A professional section, to retain the name of THE CLINICAL CHEMIST, will contain local section news, Executive Committee proceedings, national meetings, and other items of interest. Literature abstracts shall be included. The bulk of editorial material will of course consist of original articles pertaining to clinical chemistry, and will be accepted from members and non-members alike. Manuscripts for Volume 1 are now being reviewed and should be sent to the address of THE CLINICAL CHEMIST.

Subscriptions to CLINICAL CHEMISTRY have been set at $8.00 per year. Members will be obligated to subscribe at a cost of $3.50 per year, which amount shall be added to the present dues beginning with the calendar year 1955. The success of this, your journal, will of course depend on the cooperation by the members of the AACC. The project has been gradually developed by past administrations, but it is to the Executive Committee of 1953-54 that we must credit its final culmination.

There are two immediate ways, among others, that the membership could be of assistance in the launching of this publication. Submit now to your own journal any manuscripts you may feel are appropriate to CLINICAL CHEMISTRY. We might well expect that the first volume will be critically examined by the scientific world and we should certainly put our best foot forward. Secondly, it is imperative for us to obtain a reasonable number of non-member subscriptions between now and publication time to avoid any delay. Every member is strongly urged to encourage prepublication subscriptions from his laboratory or library. We must bear in mind that copies of the members at reduced price are for personal use.

The need for such a periodical is obvious. With the publication of CLINICAL CHEMISTRY we may well feel that our specialty has come of age.

## SIXTH ANNUAL MEETING
### September 13 - 16

The sixth annual meeting of the AACC will be held in New York City in conjunction with the 126th national meeting of the American Chemical Society. The scientific program for clinical chemistry will be announced in the August 9th issue of Chemical and Engineering News. It will contain, among other features, a symposium on Lipid Metabolism which has been arranged by Dr. Arthur Knudson in cooperation with the Division of Biological Chemistry of the ACS. There will also be the annual dinner at which the Ernst Bischoff Award will be presented, as well as the membership meeting.

## FREEMAN ELECTED PRESIDENT

Lt. Col. Monroe E. Freeman, Assistant Chief of the Medical Service Corps of the Department of the Army, has been elected President of the Association for 1954-55. Colonel Freeman has served as Vice-President of the AACC for the past year and is also the United States representative to the International Federation of Clinical Chemists. The other elected officers are Vice-President, Otto Schales of the Ochsner Clinic, New Orleans; National Secretary, Max M. Friedman of Lebanon Hospital, New York; National Treasurer, Louis B. Dotti of St. Luke's Hospital, New York.

The Executive Committee will consist of Hugh J. McDonald of Loyola University, Chicago; Robert M. Hill of University of Colorado, Denver; Marschelle H. Power of Mayo Clinic, Rochester; Miriam Reiner of D.C. Hospital, Washington, D.C.; and Albert B. Sample of Bryn Mawr Hospital, Bryn Mawr, Pa.

The Nominating Committee elected for 1954 consists of John G. Reinhold, chairman; Harry Sobotka, Joseph I. Routh, Warren M. Sperry, Samuel Natelson, Oliver H. Gaebler, and Joseph Benotti.

## PROGRAM FOR SIXTH ANNUAL MEETING

Activities of the AACC have been scheduled for Thursday and Friday, Sept. 16 and 17 of the week long meeting of the ACS in New York City. Thursday will feature a symposium on LIPIDS, sponsored jointly with the Division of Biochemistry and Friday will be devoted to scientific papers on Clinical Chemistry. The Annual Meeting will take place Thursday afternoon following the symposium. The Annual Dinner will take place Thursday evening. The full program will be published in the August issue of The Clinical Chemist and the August 9th issue of Chemical and Engineering News.

## CLINICAL CHEMISTRY

With the announcement that the AACC will sponsor a new journal "Clinical Chemistry" to begin publication January 1954, papers on original research

*(Continued on Page 41)*

## EXECUTIVE COMMITTEE MINUTES
## April 14, 1954

The National Executive Committee met at the Hotel Jefferson in Atlantic City on Wednesday, April 14, 1954 at 9:00 P.M. Those present included Hugh J. McDonald, President; Max M. Friedman, National Secretary; Louis B. Dotti, National Treasurer; Robert M. Hill, Arthur Knudson, Cecelia Riegel, Joseph I. Routh, Albert E. Sobel, Harold D. Appleton (by invitation), Marschelle H. Power (by invitation), Miriam Reiner (by invitation), Otto Schales (by invitation), Harry Sobotka (by invitation), Ellenmae Viergiver (by invitation).

The main part of the evening was devoted to the discussion of a journal for the Association. Mr. Paul B. Hoeber of the Medical Book Department of Harper & Brothers was present for these discussions. It was moved, seconded, and approved unanimously that the American Association of Clinical Chemists shall enter into a contract with Paul B. Hoeber, Inc. for a journal with the following conditions:

1. The publisher shall undertake the financial risks pertaining to this journal, with a five year contract.
2. The publisher shall undertake full responsibility for printing, distribution, and advertising.
3. The Association shall maintain complete control of editorial and advertising policy.
4. There shall be a minimum of 430 pages of editorial text per year, issued bimonthly, and expanded as material and advertising permits.
5. The subscription shall be $8.00 per year and each member shall be obliged to subscribe at a considerably reduced rate.
6. When the circulation reaches 1500 (including member subscription) the Association shall receive a royalty on the paid subscriptions (not including member subscriptions) and from advertising.
7. The name of the journal shall be CLINICAL CHEMISTRY, OFFICIAL PUBLICATION OF THE AMERICAN ASSOCIATION OF CLINICAL CHEMISTS, INC. with a professional section to be called THE CLINICAL CHEMIST to carry on newsletter material.

It was moved, seconded, and approved that all appointments to CLINICAL CHEMISTRY shall be made by the Executive Committee.

It was moved, seconded and approved that there be appointed a Board of Editors to consist of eight individuals, including a chairman, and each individual is to serve for a term of four years. No member of the editorial board may immediately succeed himself, but could be reappointed for

*(Continued on Page 49)*

# THE DETERMINATION OF ERYTHROCYTE SEDIMENTATION RATE WITH BLOOD SPECIMENS COLLECTED FOR PROTHROMBIN TIME ESTIMATIONS

by

**Murray Weiner and George Simson**

*From the Third (New York University) Medical, Clinical and Research Services, Goldwater Memorial Hospital, New York, N.Y.*

Serial estimations of the erythrocyte sedimentation rate are commonly employed as an index of progress in patients under treatment for acute myocardial infarction. It has been adequately demonstrated that changes in prothrombin activity induced by Dicumarol therapy do not influence the sedimentation rate (1). Since patients with coronary occlusion are frequently treated with the coumarin anticoagulants, repeated estimations of both sedimentation rate and prothrombin activity are often necessary.

In present practice each of these two tests requires blood collected in a different form of anticoagulant, i.e., a measured amount of dry oxalate for sedimentation rate by the Wintrobe technique, and of 0.1 Molar oxalate solution for prothrombin estimation by the one stage method. This note describes a comparison of sedimentation rate with the above two types of blood specimens in the standard Wintrobe tube.

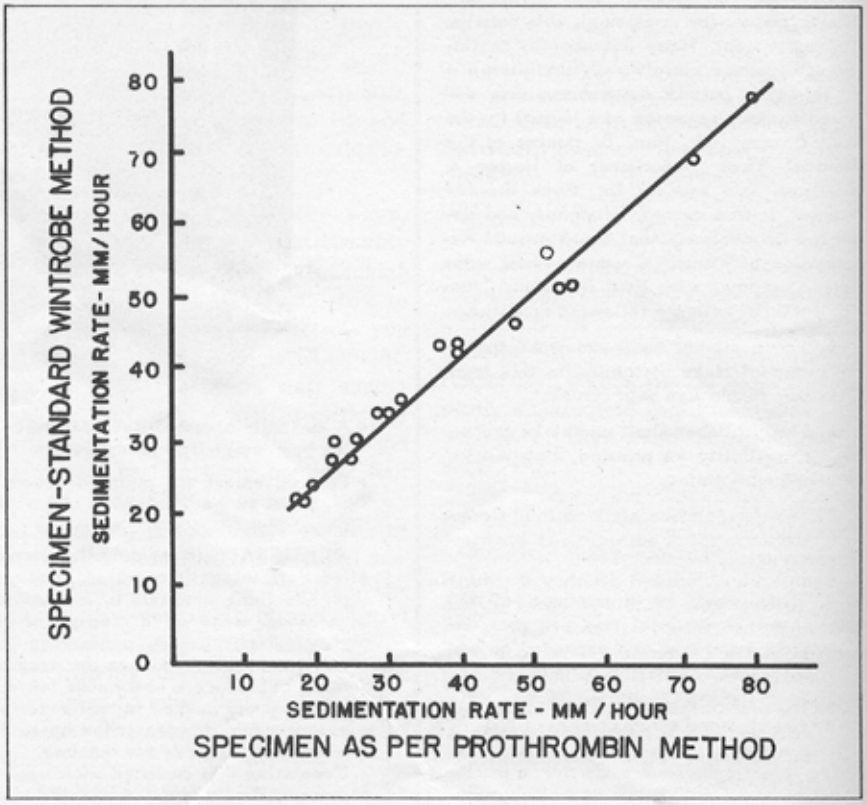
## METHOD

Five ml. aliquots of venous blood were added to dry bottles containing 6.0 mg. ammonium oxalate and 4.0 mg. potassium oxalate as per the method of Wintrobe (2), and 4.5 ml. aliquots of blood were added to 0.5 ml. 0.1 Molar Na oxalate solution as per the prothrombin time method of Quick (3). Proper aliquots of each of these specimens were entered into standard Wintrobe tubes and the erythrocyte sedimentation was read after one hour. The hematocrit of each specimen was then determined.

## RESULTS

Figure 1 demonstrates the relationship of the sedimentation rate as determined by the two types of blood samples in 22 subjects with a rate in excess of 20 mm/hr. It is clear that the sedimentation rate is slightly slower in the "prothrombin" specimen diluted by oxalate solution. The true hematocrits in this group ranged from 18 to 48. The "hematocrits" as measured by the "Prothrombin" specimens in every instance could be converted to the true hematocrit by the formula: True hematocrit = 10/9 × "prothrombin" hematocrit. The sedimentation rate could thus be corrected for hematocrit in the usual way.

In addition to the above specimens, blood from 3 polycythemic subjects with hematocrits over 51 were studied. Unlike the others, these 3 consistently showed a faster sedimentation rate in the "prothrombin" samples than in the standard.




## DISCUSSION

Erythrocyte sedimentation is known to occur in 3 phases: (1) an initial phase during which the cells have not yet formed into aggregates. During this period the red cell presents the largest surface volume per unit mass, resulting in a very slow rate of sedimentation; (2) a phase of constant relatively rapid rate of sedimentation, which is largely a function of the degree of cell aggregation, this in turn being a function of the nature and concentration of plasma proteins; (3) a final slowed rate due to the packing of cells as settling approaches the hematocrit.

The slower rate found for blood taken into oxalate solution is consistent with the fact that all plasma components, including the proteins which are responsible for aggregate formation, are diluted by about 15 to 20%. A significant degree of packing of red cells within one hour in rarely encountered except with polycythemic blood, in which case the specimens diluted with oxalate solution may have a more rapid rate of sedimentation.

## CONCLUSIONS

1. Erythrocyte sedimentation rate as determined with "prothrombin" type oxalated plasma differs very little from that obtained with the standard Wintrobe technique using dry oxalate. In subjects with normal or low hematocrit, the sedimentation rate of the "prothrombin" blood specimen is somewhat slower than by the standard Wintrobe method. This situation is reversed with polycythemic blood.

2. For practical purposes, sedimentation rate can be estimated and followed with "prothrombin" blood specimens.

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# PREPARATION OF A STABLE SOLUTION OF POOLED HUMAN SERUM

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*Fort Baker, California*

Modern clinical biochemistry has produced a wealth of methods so that for any one clinically significant chemical entity in a body fluid there exists a number of possible procedures for its quantitative determination. This has produced considerable confusion both for the physician who must interpret the results and for the clinical biochemist who must select the procedures. Many groups of clinical scientists, recognizing this problem, have instituted various forms of evaluation surveys in order to obtain some estimate of the relative merit of various procedures as well as to gain some insight into the proficiency of member laboratories. The literature reflects current interest in this problem (1-10). It is the hope of the author that this current wave of interest in the problem will ultimately result in suitable standardization of procedures in clinical biochemistry analogous to the standardization presently available in agricultural chemistry and in industry.

The prime requisite for an intelligent evaluation survey is suitable medium for preparation of survey specimens. Such a specimen should approximate the composition of the material the analyst encounters in routine work, it should be stable on storage and finally, it should be readily available and reasonably easy to prepare. Since a large majority of determinations in clinical biochemistry are routinely performed on blood serum or plasma and since this material meets the requirements listed above, attempts were made over a period of two and a half years in this laboratory, to utilize pooled human serum for preparation of evaluation survey specimens in clinical biochemistry. The purpose of this paper is to describe the method used here in the preparation of such a sample.

This laboratory conducts clinical biochemistry evaluation surveys among 25 to 35 Army and Air Force laboratories in the Sixth Army Area at quarterly intervals. Nine larger laboratories participate as controls. Prior to 1952 synthetic solutions containing most of the chemicals found in reasonably large concentration in serum (with the exception of proteins and lipoproteins) were employed in this work. This material was considered inadequate for three reasons: (1) Since it contained no protein it failed to evaluate the proficiency in preparation of a protein-free filtrate, so often an integral part of determinations in clinical biochemistry. (2) It failed to provide the means for evaluating proficiency in the determination of proteins and fats. (3) Finally, it failed to provide the buffer capacity of human plasma or serum. Attempts to overcome these objectionable features by adding bovine or egg albumin and using a phosphate buffer system met with failure. Next the use of commercially available preparations of lyophylized human blood plasma was attempted. When this material was reconstituted by adding sterile, distilled water, a murky, rather unhomogenous mixture resulted which was quite unsuitable for our purposes. The manufacturer (Cutter Laboratories) informed us that this turbidity was due to the instability of certain lipoprotein complexes when reconstituted. Finally we resorted to the use of pooled, human serum which has produced a suitable medium for preparation of survey specimens.

The greatest problem encountered in the preparation of this material was sterilization. We purposely avoided the addition of any bacteriostatic agents since this might interfere with the determinations to be evaluated.

The apparatus employed is illustrated in figure 1 and consists of a Seitz filter, a 1 liter bottle with vacuum-type rubber stopper (the "Vacoliter" bottle proved quite satisfactory for this purpose), 5, 10 or 15 ml serum vials with vacuum-type rubber stoppers, thick-walled, gum rubber tubing for air and vacuum line, one large spinal needle and three short hypodermic needles, a ring stand with three clamps, a screw-type pinch clamp to control vacuum, a vacuum pump and a disposable, polyethylene, intra-venous tube. This apparatus is grouped into four assemblies prior to sterilization (see figure 2). The Seitz filter with pad inserted is fitted with a length of gum-rubber tubing the other end of which is fitted with a spinal needle. To protect the needle it is convenient to insert it through a rubber stopper into a small, glass test tube. The 1 liter bottle and the small vials are chemically cleaned and dried. To the bottles are added very small amounts of distilled water, just enough to insure saturation of the interior with water vapor during sterilization (about 6 or 7 drops in the case of the 1 liter bottle and about ½ drop in the case of the vials). The mouths are closed with very snug-fitting, vacuum-type, rubber stoppers.

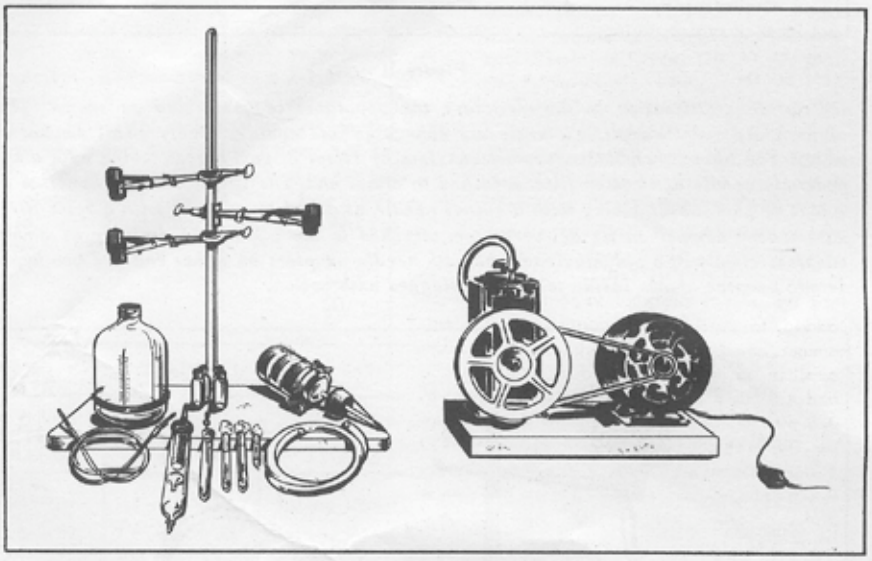

**Figure 1**

*The apparatus required consists of a vacuum pump, a ring stand with three clamps, a 1 liter "Vacoliter" bottle and 5, 10 and 15 ml. serum vials with snug-fitting, vacuum-type, rubber stoppers, a disposable, polyethylene, IV tube, a cotton filter, 1 spinal needle, 3 hypodermic needles, and 2 to 3 feet of gum rubber tubing.*

Vacuum is applied through a small hypodermic needle for about 1 hour in the case of the 1 liter bottle and for about 1 minute in the case of the small vials (equivalent to approximately 1 mm Hg). This insures a tight seal around the mouth and provides for rapid transfer of the serum from the large bottle to the small vials later in the procedure. The vacuum line which also serves as air entry during transfer of the serum from the 1 liter bottle to the small vials, consists of a length of gum-rubber tubing, long enough to reach from the mouth of the 1 liter bottle to about an inch or more above the base of the bottle when it is inverted. One end of this tube is fitted with a cotton filter (the Baxter Co.

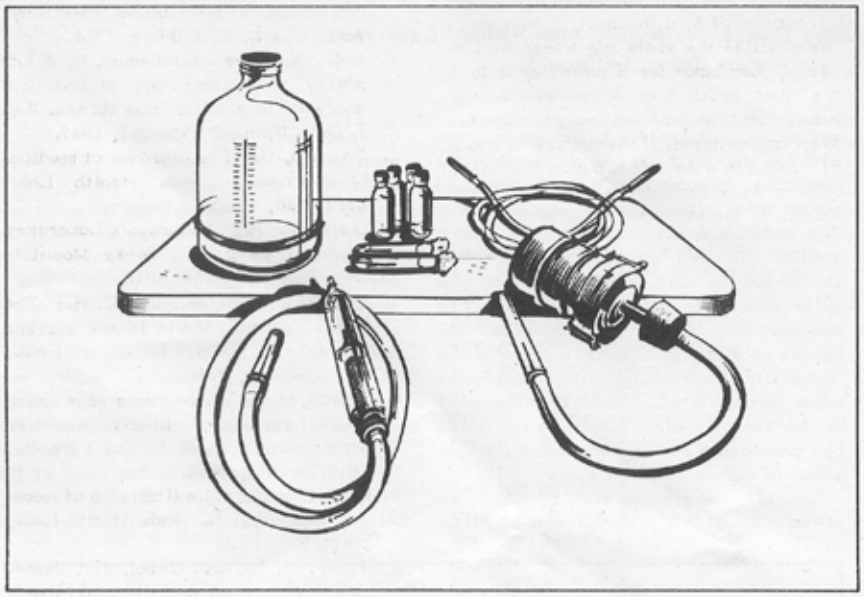

Figure II

*Prior to sterilization in the autoclave the apparatus is assembled as shown: The serum vials and "Vacoliter" bottle are evacuated and contain a very small amount of water. The air-vacuum line assembly consists of 1 foot of gum rubber tubing with a hypodermic needle and cotton filter attached to either end. The filter assembly consists of 1 foot of gum rubber tubing with a spinal needle attached to one end with a Seitz filter with rubber stopper on its delivery stem attached to the other. The delivery assembly consists simply of a polyethylene tube with needle adapters on either end and two hypodermic needles shown inside test tubes plugged with cotton.*

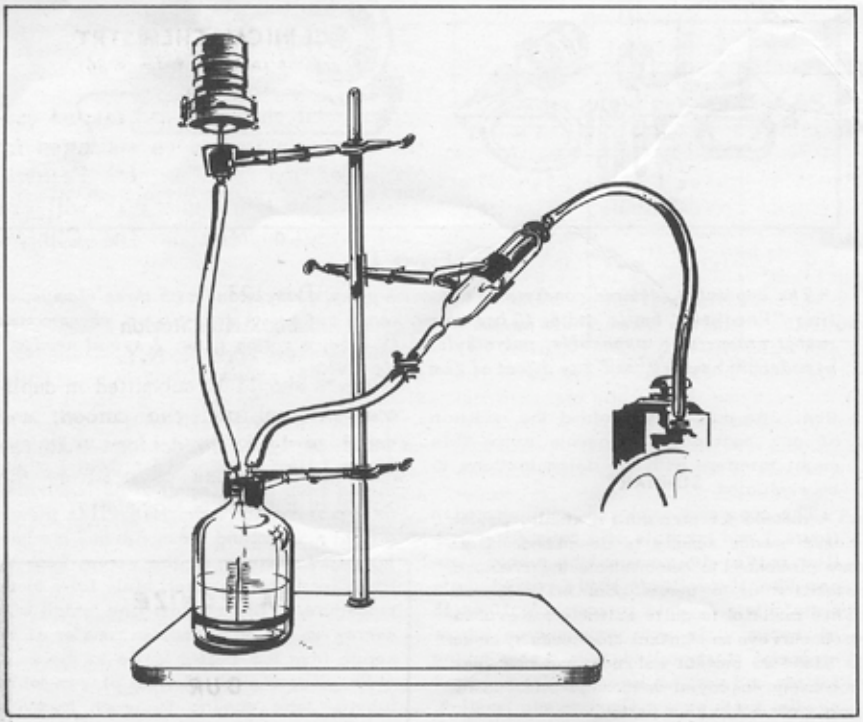

Figure III

*Showing the assembly during filtration. Note the screw-type pinch clamp on the vacuum line permitting control of the rate of filtration and of "frothing".*

"Filterdrip" tube serves this purpose nicely) while the other is fitted with a short, large diameter, hypodermic needle. The delivery assembly is simply a disposable, polyethylene tube, with hard plastic adapters on either end for insertion of hypodermic needles. This assembly is commercially available and comes already sterilized. If unavailable, a substitute can be readily made up using small diameter, gum-rubber tubing and 1 ml syringes cut off about 1 inch from the tip to serve as adapters for the hypodermic needles. The four assemblies described above are transferred to an autoclave and sterilized at 15 lb/sq. in. for at least 30 minutes.

The serum used in this work is obtained from a syphilis serology laboratory. Only sera negative to the standard test for syphilis are used in the pool. If the serum pool looks rather turbid a preliminary centrifugation and/or filtration through glass wool is indicated.

Next the sterile apparatus illustrated in figure 3 is assembled. The vacuum line assembly is pinched off near the filter end with a screw-type pinch clamp and the open end of the filter is connected to the vacuum pump. The needle on the other end of the vacuum line is rapidly removed from its test tube cover and aseptically inserted through the rubber stopper in the mouth of the 1 liter bottle which is first swabbed with 70% ethanol. Vacuum is applied and the screw-type pinch clamp on the vacuum line is opened. The Seitz filter is mounted on the ring stand as illustrated in figure 3 and filled with serum. The stopper of the 1 liter bottle is again swabbed with 70% ethanol and the spinal needle rapidly and aseptically inserted. A spinal needle is used here in order that the serum leaving the end of the needle is as far removed from the vacuum outlet as possible to prevent sweeping the serum into the vacuum line. The vacuum can now be adjusted to control frothing of the serum inside the 1 liter bottle. It is necessary to watch this frothing continually during the course of the filtration. If the rate of filtration becomes too slow a fresh, sterile, Seitz filter can be substituted. This is done by applying a pinch clamp to the rubber tube about 2 inches below the delivery tip of the Seitz filter. A scissors is then flamed over a Bunsen burner and used to cut the rubber tube, which is first cleansed with ethanol, as close to the delivery tip of the Seitz as possible. The tip of the fresh, sterile, Seitz filter is then flamed, rapidly inserted into the open end of the cut rubber tube, mounted in place of the used filter on the ring stand and filled with serum. The pinch clamp on the rubber tube is then released.

When the amount of serum desired has been filtered, addition of an aqueous solution of clinically significant chemicals may be made through the filter. Of course this dilutes the serum proteins as well, making the total protein lower, while still maintaining the same A/G ratio.

When the filtration is completed the pinch clamp on the vacuum line is closed and the spinal needle removed from the 1 liter bottle which is transferred to a 37° C incubator for a three to five day period. The sterility is checked by culturing a small aliquot. If this culture is negative at the end of a three day incubation period the serum pool is considered sterile and transfer to the vials is begun.

To effect this transfer the assembly illustrated in figure 4 is set up. With the 1 liter bottle in an upright position the pinch clamp on the vacuum line is slowly opened to allow the air to enter the bottle slowly without passing through the serum. Next the stopper on the 1 liter bottle is swabbed with 70% ethanol and one end of the polyethylene tube is aseptically fitted with a sterile, hypodermic needle which is rapidly inserted through the rubber stopper. The next step is best performed by two people. One handles the 1 liter bottle and polyethylene delivery tube which he keeps pinched closed while carefully inverting the bottle and securing it to the ring stand as illustrated in figure 4. The other handles the air entry tube with the cotton filter attached which he keeps always above the level of the serum in the 1 liter bottle to prevent the serum from contacting the cotton. When the 1 liter bottle is inverted and secured to the ring stand the second operator fastens the filter to the ring stand as illustrated in figure 4. A sterile, hypodermic needle is aseptically fitted to the delivery end of the polyethylene tube. The rubber stoppers on the vials are swabbed with 70% ethanol just before inserting this needle. Transfer is rapidly effected due to the vacuum in the small vials which, if adequate, should effect transfer of 5 ml in about 15 seconds.

When filled the vials are transferred to a 37° C incubator for a period of 3 to 5 days after which 3 or 4 are selected at random, and an aliquot aseptically removed and cultured. If the culture is negative after 3 or 4 days incubation the serum in the vials is considered sterile and distribution to the laboratories is begun.

This serum makes an excellent standard protein solution as well and is used routinely as such in this Army Area in the "Biuret" method for serum protein analysis.

Table I demonstrates the chemical stability of a pool of human serum prepared at this laboratory as described above. It is our opinion that the discrepancy noted in the case of non-protein nitrogen does not reflect sample instability but is referrable rather to the inherent difficulty of this procedure. This serum was stored in the refrigerator between the analyses reported. There is some question as to the advisability of refrigerating this material as this may sometimes produce a very small amount of fine sediment in the bottom of the container, which may represent material whose solubility constant in serum has been exceeded due to the differential between room temperature and that of the refrigerator (about 20 to 25° C in this case). This matter is being investigated at present.

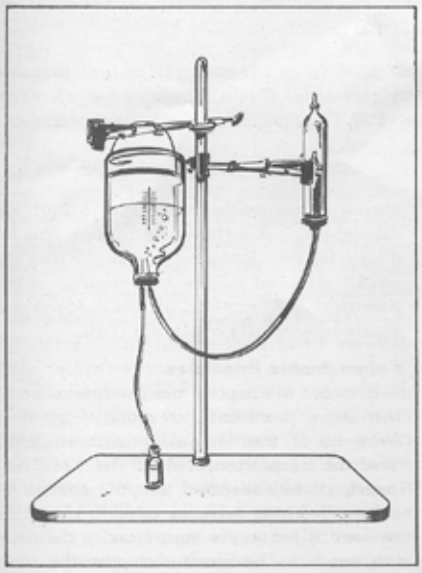

**Figure IV**

*The apparatus during the process of transfer of the serum from the "Vacoliter" bottle to the serum vials. The contents of the "Vacoliter" bottle should be swirled occasionally during this transfer to insure homogeneity.*

**TABLE I**

| DETERMINATION | RESULTS | |
|---|---|---|
| | 28 Aug 53 | 3 Nov 53 |
| TOTAL PROTEIN | 5.1 gm/100 ml | 5.2 gm/100 ml |
| ALBUMIN | 2.9 gm/100 ml | 3.1 gm/100 ml |
| A/G | 1.3 | 1.5 |
| N.P.N. | 23.0 mg/100 ml | 30.3 mg/100 ml |
| UREA N. | 12.5 mg/100 ml | 13.0 mg/100 ml |
| SUGAR* | 167 mg/100 ml | 155 mg/100 ml |

* *Total Reducing Substances.*


## SUMMARY

A method for preparing a stable, pooled human serum sample is described. Data are presented to demonstrate the chemical stability of a serum pool so prepared. This material is quite suitable for evaluation surveys in clinical biochemistry or as a standard protein solution and has been routinely employed as such in this laboratory over a two year period.



The author gratefully acknowledges the technical assistance provided by Miss Miriam Beneditz and Mr. Richard Macnair, both of the laboratory.


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## CLINICAL CHEMISTRY


*(Continued from Page 36)*


in clinical chemistry and related subjects are invited to be submitted for consideration by the new Editorial Board. The new publication will use the same address as The Clinical Chemist.

Box 123
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Papers should be submitted in duplicate, original and one carbon, and should be typed to conform with the same specifications as used by the Journal of Biological Chemistry.

# POLAROGRAPHY AND ITS APPLICATIONS TO CLINICAL PROBLEMS[1]

by

Robert L. Pecsok, Department of Chemistry
University of California, Los Angeles

The polarograph has been advantageously used for the investigation of substances of biological interest at the research level. Unfortunately, there have been surprisingly few applications to routine clinical problems. This is due partly to the fact that a gap exists between the research work with relatively pure compounds in known solutions and everyday problems involving extremely complex solutions with which the clinical chemist has to work. Perhaps equally important is the fact that the polarographic method has been developed rather recently and is not familiar to many practicing chemists. This paper is an attempt to present the principles involved, a description of the apparatus required, and a brief survey of some of the applications of present interest. The writer believes that many clinical problems could be solved successfully by means of the polarograph with relatively little investment by the practicing chemist.

## Historical Development and Scope

In the early 1920's, Heyrovsky noted anomolies in the electrocapillary curves for dropping mercury electrodes in solutions containing reducible substances. Further investigation of this phenomenon led to the development of the polarographic method. Most of the theory, apparatus, and methodology are due to Heyrovsky and his co-workers at Charles University in Prague. Shortly before World War II, investigators in this country became interested, largely through the leadership of Kolthoff and Lingane. Their monograph (14) is essential reading for any serious user of the method, although less rigorous and less complete discussions are available as well (15, 23). Interest in this method continues to increase, for example, in the field of organic polarography alone, papers are now appearing at a rate of over four hundred per year (22).

In conventional polarography, current-voltage curves are obtained for the electrolysis of dilute solutions of electroreducible or oxidizable substances between an easily polarized micro-electrode and a large area reference electrode of constant E.M.F. These curves, called "polarograms," can be interpreted to yield both the nature and concentration of the electro-active species. Complete polarograms can be recorded in ten minutes, but in many cases only a portion of the curve is required. The method is applicable to concentrations in the range $10^{-6}$ to $10^{-2}$ molar. Samples as small as 1 ml. can be easily handled, with a practical limit of about 0.1 ml. Since the amount of electrolysis required is negligible, the sample is not destroyed and can be recovered if desirable. Accuracies of 1-5% are generally obtainable provided the curve is well-defined.

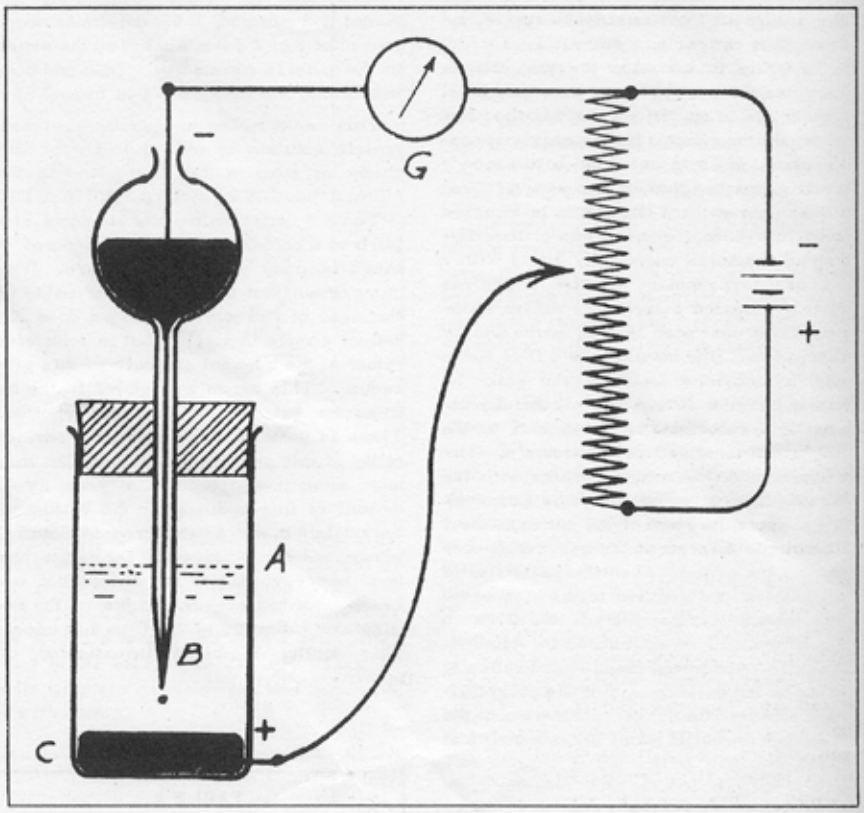


**Figure 1**

*Schematic Diagram of Polarographic Apparatus. A is an electrolysis vessel containing unknown solution. B is the dropping mercury electrode. C is a mercury pool reference electrode. G is the current measuring device. E.M.F. is supplied by batteries and adjusted with the calibrated slide-wire.*

## Apparatus

The apparatus required is simple (and inexpensive for non-recording instruments). The working electrode is a fine thread of mercury emerging in droplets from a 0.03 mm. I.D. capillary. This is opposed by a reference electrode of relatively large area, usually the saturated calomel electrode. The electrical circuit consists of a source of constant E.M.F., a slide-wire for a voltage divider, and a current measuring galvanometer sensitive to 0.01 microampere. Auxiliary equipment can be added for standardizing and regulating the sensitivity and for the convenience of the operator. A number of instruments are now commercially available (14).

## Polarographic Principles

In order to simplify the interpretation of the data, we shall arbitrarily divide a polarogram into three distinct regions. The initial portion, called the "residual current," represents a slight increase in current with increasing potential, and is caused by reducible impurities in the solution and the necessity of charging each successive drop of mercury. All observed currents must be corrected by subtraction of this current at the proper potential. The second portion of the curve exhibits a sudden increase in current. This is the "polarographic wave," and in this region the current is determined by the amount of reduction or oxidation necessary to satisfy

*1. Presented before the Southern California Section of the American Association of Clinical Chemists, Veterans Administration Center, Los Angeles, California on January 5, 1954.*

the Nernst Equation involving the E.M.F. and the concentrations existing at the electrode surface. The third region resembles a plateau and is called the "limiting" or "diffusion" current. In this region the E.M.F. is so large that the reacting species cannot move from the body of the solution to the micro-electrode at a rate sufficient to satisfy the Nernst Equation. The current is therefore determined by the rate of diffusion provided that a large excess of an inert electrolyte is present to nullify the electrical attraction or repulsion. For the dropping mercury electrode, the rate of diffusion is proportional to the concentration gradient in the diffusion layer, and the latter is directly proportional to the concentration in the body of the solution.

Thus, it is seen that qualitative polarography is based on the potential at which a wave occurs (more precisely, the "half-wave potential"), and quantitative polarography is based on the height of the wave. For practical purposes, the Ilkovic Equation, which relates the diffusion current to a number of variables, can be simplified to $i = kC$, where the constant, k, depends on the nature of the substance, the supporting electrolyte, the capillary characteristics, and the temperature.

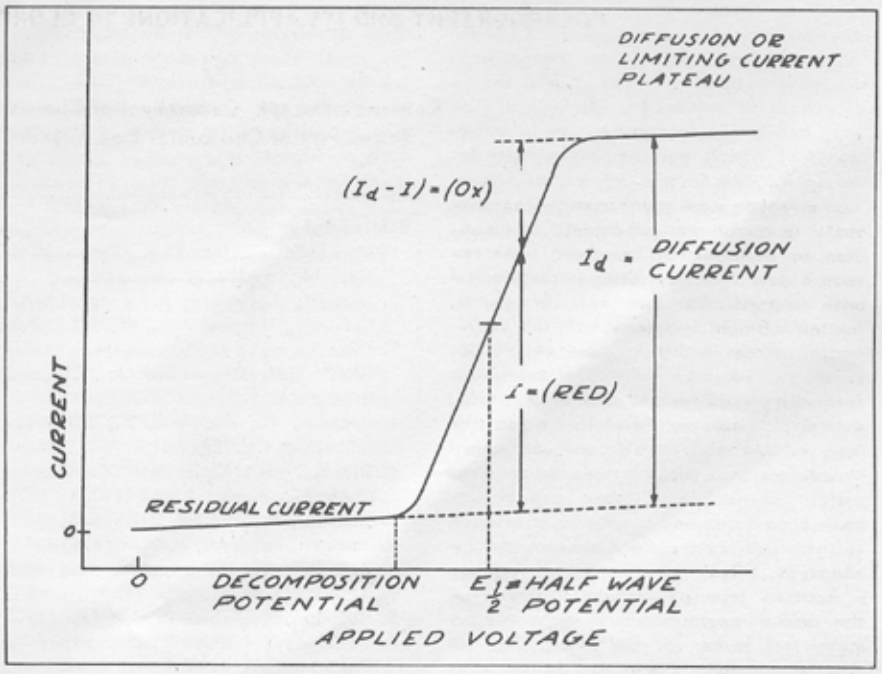


**Figure II**

*Typical Polarogram for a Reducible Substance. I is the current at any point on the curve. Id is current for points on the plateau, corrected for residual current.*

### Inorganic Polarography

There are numerous applications to metal analysis. The transition metals, in particular, yield well developed waves. In many cases, several metals can be determined from a single polarogram. If the half-wave potentials are separated by 0.2 volt or more, each wave is added to those which precede it. When interference is encountered, it is frequently possible to add a complex forming reagent, which effectively separates the waves. Organic chelating agents are becoming increasingly important for this purpose (18). Useful half-wave potential data are accumulating at a rapid rate. (3, 10).

For special cases, modified polarographs can be constructed to simplify the procedure to a "push-button" technique. For example, the "TELometer" is a direct reading polarograph for tetraethyl lead in gasoline (17). In principle it compares the diffusion current for lead to that of a known amount of antimony incorporated into the solution.

The determination of small amounts of metals in biological materials and organic preparations is within the scope of polarography. Procedures for the determination of arsenic (1) and the estimation of lead in urine or blood (21) have been described.

### Organic Polarography

The scope of organic polarography is by no means limited to ionizable substances. Recall that the electrical force of attraction due to the charge on the electrode has been minimized by the presence of the supporting electrolyte. Therefore, even uncharged substances can be polarographed provided they can be dissolved in a solution with an electrolyte. Nevertheless, the investigation of organic compounds has not been as fruitful for a number of reasons: (1) Many polarographic waves are irreversible and ill defined, possibly because of the more complex nature of the electrode reactions which may involve several intermediate species. (2) Buffers are required to maintain a constant pH at the electrode surface because hydrogen ion is usually involved in the electrode reaction. (3) Many organic compounds are not sufficiently soluble in water and require mixed or completely non-aqueous solvents. (4) Oxygen is difficult to remove from organic solvents.

The types of functional groups and compounds which have yielded satisfactory polarographic waves are as follows: (1) ethylenic double bonds when conjugated with another double bond, phenyl group, or any unsaturated group (this includes most of the stilbenes), (2) carbonyl groups in aldehydes, ketones, or quinones, (3) nitro, nitroso, and azo compounds, (4) quaternary ammonium groups, (5) halogen compounds, (6) disulfides and peroxides, (7) hydroquinones, (8) thiols, and (9) any substances which by forming insoluble or slightly dissociated mercury compounds pounds will yield anodic mercury waves. Some of the solvents which have proved useful are: (1) glacial acetic acid, (2) ethanol, (3) glycerol, (4) glycols, (5) 50% benzene-50% methanol, (6) dioxane, and (7) formamide.

### Biological Applications

Many organic compounds of biological interest have been investigated. For example, a large group of steroids yield useful waves. The 17-ketosteroids must be first converted to Girard derivatives which are more easily reduced to hydrazines (24). The derivatives give waves at about -1.4 to -1.5 V. *vs.* the saturated calomel electrode (S.C.E.) in aqueous ammonia buffers. Apparently traces of urinary extracts stabilize the hormones and sharpen the waves. Procedures for the determination of these hormones in urine have been described (2). The 3-ketosteroids can be determined in a similar fashion (19). The 3-keto-4-unsaturated steroids are reducible directly with half-wave potentials at -1.6 to -1.8 V. *vs.* the S.C.E. in a solvent of 90% ethanol containing 0.1 *N* lithium chloride (11). The latter group includes such hormones as testosterone, progresterone, cortisone, and corticosterone. Girard derivatives of these are reduced at half-wave potentials of -1.2 V. *vs.* the S.C.E., and therefore they can be determined in the presences of 17-ketosteroids.

Sulfhydryl compounds, such as proteins, give waves due to the evolution of hydrogen catalyzed by the protein at the electrode surface (7). The height of these so-called "catalytic" waves depends not only on the concentration of the protein, but also on the pH, the kind and concentration of the buffer. The sulfhydryl group is reduced to a sulfide ion and a hydrogen atom. The sulfide ion then combines with hydrogen ion from the buffer and is reduced again.

Proteins also yield catalytic waves in the presence of small amounts of cobalt (4-6). Minute traces can be determined because the waves are about five hundred times the normal height. Apparently the

reactions involve an intermediate cobalt complex ion.

A considerable effort has been made to utilize the polarograph as a tool in the diagnosis of cancer. (8, 14, 20). It has been shown that catalytic waves in an ammoniacal cobalt solution are smaller for carcinoma serum than for normal serum. The effect is more pronounced with serum that has been denatured by heat or alkali. The opposite effect has been observed with serum that has been deproteinated with sulfosalicylic acid (16). In general, the wave height increases with the development of the malignant disease. While polarographic data have been useful for following the advance or cure of individual cases, it should be stated that a positive test is not always obtained for known cases, nor is a positive test always specific. Nevertheless, some correlations have been drawn and the reader is referred to the original reports for his own conclusions. (9, 14).

Another type of application concerns the maxima commonly found on waves for metal ions in the absence of capillary active substances. These maxima are enormously high peaks at the tops of otherwise normal waves. Their cause is not completely understood, but it is possible to reduce or eliminate them completely with trace quantities (a few thousandths of a per cent) of certain materials, such as gelatin and many alkaloids (12). This phenomenon can be used to determine the alkaloid, although the method is obviously quite non-specific.

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## REVIEW OF CURRENT LITERATURE


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


ACTIVATION OF PURIFIED PROTHROMBIN WITH HEMOPHILIC PLASMA. S. A. Johnson (Wayne Univ. School of Medicine, Detroit). Am. J. Clin. Path. 23:875-880, 1953.

Extraction of hemophilic plasma, or normal serum, with ether changes the plasma or serum so that it will activate purified prothrombin at the same rate as normal plasma does. The author suggests that in hemophilic plasma, or in normal serum, the defect in activating prothrombin is not a lack of platelet co-factor but the presence of an inhibitor (antithromboplastin) which is extracted by ether.

C.R.

THE ACCURACY OF URINE SUGAR TESTS. M.H. Cook, A.H. Free and A.S. Giordano (Miles-Ames Research Laboratory, Elkhart, Ind.). Am. J. Med. Technol. 19:283-290, 1953.

A series of urine samples were tested for sugar with Benedict's qualitative test and with Clinitest (tablet test). In urines containing sugar, the quantity of sugar as determined with Clinitest showed good correlation with the amount as determined by Benedict's quantitative method. Both Clinitest and Benedict's qualitative test were completely accurate when properly performed.

E.V.

UPTAKE OF AMMONIA BY THE BRAIN IN HEPATIC COMA. S. P. Bessman, J. F. Fazekas & A. N. Bessman (Research Foundation, Children's Hospital, and the Medical Service, District of Columbia General Hospital). Proc. Soc. Exp. Biol. & Med. 85:66-67, 1954.

The cerebral arteriovenous difference of ammonia was investigated in patients both comatose and non-comatose, with and without liver disease. The arterial level of ammonia was found to be elevated in hepatic coma; the arteriovenous difference was also elevated. The hypothesis is suggested that ammonia enters the brain and combines with alphaketoglutaric acid by the reversal of glutamic dehydrogenase, thus abstracting large amounts of the keto acid which prevents regeneration of the Krebs cycle.

E.V.

A SIMPLIFIED METHOD OF ESTIMATING SODIUM. IN URINE AND IN FLUID FROM THE GASTROINTESTINAL TRACT. J. A. Pritchard (Western Reserve Univ. School of Medicine, Cleveland, O.). Am. J. Clin. Path. 23:943-945, 1953.

Sodium was determined by measuring the volume of precipitated sodium uranyl zinc acetate in a Shevsky-Stafford tube.

C.R.

DETERMINATION OF INULIN IN PLASMA AND URINE BY USE OF ANTHRONE. R.P. White and F.E. Samson, Jr. (Dept. of Physiology, School of Medicine, University of Kansas, Lawrence, Kan.). J. Lab. & Clin. Med. 43:475-477, 1954.

The method is based upon the color reaction between anthrone reagent and inulin. This method is simpler and faster than the older methods and is practical for use in both clinical and research laboratories.

E.V.

CHROMATOGRAPHIC CHANGES IN PLASMA $I^{131}$ DURING THE TREATMENT OF GRAVES' AND CARDIAC DISEASES CORRELATED WITH CLINICAL COURSE. W.E. White and W.A. Reilly (Fort Miley Veterans Administration Hospital, Radioisotope Unit, San Francisco, Calif.). J. Lab. & Clin. Med. 43:553-565, 1954.

Radioautographs of filter paper chromatographs of blood plasma were found to be valuable in predicting therapeutic results of treatment with $I^{131}$. Clinical hypothyroidism could be predicted by the disappearance of the thyroxine band, usually between two to seven days. Reduced intensity of the band during the same interval predicted euthyroidism.

E.V.

INORGANIC PHOSPHORUS IN CEREBROSPINAL FLUID IN MUMPS MENINGOENCEPHALITIS. L. Odessky, P. Rosenblatt, J.G. Loeffler and L. Landau. (Kingston Ave. Hosp., Brooklyn, N.Y.)

In 7 patients having mumps meningoencephalitis the average level of inorg. P was 2.1 mg% as compared to a normal of 1.4 mg%. This statistically significant average increase is attributed by the authors to 1) action of the virus on P-contg. components of the central nervous system; or 2) diffusion from serum because of altered permeability of the membrane from the inflammatory reaction; or 3) diffusion from serum due to alteration in the Donnan equilibrium; or 4) requirement of additional inorg. phosphate ions to maintain pH.—C.R.

INTERFERENCE BETWEEN ISONICOTINYL HYDRAZIDE AND SUGARS. P. Preziosi (Univ. Naples). Boll. soc. ital. biol. sper. 28:1288-90, 1952.

It is pointed out that in the presence of isonicotinyl hydrazide erroneous sugar values may be obtained due to the reducing action of the hydrazide. H.A.

SERUM POTASSIUM CHANGES IN BLOOD CLOTS. J.R. Goodman, J. Vincent, and I. Rosen. (Vet. Admn. Hosp., Long Beach, Calif.) Am. J. Clin. Path. 24, 111-3, 1954.

K values of serum from clotted blood were found to increase gradually over a 24 hr. period, if the serum remained in contact with the clot. The increase was greater if the blood was kept at 4 deg. C than if it was at 25 deg. C. The authors believe the greater inc. at low temp. is due to inhibition of enzyme activity carrying K into the cells. C.R.

A SIMPLE TEST FOR URINE BILIRUBIN. A.H. Free and H.M. Free (Miles-Ames Research Laboratory, Elkhart, Ind.). Gastroent: 24:414-421, 1953.

A tablet test is described for the detection of bilirubin in urine. It is based on the adsorption of bilirubin on the surface of an asbestos-cellulose mat. When a reagent tablet containing a stable diazonium salt is placed on the mat and moistened with water, the diazonium salt couples with the bilirubin to give a characteristic blue or purple color. Advantages of the test are discussed. E.V.

MICRODETERMINATION OF POLYVINYLPYRROLIDONE IN AQUEOUS SOLUTION AND IN BODY FLUIDS. G.B. Levy and D. Fergus (Schenley Labs., Inc., Lawrenceburg, Ind.). Anal. Chem. 25:1408-10, 1953.

A rapid and simple method for the determination of PVP is described. H.A.

AN IMPROVED BIURET REAGENT IN THE DETERMINATION OF SERUM PROTEIN. Wataru Mizuta (Yamaguchi Prefectural Med. College). Igakuto Seibutsugaku (Med. and Biol.) 27:185-9, 1953.

To 0.1 ml. serum add 2 ml. water and 4 ml. biuret reagent. Read with a 57 filter after 5-15 min. The biuret reagent is prepared by dissolving 1.7 gm. $CuSO_4 \cdot 5H_2O$ in a minimum amount of water, add 150 ml. conc. $NH_4OH$, 150 ml. $H_2O$ and 100 ml. saturated NaOH in that order; make up to 500 ml. H.A.

DETERMINATION OF GASTRIC SECRETORY FUNCTION BY MEASUREMENT OF SUBSTANCES EXCRETED BY THE KIDNEYS. I. UROPEPSIN EXCRETION IN HEALTH AND DISEASE. R.J. Bolt, H.M. Pollard and A. Carballo (Dept. of Internal Medicine, University Hospital, University of Michigan Medical School, Ann Arbor, Mich.). J. Lab. & Clin. Med. 43:335-339, 1954.

The urinary pepsinogen excretory rate of patients with duodenal ulcers was found to be approximately twice that of normal individuals. 83% of the controls excreted less than 700 mg% "tyrosine" per 24 hrs., while only 27% of the duodenal ulcer group excreted less than that amount. However, because of the overlap of the values for the two groups, the excretion by a given individual cannot be considered conclusive evidence, although it may be suggestive evidence, for the presence or absence of a peptic ulcer. The test is not proposed as a routine procedure because of the difficulties of analysis and the need for repeated accurate urine collections. E.V.

DETERMINATION OF GASTRIC SECRETORY FUNCTION BY MEASUREMENT OF SUBSTANCES EXCRETED BY THE KIDNEYS. II. AN EVALUATION OF THE TUBELESS METHOD OF GASTRIC ANALYSIS. H.M. Pollard, A. Carballo and R.J. Bolt (Dept. of Internal Medicine, University Hospital, University of Michigan Medical School, Ann Arbor, Mich.). J. Lab. & Clin. Med. 43:340-346, 1954.

Urinary quinium determinations in 76 patients compared favorably with the tube method of gastric analysis. Although the quinium method is qualitatively reliable for the detection of free acid, neither the concentration nor the quantity of free acid can be accurately predicted on the basis of the present grading system. E.V.

METHODS FOR QUANTITATIVE ESTIMATION OF HYDROLYTIC ENZYMES IN SERUM AND OTHER BIOLOGICAL FLUIDS. G. Gomori. (Dept. of Med., Univ. of Chicago, Chicago, Ill.). Am. J. Clin. Path. 24, 99-110, 1954.

A critical review of procedures for phosphatases, esterases, and amylase. C.R.

POLYPHENOL OXIDASE OF HUMAN SERUM. O. Homyklewicz and G. Niebauer (Univ. of Vienna). Naunyn-Schmiedebergs Arch. exptl. Pathol. u. Pharmakol. 218:449-56, 1953.

A copper containing enzyme was found in human serum. From the specificity with reference to the substrate and the behavior towards inhibitors it is concluded that the enzyme is a p-polyphenol oxidase. H.A.

NEPHROCALCINOSIS: A REVIEW. J.D. Mortensen and A.H. Baggenstoss. (Mayo Clinic, Rochester, Minn.) Am. J. Clin. Path. 24, 45-63, 1954. C.R.

METABOLISM OF ADIPOSE TISSUE. I. INCORPORATION OF ACETATE CARBON INTO LIPIDES BY SLICES OF ADIPOSE TISSUE. D.D. Feller. (Radioisotope Unit, Veterans Administration Hospital, Seattle, Washington) J. Biol. Chem., *206*, 171-180, 1954.

Adipose tissue actively synthesizes fatty acid from $C^{14}$ labeled acetate. The rate of synthesis is equal to or greater than that found for liver slices.

The oxidation rate of acetate by adipose tissue is about one third or one fourth of that found for liver.

C. vF. D.

CRITICAL STUDIES ON THE SPECTROMETRIC CARBON MONOXIDE DETERMINATION. K. Seller (Univ. Bonn, Ger.). Klin Wochschr. 31:1006-8, 1953.

The spectrophotometric determination of CO hemoglobin according to Oettel is invalid, owing to considerable methodical errors. H.A.

DIRECT MICRODETERMINATION OF SODIUM, POTASSIUM, AND CALCIUM IN A SINGLE BIOLOGICAL SPECIMEN. G.R. Kingsley and R.R. Schaffert (Univ. of California, Los Angeles). Anal. Chem. 25:1738-41, 1953.

A method employing the Beckman DU with photomultiplier tube attachment is described. Excellent results even in the presence of extreme concentrations are claimed. H.A.

A MICROCOLORIMETRIC METHOD FOR THE DETERMINATION OF GLUCOSE. S.C. Harvey and V. Higby (Univ. of Texas School of Dentistry, Houston). Texas Repts. Biol. Med. 11:489-93, 1953.

Glucose reacts with catechol when heated in $H_2SO_4$ at 140-5° to give a red color. Glycerol and certain carbonyl compounds interfere and are removed by extraction with EtOAc; many reducing substances which affect the usual methods for glucose do not interfere. H.A.

## AACC MEMBER HONORED

*Dr. C. B. Pollard (left) receiving the plaque indicating his election to the Florida Award from Dr. John V. Vaughen.*

Dr. Cash Blair Pollard, Professor of Chemistry at the University of Florida and Consulting Toxicologist at the Alachua General Hospital, Gainesville, Florida, received the annual Florida Section Award at the Meeting-in-Miniature in St. Petersburg on May 15. The award is presented each year to a Southern chemist selected by the membership of the Florida Section of the American Chemical Society.

Dr. Pollard came to the University of Florida in 1930. One of the reasons for bringing Dr. Pollard to the University was to establish a program of graduate study in the Department of Chemistry. The first department on the campus to award a Ph.D. degree was the Department of Chemistry. The program began in 1930, and the first degree was taken under Dr. Pollard and awarded in 1934. Since that time, twenty-six other Ph.D. degrees have been under Dr. Pollard's direction (more than any other staff member on the entire campus). In addition, eighteen M.S. degrees have been taken under Dr. Pollard's direction.

Dr. Pollard obtained his A.B. degree from William Jewell College in 1921. His M.S. and Ph.D. degrees were obtained at Purdue while working on a part-time basis as baseball coach, graduate assistant and instructor in chemistry. His interest in scientific criminal investigation began before he came to Florida and has been one of his most important fields of endeavor. Many members of the legal profession and several law enforcement agencies in Florida consult with him on matters pertaining to chemistry. Since 1930 he has been consultant to the State's Attorney and is qualified as an expert witness in State and Federal courts. As a toxicologist, he has been a consultant to many physicians and hospitals, and has appeared on many programs of physicians and allied professions.

Dr. Pollard has served as councilor, secretary, vice-chairman and chairman of the Florida Section of the A.C.S. His many publications include seventy-five journal articles, co-authorship of two books and several patents. He serves as reviewer for several journals and handbooks. He is a fellow of the American Institute of Chemists, member of the American Association for the Advancement of Science, the Florida Academy of Science, and the American Association of Clinical Chemists. In 1945, with John Pomeroy, he received the annual award of the Florida Academy of Science for a paper on the sensitivity of aldehyde reagents.

While he is widely recognized for his research on quinine and infant deafness, piperazines, organic nitrogen compounds, and snake venoms, he has maintained his interest in fundamental organic chemistry. His feeling for and interest in undergraduate students has never become secondary during his many contacts with graduate students. He is deeply interested in good teaching at all levels.

The subject of Dr. Pollard's address at the annual banquet during the Florida Section's Meeting-in-Miniature at St. Petersburg, was "Snake Venoms and Snake Bite Treatment". A recent article in the Journal of the Florida Medical Association reports the findings of Dr. Pollard and Dr. E. H. Andrews, prominent Gainesville surgeon, in a new technique for treating snake bites.

## BOOK REVIEWS

METHODS OF BIOCHEMICAL ANALYSIS. VOL. I. Edited by David Glick, Interscience Publishers, New York, X + 521 pp. $9.50

*Reviewed by Harry Sobotka, Chemistry Department, Mt. Sinai Hospital, New York, N.Y.*

Numerous annual volumes of Advances in the various branches of chemistry and allied sciences are intended to relieve scientists of the necessity of scanning "Chemical Abstracts" and of reading the original literature. In contrast to these series, "Methods of Biochemical Analysis" strikes across the entire field of physiological chemistry and its medical applications from the analytical point of view.

Thirty contributors describe in 17 articles the rationale and the techniques of chemical and of some microbiological methods for the analysis of important groups such as sulfhydryl, phenols, nucleic acids, and antibiotics, and for the estimation of individual substances such as cobalamine, choline, raffinose, etc. A number of articles are of specific interest for the clinical chemist: chromatography of radioactive iodine compounds and of adrenal steroids, and the assay of 17-keto steroids in urine. The descriptions of the methods, while varying in style, are uniformly given in sufficient detail so that they may be carried out without further reference to the original literature.

This reviewer liked in particular the articles on -SH groups, on nucleic acids, on ascorbic acid by Roe, and the excellent presentation of the present state of $B_{12}$ assays by Hoff-Joergensen, but he is aware of the arbitrariness of this selection amongst a multitude of others of equally high calibre.

A similar attempt, made by Abderhalden a generation ago, resulted in the many volumes of the "Handbuch der biochemischen Arbeitsmethoden", which tried to be definitive and, thus, never could reach completion. We greatly prefer Prof. Glick's plan of Annual volumes "comprising a self-modernizing encyclopaedia".

It is hoped that the publishers will keep the price of future volumes at the present level which, although high, is justified by contents and form. We wish them and the Editor good luck in their ambitious enterprise.

## PUBLISHER'S CORNER

"X-Ray Diffraction Procedures" by Harold P. Klug and Leroy E. Alexander was published in June by John Wiley & Sons.

The result of more than twenty-five years of research, the new book deals with procedures for polycrystalline and amorphous materials. The major procedures and applications are thoroughly examined by the authors, who have tested the majority of these procedures in their own laboratories.

In the first three chapters, Klug and Alexander supply a well-rounded introduction to elementary crystal geometry, the production and properties of x-rays, and the interaction of x-rays and crystals. Succeeding chapters bring together previously scattered information on the Debye-Scherrer method and powder techniques in general, the Geiger-counter spectrometer, small-angle scattering methods, and radial-distribution techniques. An individual discussion is also devoted to qualitative and quantitative analysis of powder mixtures.

Both authors are at the Mellon Institute where Dr. Klug is a senior fellow in x-ray diffraction and Dr. Alexander is head of the department of research in chemical physics. Closely associated for the past fifteen years, the authors share distinguished teaching records. Dr. Alexander was also with the General Electric Company as a research chemist. In addition to his duties at the Mellon Institute, Dr. Klug is an associate member of the graduate faculty in chemistry and a lecturer at the University of Pittsburgh.

"X-Ray Diffraction Procedures" contains 716 pages and is priced at $15.00

On August 16th, APPLETON-CENTURY-CROFTS, INC. will publish the new 2nd edition of LEGAL MEDICINE, PATHOLOGY AND TOXICOLOGY by Gonzales, Vance, Helpern and Umberger. This enlarged and profusely illustrated text discusses the medicolegal investigation of deaths due to violence or poisoning, known or suspected.

The general discussion of forensic toxicology is covered in 8 chapters totalling 212 pages.

The 350 page concluding section is a complete working manual for the isolation, detection, quantitative and qualitative (emphasis on the latter) of poisonous substances that are not normal components in the biologic material obtained at autopsy to aid in establishing the cause of death or in elucidating the circumstances of the death.

Here, for the first time, are descriptions of systematic methods for attacking the analytic problem of the "general unknown" (when any poisonous substance may have been the cause of death, and a complete examination is indicated). Prior toxicologic literature gives only the physical and chemical properties of poisonous substances under the name of the known compound.

In describing the methods used and developed in the Microchemical and Physical Laboratory of the Chief Medical Examiner of the City of New York, the newer methods are given preference, and the author has assumed that adequate personnel is available and has access to equipment such as the spectrograph, the ultraviolet and infrared spectrophotometers and x-ray diffraction when needed.

Also included in this section is a 12 page chart listing 183 compounds with their reactions, if any, to 22 chemical agents.

## BOX 123

Gentlemen:

Miss Reiner's problem (Letters From Members; February issue of The Clinical Chemist) in connection with establishing some reference for normal workloads in the Clinical Chemistry Laboratory is well appreciated.

Perhaps some statistics from a large City General Hospital may contribute toward a concept of a reasonable workload. One cannot become too detailed due to the variety of analytical methods used for a given analysis. Also, the larger the repertoire offered, the smaller the per-analysis efficiency becomes. We are the Chemistry section of the Clinical Laboratories, performing the common blood chemical examinations. We also extend the methods to some urine, cerebrospinal fluid, transudate and exudate, and duodenal fluid tests.

We have the equivalent of four full-time Technicians available for this routine work, and a medical student on duty for night and Sunday work.

Total Chemical Tests per Year

| | |
|---|---|
| 1951 | 43,300 |
| 1952 | 46,000 |
| 1953 | 56,100 |

In 1953 the work was divided among the four Technicians as follows:

| | |
|---|---|
| First Technician: | |
| $CO_2$ content-Sugars-Amylase | 12,848 |
| Second Technician: | |
| Urea-N -Creatinine-Uric Acid-NPN-pH | 16,400 |
| Third Technician: | |
| Na-K-Prothrombin-Proteins-Bilirubin | 16,823 |
| Fourth Technician: | |
| Cl-Ca-P-P'tase-Cholesterol-Sulfa, etc.* | 10,045 |
| Total: | 56,116 |
| Average analyses per Technician: | Approximately 14,000 |

* The fourth Technician was actually a half-time Technician and the Biochemist alternating on the routine work. An additional fifteen tests not listed were done in small quantities (less than 100 each during the year) and included in the total for this job.

The emergency work done by the night and Sunday medical Student averaged 10% of the total, and so the corrected average for each Technician is 14,000 −10% or *12,600 analyses per Technician per year.* A two-week vacation period is given our personnel each year. Otherwise this amount of work is done while working a forty-three hour work week.

The remarkable growth in demands made upon the clinical lab within the past five years, coupled with the financial difficulties of all hospitals, both public and private, creates a situation where Technician morale and efficiency deteriorates. Clinical Chemists realize the necessity to improve the accuracy and precision of clinical laboratories (recent surveys have indicated areas of deplorable lack of analytical accuracy), but we labor under increasing volumes of work and decreasing or statis budgets. I am also quite concerned about the need for some effective yard-stick for use in discussions with Administrators.

Sincerely,
*Norman A. Keller*, Biochemist
Cincinnati General Hospital
Cincinnati, Ohio

## NEW APPARATUS

**ELECTROPHORESIS APPARATUS,** Paper Strip Model, for preparing a series of horizontal paper electrophorograms of micro quantities of mixtures whose components are separable by migration upon application of an electric potential. With separate variable power supply, the apparatus consists of a compact, lightweight cabinet of transparent plastic, including a phenolic plastic paper strip carrier, which permits use of a single paper sheet or multiple strips up to a maximum width of 7-3/8 inches; and a removable glass cover, featuring a recessed safety interlock switch which breaks the current to both electrodes when the cover is lifted. Separation of normal human serum can be completed in approximately 6 hours, using a 250-volt poten-

*cont. p. 49*

## BOSTON SECTION

The Boston Section, held its 7th meeting of the current season on April 21st, at the New England Center Hospital.

Dr. David Skinner, pathologist at the Newton Wellesley Hospital, spoke on prothrombin time determinations.

Prefacing his talk with an explanation of the mechanism of blood coagulation, Dr. Skinner went on to deal with the practical aspects of prothrombin time determinations.

Although several different methods exist, all employ a thromboplastic material and produce coagulation of normal plasma in 11-15 seconds. The speaker stressed the importance of carrying out the test within an hour of obtaining the specimen, or, if refrigerated, within four hours.

The reporting of results varies, and at least four different systems are in use. The most readily understandable however, is to report both control and test in seconds, and this expression is apparently coming once again into wider usage. If prothrombin concentration is read from a curve of serially diluted normal plasma, Dr. Skinner recommended using prothrombin free plasma as the diluent, rather than saline. This, by maintaining a constant amount of fibrin in all tubes, enables quick recognition of clotting in even the greater dilutions.

Apart from the use of the prothrombin determination in cardiovascular disease, the speaker also dealt with its diagnostic value as a liver function test, and as a tool in the differential diagnosis of jaundice.

## NEW YORK SECTION

The New York Section held the last of the spring meetings on May 25 at the New York Academy of Sciences. The scientific Session was headed by Abraham W. Freireich, Toxicologist for Nassau County, N.Y. and featured discussions on "Chemical Aspects In Toxicology". Leo R. Goldbaum, Toxicologist, Army Medical Center, Washington, D.C., spoke on "Barbiturate Poisoning, Analytical and Clinical Aspects", and Joseph Umberger, Toxicologist, New York Medical Examiner, spoke on "Metal Poisoning, Analytical and Clinical Aspects". A discussion period followed the speakers.

## INSTRUMENTATION TECHNIC – SYMPOSIUM

The Washington Sections of the American Chemical Society, Instrument Society of America and the American Association of Clinical Chemists sponsored a three day symposium on "Recent Developments In Instrument Techniques and Applications". The symposium was held May 24-26 at the National Institutes of Health, U.S. Department of Health, Education and Welfare, Bethesda, Md.

The Symposium was in four sections: Electroanalytical Techniques"; "Spectroscopy And Molecular Structure"; "Electrophoresis And Chromatography"; and "Methodology And Instrumentation In Microanalysis".

## SOUTHERN CALIFORNIA SECTION

Rex D. Sterling, Ph.D., Biochemist at the Los Angeles County Hospital, discussed the "Determination of Heavy Metals in Biological Materials" March 2 at the Los Angeles County Hospital.

William R. Bergren, Ph.D., Assistant Professor of Biochemistry and Nutrition at the University of Southern California Medical School, discussed "Filter Paper Electrophoresis with Particular Reference to the Determination of Abnormal Hemoglobins" April 6 at the Hollywood Presbyterian Hospital. Dr. Bergren described his simple, inexpensive apparatus and procedure that bring the determination of abnormal hemoglobins within the ability of the smallest laboratory.

Reports on original research were presented by four members of the local section May 4 at the Veterans Administration Center, Los Angeles.

A. L. Chaney, Ph.D., A. L. Chaney Chemical Laboratory, presented his "Method for Increased Sensitivity in the Determination of Mercury". This method can determine several hundreths of a microgram and requires only 5 to 10 ml. of normal urine, one ml. of blood or one half gram of tissue. Dr. Chaney observed that, in his iodine method, mercury inhibits the iodine catalysis of cerium reduction by arsenite and proceeded to determine mercury by measuring this inhibition under standard conditions.

G. R. Kingsley, Chief Biochemist of the Laboratory Service, Los Angeles Veterans Administration Center, appraised earlier work and reported improvements in the "Determination of Serum Iron". He has developed and evaluated a modified Peterson method (Peterson, *Anal Chem.* **25** 1337 (1953)), which he has also adapted for determining iron-binding capacity of serum.

R. R. Schaffert, Biochemist in Mr. Kingsley's laboratory, reported on his "Rapid Method for the Determination of Ascorbic Acid". By modifying the method of Roe and Kuether (*Jour. Biol. Chem.* **147** 399 (1943)), particularly by heating on a boiling water bath instead of using 37° incubation, he can speed up the determination considerably.

Otto E. Lobstein, Ph.D., Chem-Tech Laboratories, Beverly Hills, described his investigations on "Lysozyme and Its Effect on Malignancy", with attention to the possibilities of (1) "digesting" malignant tissue and (2) changing malignant metabolism back to normal. Mice were divided into five groups: (1) normal control mice; (2) normal mice given buffered lysozyme; (3) tumor-bearing mice (having lymphosarcoma); (4) tumor-bearing mice given buffer alone; and (5) tumor-bearing mice treated with buffered lysozyme. Whereas the effects of buffered lysozyme on normal mice and of buffer alone on tumor-bearing mice were apparently insignificant, buffered lysozyme substantially prolonged the life of tumor-bearing mice, in which autopsy also showed less tumor tissue. Dr. Lobstein also displayed some photographs evidencing marked tumor regression after buffered lysozyme treatment.

More detailed descriptions of some of the foregoing research activities will appear later in THE CLINICAL CHEMIST.

## EXECUTIVE COMMITTEE MINUTES

*(Continued from Page 37)*

another four year term after an interim of at least one year. The editorial board shall be so constituted that one-fourth of the members shall be replaced each year. With the necessity of starting an orderly rotation it was decided to begin by the appointment of two members to a six year term, two members to a five year term, two members to a four year term, and two members to a three year term.

It was moved, seconded, and approved that an advisory board to CLINICAL CHEMISTRY shall be appointed, the number of individuals on this board to be de-

termined by the Executive Committee, but it shall include at least two medical practitioners who are also accomplished in clinical chemistry, and at least one scientist not residing in the United States.

It was moved, seconded, and approved that Harold D. Appleton be appointed as Chairman of the Board of Editors to serve for a term of six years.

It was moved, seconded, and approved that the National Secretary shall be editor of THE CLINICAL CHEMIST, the professional section.

It was moved, seconded, and approved that Ellenmae Viergiver shall be editor of the abstract section for a term of four years.

The following committee was appointed to select candidates for the editorial and advisory board: Harry Sobotka, chairman; Harold D. Appleton, Max M. Friedman, Hugh J. McDonald, Joseph I. Routh, and Albert E. Sobel. This committee shall submit to the Executive Committee at least two names for each vacancy. The Executive Committee shall select the editorial and advisory board.

It was moved, seconded, and approved that each member of the Association shall be assessed $3.50 for the journal.

The matter of regional versus local sections was discussed. It was the sense of the Executive Committee that regional sections may be chartered in those areas where the density of membership is low, but such regional sections shall not in the future prejudice the formation of local sections in such regions.

It was moved, seconded, and approved that the individual receiving the highest number of votes for the Nominating Committee shall serve as chairman of this committee.

It was the sense of the Executive Committee that associate members who have remained in that classification for five years shall be assessed the same dues as full members. This recommendation shall be made to the membership at the Stated Annual Meeting.

It was moved, seconded, and approved that in order to help support activities of the Association, contributors may be permitted as "Friends of the Association".

The meeting was adjourned at 11:45 P.M.

Respectfully submitted,
*Max M. Friedman, National Secretary*

## MIT SPECIAL SUMMER PROGRAM

The Massachusetts Institute of Technology, announces that it will again offer two one-week Special Summer Programs in "Instrumental Chemical Analysis", to enable chemists in industry and other laboratories to study the application of new instrumental techniques and methods in the field of applied analytical chemistry. The sessions will be held from August 16 to 20 and 23 to 27.

The purpose of these Programs is to provide, for both electrical and optical methods of instrumental analysis: (1) adequate background in the fundamental principles and theory involved; (2) understanding of representative practical applications; and (3) demonstrations of typical commercial instruments. The practicing analyst will find the evaluation of the various methods beneficial in approaching his problems. Information on the comparative performances of different instruments will be of interest to those concerned with establishing or expanding an instrumental laboratory.

The Programs are designed as broad surveys for those having a Bachelor's degree or its equivalent in experience. They will consist of a lecture-demonstration and discussion period each morning and afternoon.

The first Program, beginning August 16, will be devoted to Electrical Methods of Instrumental Chemical Analysis and will emphasize polarography, potentiometry, conductimetry (audio- and radio-frequency), amperometric titrations, automatic titration methods, and applications of thermistors and self-balancing recording potentiometers. A discussion of the principles of non-aqueous titrimetry is included.

The second Program, beginning August 23, will be devoted to Optical Methods of Instrumental Chemical Analysis and will emphasize spectrophotometry (visible and ultra-violet), colorimetry, fluorimetry, turbidimetry, nephelometry, photometric titrations, reflectance techniques, and flame photometry.

*Further information about the 1954 Summer Session at M.I.T. may be obtained from: Professor Ernest H. Huntress, Director of the Summer Session, Room 7-103, Massachusetts Institute of Technology, Cambridge 39.*

## NEW APPARATUS *(continued)*

tial, and the entire procedure, including fixing, staining, drying and evaluating, can be accomplished in from 7 to 10 hours. The apparatus is readily portable and easily inserted and operated in refrigerators or incubators. For complete information, write for illustrated, 2-page *Bulletin 115.* Arthur H. Thomas Company, 230 South 7th Street, Philadelphia 5, Pa.

## MANOMETRIC BLOOD GAS APPARATUS

The new Thomas-Van Slyke Magne-Matic Model produces agitation by means of a magnetically driven stirring bar in the extraction chamber, thus reducing costly breakage of glassware formerly caused by violent mechanical shaking. The chamber is held stationary and constantly vertical, permitting rapid adjustment of the meniscus, convenient addition of reagents, etc. Other innovations include: ball-and-socket ground glass joints for quick removal of glassware for cleaning; built-in automatic timer for reproducible periods of agitation; corrosion-resistant housing of Formica and Stainless steel and acid-resistant hardwood base with trough for spilled mercury; clear plastic column which supports and illuminates the manometer; etc. For complete information, write for 4-page, illustrated Bulletin 120. Arthur H. Thomas Company, 230 South 7th St., Philadelphia 5, Pa.

## BOOKLET ON LABORATORY OVENS

Precision Scientific Company has published a new "Catalog No. 331" describing Precision-Freas and Thelco Ovens, Sterilizers, Incubators and related equipment. The 36-page illustrated booklet describes the latest additions to the Freas line of constant temperature cabinets, including recessed-in-the-wall ovens and sterilizers, a re-designed Low Temperature Incubator, and new, frameless glass inner doors.

For all 54 models, the essential information — dimensions, temperature range, power requirements, weight, and accessories—is presented in convenient tabular form. The catalog greatly simplifies the problems of laboratory or hospital managers who require constant temperature cabinets.

A free copy will be mailed to those requesting "Catalog No. 331." Precision Scientific Company, 3737 West Cortland Street, Chicago 47, Illinois.

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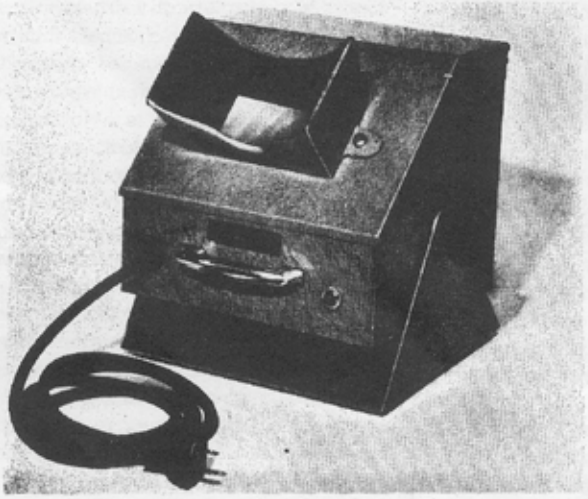
# EBON-SCOPE

## For All Routine and Research Fluorometric Comparisons and Observations



**A compact, portable, versatile "Black-Light" fluorescent viewing unit.** Eliminates the necessity of a dark room. Gives full protection to the viewer from direct or reflected ultraviolet rays. Ideal for the Diagnex* Test for Achlorhydria. Can be used as a shadow box unit or can be easily disassembled and the lamp unit utilized alone as a microscope lamp or illuminator. The handsome grey hamerloid and chrome case is furnished on a tilting leg stand.

### CAN BE USED FOR THE

1. Detections of Quinine Compounds in the latest tests for Achlorhydria, (Squibb "DIAGNEX*" assay procedure) eliminating the necessity for intubation.
2. Examination for the presence of Porphyrins in Organic and Clinical chemistry.
3. Detection of Milkstone and impurities in the Dairy and Food industries.
4. Demonstrations of Fabrics, Dyes and Starches in the Textile industry.
5. Detection of Thiamine (B-1), Riboflavin (B-2) etc., as used in chromatography and Fluorochemical research.
6. Examination for fluorescent tracer materials in Criminology and Toxicology.
7. Clinical determination of Porphyrin.
8. Has application in Minerology and Oil analysis.
9. Can be applied to the activation of an extensive group of fluorescent materials, including many organic materials, dyes and inks, used in fabric, paper, plastics, lacquers and paints.

The "Ebon-Scope" measures 10¼" wide, 9¾" high by 8" deep, supplied complete with 6 "Pyrex Brand" culture tubes 18x150 mm and rack. Operates on 110/115 volts, 60 cycle A.C.

### DETACHABLE LAMP UNIT

The lamp unit can be removed from the viewing chamber and mounted on the tilting leg stand for use as a microscope illuminator or may be used as a hand lamp for fluorescent tracing. The lamp unit is supplied with a special 6 inch, 4 watt, 60 cycle, 110/120 volt longwave fluorescent ultraviolet tube. This tube has an energy peak of over 3600 angstrom units with practically no radiation shorter than 3000 angstrom units. This instant starting "Black-Light" tube is made of a special high transmitting self-filtering Corning glass to give maximum intensity. A special aluminum compound reflector gives maximum ultraviolet reflection. The reflector is constructed so as to shield the user from direct ultraviolet rays.

1. The Ebon-Scope can be equipped with a shortwave ultraviolet germicidal tube for sterilizing small objects inside the viewing compartment. Eliminates the necessity of wearing protective equipment.
2. The Ebon-Scope can be equipped with an intense type white light tube. By detaching the viewing unit, this light can be used as an adjustable light for fluorescent microscopy.
3. The Ebon-Scope can be equipped with a daylight fluorescent tube and by removing the yellow filter in the viewer used for making color matches.

### VIEWING CHAMBER

The viewing chamber is lightproof and has a satin black finished interior. The removable rack holds 6 "Pyrex Brand" test tubes 150 x 18 mm and is adjustable through nine positions. Rack can be slanted to hold Petri dishes. The rack is provided with a special aluminum compound mirror with maximum reflecting properties that redirects all escaping transmitted light back through the sample tubes giving in effect double intensity. Tubes and rack are placed in the "Ebon-Scope" through the front of the instrument for ease in operations. Tubes, dishes, flasks or any object less than 6" wide by 5" high by 4" deep can be placed inside the compartment. The form cast viewer eliminates the necessity of making examinations in a dark or even dimly lit room. The viewing head is equipped with a removable, complementary, yellow filter to eliminate all visible light reflections, thus permitting sharp and detailed examination and comparison of the samples' fluorescent properties.

**Cat No. 70985 .....EACH 74.50**

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