VOL. 5
NO. 2

April 1953

The CLINICAL Chemist

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Official Publication
of
AMERICAN ASSOCIATION
of
CLINICAL CHEMISTS,
INC.

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

The CLINICAL Chemist

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

VOL. 5, NO. 2 APRIL 1953

THE SECRETARY REPORTS

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

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

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

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

Max M. Friedman, National Secretary

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

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

VOLUME 1, June 1953

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

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

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

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

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

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

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

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

QUID NUNCS

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

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

ABSTRACTS OF PAPERS ON CLINICAL CHEMISTRY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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