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The CLINICAL Chemist

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The CLINICAL Chemist

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VOL. 6 NO. 5-6 Nov.–Dec. 1954

The past six years of publishing THE CLINICAL CHEMIST has shown the need for a publication that truly represents the profession of clinical chemistry. The Editorial Committee has tried and believingly achieved its purpose, to bring to the members of the AACC and to the profession a publication that would enhance professional stature in the scientific community and to place the profession and practice of clinical chemistry on the highest scientific plane.

We are very pleased that our efforts, from the very beginning, were very well received both among our membership and throughout the world. A number of our members, returning from visits to foreign countries, tell of the amazingly wide circulation of the limited copies available, from laboratory to laboratory. In our own country, the publication has given our members a certain pride in their profession and has improved the relationship between the clinical chemistry laboratory and medical practice.

It is particularly gratifying to realize that our Association recognized the need and the objectives of a professional publication very early in the Association's organization. The publication budget always has made up a large percentage of the Association's income. It is to the credit of the various National Executive Committees that they have always unanimously approved the constant growth of their publication and at a very early date set as a goal the eventual organization of an official Journal.

This is the last issue of THE CLINICAL CHEMIST. Our six years of effort has been rewarded by the establishment of an Official Association Journal, CLINICAL CHEMISTRY, which will make its debut next month. As Chairman of the Editorial Committee for the past five years, I wish to take this opportunity to publicly thank Dr. Ellenmae Viergiver and her abstracting group for their efforts in making that section one of the features of our publication; Dr. Clyde A. Dubbs, of the Southern California Section, for his work in arranging to bring to our readers the excellent scientific papers which were presented before the scientific sessions of his section; and to the secretaries and section correspondents for transmitting all the local section news.

During the past month every member received a letter from Dr. Max M. Friedman, National Secretary, explaining how every member having an interest in his profession has an interest in the Association's Journal, CLINICAL CHEMISTRY. It may be well to quote the last few paragraphs of that letter to emphasize how every member can help his Association and profession.

".....CLINICAL CHEMISTRY belongs to our Association and so we are all interested in gaining the widest circulation for it.

There are also a few other ways in which you might help support your journal. It is of first importance that there be a continuous flow of good scientific material in order to maintain the necessary standards. We therefore respectfully urge you to consider CLINICAL CHEMISTRY when preparing manuscripts for publication. It would also be appreciated if you called the attention of prospective advertisers to this journal.

Printing and distribution costs of a scientific periodical are borne mainly by circulation, advertising, and subsidy. We have no source of subsidy at this time except that the publishers have trustfully assumed the risk and initial expenses of this journal for a period of five years. Under these circumstances each member must assume some part of these responsibilities. A successful journal will return to the Association a liberal royalty and allow us funds for other important projects in the interest of clinical chemistry.

This much-needed scientific periodical is the result of several years of planning and industry by many individuals in the Association, and it is with much enthusiasm that we look forward to the first issue of CLINICAL CHEMISTRY in January, 1955."

Harold D. Appleton, Chairman
Board of Editors

NATIONAL SCIENCE FOUNDATION TRAVEL GRANTS

The National Science Foundation, the American Society of Biological Chemists, and the Division of Biological Chemistry of the American Chemical Society, acting jointly, will award individual grants to defray partial travel expenses of a limited number of scientists who will attend the Third International Biochemical Congress to be held in Brussels, Belgium, August 1-6, 1955. Applications will be considered in two groups: (1) those from scientists under 40 years (about 20 grants); and (2) those from more senior scientists (about 5 grants). Primary consideration will be given to scientific merit in the selection, but preference will be given to those scientists who have not previously attended an international scientific congress or studied in Europe, and to those who are unable to attend without the aid of a grant. Applications for grants to any of the three organizations will be considered together.

Application blanks may be obtained from the National Science Foundation, Washington 25, D.C. *Completed forms must be received by the Foundation by January 3, 1955.* Announcement of the award of travel grants will be made on or about March 1, 1955.

BROOKLYN POLYTECH GIVES RADIOCHEMICAL ANALYSIS COURSE IN SPRING 1955

Polytechnic Institute of Brooklyn will give a graduate course in "Radiochemical Analysis" in the spring semester, 1955. This course, #1112, is designed to familiarize chemists with a descriptive account of the nature and structure of nuclei, and the types of nuclear reactions. Considerable attention is given to types of radiation, technics of quantitative measurement and the principles and operation of instruments used for radiation measurements. Tracer technics in chemical systems will be discussed and illustrated. Laboratory work will be flexible in character. After certain fundamental technics are acquired, experiments will be designed to meet the needs of the individual student.

Pre-requisite (for degree students) Chem. #1101 or equivalent. Students who are not on a degree program may be admitted to the course without formal requirements. An interview with the instructor is suggested.

Registration will be held the week of January 31, 1955. Professor Joseph Steigman is in charge of the course.

INSTRUMENTATION ASSOCIATES

Instrumentation Associates announces the opening of its new office located at 17 W. 60 Street, New York 23, which will include showroom facilities for the introduction and demonstration of newly developed scientific and medical apparatus. Special emphasis will be given to instrumentation as applied to the medical, biological and industrial application of measurement. An extensive file on instruments produced throughout the world will be maintained; stressing the produce of the United States, Great Britain, Switzerland, Germany and other European countries. The purpose of this file, is to facilitate the solutions to the many problems faced by the scientist today.

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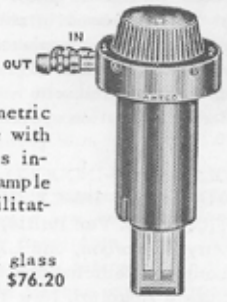

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ABSTRACTS OF PAPERS PRESENTED AT THE SCIENTIFIC SESSIONS OF THE 1954 ANNUAL MEETING

The Scientific Sessions Were Held In Participation With The Division of Biological Chemistry, at The 126th National Meeting of The American Chemical Society, New York

STUDIES ON THE MECHANISM OF INHIBITION OF INORGANIC PHOSPHATE LOSS FROM ERYTHROCYTES. Allen F. Reid, Jack K. Jeanes, Richard C. Gilmore, Jr., and Margaret C. Robbins, Biophysics Department, Southwestern Medical School, University of Texas, Dallas, Tex.

Dextrose and another unidentified component of plasma when incubated with human erythrocytes markedly decrease their subsequent rate of inorganic phosphate loss. When labeled phosphate is incubated with human erythrocytes and then the loss rate of the bound labeled phosphate is measured, it is found that the presence of the above inhibitors in the incubation medium containing the phosphorus-32 causes a more pronounced decrease in phosphorus-32 loss than in total inorganic phosphate loss. Phosphate decrease may be the result of a decrease in ATPase activity or the result of inhibition at one of the steps in the formation of ATP.

The concurrent specific activity decrease is interpreted to mean that there is slowing of the diphosphoglycerate-ATP transphosphorylation, as well as slowing of ATP hydrolysis. If methylene blue is included in the phosphorus-32 uptake media, the inhibitors cause a decrease in phosphate loss but not the pronounced decrease in specific phosphorus-32 activity. If glyceraldehyde is included in the media, the inhibitors do not cause adecrease in phosphate loss or a decrease in the fraction of labeled phosphate lost. It is suggested that the decreases in phosphate loss are the result of the complexing of ATP with a glycolytic precursor of diphosphglycerate. This is compatible with other studies using different inhibition systems.

GLUCAGON-INDUCED HYPERGLYCEMIA AS AN INDEX OF LIVER FUNCTION. T.B. Van Itallie, W.B.A. Bentley, Mary C. Morgan, and L.B. Dotti, Departments of Medicine and Biochemistry, St. Luke's Hospital, New York, N.Y.

Glucagon (HGF) raises blood sugar by stimulating hepatic glycogenolysis. Highly purified glucagon (0.033 mg. per kg.) was given intravenously over a 30 minute period to normal subjects and to patients with liver disease, and blood glucose levels were measured before, during, and after glucagon infusion. The hyperglycemic effect of glucagon alone was compared with the hyperglycemic effect of the same quantity of glucagon in conjunction with epinephrine (0.0033 mg. per kg.) administered subcutaneously to the same subject.

In patients with parenchymal liver disease the hyperglycemic response to glucagon and epinephrine given together was markedly decreased in height and delayed. In normal individuals pretreated with epinephrine the blood glucose at the end of glucagon infusion was approximately 65 mg. % above the control level, while blood glucose of patients with liver disease rose approximately 20 mg. % in the same time. Administration of epinephrine in conjunction with glucagon permitted a more consistent distinction to be made between the responses of the normal and pathologic groups, than when glucagon was given alone.

THE METABOLISM IN THE MOUSE OF 3,4,5–TRIMETHOXYBENZOYL (CARBOXYL-C-14) METHYL RESERPATE [RESERPINE]. Paul Numerof, Maxwell Gordon, and Jacques M. Kelly, Squibb Institute for Medical Research, New Brunswick, N.J.

Reserpine, labeled with carbon-14 in the carboxyl group of the 3,4,5-trimethoxybenzoic acid moiety, has been prepared and its fate in the mouse investigated. The 3,4,5-trimethoxybenzoic acid was prepared by carbonation of 3,4,5-trimethoxyphenyl lithium. Conversion of the acid to the acid chloride and condensation with methyl reserpate, in pyridine, gave labeled reserpine.

Ninety six micrograms of labeled reserpine were given orally to mice; the animals were sacrificed four and 24 hours later. Extracts of urine, feces, and selected tissues were examined by paper chromatography for both intact reserpine and free trimethoxybenzoic acid was further substantiated by the addition of unlabeled carrier and crystallization to constant specific activity.

After four and 24 hours, about 35 and 70%, respectively, of the administered radioactivity appears in the urine. The respective fecal values are about 1 and 15%. The major part of the radioactivity in both urine and feces is present as trimethoxybenzoic acid.

THE ESTIMATION OF SUBMICROGRAM QUANTITIES OF RESERPINE IN BIOLOGICAL MEDIA. Raymond B. Poet and *Jacques M. Kelly*, Squibb Institute for Medical Research, New Brunswick, N.J.

The alkaloid reserpine is pharmacologically active in microgram quantities. Hence, the delineation of its absorption, distribution, and excretion by chemical means requires sensitive methods. The compound is strongly fluorescent in mineral acids and this property has been utilized in the development of a quantitative method which is sensitive to concentrations of 0.02 y per ml. In sulfuric acid alone the limit of detection of the alkaloid fluorimetrically is of the order of 0.1 y per ml. The sensitivity can be heightened by heating sulfuric acid solutions in the presence of selenious acid. Under these conditions the fluorescent response is linear over the concentration range 0.02 to 1.0 y per ml.

In practice, reserpine is extracted from biological samples adjusted to pH 8.5 into petroleum ether containing 1.5% isoamyl alcohol. It is then extracted from the organic phase into sulfuric acid in which the fluorophor is measured after heating in the presence of selenious acid. With highly purified reagents, dog plasma blanks are approximately the same as reagent blanks. The specificity of the method was studied by noting the response of several possible metabolic fragments available in pure form. Trimethylgallic acid and reserpic acid are not extracted in the specified system. Methyl reserpate has a distribution coefficient of 0.3 and gives a fluorescence intensity about 1.2 times that of reserpine on an equivalent basis. However, it can be separated from reserpine by re-equilibrating the organic phase with pH 8.5 buffer.

The details and application of the method in metabolic problems are discussed.

ON THE SULFHYDRYL CATALYZED ALKALINE HYDROLYSIS OF p-NITROPHENYL SULFATE. Philip Feigelson and Margaret Been, Department of Biochemistry, Fels Research Institute, Antioch College, Yellow Springs, Ohio.

The procedure for the assay of serum phenolsulfatase [J. Biol. Chem., *170*, 391 (1947)] calls for alkalinization of the reaction mixture to halt enzyme activity and

develop the color of the released p-nitrophenol. It was found, however, that subsequent to alkalinization in the presence of serum, a rapid hydrolysis of p-nitrophenyl sulfate ensued. This artifact could be eliminated by protein precipitation or chilling the reaction mixture prior to alkalinization. With these precautions observed, no significant phenol-sulfatase activity was noted in human serum.

The question arises as to the nature of the substances in serum responsible for the alkaline catalytic hydrolysis of p-nitrophenyl sulfate. The degree of hydrolysis was demonstrated to be a function of the amount of serum and p-nitrophenyl sulfate and incubation temperature. The catalyzed hydrolysis is negligible below pH 12 and increases exponentially above this pH; the catalyst is heat labile and is precipitated by zinc sulfate-barium hydroxide. Paper ionography of serum demonstrated that the active catalytic areas correspond with those of blood proteins. Testing a variety of purified proteins, polypeptides, and amino acids indicated that only those containing cysteine possessed catalytic activity. Solutions of glutathione or cysteine (0.008M) were potent catalysts, cystine being a weak catalyst with cysteic acid, methionine, other amino acids, and reducing reagents such as sodium hydrosulfite and ascorbic acid possessing no significant catalytic activity; nonamino acid mercaptans are catalytically active demonstrating that free sulfhydryl groups are responsible for the catalysis.

THE FORMATION OF L-XYLULOSE BY GUINEA PIGS AND BY A NORMAL MAN. Oscar Touster and Ruth M. Hutcheson, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tenn.

L-Xylulosuria may resemble other genetic metabolic abnormalities in being characterized by the excretion of a normal metabolic intermediate. The origin of the pentose is unknown. However, Enklewitz and Lasker [J. Biol. Chem., *110*, 443 (1935)] reported that glucuronolactone, and substances excreted as glucuronides, cause excretion of increased amounts of L-xylulose in pentosuric subjects but do not yield urinary xylulose in normal individuals. The authors have attempted to induce L-xylulose excretion in guinea pigs and in normal man. Urine was fractionated on Dowex 1 (borate) columns and then on paper chromatograms.

Guinea pigs and a male human being fed large amounts of glucuronolactone have yielded fractions which contain a substance behaving identically with xylulose in various chromatographic and colorimetric tests. From the urine of the glucuronolactone-fed man an osazone, melting point, 161° to 162.5° (cap.) has been obtained, which, on recrystallization with an equal amount of D-xylosazone, gave the characteristic crystals of DL-xylosazone, melting point, 198° to 198.5° (cap.). Urine fractions of the human subject on a normal diet also gave chromatographic evidence for the pentose. These findings, together with experiments indicating rapid utilization of L-xylulose by the mouse and by guinea pig liver slices, strongly suggest that this pentose may have a normal metabolic role.

COMPETITIVE INHIBITION OF DRUG-PRODUCED EXPERIMENTAL PSYCHOSIS. Roland Fischer and Neil Agnew, Saskatchewan Department of Public Health, General Hospital, Munroe Wing, Regina, Saskatchewan, Canada.

Wool was used as a model for the protein component of receptors involved in drug-produced experimental psychoses (Fischer, R., J. Mental Sci. London, July 1954). Any one of 500 mg. of mescaline, 1 mg. of lysergic acid ethylamide (LAE), or 100 γ of lysergic acid diethylamide (LSD) cause schizophrenia-like experimental psychoses of similar intensity and duration in healthy volunteers [Fischer, R., *et al.*, Schweiz. Med. Wochschr., *81*, 817 (1951)]. This decreasing order of dosage is correlated with the increasing affinity (absorption) for wool of the same compounds (0, 1.1, 2.6 x 10^{-2}mM respectively per gram of wool) as well as with their increasing adrenergic blocking activity.

It was attempted to prevent an LSD-caused psychosis by previous administration of a competitive inhibitor. Suitable compounds were found in the phenothiazine series: methylene blue, N-(2-diethylamino-n-propyl)-phenothiazine, 3-chloro-10-(-3-dimethylaminopropyl) phenothiazine, and β-diethylaminoethyl-N-phenothiazine which display a gradually increasing affinity for wool (3.3, 4.8, 5.3, 5.5 x 10^{-2}mM per gram of wool respectively) as well as modify and inhibit the psychotic experience otherwise caused by LSD. Preliminary experiments suggest that a gradual increase in affinity for wool of a compound might be associated with a more complete inhibition of the experimental psychosis. These inhibitors also display a gradually increasing adrenolytic action.

A curvilinear relation exists between the gradually increasing affinity for wool protein of the seven compounds (from mescaline to β-diethylaminoethyl-N-phenothiazine) and the log of their relative toxicity (0, 1.26, 2.54, 2.9, 3.35, 3.43, 3.43) towards 14 day old tadpoles of *Xenopus levis* [Fischer, R., Science, *118*, 409 (1953)].

IMPROVED DETECTION OF LIPOPROTEINS IN HUMAN SERA. Hugh J. McDonald and Edward R. Marbach, Department of Biochemistry, Stritch School of Medicine and the Graduate School, Loyola University, Chicago, Ill.

In the study of lipides and lipoproteins by the method of ionography in paper-stabilized media, the chief difficulties encountered are in the detection of the zones on the ionogram, in the adsorption of the lipide or lipoprotein on the paper and in the retention of the lipide stain by the paper itself. The various techniques for detecting lipoproteins which are mentioned in the literature were tested for sensitivity and practicality, and an improved procedure was developed for lipoproteins in blood serum. The conditions for separating the lipoprotein fractions were as follows: 0.002 ml. of blood serum was applied to the Eaton-Dikeman No. 613, filter paper strip, 8mm. in width, and allowed to migrate for 3 hours, under a potential gradient of 8 volts per cm. A veronal buffer at room temperature, at a pH of 8.6 and an ionic strength of 0.05 was used.

The staining procedure was as follows: 100 ml. of a 75% solution of ethanol was boiled with 0.1 gram of Sudan Black B for 3 minutes. The solution was cooled to room temperature and filtered repeatedly using a hard close-textured filter paper (Whatman No. 42). The solution was then kept at 40° C. in a constant temperature bath. The paper strips were stained in this solution for 20 minutes and rinsed in 50% acetone for 2 minutes at room temperature. The rinsing procedure was repeated, usually three to four times, until the paper background was a very light blue, in contrast to the dark blue areas which corresponded to the lipoproteins. The dark blue zones on the ionograms could be changed to a dark reddish color by placing the developed paper strip in a dilute solution of nitrous acid.

STUDIES OF SERUM LIPOPROTEINS AND PLASMA LIPIDE CLEARANCE IN MULTIPLE MYELOMA. I.F. Greenblatt, R. Wayne, D.M. Spain, and I. Snapper, Messinger Research Laboratories, Beth-El Hospital, Brooklyn 12, N.Y.

Eleven human subjects with multiple myeloma were studied as to their ability to clear their plasma of a 70 to 100 gram fat meal in the form of sweet cream. To measure their lipide transport following an overnight fast, blood was drawn for lipoprotein, cholesterol, and opacity of the plasma. A 70 to 100 gram fat meal was ingested and bloods were drawn every hour for the following five hours. No supplementary feeding was permitted during this period.

Because multiple myeloma subjects frequently have very low serum cholesterol levels for their age (often below 125 mg. %), the authors were interested in the relationship of the disease with the presence of atherosclerosis as determined by the serum studies.

In six of the patients, the degree of lipemia was extremely small when compared with a group of similar age and no evidence of disease. The lipoproteins in this group were also very low as measured by ultracentrifugation.

SERUM LIPOPROTEIN STUDIES IN ATHEROSCLEROSIS. S.P. Gottfried, R.H. Pope, N.H. Friedman, and S. DiMauro, Biochemistry, Pathology, and Medical Departments of Bridgeport Hospital, Bridgeport, Conn.

A simple method has been devised for the quantitative estimation of serum lipoproteins. This consists of streaking filter paper with 0.04 ml. serum and running for 24 hours in a paper electrophoresis apparatus at a pH of 8.55 and an ionic strength of 0.125. The pattern is visualized by staining with Sudan III, obtaining the absorbance readings by means of a densitometer, plotting these readings against the distance migrated from the point of application, and measuring the area under the respective curve by means of a planimeter.

Thus far a total of 33 determinations have been performed on normal males and females 20 to 55 years of age. The α- and β- lipoproteins have not been found to vary with age and sex. Seven atherosclerotics have been studied for periods up to six months. The studies were initiated as soon after an immobilizing myocardial infarction as possible. The studies were then continued at weekly intervals while the patient was still immobilized, and at monthly intervals when he had once more become ambulatory. In all cases, it has been observed that the β- lipoproteins were elevated soon after the heart attack, but tended to decrease as the patient became ambulatory, reaching normal levels. The α- lipoproteins revealed no distinctive trend.

In addition, total cholesterol and total lipide determinations were performed by chemical means, and attempts were made to correlate them with the serum lipoproteins. These have been unsuccessful.

DETERMINATION OF CHOLINE CONTAINING PHOSPHOLIPIDES IN BIOLOGICAL MATERIAL. Betty B. Levy, *Harold D. Appleton*, Bernard B. Brodie, and J. Murray Steele, New York University, Research Service, Goldwater Memorial Hospital, New York, N.Y., and Laboratory of Chemical Pharmacology, National Heart Institute, Bethesda, Md.

A procedure for the determination of free choline in plasma [J. Biol. Chem., *205*, 803 (1953)] has been extended to include the determination of the individual choline containing phospholipides in plasma and tissue homogenates.

The material containing phospholipides is extraxted and deproteinized by treatment with alcohol-ether and further purified by reextraction with petroleum ether. Separation of the two major choline containing phospholipides is achieved by taking advantage of the different rates of alkaline hydrolysis. Easily hodrolysable phospholipide (lecithin) is completely hydrolysed in 1N potassium hydroxide at 37° C. in 18 hours, while the difficulty hydrolysable phospholipide (sphingomyelin) remains unchanged and is separated from the lecithin hydrolysate by precipitation with acid-acetone. The precipitated sphingomyelin is then hydrolysed by 20% potassium hydroxide at 100° C. for 4 hours. The choline freed from each phospholipide fraction is determined by treating the hydrolysates according to the procedure for free choline. The quantity of the individual phospholipides is calculated from the choline value on a molar basis.

The specificity of the choline assay in the hydrolysates was determined by counter current distribution.

MUCOPROTEIN DETERMINATION IN BLOOD AND URINE. Joel R. Stern, Molly Lillien, and B.M. Kagan, Kundstadter Laboratories for Pediatric Research, Michael Reese Hospital, Chicago 16, Ill.

Investigations were conducted regarding the determination of mucoprotein in urine, and its semimicro determinatinn in blood serum, both in man and rat. Attempts to determine urinary mucoprotein by precipitation with 0.58M sodium chloride were unsuccessful. With some modifications, the method of Winzler for blood serum was found to be satisfactory. This involved removal of other proteins with perchloric acid, precipitation, solution, and reprecipation of mucoprotein with phosphotungstic acid, and its estimation on the basis of tyrosine. The effect of various concentrations of perchloric acid on mucoprotein concentration was studied. Mucoprotein was found in urine of rat and man at levels ranging from 2 to 8 mg. of mucoprotein tyrosine per 100 ml. of urine. Results of semimicro determination of mucoprotein in serum checked well with the macro method. By this method, mucoprotein in serum of normal rats ranged from 3.5 to 6.8 mg. of mucoprotein tyrosine per 100 ml.

A SPECIFIC COLORIMETRIC TEST FOR GLUCOSE USING GLUCOSE DEHYDROGENASE. Jacques M. Kelly and Raymond B. Poet, Squibb Institute for Medical Research, New Brunswick, N.J.

In many experimental situations a simple and rapid, specific test to establish the presence of D-glucose is desirable. The reagents commonly used which respond to the reducing properties of D-glucose suffer from lack of specificity, especially in complex biological media.

Glucose dehydrogenase, an enzyme of wide occurrence, is specific in catalyzing the aerobic oxidation of β-D-glucose to gluconic acid and hydrogen peroxide. The enzyme mediates the transfer of hydrogen from glucose to oxygen and is operative in the presence of hydrogen acceptors other than oxygen, e.g., 2, 6-dichlorophenolindophenol and methylene blue. The use of the enzyme in specific quantitative methods for glucose has previously been described wherein either oxygen uptake or gluconic acid formation is measured.

In order to retain the desirable specificity of the enzyme and obviate the need for specialized techniques or long incubation periods a semiquantitative colorimetric test using glucose dehydrogenase has been developed. The reagent consists of a mixture of a commercial preparation of the enzyme (containing some catalase), buffer salts, and a suitable dye which can act as a hydrogen acceptor in the system. For a qualitative demonstration, a portion of the dry reagent is added to an aqueos solution of the unknown and allowed to stand a few minutes at about 40° C. When glucose is present, the reduction of the dye will be indicated by a color change. The reagent can be made semiquantitative by changing the relative amounts of enzyme and the pH of the buffer salts in the mixture while holding the volume of test solution constant. Thus, the sensitivity of the reagent for glucose is varied and positive response can be related to concentration.

The details of the method are outlined and its application in biological and clinical chemistry is discussed.

THE APPLICATION OF THE CHROMOTROPIC-SULFURIC ACID REAGENT TO POLYSACCHARIDES. Bernard Klein and Milton Weissman, Laboratory Service, Veterans Administration Hospital, Bronx, N.Y.

In an earlier paper it was demonstrated that 5-hydroxymethyl-2-furaldehyde in 15M sulfuric acid loses formaldehyde, which forms a color with chromotropic acid. This reaction was used to distinguish hexoses and hexose disaccharides in the presence of pentoses. The prediction was made that hexose polysaccharides and other hexose derivatives capable of forming these intermediates with 15M sulfuric acid would also react satisfactorily with the color reagent. This prediction has been put to test and confirmed. Thus, glucose-1-phosphate, glucose-1, 6-diphosphate, glycogen, raffinose, salicin, trehalose, inulin, dextrin, and starch all react positively with the chromotropic-sulfuric acid reagent. On the other hand, glucosamine, dulcitol, adonitol, sorbitol, inositol, and mannitol, which would not be expected to react, do not. Dextran and the mucopolysaccharide from hog mucin gave equivocal results.

SEPARATION OF α-KETO ACID DINITROPHENYLHYDRAZONES BY PAPER ELECTROPHORESIS AND THEIR COLORIMETRIC DETERMINATION. Henry Tauber, Venereal Disease Experimental Laboratory, U.S. Public Health Service, School of Public Health, University of North Carolina, Chapel Hill, N.C.

A method is presented for the separation of the 2, 4-dinitrophenyl hydrazones of α-

ketoglutaric acid and pyruvic acid by electrophoresis on paper. The separated bands are extracted with sodium hydroxide and their color intensities are determined colorimetrically. Commercially obtainable apparatus is employed. The method has been applied to the analysis of protein-free blood filtrates. A study of the blood α-ketoglutaric acid and pyruvic acid content of various mammals is presented.

IMPROVED SPECIFICITY OF CREATINE DETERMINATIONS IN URINE BY THE JAFFE REACTION. Hertha H. Taussky, Russell Sage Institute of Pathology and The Department of Medicine, Cornell University Medical College and The New York Hospital, New York, N.Y.

In 1934 Linneweh and Linneweh pointed out the presence in urine of undetermined substances other than creatine which give a color in the Jaffe reaction when urine is heated with acid to convert creatine to creatinine. This nonspecific interference could be eliminated by prolonged extraction (10 to 30 hours) with either in which neither creatine nor creatinine are soluble. Their observation suggested an additional step in the recently described micro-method for the determination of creatine and creatinine in urine [J. Biol. Chem., *208*, 853 (1954)].

In brief, equal amounts of diluted urine acidified with picric acid (1) for the determination of preformed creatinine and (2) after conversion of creatine to creatinine in the water bath, are extracted with ether by shaking for 1 minute in small glass-stoppered bottles. Aliquots of the aqueous phase are then pipetted directly into colorimeter tubes for the Jaffe reaction. Recovery experiments of creatine and creatinine are from 95 to 105%. There is little reduction in preformed creatinine with this procedure, but appreciably lower creatine values are found in most urine specimens. These lower values are attributed to the elimination by the ether extraction of nonspecific interfering substances.

ON THE COLORIMETRIC DETERMINATION OF UREA AND DIACETYL MONOXIME. Harold L. Rosenthal, Clinical and Pathological Laboratories, Rochester General Hospital, Rochester, N.Y.

The condensation of urea with diacetyl monoxime and oxidation by arsenic acid (Kawerau) has been extensively studied in an effort to improve the reproducibility and linear response of the reaction. The concentration of mineral acid and aresenic was found to be criticdl. With the modified procedure aqueos solutions and blood filtrates containing 10 to 100 γ of urea yield a straight line when measured in a Klett colorimeter with No. 47 filter. The line does not go through the origin. The reaction is performed in 10-ml. volume as follows:

To an aqueos solution containing urea add 0.8 ml. of saturated arsenic acid in concentrated hydrochloric acid, 2.2 ml. of concentrated hydrochloric acid, and 1 ml. of 5% diacetyl monoxime in 5% acetic acid. Dilute to 10 ml. Cap tubes and place in boiling water bath for 30 minutes. Cool, dilute to 20 ml., determine absorbance, and compare with a standard solution of urea. Recovery studies of urea added to blood and urine ranged between 100 and 103% with standard deviation of 3.3 to 4.9. This method will detect 5 γ of urea with an error less than 25%. The procedure is adaptable to the determination of 1.0 to 10 γ by microanalytical procedures.

USE OF 1,2-DICHLOROETHANE IN THE LIEBERMANN-BURCHARD REACTION FOR THE DETERMINATION OF CHOLESTEROL. Augusta B. McCoord, Department of Pediatrics, The University of Rochester School of Medicine and Dentistry, Rochester, N.Y.

Chloroform may be replaced by 1,2-dichloroethane (Eastman Organic Chemical No. 132) in the Liebermann-Burchard reaction for the determination of cholesterol. The cholesterol was dissolved in 5 ml. of either chloroform or 1,2-dichloroethane, and 1 ml. of acetic anhydride followed by 0.1 ml. of concentrated sulfuric acid added, and the mixture shaken. The reaction was carried out at room temperature. The amount of green color produced, which depends on the amount of cholesterol present, was determined by a Bausch and Lomb monochromatic photoelectric colorimeter.

When 1,2-dichloroethane was used as the solvent, the green color tended to develop more rapidly than when chloroform was the solvent and was maximum in about 4 minutes. The green color was of the same intensity and was equally stable in both solvents. The use of 1,2-dichloroethane in place of chloroform considerably shortens the time involved in carrying out a cholesterol determination. Some methods based on the Liebermann-Burchard reaction for the determination of cholesterol require up to 45 minutes for the development of the maximum green color.

Addition of a small amount of hydroquinone to the reaction mixture increased the intensity of the green color. The cost of 1,2-dichloroethane is about half that of chloroform.

A NEW METHOD FOR THE DETERMINATION OF SULFHYDRYL LEVELS AND THEIR VARIATION IN THE BLOOD OF RATS. Priscilla Teitelbaum, Institute of Applied Biology, Brooklyn, N.Y., and M. Bier, Fordham University, New York, N.Y., and Institute of Applied Biology, Brooklyn, N.Y.

A new method for the estimation of sulfhydryl groups in biological fluids was developed. The method is based on the sulfhydryl catalyzed oxidation of sodium azide by iodine. The liberated nitrogen is determined in a Warburg apparatus. The evolution of nitrogen is virtually completed within 13 minutes, and 0.05 ml. of blood, or 1 ml. of a 3 x 10^{-4}M sulfhydryl containing solution are amply sufficient for a determination. The reagents are 1 ml. of 0.2M sodium azide and 1 ml. of 0.1M iodine-potassium iodide solution.

The advantages of this method are its rapidity and the small volumes required. By repeated bleeding, serial determinations can be carried out on small laboratory animals. The shortcoming of the method is that the reaction is not stoichiometric, and the nitrogen liberated varies with different sulfhydryl compounds.

Consistent and reproducible results were obtained on a large number of rats. The sulfhydryl level of blood increased significantly with the age of the animal, female rats having, on the average, higher levels than males. The sulfhydryl content decreased in pregnancy, as well as on repeated bleeding of the animal. Other factors affecting the sulfhydryl levels were investigated, and the rate of adsorption into the blood-stream of various sulfhydryl-containing compounds, injected intraperitoneally, was determined.

MICRODETERMINATION OF CHROMIUM IN VARIOUS BIOLOGICAL MEDIA. Charles H. Grogan, *H.J. Cahnmann*, and Elizabeth Lethco, Environmental Cancer Section, National Cancer Institute, Bethesda, Md.

It has been demonstrated in recent years that certain metals, among them chromium, present a cancer hazard. Studies of certain aspects of chromium metabolism were therefore undertaken. One of the prerequisites for such a study was a rapid and sensitive method for the microdetermination of chromium in a variety of biological media. Such a method, permitting the assay of 10 to 60 samples per person per day with good precision and accuracy has been developed.

The method described consists essentially of the basic steps: a wet or dry ashing of the sample; hypobromite oxidation of chromium (III) to chromium (VI); and spectrophotometric determination of chromium (VI) as the pink compound that it forms with 1,5-diphenylcarbohydrazide. By means of this method the microdetermination of chromium in buffer solutions, protein solutions, plasma, sera, paper strips, and urine is performed routinely with recoveries of 95% or better. With liquid samples ranging from 10 μ l. to 10 ml., or filter paper strips from 1 to 100 sq. cm., containing from 0.1 to 5 γ of chromium, the standard deviations for the method fall for all the biological media listed within the range of 0.02 to 0.06 γ of chromium.

Increasing emphasis is being placed on trace metals in biological processes. Although no demonstrable biological function of chromium is known, trace amounts of this metal are widespread in plant and

animal tissues, foods, and water. The procedures outlined for the microdetermination of chromium proved to be of great value in the authors' biological investigations of chromium and should be useful to other investigators in this field.

USE OF 2-(O-HYDROXYPHENYL)-BENZIMIDAZOLE AS A SPECTROPHOTOMETRIC METHOD FOR DETERMINING IRON(III). M. Ann Wahl and B.H. Armbrecht, Georgetown University Medical Center, Washington 7, D.C.

In recent reports, Walter and Frieser [Anal. Chem., *25*, 126 (1953); *Ibid.*, *26*, 217 (1954)] employed 2-(2-pyridyl)-benzimidazole (I), 2-(2-pyridyl)-imidazoline (II), and mentioned 2 o-hydroxyphenyl)-benzimidazole (III) as reagents for the spectrophotometric determination of iron. The authors have prepared III by their method and the method of Hubner and Menschning [Ann., *210*, 345 (881)]. Both products, purified by sublimation, are identical by infrared and ultra-violet analysis and data are presented to substantiate the postulated structure.

The iron chelate was prepared from iron (III) nitrate dodecahydrate and compound III. On analysis this material best fitted the formula $C_{26}H_{19}N_5O_6$ Fe. Its spectra showed maxima at 500 μ, log E 4.43; and 349 μ, log E 5.02. Beer's law is followed over a 1 to 12 γ range with optimum pH at 5.5 + 1.0. Applications of compound III to iron determination in body fluids are discussed.

THE IRON CONTENT OF TISSUES IN ENDOGENOUS AND EXOGENOUS HEMOCHROMATOSIS. Bernard Klein, Benjamin S. Gordon, Irving Graef, Renzo Olivetti, and Walter Newman, Veterans Administration Hospital, Bronx, N.Y.

Hemochromatosis is a disease marked pathologically, among other findings, by the appearance of multiple lesions with iron pigment deposits and fibrosis of the liver, pancreas, and myocardium, endocrine glands, etc. The disease occurs in three forms: (a) the endogenous or idiopathic type, presumably resulting from a derangement of the iron absorption or transport mechanism, (b) the exogenous type, presumably resulting from multiple blood transfusions, and (c) the dietary type.

A study of the iron content of tissues of types a and b was undertaken to ascertain differences in the amounts deposited in order to differentiate between the two forms. None was found. Similarly the iron content of tissues from several cases of refractory anemias, also treated with multiple transfusions but which did not develop the fibrosis seen in hemochromatosis, overlapped the values found in the hemochromatotic states.

The tissues were prepared for analysis by a combined azeotropic distillation of water and fat extraction in preference to the usual oven drying method. An apparatus was designed to permit in one operation the simultaneous drying and extraction of up to six tissue specimens representing a complete autopsy. This drying procedure produced specimens which could be more easily handled, pulverized, and weighed than the usual oven dried specimens. The analyses performed after this method of drying and extraction, reported on a dry, fat-free basis were more reproducible and precise than the usual procedures.

SPECTROPHOTOMETRIC DETERMINATION OF TOTAL HEMOGLOBIN IN PLASMA. Keith B. McCall, Division of Laboratories, Michigan Department of Health, Lansing, Mich.

A method has been developed for the spectrophotometric determination of total hemoglobin in irridiated liquid plasma containing 5% glucose as a stabilizer. Hemoglobin is converted to methemoglobin by the use of ferricyanide in a buffered solution (pH 6.6) and the absorbance is determined at a specific wave length (540 to 550 μ range). Hemoglobin concentration is proportional to the change in absorbance at this wave length through the conversion of all methemoglobin to cyanmethemoglobin.

Primary calibration was based upon the oxygen capacity (manometric) method of Van Slyke but methods based on iron content are suitable. The absorbancy indices for methemoglobin and cyanmethemoglobin were evaluated using whole blood in water and in plasma in the concentration range of 10 to 100 mg. % hemoglobin in the final solutions in a 1-cm. cell.

The specific advantages include the use of stable reagents, the production of stable colors and the elimination of interference in accuracy or precision by a varied color which is inherent in all blood plasma.

SPECTROPHOTOMETRIC TITRATION OF VERSENE IN URINE USING ARSENIOUS ACID AS THE TITRANT. Bernie Zak, Paul J. Cherney, and Eleanor G. White, Department of Pathology, Wayne University College of Medicine and Detroit Receiving Hospital, Detroit, Mich.

The use of Versene in the various fields of medicine and the fact that it is excreted only via the kidney combine to make an accurate determination of Versene in urine of considerable value.

The reduction of chromate by arsenious acid in the presence of Versene results in the formation of a stable purple chelate of chromium Versenate. Titration with a standard arsenious acid solution using a Coleman Jr. spectrophotometer and ordinary cuvettes for the spectrophotometric measurement of the constantly increasing absorbance enables one to accurately analyze for Versene in small amounts. The use of pretreatment urine as a blank is obviated, since each sample titrated is used as its own blank, an important consideration since urines are variable.

The effect and possible interference of the more common urinary anions on the reaction were studied. Under the conditions of the analysis and in amounts greatly exceeding those found in normal urine, nitrate, phosphate, chloride, and sulfate showed no appreciable effect on the accuracy of the determination.

Graphs of corrected absorbance vs. milliliters of titrant show steep slopes with almost perpendicular intersections making interpretation of results simple.

INVITE MANUSCRIPTS

CLINICAL CHEMISTRY, the new publication of the AACC will begin publication in January, 1955. Papers on original research in clinical chemistry and related subjects are invited to be submitted for consideration by the new Board of Editors. The publication will use the same address as *The Clinical Chemist.*

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

Preparation of the manuscripts should follow the specifications outlined in "Instructions to Authors" published on page 59 of this issue.

MEETING-IN-MINIATURE ACS NEW YORK SECTION

The Metropolitan-Long Island Sub-Section of the New York Section of the American Chemical Society announces a Meeting-In-Miniature to be held in Brooklyn on Friday, February 25, 1955. Papers are invited in the fields of clinical, analytical, organic, physical, inorganic, polymer and industrial and engineering chemistry. There will also be sections devoted to biochemistry and chemical education. A two hundred word abstract (original plus carbon) should be submitted by December 27, 1954, to Professor Joseph Steigman, Department of Chemistry, Brooklyn Polytechnic Institute, 85 Livingston Street, Brooklyn 1, N.Y.

LOCAL SECTION NEWS

BOSTON SECTION

The Boston Section held its first meeting of the current season on October 27 at the New England Center Hospital.

The speaker of the evening was Dr. J.T.R. Nickerson, Professor of Food Processing at the Massachusetts Institute of Technology. His topic, "Chemical and Bacteriological Changes in Food Products," dealt with the changes in flavor, texture and color which can occur.

Fats are subject to several causes of breakdown the speaker asserted. Hydrolysis may occur by lipase present, or by certain bacteria. Such hydrolysis may not however produce off-flavor unless shorter-chain fatty acids are present, such as butyric, capric or caprylic acids. In the case of butter, prevention of such breakdown may be accomplished by maintaining suitable acidity and by the addition of salt.

Molds, by the mechanism of beta oxidation of the fatty acids, may cause ketone rancidity in dairy products. This, however, in the case of certain cheeses (Roquefort) is not always undesirable.

Radiant energy may also be responsible for fat breakdown, hence brown bottles are often used in packaging.

Foods containing protein and carbohydrates are also subject to characteristic alterations, Dr. Nickerson said. Condensation between amino groups and carbonyl groups cause yellowing. Retardation of this reaction, when feasible, is accomplished by removal of water to below 2%.

Following the speaker, the annual election of officers was held, the following being elected:

Joseph Annino (Massachusetts Memoeiral Hospital), Chairman
Eli Dubinsky (New England Center Hospital), Vice-chairman
Esther Thomas (Boston Medical Laboratory), Secretary-Treasurer

WASHINGTON-BALTIMORE-RICHMOND SECTION

The last meeting of the 1953-54 year for the Washington-Baltimore-Richmond section was held June 10 at George Washington University School of Medicine. The program consisted of four discussions on clinical chemical techniques. They were:

"Use of Anthrone in Determination of Carbohydrates" by Joseph H. Roe.

"Modified Method for Determination of Lead" by Sam Bessman and E.C. Layne, Jr.

"Determination of Serum Proteins" by Dan Sanchuk.

"Determination of Serum Copper with bis-cyclohexanone oxalyldihydrazone" by R.E. Peterson and M.E. Bollier.

The officers elected for the 1954-55 year are the following:

Chairman: Martin Rubin
Secretary-Treasurer: Marion Webster
Counselor for the three-year term: Miriam Reiner
Counselor for the two-year term: Henry Wishinsky

PHILADELPHIA SECTION

The Philadelphia Section of the American Association of Clinical Chemists, held their first meeting of the current year on October 26, 1954. The new officers, Ellenmae Viergiver – President; Harry B. Lockhead – Vice-President; and O. C. Beckord – Secretary-Treasurer were introduced.

After an informal dinner in honor of the speaker, Dr. Harry B. Lockhead, the group moved to the University of Pennsylvania Hospital to hear a discussion on: Techniques of Hemoglobin Measurement. Dr. Lockhead presented various methods for the measurement of hemoglobin and several techniques for the preparation of a standard curve. Commonly encountered errors and mistakes were briefly mentioned.

SOUTHERN CALIFORNIA SECTION

The first scientific section of the new season, held October 5, 1954 at the Los Angeles County Hospital, was addressed by Frank W. Cranz, Sales Engineer and Elwood Blondfield, Sales Training Supervisor, Beckman Instruments, Inc. Mr. Cranz previewed the new Beckman Direct Reading Flame Photometer, an instrument that has been designed for the specific purpose of performing simple and rapid sodium and potassium determinations. Mr. Blondfield described the new Beckman Model C Colorimeter. Short author abstracts of these addresses will appear elsewhere in this issue.

Previously, on September 7 at the Carolina Pines Restaurant, Los Angeles, a dinner and business meeting had opened the season.

Abstract of a talk describing "The Beckman Model C Colorimeter" given by Elwood Blondfield, Sales Training Supervisor of Beckman Instruments, Inc., before the local chapter of the American Association of Clinical Chemists, on October 5, 1954 at the Los Angeles County Hospital.

The Beckman Colorimeter was designed to develop speed of operation with greater accuracy.

The sample tube opens and closes the shutter automatically, thus allowing a "zero" check between each measurement without the manipulation of any control. If the needle ever drifts from zero, the operator simply turns one knob to reposition the needle. There are only two steps to a measurement. Put the reference tube in the holder and turn a knob until the needle reads 100%. The needle movement is instantaneous so there is no time wasted waiting for the reading to settle down. Next put the sample tube in and read the meter.

A new optical system focuses the light beam on the center of standard test tube, thus minimizing reflected and stray light. Good accuracy may be obtained with ordinary unselected tubes. The low-wattage light source does not heat the sample and lasts in operation well over 2000 hours. One inch round or square filters up to one-half inch thick are placed between the sample and vacuum phototube to eliminate most of the stray light.

The test tube holder is designed with elastic grommets which automatically center the test tube. It also has a rubber pad on the bottom to protect the test tubes from breakage. If a test tube should accidentally break, the holder may be easily removed. A hole in the bottom allows drainage of spilled solution.

The entire optical system is housed in a single casting to maintain optical rigidity. It is thermally insulated from other instrument components.

A simple sturdy electronic amplifier with built in voltage regulation provides superior no-noise performance.

continued on page 72

NEW APPARATUS

AUTOMATIC-RECORDING TOTALIZING DENSITOMETER

A component distribution curve and an accompanying saw-tooth totalizing curve are produced simultaneously in the new Spinco Analytrol as a variable density strip — such as a paper electrophoresis pattern — is automatically drawn through the scanning mechanism. Alternatively, the automatic totalizing curve can be produced from any existing distribution curve by manual following of the original curve.

Auxiliary mechanisms are available to permit operation with ultraviolet rather than visible light and to adapt for the colorimetric recording of stationary or moving fluids in tubes. Accessories are under development for the production of similar traces derived from strips containing patterns with various concentrations of radioactive materials.

Functionally, the Analytrol is a null or unbalanced device utilizing a system of two barrier-layer photocells illuminated from a single light source. The material to be scanned is passed before one of the cells, causing an output current drop and unbalancing a bridge circuit. The unbalanced signal is amplified and used to drive a motor which interposes a light-shielding cam in front of the other photocell until balance is restored. This same mechanism drives the curve-drawing and integration pens.

Because of this light-shielding cam principle, various mathematical functions can be introduced to accommodate for differences in response between the optical density and the actual concentration of material. For example, in paper electrophoresis analysis, a special cam is available to coordinate with a bromphenol blue dyeing technique. This can be changed to accommodate the use of other dyes.

Other features of the photocell-balance principle are that line voltage variations and aging of the light source or amplifier components are cancelled out and uniform accuracy is maintained under varying conditions.

Maximum width of the curve-drawing paper-feed is 12 inches. Maximum width of the scanned strip is 2 inches. Maximum height of the finished distribution curve is 6½ inches. The integration curve is drawn in such a way that every tenth pip in the saw-tooth configuration is extended for convenience in counting and tabulation. The complete instrument has dimensions of 16 5/8 by 15 1/2 by 10 7/8 inches; weighs a total of 50 pounds. Operation is from a standard 115-v 50/60 cps source drawing 125 watts. Specialized Instruments Corporation, 682 O'Neill Avenue, Belmont, California.

ROTO-CELL, THOMAS

(Accessory for Bausch & Lomb "Spectronic 20" Colorimeter)

Arthur H. Thomas Company, Philadelphia, Pa., has developed a liquid-cooled double cell carrier for use with the new B. & L. "Spectronic 20" Colorimeter to make it suitable for rapid spectrophotometric scanning at controlled temperatures.

The Roto-Cell is readily interchangeable with the single-place sample holder regularly supplied with the instrument and provides for instantaneous interchange *within the instrument* of a 1 ml sample with the blank or standard into the light path, thereby greatly facilitating preparation of spectral transmission or absorption curves.

Consists of a swivel-action, water-jacketed holder, with lightproofing collar, slotted carrier for two 1 ml cells, each with 10 mm light path, and control knob with stops for positioning either cell for completely blocking the light beam for checking dark current adjustment without removing the cells. The holder is provided with suitable inlet and outlet tubulatures for attachment to a properly controlled, external constant cooling system to maintain the cells at optimum working temperature. A partitioned cell of Corex glass has been specially designed for use with this carrier. Descriptive Bulletin 121 sent upon request.

SHAKING INCUBATOR

Precision Scientific Company has published "Data Sheet #11482," describing the redesigned Precision-Dubnoff Metabolic Shaking Incubator. The illustrated folder describes how the chamber has been enlarged for more rapid cooling, and includes a table showing evaporation rates.

Essential information about capacity, temperature range, accuracy, shaking speed and power requirements is presented in concise form. Improvements of the instrument are described in detail. A free copy of "Data Sheet #11482" will be mailed on request. Precision Scientific Company, 3737 W. Cortland Street, Chicago 47, Illinois.

BOOKS TO BE REVIEWED

The following books have been received from various publishers and are of interest to clinical chemists. A critical review of these publications will appear in the Book Review Section of CLINICAL CHEMISTRY.

Practical Clinical Biochemistry, by Harold Varley vi + 551 pages. Interscience Publishers, Inc., 250 Fifth Avenue, New York 1, N.Y. $6.50

CLINICAL CHEMISTRY IN PRACTICAL MEDICINE, by C. P. Stewart and D. M. Dunlop, 4th Edition, vi + 320 pages, The Williams and Wilkins Co. Baltimore 2, Md. $5.00.

A PRACTICAL MANUAL OF MEDICAL AND BIOLOGICAL STAINING TECHNIQUES, by Edward Gurr, xix + 320 pages, Interscience Publishers, Inc., 250 Fifth Avenue, New York 1, $4.00.

STATISTICAL ANALYSIS IN CHEMISTRY AND CHEMICAL INDUSTRY, by Carl A. Bennett and Norman L. Franklin, xvi + 724 pages, John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, $8.00.

CHEMOTHERAPY OF INFECTIONS, by H.O.J. Collier, xvi + 248 pages, John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, $4.00.

LEGAL MEDICINE PATHOLOGY AND TOXICOLOGY, by Thomas A. Gonzales, Morgan Vance, Milton Helpern, and Charles J. Umberger, 2nd Ed. xii + 1349 pages, Appleton-Century-Crofts, Inc., New York, $22.00

HUMAN BIOCHEMISTRY, by Israel S. Kleiner, 4th Ed. 746 pages, The C. V. Mosby Co., St. Louis, Mo., $7.50.

LABORATORY INSTRUCTION IN BIOCHEMISTRY, by Israel S. Kleiner and Louis B. Dotti, 4th Ed. 285 pages, The C.V. Mosby Co., St. Louis, Mo., $3.50.

SO. CALIFORNIA SECTION *(cont'd)*

The lamp is not subjected to line voltage or frequency variations.

The combined advantages of current regulation to the light source, voltage regulation and a superior optical system give the clinical technician a new accuracy which has never been previously achieved by instruments of this type.

Abstract of a talk describing "The New Beckman Direct Reading Flame Photometer", given by Frank W. Cranz, Sales Engineer of Beckman Instruments, Inc., before the local chapter of the American Association of Clinical Chemists, on October 5, 1954 at the Los Angeles County Hospital.

The new Beckman Flame Photometer was designed for the rapid determination of sodium and potassium. The instrument is a completely self-contained direct reading instrument calibrated directly in milliequivalents of sodium and potassium on separate scales. The calibration covers a range of 0 to 2.8 milliequivalents per liter of sodium and 0 to .14 milliequivalents per liter of potassium. The meter readings are multiplied by the dilution factor to obtain the concentration of sodium and potassium in the serum or other sample. The scales are linear and may be easily calibrated by a single external standard supplied with the instrument.

The instrument uses compressed air from a laboratory supply line, portable compressor, or tank, and natural gas or propane as fuel. A small pilot flame allows for instantaneous operation by moving a single lever. An external compressor, if used, is also controlled by this same lever. A single stage air regulator and filter are built in, providing freedom from interferences caused by air pressure variations and air contamination.

Samples are atomized directly from small beakers through an inexpensive, replaceable stainless steel atomizer. The nebulizer provides a dry sample spray so that the entire sample system remains free from contamination for long periods of operation. The entire burner unit can be easily dismantled for cleaning when necessary by removing one screw. There are a minimum of controls, all of which are on the front panel for ease of operation. A single lever moves the separate calibration controls for sodium and potassium, as well as the correct filters into position so that the wrong controls cannot be used during a determination. A stable industrial amplifier with vacuum phototube is used to provide the utmost in stability and accuracy.

Approximately fifty samples may be run per hour, and no rinsing with distilled water between samples is required. Analysis for sodium and potassium may be easily made on less than two drops of serum diluted 100:1. Sample consumption is approximately four milliliters per minute. Sensitivity of the determinations is .02 milliequivalents per liter for sodium and .002 milliequivalents per liter for potassium. Reproducibility is .01 milliequivalents per liter of sodium and .001 milliequivalents per liter of potassium. The operation of this instrument is so simple that inexperienced personnel can be taught to operate it in a few minutes. Simplicity, speed of operation, and accuracy are the prime features of this instrument.

INSTRUCTIONS TO AUTHORS "CLINICAL CHEMISTRY"

1. Except for invited review articles, submission of a manuscript to the Board of Editors is with the authors' assurance that no similar paper, other than an abstract or preliminary report has been published by the author.

2. Manuscripts should be typed with triple spacing and the original plus the first carbon copy should be submitted. Only one copy of drawn figures should be submitted. These should be attached to the original copy. All errors in typing should be corrected, and the spelling of all proper names, correctness of analytical data, values presented in tables, mathematical calculations, all references, etc., should be carefully verified by the author. Variation from standard nomenclature and all abbreviations should be explained in the text. For chemical terms, the usage of the American Chemical Society, as published in the indexes of *Chemical Abstracts* should be used. Style of the manuscript should conform to that used by other scientific journals. Separate pages should be used for title page, references, footnotes, legends for figures, tables and other inserts and should follow the text.

3. The title page should carry the title of the paper, authorship, and the name of the institution or laboratory. The latter should contain enough information for use as the author's address.

4. The title of the paper should give a clear indication of the subject matter and should be concise. Chemical symbols may be used in the title only to indicate isotopically labelled compounds. A short running title should be provided (38 characters and spaces).

5. The manuscript should be organized as to provide a clear and concise presentation of the subject matter. Approximate location of the figures and tables should be indicated in the text. New methodology should be presented in entirety. Reference to published procedures, unless extensively modified, should be referred to by citation in the reference section.

6. Organization of charts and tables should be such as to require a minimum of discussion in the text. Discussion should be limited to the significance of the data presented. Unsupported hypotheses should be avoided. The paper should be concluded with a brief summary in which the essential results of the research are outlined.

7. The references should follow the text and should conform to the following example:

(5) Doe, J., Bruce, J., and Doe, H., J. Biol. Chem. *23*, 847 (1950)

The abbreviated name of the reference journal should conform to the abbreviations used in *Chemical Abstracts*.

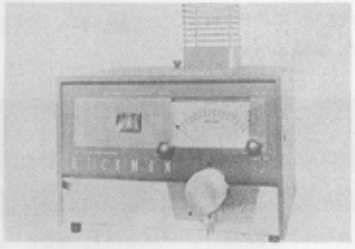
In references to books, the author's name, full title, publisher, edition, page and year of publication should be cited in the order given.

RESPONSIBILITY FOR THE ACCURACY OF THE REFERENCE LIST IS THE AUTHOR'S.

8. Tables and Illustrations. Reference is made to "Instructions to Authors", paragraphs 6-7 *The Journal of Biological Chemistry*, for detailed instructions on the preparation of tables and illustrations.

9. Authors are responsible for reading of galley and page proof. The cost of changes, other than correction of printer's errors, will be charged to the author.

10. The total number of reprints must be ordered when the galley proofs are returned to the publisher.



QUID NUNCS

William Gruen, specialist in instrumentation, was elected president of Instrumentation Associates, an organization devoted to the introduction and distribution of new apparatus for laboratory, medical and industrial organizations.

William Gruen will continue his present association with the J. Beeber Company.

NEW!

Coleman Flame Photometer

measures Sodium, Potassium AND CALCIUM directly, easily and accurately

New and Exclusive Atomizer-Burner . . . Burns common illuminating gas with oxygen for a stable high-temperature flame. No explosive tank gas required. Safe in the most inexperienced hands.

Simple Direct-Coupled Optics . . . No slits to adjust . . . no mirrors or prisms to align. Optical filters provide spectral isolation.

Direct Reading . . . Without internal standards. High temperature flame gives full excitation . . . eliminates need for chemical additives such as lithium.

Easy to Use . . . Safe in any laboratory. The Coleman Flame Photometer is as safe and easy to use as a Bunsen burner. Flame will not blow out or flash-back, and pressure adjustments are not critical.

Lowest Cost . . . Highest Value . . . The Coleman Flame Photometer is ready to operate with any modern Coleman Photometer . . . Junior Spectrophotometer, Universal Spectrophotometer, Electric Colorimeter, Photo-Nephelometer, or Nepho-Colorimeter .

For users who prefer to conduct flame analysis independent of other procedures or who do not have available one of the above Coleman instruments, a separate indicating instrument . . . the Coleman Galv-O-Meter is offered.

Cat. No. 91280 — Flame Photometer, ea. $450.00

Cat. No. 91282 — Galv-O-Meter, ea. . . $150.00

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