

The CLINICAL

Chemist

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THE CLINICAL Chemist

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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 believably 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 publish-

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.

ers 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

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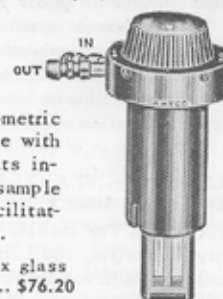
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ABSTRACTS OF PAPERS PRESENTED AT THE SCIENTIFIC SESSIONS OF THE 1954 ANNUAL MEETING

*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 a decrease 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 diphosphoglycerate. 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 carbonylation 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 μ per ml. In sulfuric acid alone the limit of detection of the alkaloid fluorimetrically is of the order of 0.1 μ 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 μ 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 alkalization 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 alkalization. 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, cysteine being a weak catalyst with cysteic acid, methionine, other amino acids, and reducing reagents such as sodium hydro-sulfite and ascorbic acid possessing no significant catalytic activity; nonamino acid mercaptans are catalytically active demonstrating that free sulphydryl 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, melt-

ing 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 PSYCHOSES. 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 μ 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 $\times 10^{-2}$ 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 $\times 10^{-2}$ 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 adrenergic 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 en-

countered are in the detection of the zones on the ionogram, is 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.

