Formation of Midwest Section.

VOL. 6
NO. 1

February 1954

The CLINICAL Chemist

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Official Publication
of
AMERICAN ASSOCIATION
of
CLINICAL CHEMISTS,
INC.

Election of National Officers Ballots Due Before – May 10, 1954 –

The CLINICAL Chemist

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

VOL. 6, NO. 1 FEBRUARY 1954

THE SECRETARY REPORTS

The 1954 Directory of the American Association of Clinical Chemists is being distributed with this issue of THE CLINICAL CHEMIST. Every effort was made to keep the listings as accurate as possible, but with the many changes of address that occur during a year some errors may have been made. It would be appreciated if any such were called to our attention for correction. Also there may be listings of home addresses for individuals who would prefer their institution addresses.

This directory for the first time includes a geographical listing by States as well as the year of admission of the member. The growth of the AACC may be seen from a compilation of the now active members by their year of affiliation.

1948	8
1949	129
1950	155
1951	69
1952	66
1953	87
1954	8

Excepting 1948, when only the founders are included, and the first fifteen days of 1954, one may readily see a steady growth that followed the first two years of large enrollment. Our loss from deaths and resignations has thus far averaged under 3 per cent per year. One may also note a concentration of members in areas where local sections exist.

Scientists are notorious for their promptness in failing to return mail ballots in the election of their officers. The AACC has had an unusually good "batting average" thus far, and it is sincerely hoped that the 1954 response will be as encouraging as the past ones. Please mark and return your ballot as soon as convenient.

Max M. Friedman National Secretary

ERNST BISCHOFF AWARD

The 1954 Ernst Bischoff Award of the American Association of Clinical Chemists is scheduled for the annual meetings in September, 1954. This Award consists of a scroll, medal, and five hundred dollars presented by the Ernst Bischoff Company of Ivoryton, Conn. and under the auspices of the Association.

The selection of a recipient is made from recommendations of the membership to the Award Committee, and the final choice determined by the Honorary Members.

The Award Committee consists of Otto Schales, chairman, Fritz Bischoff and Joseph H. Roe. Members are invited to submit their recommendations to Dr. Otto Schales, 3503 Prytania Street, New Orleans 15, La. and must be received by April 20, 1954. The summaries should be as complete as possible, and in triplicate. By vote of the Award Committee three candidates are selected and submitted to the Honorary Members. The latter then select the Award recipient.

SIXTH ANNUAL MEETING NEW YORK CITY SEPTEMBER, 1954

The Sixth Annual Meeting of the AACC will be held in New York City during the week of September 12. The meetings will be held during the 126th National Meeting of the American Chemical Society. Scientific sessions will be sponsored jointly with the Division of Biological Chemistry.

Members are urged to plan for this meeting both for attendance and preparation of scientific material. The program will be announced in the CHEMICAL and ENGINEERING NEWS and in the August issue of THE CLINICAL CHEMIST.

ELECTION OF NATIONAL OFFICERS

The Nominating Committee consisting of Joseph Benotti, Clarence Cohn, Samuel Natelson, Miriam Reiner, John G. Reinhold, Harry Sobotka, and Warren M. Sperry met in New York and proposed the following slate of officers for the National Executive Committee to serve from July 1, 1954 to June 30, 1955:

President — Monroe E. Freeman
Vice-President — Otto Schales
National Secretary — Max M. Friedman
National Treasurer — Louis B. Dotti

Executive Committee

Robert M. Hill
Hugh J. McDonald
Marschelle H. Power
Miriam Reiner
Albert B. Sample

The procedure for elections is determined by Article XI of the constitution. All members in good standing as of January 15, 1954 are eligible to vote. A ballot is included in this issue of THE CLINICAL CHEMIST. The names of any members of the Association listed in the 1954 Directory may be substituted for any name on the ballot by using the blanks provided therefor. In addition, seven members of a Nominating Committee are to be selected from among the membership to serve from January 1, 1955 to December 31, 1955.

Ballots must be received at the National Secretary's office by May 10, 1954 to be counted.

BACK NUMBERS

The Editorial Committee has available a limited number of back issues of THE CLINICAL CHEMIST for the years 1952 and 1953, Vols 4 and 5. Members that do not have complete volumes for those years or are missing single issues can obtain them by sending a post card to the committee at **Box 123, Lenox Hill Station, New York 21, N.Y.** The card should state the date of election to membership in the AACC as preference will be given to those members that were elected during the year. The number of available copies are limited and they will be distributed according to date of request.

MOUNT SINAI HOSPITAL HONORS HARRY SOBOTKA

by Max M. Friedman

It was at the Mount Sinai Hospital in New York City that the American Association of Clinical Chemists was founded on December 15, 1948. This writer well remembers the courtesies extended to us at that time. The host at these first meetings was Harry Sobotka, and he has subsequently contributed in large measure to the Association in the many offices held by him.

On January 14, 1954 several members of the Association again had the pleasure of an invitation from the Mount Sinai Hospital, this time to attend a dinner in honor of Doctor Harry Sobotka upon the occasion of the completion of his 25th year of service to the Hospital. In the after-dinner talks of tribute to the honored guest, various authorities in the different medical disciplines, including John G. Reinhold for the clinical chemists, noted the importance and the influence of chemistry in present-day medical practice.

In honoring Harry Sobotka the Mount Sinai Hospital not only acknowledged a great debt to an individual but also to the science he represents. Twenty-five years ago, when in most hospitals clinical chemistry was largely in the domain of some resident pathologist, this Institution already recognized the role that chemistry was to play in clinical medicine by appointing to its staff a chemist with recognized talents and making available to him the tools and facilities of his specialty. Whenever the progress of clinical chemistry is to be evaluated, that quarter of a century span at the Mount Sinai Hospital must surely enter into the calculations.

Our cordial respects and best wishes go to Doctor Harry Sobotka, the recipient of this honor.

CHEMICAL EVALUATION OF THE FUNCTIONS OF THE LIVER

by

John G. Reinhold

William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pa.

PART II

(C) (3) Nitrogen Metabolism (Continued from Part I)
(D) Hormone Metabolism
(E) Disturbances of Water and Electrolyte Balance
(F) Detoxification Reactions
(G) Bile Pigment Metabolism

Cephalin-Cholesterol Flocculation.
(Hanger, 1938)

Principle.— Suitably diluted serum of patients suffering from liver disease forms a flocculent precipitate when treated with a suspension of cephalin and cholesterol in water.

Reagents.— Cephalin-cholesterol stock solution: The purchase of the reagent ("antigen") is recommended, (Difco and Wilson brands have been used by the writer). It is supplied in vials containing 100 mg. of cephalin, prepared from brain and aged, and 300 mg. of cholesterol. The contents of a vial are dissolved according to directions in ethyl ether of anesthesia quality, after addition of one drop of distilled water. The stock solution so obtained may be kept for several months if refrigerated and tightly stoppered.

Cephalin-Cholesterol reagent: Measure 32 ml. of water into an erlenmeyer flask of 50 ml. capacity with a mark scratched on it to indicate 27 ml. Place the flask in a water bath on an electric heater, and heat to 65 or 70°. While the water in the flask is kept at this temperature, add drop by drop 1 ml. of the stock cephalin-cholesterol solution, rotating the flask at the same time. Place the flask directly on the hot plate at low heat and boil gently to remove the ether until the volume is reduced to 27 ml. When cool, add 3 ml. of merthiolate solution, aqueous, 1:1000, as a preservative. This reagent retains its activity unchanged for at least a week if stored in a refrigerator.

Sodium chloride solution, 0.85 per cent. Reagent quality sodium chloride should be dissolved in distilled water of high purity.

Procedure.— It is advantageous to use conical centrifuge tubes of 15 ml. capacity. Measure into such a tube 4 ml. of sodium chloride solution. Add 0.2 ml. of serum and mix well by tapping the tube vigorously 5 times. Add 1 ml. of cephalin-cholesterol reagent and again mix by tapping the tube vigorously at least 15 times. Place the tube in a water bath at approximately 25°C. in the dark and away from chemical fumes until the following day. Examine the tube for presence of flocculation after 24 hours and again after 48 hours (optional).

Grade the reaction as one plus to four plus as described under thymol flocculation taking into account the amount of precipitate and the transparency of the supernatant. If desired, the precipitate can be removed by centrifugation and the supernatant decanted and its absorbency measured in a photometer at 660 mu. using a tube containing sodium chloride solution and reagent, but without serum, as a control (Kibrick, et al 1952). Saifer (1948) has described a quantitative method based on measurement of the cholesterol content of the flocculum.

Precautions.— Preparations of the cephalin-cholesterol reagent as purchased or prepared have been found to vary widely in their sensitivity, some being excessively sensitive and others insensitive. Each lot of reagent must be assayed by testing serum of healthy individuals and of a sampling of patients showing positive reactions. Acceptable reagents do not produce more than an occasional one plus flocculation when the sera of healthy subjects are tested, and on the other hand, yield a high proportion of positive flocculations in patients suffering from liver disease. Once a suitable reagent has been obtained, new lots can be tested by comparing them with previous ones. In the writer's laboratory tests are done in duplicate using two different preparations. Difficulties of standardizing and of controlling this test make it practical only when a considerable flow of samples through the laboratory may be expected.

Interpretation.— It is essential to know the sensitivity of the reagent used, since in different laboratories the serum of healthy individuals and of patients without liver involvement may vary from negative to two plus or even three plus. Using the criteria described in the preceding paragraph, reactions of two plus or greater offer evidence of disturbed liver function. Hanger believes that a positive test is an indication of active parenchymal disease, and in support of this may be cited the high proportion of patients suffering from acute viral hepatitis who exhibit positive cephalin cholesterol flocculation. Positive tests occur within a few days of onset of clinical illness, frequently before jaundice or significant elevation of bilirubin appears. Thus it offers valuable support for a diagnosis of viral hepatitis, particularly in hepatitis without jaundice. However, failure of the cephalin cholesterol test to become abnormal does not exclude the diagnosis. Neefe, Gambescia, Gardner and Knowlton (1950) found that 20 per cent of a large group of patients ill of viral hepatitis gave negative tests. Abnormal cephalin cholesterol flocculation readings may or may not persist into the stage of recovery. In this respect it differs from the thymol test which tends to remain positive for a considerable time.

A high proportion of positive tests is found in cirrhosis. Positive tests are prevalent in a variety of diseases such as malaria, pneumonia, infectious mononucleosis, and others causing damage to the liver. Biliary obstruction of short duration is characterized by negative cephalin cholesterol flocculations, and it may be used along with other observations to aid in determining whether jaundice is due to causes that may require surgical intervention. Inflammatory disease of the bile ducts usually is accompanied by positive tests.

The mechanism producing flocculation of the cephalin-cholesterol reagent appears to differ somewhat from that of the thymol test. Moore, Pierson, Hanger and Moore (1945) showed that the change in serum responsible was associated primarily with the albumin fraction. Addition of normal serum albumin to serum giving a positive test may alter the response to negative, hence care must be taken to avoid collection of serum for testing during the 12 to 24 hours after administration of albumin parenterally.

Abnormalities in blood clotting in liver disease.— These are another manifestation of the disturbances in protein metabolism although associated disturbances in lipid metabolism may be of equal importance. It is now known that the delayed clotting of blood of many patients with liver disease is the result of a combination of defects. Besides the diminished prothrombin that commonly is found in both acute and chronic liver disease, deficiency of accelerator globulin (Ac-globulin, Factor V, labile Factor, proaccelerin, etc.), and probably of other factors occurs. Alexander and Goldstein (1950) report not only a lowering of labile factor but of serum prothrombin conversion factor. Fibrinogen concentration may be low, as already mentioned. Fibrinolysin activity of plasma may be increased. Hepatectomy causes a decrease in fibrinogen, "thrombogen," and antifibrinolysin (Nolf and Adant, (1951) and in prothrombin, cothromboplastin, labile factor as well as fibrinogen (Mann, Shonzo, and Mann, 1951). The complexity of the disturbance of clotting function in liver di-

sease is apparent in recent reviews of this topic (Harrington, 1950), (Stefanini, 1953).

The depletion of prothrombin in biliary obstruction is related to impaired vitamin K absorption. This in turn is due to the failure of bile to reach the intestine in sufficient amounts to maintain absorption of fats and fat soluble vitamins. Perhaps a similar mechanism contributes to the lowered prothrombin activity in parenchymal liver disease. Vitamin K administered parenterally in large amounts usually has little effect on the lowered plasma prothrombin activity of patients suffering from parenchymal liver disease. On the other hand, it specifically corrects the defect in patients with biliary obstruction provided liver damage is not excessive.

Methods for evaluation of clotting function.— These include studies of plasma prothrombin activity, accelerator factor, fibrinolysin, and, at times, fibrinogen. Numerous techniques for prothrombin and fibrinogen measurements are described in the literature and their merits will be discussed in a review to appear in this journal.

Estimation of prothrombin activity is an patient with disease of biliary tract, liver, or small intestine — and to needle biopsy of the liver. It is used also to evaluate the response to vitamin K administered to jaundiced patients. A substantial rise in prothrombin activity to values that are within or approaching the normal range suggests that liver function is not greatly impaired: thus the jaundice may be presumed to originate from a lesion of the biliary tract (Shapiro and Richards, 1945). One of the important shortcomings of this method is the lack of precision of prothrombin measurements, as ordinarily measured, in the 30 to 100 per cent range.

Many patients with parenchymatous liver disease maintain prothrombin activities within normal limits throughout the course of their illness (according to Stefanini (1949), roughly half). This is true especially if liver involvement is of minimal or moderate severity. For this reason the measurement of prothrombin activity is not as efficient for detection of liver damage as are a number of the other procedures described in this review. However, an occasional patient will have a significantly lowered prothrombin activity due to liver disease when other simple tests, including the serum bilirubin concentration and flocculation and turbidity group, fail to give an abnormal response.

Impairment of energy production and storage.— Liver disease is associated with increased concentrations in blood and urine of the di- and tri-carboxyl acids that constitute pathways by which foods are converted into energy. Considerable attention has been given, by Scandinavian workers especially, to the elevation of plasma citrate in patients suffering from liver disease. These studies have been reviewed by Sjostrom (1947). Others have found that lactate (Snell and Roth, 1932), pyruvate (Amatuzio and Nesbitt, 1950), alpha ketoglutarate (Seligson, McCormick and Sborov, 1952) and succinate (Emmrich, 1948) concentrations also are elevated. These findings suggest the occurrence of a general increase in concentration of such substances. The occurrence of such an increase in turn suggests that the efficiency with which these transformations are being made is impaired in the presence of severe parenchymol liver disease. Thus, the supply of energy available to the cells is decreased. The effects on cellular metabolism would be widespread and important, and would be manifested by decreased rates of synthesis of the various products made by the liver cells as well as impairment of regulatory and other functions. Saltzman and Caraway (1953) have described a method for measurement of the efficiency of an oxidation, the conversion of cinnamic to benzoic acids, in vivo. They find a reduced rate in patients with liver disease.

Studies of certain components of the phosphate cycle have been made. Helve (1946) reports no change in major fractions of the blood cell phosphate. However, it is difficult to establish impairment in functioning of the phosphate cycle in vivo by analysis of concentrations because its efficiency is a function of the rate of turnover rather than of concentration. Smith, Ettinger and Seligson (1953) found that a decrease in serum inorganic phosphate following injection of glucose and fructose did not differ markedly in patients with liver disease from that in normal individuals, while in diabetes mellitus the decrease was less marked. The marked rise in serum creatine concentration and increase in creatine excretion previously described suggests that phosphocreatine is being hydrolyzed and presumably not rapidly reformed. As a result, stores of high energy phosphate are being depleted.

Waterlow (1953) has studied the enzyme activity of samples of liver removed by punch biopsy from infants suffering from liver disease of nutritional origin. His material included specimens from patients with Kwashiorkor or the closely related nutritional liver disease of Jamaica infants as well as samples obtained from control subjects. He found a wide variation in susceptibility of various respiratory enzymes to severe malnutrition and associated liver damage. His work, and that of others using animals, emphasizes the importance of low protein intake in causing depletion of certain enzymes.

Hormone metabolism.— Severe chronic liver disease is often accompanied by enlargement of the breasts in males and other evidences of altered sex hormone metabolism. A number of studies have been made recently of the excretion of various hormones in liver disease. Thus Dohan et al (1952) found a significant increase in estrogen excretion in cirrhotic male patients. This was especially marked if they showed signs of gynecomastia. Spider nevi were associated with increased estriol excretion. 17-Ketosteroid excretion was significantly decreased. A decrease in gonadotrophin (FSH) was associated with testicular atrophy.

The liver produces enzymes that oxidize testosterone and related steroids. Injected testosterone is inactivated less rapidly in hepatectomized dogs than in intact dogs (West, 1951). Even mild liver damage decreased the proportion of injected testosterones excreted as 17 ketosteroid by patients (West and associates, 1951). Cantarow and associates, (1951) found that the conjugation of injected testosterone was decreased in patients with liver disease. Adrenocorticotrophic hormone is inactivated by liver tissue (Eversole and Giere, 1951; Geschwind and Li, 1952).

Disturbances of water and electrolyte balance.— Retention, loss or maldistribution of electrolytes and the resulting disturbances of water metabolism are among the most important and troublesome encountered in patients with liver disease. These are caused in part by impairment of those functions of the liver related to water and salt metabolism and in part by the effects of liver disease on the function of the kidney and other organs regulating salt and water balance. Included among the contributions of the liver in this connection are synthesis of albumin and inactivation of hormones concerned with regulation of salt and water balance.

Salt and water retention is one of the characteristic complications of Laennec's cirrhosis. It is of complex etiology. Mechanical factors such as increased pressure in the portal venous system are important. Resulting alterations in renal hemodynamics impair the efficiency of renal function. The lowered serum albumin concentration, while favorable to glomerular filtration, also favors transudation from the capillaries and thus accumulation of water in the tissues. Hormonal factors also are known to be important. Shorr and associates have described a vasodepressor substance, ferritin (or VDM), whose concentration in the body fluids, they claim, is regulated in large measure by the liver. In addition to ferritin, it is possible that the antidiuretic hormone of the posterior pituitary contributes to the oliguria and hyposthenuria that precedes or accompanies salt and water retention. Still other factors may be involved. Plasma volume generally is increased in Laennec's cirrhosis (Bateman, Shorr, and Elquin, 1949).

Rigorous restriction of sodium intake appears to be the most effective method of preventing accumulation of salt and water in excessive amounts by the cirrhotic patient. The patient on such a regime, in turn, requires regular and frequent chemical study because of the threat of the salt-depletion syndrome. The hazard of the latter is greatest when large amounts of fluid, two to ten liters or more, are removed by paracentesis. If the patient subsequently dilutes the remaining extra-

cellular fluid by consumption of water, sodium concentrations may fall sufficiently to bring about the abnormalities in blood flow and blood supply to the tissues that characterize the low salt syndrome (Nelson, Rosenbaum, Strauss, 1951; Holly and McLester, 1950). Serum sodium concentrations as low as 110 m. Eq. per liter have been observed. Circulatory failure, in such circumstances may lead to coma and death.

Depletion of serum potassium and magnesium together with other electrolytes have been reported in some patients with severe cirrhosis and impending hepatic coma (Amatuzio, et al 1952). Elevation of serum potassium also must be guarded against, especially in those patients whose liver disease is accompanied by marked impairment of kidney function.

Serum iron in liver disease.– Serum iron concentrations are markedly affected by liver disease. In hepatitis, serum iron rises to two or three times the highest concentration ordinarily found in health. (Peterson, 1952; Matassarin and Delp, 1952). Low concentrations occur in serum of some cirrhotic patients (Howard, 1950). Serum iron determinations and especially determinations of serum iron binding capacity, are of special interest in hemochromatosis. In this condition, accumulations of exceptionally large amounts of iron in the body lead to its deposition in liver, skin, and other tissues. The architecture of the liver is distorted as in cirrhosis. Serum iron concentrations are elevated, although not necessarily to concentrations higher than those occurring in in health or in hepatitis. However, the measurement of iron binding capacity in hemochromatosis consistently shows that the serum is almost completely saturated with iron. Gitlow, Beyers and Colmore (1952) cite saturation values of 74 to 99 per cent as compared with 14 to 69 per cent in cirrhosis and 28 to 58 per cent in normal individuals. In hepatitis and all other conditions, at least a third of the total iron binding capacity remains unsaturated.

Methods for study of iron and iron-binding capacity.– Among several practical methods for serum iron are those of Barkan and Walker (1940) and of Kitzes, et al (1944). Iron-binding capacity can be determined by the method of Cartwright and Wintrobe (1949) or of Ventura (1952).

Copper and liver disease.– Interest in serum and urine copper recently has been stimulated by the finding that Wilson's disease (hepato-lenticular degeneration) is associated with abnormalities in metabolism of copper (Glazebrook, 1945; Denny-Brown and Porter, 1951). The copper content of brain, liver and urine is increased. After treatment with BAL (2.3 dimercaptopropanol) urine copper concentration decreases. Changes may occur in serum copper but these appear to be less consistent or dependable than are the changes in excretion of copper in the urine. Scheinberg and Gitlin (1952) have found lowered concentrations of caeruloplasmin in blood plasma of patients suffering from Wilson's disease. Caeruloplasmin is a specific copper containing protein found in blood plasma.

Detoxification reactions of the liver.– Numerous studies have demonstrated that in liver disease various reactions associated with detoxification are impaired. The best known example is the synthesis of hippuric acid following administration of benzoic acid, extensively studied by Quick (1940). Whereas at least 70 per cent of injected benzoic acid is excreted as hippuric acid within one hour by healthy individuals, less than this amount is excreted by patients with liver damage. Quick believes that the factor limiting hippuric acid synthesis in liver disease is inability to mobilize glycine, however this has been disputed (Voight, 1951). The conjugation of benzoic acid with glycine has been demonstrated in liver homogenates by Borsook and Dubnoff (1947). It occurs also in kidney, spleen and probably other tissues.

Deiss and Cohen (1950) have described a similar test in which para-aminobenzoate replaces benzoate, and the conjugation is evaluated by analysis of serum. The para-aminohippurate formed is estimated colorimetrically in a sample of blood collected one hour after the test dose.

Conjugation of various substances with glucuronic acid also is impaired in liver disease (Wagreich et al, 1941; Ottenberg et al, 1943). Snapper and Saltzman (1949) have described a test of liver function that measures glucuronate conjugate excretion after administering sodium cinnamate. Its application to the study of liver disease is evaluated by Sharnoff, et al (1951). Saltzman and Caraway (1953) have described a refinement of the cinnamic acid test in which blood cinnamic acid levels are measured.

Deamination of tyramine, a pressor amine, occurs in the liver (Hare, 1928) and it is thought that other substances of this type are similarly inactivated.

Methods for the study of detoxification function: Measurement of hippuric acid excretion is most frequently used for this purpose, however, availability of more simple procedures for study of liver disease appears to have decreased the use of the hippuric acid test. The technique of Quick (1940) may be used and intravenous administration of hippuric acid is preferred. The conditions under which the test is done are important. The patient should ingest sufficient water to insure adequate urine flow (Machella, Helm, and Chornock, 1942). He must be physically and mentally relaxed. Persky and associates (1950) found the output of hippuric acid to be increased by anxiety. A similar effect was described by Deiss and Musser (1950) with respect to para aminohippurate excretion. Hippuric acid is readily excreted by the kidney but marked impairment of kidney function lowers the output. Body weight and sex should be taken into account in interpretation of results (Hepler and Girley, 1942).

The para aminohippurate test of Deiss and Cohen (1950) has the great advantage of avoiding the necessity for urine collections, a major source of difficulty in the hippuric acid test. Trial of this procedure is desirable. The same may be said of the new cinnamic acid test of Saltzman and Caraway (1953).

Serum Alkaline Phosphatase.– Measurement of alkaline phosphatase activity of serum often assists in the differentiation of parenchymal liver disease from that due to obstruction and other lesions of the biliary tract. Biliary obstruction is characterized by an increase in phosphatase activity to two or more times the maximum found in healthy individuals (Roberts, 1933), and by the persistence of such high activities. Some increase occurs also in patients suffering from viral hepatitis, however, the rise is moderate and transitory. Toxic hepatitis caused by chemicals or drugs is usually accompanied by elevated phosphatase activities. In cirrhosis of the Laennec type no marked rise in alkaline phosphatase activity occurs ordinarily, but at times, high activities may be encountered.

The rise in phosphatase associated with obstruction of the bile ducts occurs regardless of the nature of the obstruction, whether due to calculus, stricture, or neoplasm. Elevation of serum alkaline phosphatase may provide one of the few clues to the presence of neoplastic growth in the liver (Rothman et al, 1936; Mendelsohn, 1952). Inflammatory disease of the bile ducts (cholangiolitic hepatitis) causes striking increases in serum alkaline phosphatase. The chemical changes occurring in this condition are indistinguishable from those observed in many patients with extra-hepatic biliary obstruction.

The cause of the increase in serum alkaline phosphatase activity occurring in patients suffering from biliary obstruction has provoked much discussion. The liver appears to be able to remove phosphatase from blood plasma and excrete it in the bile. However, hepatectomized animals show, at most, a moderate rise in phosphatase activity of serum (Freeman, 1951; Flock et al, 1952). This suggests that failure of the liver to excrete phosphatase produced in other organs is responsible only to a limited extent. Histochemical studies show high concentrations of phosphatase in the cholangiolar epithelium (Burke, 1950) in cholangiolitic hepatitis. Sherlock and Walshe (1947) found increased amounts in the hepatic cells and nuclei in hepatitis and in bile duct obstruction. The amount in the sinusoidal walls also was found increased. Thus it appears possible that the excess phosphatase in plasma

may have several sources. The predominant source may be the cholangiolar epithelium in biliary tract disease and the liver parenchyma in hepatitis. Rosenthal and associates (1952) have shown that regenerating liver parenchyma is very rich in phosphatase.

Applications of phosphatase activity measurements include not only the detection of involvement of the biliary tract but also the evaluation of the clinical course of patients with such lesions. Ulevitch et al (1951) have pointed out that phosphatase activity and serum bilirubin may change independently and that phosphatase activity is the more sensitive indicator of change in degree of biliary obstruction.

Bile pigment metabolism.— Jaundice is such a conspicuous sign of liver damage or bile duct blockage that it has attracted more than its share of attention, often to the neglect of other and more important aspects of liver or biliary tract disease. A number of excellent reviews of bile pigment chemistry and metabolism have appeared within the last few years (Watson, 1946; Lemberg and Legge, 1949; Gray, 1953). Aschoff, Whipple and Mann demonstrated that bilirubin is formed from hemoglobin by the reticulo-endothelial cells throughout the body. The bilirubin is transported to the liver and excreted in the bile. Certain peculiarities in the behavior of bilirubin suggest that it may exist in serum in two forms. These are differentiated by means of their rates of reaction with diazotized sulfanilic acid (Hijmans van den Bergh and Mueller, 1917). A rapidly reacting form of bilirubin, (direct or prompt reacting bilirubin) is considered to have been secreted into the bile by the parenchymal cells and subsequently to have returned to the blood stream because of disruption of the liver structure by parenchymal disease or because of biliary obstruction. This form of bilirubin (cholebilirubin) is distinguished from a second (hemobilirubin) considered to be in transport from extrahepatic sites of hemoglobin breakdown to the liver. The latter reacts with the diazo reagent slowly and yields additional color after alcohol, caffeine, or other catalysts are added to the reaction mixture.

The cause of the difference in behavior remains unexplained despite numerous efforts to find it. All of the bilirubin in serum is bound to protein, but it is possible that a portion is more firmly bound. Plasma protein fractionation by Cohn and associates (1950) has yielded an alpha globulin which, it is stated, binds bilirubin firmly and specifically. Martin (1949) has described its characteristics, among them a delaying action on the diazo reaction of bilirubin bound by it. Attempts to establish a structural change in the bilirubin molecule that might provide a basis for the observed difference have led to negative or equivocal results. However, Najjar (1951) recently described the preparation from serum of two bilirubins differing in crystal form and solubility and in their reactivity with the diazo reagent. Other factors influencing the rate of reaction include the concentration of bilirubin and its degree of dispersion. There is reason to believe that the serum lipid may be involved. For a review of the various hypotheses concerning the diazo reaction of serum bilirubin the reader is referred to Gray (1953) Chapter VIII.*

Regardless of the cause, distinct differences in the rate of the diazo reaction of serum bilirubin occur. These are demonstrated more successfully by means of the one minute reading technique introduced by Ducci and Watson (1945) than by older methods. Sera obtained from patients ill with hemolytic anemias or pernicious anemia when tested with the diazo reagent produce relatively little color in one minute compared to that produced after addition of methanol. This is true also after administration of aged or incompatible blood, and of the serum of newborn. On the other hand, approximately 50 per cent of the total color appears in one minute when the sera of patients with biliary obstruction or moderate to severe parenchymal damage are tested.

Bilirubin is present in the urine of healthy individuals in concentrations so low that it is not detected by ordinary methods. Positive tests for bilirubin in urine thus indicate the existence of liver damage or biliary obstruction. They occur, at times, before serum bilirubin is elevated. It is for this reason that urine bilirubin tests are useful for the detection of acute viral hepatitis in its early stages (Neefe et al, 1944; Swift et al, 1950). Later in this disease, urine bilirubin tests may become negative while the disease is still active and serum bilirubin concentrations remain above normal limits. The means by which bilirubin passes the renal barrier is far from clear, since it is firmly bound to protein in serum and non diffusible or only slightly so. Some may enter the urine combined with protein which is a fairly regular constituent of urine of patients suffering from hepatitis. The remainder must be split from protein by a renal mechanism still unknown.

After the presence of liver disease has been established and if serum bilirubin concentrations are being measured urine bilirubin tests offer little useful information.

Method for measurement of the concentrations of direct reading and total bilirubin in serum.— Ducci and Watson (1945) modification of the method of Malloy and Evelyn (1936).

Principle.— Bilirubin treated with diazotized sulfanilic acid forms "azobilirubin".** The density of the red color of the latter is proportional to the concentration of bilirubin. The rate at which this reaction occurs has clinical significance and it is measured by relating the reading made one minute after adding reagents to the reading made at final equilibrium after addition of a catalyst.

Reagents.— Methanol: This should conform to ACS specifications for reagent methanol. Occasionally methanol contains impurities that form color with the diazo reagent. Other impurities may inhibit color development. Questionable methanol should be redistilled. New lots of methanol should be tested by carrying out the procedure but omitting serum.

Dilute hydrochloric acid: 15 ml. concentrated HCl are diluted to 1 liter with water.

Sodium nitrite: 0.5 g. pure sodium nitrite is dissolved in 100 ml. water. This solution should be renewed at least once a week. Sodium nitrite deteriorates on storage, and supplies should be renewed after 6 to 12 months. Discolored preparations must be rejected.

Sulfanilic acid: Place 1.0 g. of sulfanilic acid in a mixing cylinder of 1 liter capacity. Add 15 ml. of concentrated HCl and dilute to 1 liter with water. Mix until dissolved. It keeps indefinitely.

Diazo reagent: Prepare just before use by adding 0.3 ml. of sodium nitrite solution to 10 ml. of sulfanilic acid solution. Mix.

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*Cole and Lathe (1953) have succeeded in separating serum bilirubin into two components by means of reverse phase chromatography using silicone-treated kieselguhr as adsorbent. A rapidly moving type is more soluble in water and gives the direct diazo reaction (without addition of alcohol). The other is more soluble in organic solvents and it reacts only in presence of alcohol. The latter is thought to be bilirubin. The nature of the fast moving component is not established, but it predominates in serum in obstructive jaundice. By contrast in hemolytic jaundice the slower component predominates. No protein was present in the extracts of serum studied, hence they conclude that differing degrees of association of bilirubin with protein cannot be responsible for the differences in behavior of various serums with the diazo reagent. The presence of several pigments in bile, some of which do not react with the diazo reagent, was demonstrated.

**Pyrroles couple with diazotized sulfanilic acid if they have free alpha or beta positions or substituted groups that are readily displaced, such as carboxyl. (The alpha carbon is that adjacent to the N in the ring). It is believed that bilirubin is split into two dipyrryl compounds, neoxanthobilirubic acid and isoneoxanthobilirubic acid, by the diazo reagent. Each of these has a free alpha position which permits coupling to occur. The term azo-bilirubin is loosely used to describe the mixture of azo-pigments produced. For further information consult Lemberg and Legge (1949) or Gray (1953).

Procedure.– Measure into a test tube (A) 1 ml. of fresh serum and 9 ml. of water. (If bilirubin is high, use 0.5 ml. and 9.5 ml. of water). Mix. Transfer 5 ml. to a second test tube, (B).

Direct "one minute" reaction.– Add 1 ml. of the dilute HCl to tube A. Add 1 ml. of diazo reagent to tube B. Mix at once. Using (A) for zero setting, read (B) in one minute exactly. The wavelength used is 540 mu.

Total bilirubin.– Add 6 ml. of methanol rapidly to each of the two tubes. Mix vigorously at once. Read in 20 minutes again using tube (A) for the zero setting. When new reagents are used verify the reading at 25 minutes to make certain that color development in maximal. If it is not, make additional readings. Make a reading of the reference standard.

Calculation.– Direct bilirubin = $U \times \frac{C}{2S}$

(or $U \times 2 \times \frac{C}{2S}$, depending on the dilution of the serum).

Total bilirubin = $U \times \frac{C}{S}$ (or $U \times 2 \times \frac{C}{S}$, depending on the dilution of the serum).

Where U is the maximal absorbency of the serum, S the absorbency of the stable reference standard and C the bilirubin equivalent of the latter. Report as Direct (one minute) and Total bilirubin.

Standardization.– Bilirubin is unstable in solution and standard solutions may deteriorate appreciably within a few hours. The bilirubin solution is used to calibrate an artificial standard. One or more dilutions of such a standard are calibrated by means of the following procedure:

Weigh 25 mg. of bilirubin (That sold by Armour Laboratories is satisfactory). Transfer to a 250 ml. volumetric flask with the aid of 2 ml. of 0.2 N. NaOH. Dilute to 250 ml. with water. One ml. of the resulting solution contains 0.1 mg. of bilirubin. Addition of 50 mg. of ascorbic acid may delay but does not prevent deterioration. This solution must be freshly prepared and used immediately. Protect it from the light. Solutions in chloroform may be used but offer no advantage, whereas impurities in the chloroform may accelerate the decomposition of bilirubin.

Dilute 5 ml. of the above solution to 50 ml. and measure the following amounts of bilirubin solution and water into two sets of tubes (a) and (b) of about 20 ml. capacity to obtain the bilirubin equivalents shown.

Tube	Bilirubin sol. in ml.	Water ml.	Bilirubin content in mg.	Concentration mg./100 ml. serum
1	0.2	4.8	0.002	0.4
2	0.5	4.5	0.005	1.0
3	1.0	4.0	0.010	2.0
4	2.0	3.0	0.020	4.0
5	3.0	2.0	0.030	6.0
6	5.0	0.0	0.050	10.0

Add to each tube of the (a) set, 1 ml. of dilute HCl and to the other (b) set, 1 ml. of diazo reagent. Mix and add 6 ml. of methanol to each. Measure the absorbancy of each tube in 20 minutes and repeat the readings at intervals until maximal values are obtained. Use the (a) series for zero settings. Several bilirubin solutions should be prepared and tested.

Plot the absorbancies on the ordinate of rectilinear graph paper against bilirubin concentration on the abscissa. Beer's law is obeyed. Readings may be converted to bilirubin concentrations from the graph. As an alternative method of calculation, the ratio of C/S may be calculated for each standard and the average ratio used to multiply the absorbancy of the unknown. Using an Evelyn colorimeter the mean of C/S has been found to be 14.3.

Permanent standards.–A solution of methyl red indicator in acetate buffer has been found by King and Coxon (1950) to reproduce closely the spectral absorption curve given by bilirubin treated with diazo reagent. It is prepared by dissolving 0.145 g. of pure methyl red (ortho carboxy benzine azodimethylalanine) in 50 ml. of glacial acetic acid. 1 ml. of this stock solution is transferred to a volumetric flask of 1000 ml. capacity. 5 ml. of glacial acetic acid and about 500 ml. of water are added followed by 14.4 g. of crystalline sodium acetate (or 8.6 g. of anhydrous). After the acetate has dissolved, the volume is made to 1000 ml. with water. This solution represents 7.0 mg. of bilirubin per 100 ml. in the method described, however, it is desirable that this figure be verified in each laboratory by means of the calibration technique described above.

Interpretation.–Total serum bilirubin of 95 per cent of healthy individuals is below 1.10 mg. per 100 ml. of serum. Occasionally individuals who appear to be in good health will have concentrations as high as 1.50 mg./100 ml.

The direct reacting, "one minute" bilirubin reading estimates bilirubin reacting with the Ehrlich diazo reagent promptly in the absence of ethanol, methanol or other catalyst. This fraction represents 10 to 20 per cent of the total bilirubin of normal serum. In 95 per cent of healthy individuals it does not exceed 0.18 mg./100 ml. and in 99 per cent is below 0.25 mg./100 ml. In the presence of hyperbilirubinemia due to any cause other than increased hemolysis, the one minute direct reading increases to approximately 50% of the total, and ranges from 30 to 70%. The direct bilirubin may be elevated in serum of some patients whose serum total bilirubin is within normal limits and it is somewhat superior to the latter for detection of liver involvement. When hemolysis occurs with severity sufficient to cause hyperbilirubinemia, the total serum bilirubin consistently is elevated to a greater degree than is the prompt reacting portion. However, the behavior of the serum bilirubin in jaundice caused by biliary obstruction does not differ from that occurring in jaundice caused by injury to the hepatic parenchymal cells, at least not to an extent that enables conclusions to be reached in a given patient regarding the etiology of the jaundice. Measurement of the rate of the diazo reaction of bilirubin adds little of value for differential diagnosis because hemolytic jaundice, the one condition it distinguishes, generally can be recognized by other more specific findings. The significance of serum bilirubin determinations with special reference to the one minute reading has been discussed by Zieve et al (1951).

Studies of total serum bilirubin are useful for following the clinical course of a patient. Rising serum bilirubin concentrations in general have unfavorable implications; falling values are characteristic of remission of liver disease or biliary obstruction. A stabilized serum bilirubin concentration is considered a highly desirable prerequisite to operation for relief of biliary obstruction. ACTH and cortisone given to patients with increased serum bilirubin cause a marked decrease in bilirubin concentration of serum.

Tests for bilirubin in urine.–Many of the procedures widely used for detection of bilirubin in urine are not sufficiently sensitive to be useful for detection of early hepatitis or moderate liver damage. Foord and Baisinger (1940) compared many of the commonly used procedures and found that the Harrison-Fouchet method as described by Godfried (1934) was one of the most dependable, and the experience of many workers has confirmed the value of this procedure. Several modifications of this method in which the reagents are applied to paper strips (Watson and Hawkinson, 1946) or to tablets of plaster of Paris have proven to be convenient although

less sensitive than the original Harrison-Fouchet method. The latter concentrates the bilirubin in urine by adsorption on freshly precipitated barium phosphate and sulfate. The blue-green color produced by treating the precipitate with acid ferric chloride is quite specific for bilirubin and closely related pigments. Urobilinogen or urobilin do not react.

Other methods for testing urine for bilirubin include the methylene blue test which depends upon the extinction by additional methylene blue of the green color produced by interaction of bilirubin and methylene blue (Reinhold and Fowler, 1947). This test is easily applied, but it measures also other yellow pigments that may be present. It gives a roughly quantitative estimate of bilirubin concentration.

Recently, Free and Free (1952) described a rapid and sensitive diazo test for bilirubin which appears to compare favorably with the Harrison-Fouchet method and which may prove to be superior, (Klatskin and Bungards, 1953; Sobotka and others, 1953; Giordano and Boyle, 1953).

The Harrison-Fouchet Spot Test
(Godfried, 1934)

Reagents.—Fouchet's reagent: Dissolve 25 g. of trichloracetic acid in 100 ml. of water. Add 10 ml. of a 10 per cent solution of ferric chloride.

Barium chloride solution. — 10 g. of $BaCl_2 2H_2O$ in 100 ml. of water.

Procedure.—Measure approximately 10 ml. of urine into a test tube. Add 5 ml. of the barium chloride solution. Mix by tapping. Filter through a retentive paper. Unfold the filter containing the precipitate and place it on a dry filter paper. Add 1 or 2 drops of Fouchet's reagent. The appearance of a green or blue color indicates the presence of bilirubin.

Para-nitrobenzenediazonium Para-toluene Sulfonate ("Bilazo") Test
(Free and Free, 1952)

Principle.—Bilirubin couples with the bilazo reagent to form a blue color. An asbestos mat adsorbs and concentrates the bilirubin.

Procedure.—Place 5 drops of urine on a test mat of cellulose-asbestos. The reagent is placed on the spot in the form of a tablet and moistened with two drops of water. If bilirubin is present in an amount exceeding 0.04 mg. per 100 ml. a blue color appears and is read at 30 seconds.

Reagent.—The bilazo reagent tablets and asbestos pads are obtainable from the Ames Co., Inc., Elkhart, Indiana.

Urobilinogen in urine and feces.—Urobilinogen is normally present in urine. It is formed by reduction of bilirubin by bacterial action in the cecum and colon. Urobilinogen is reabsorbed from the intestine and excreted mainly by way of the bile. Impaired excretory ability of the liver leads to an increase in its output in the urine. Thus, measurement of urobilinogen concentration in urine is a test of liver function. However, when the liver is damaged to such an extent that the secretion of bile is suppressed, no urobilinogen will be formed or detected. It is absent also if obstruction of the bile ducts prevents entry of bile into the intestine. Patients receiving (a) certain diets not conducive to maintenance of the usual bacterial flora, (b) antibiotics, or (c) other substances altering the bacterial flora of the intestine may excrete little or no urobilinogen in their feces or urine.

Urobilin is formed by oxidation of urobilinogen. Whereas urobilinogen is colorless, urobilin is yellow. Urobilinogen is converted to urobilin on standing in the presence of oxygen, or by oxidizing agents. This change is delayed by protecting urine from light and air and by alkaline reactions. Urobilin may be reconverted to urobilinogen by treating the former with ferrous sulfate and sodium hydroxide.

The presence of increased amounts of urobilinogen in urine is a characteristic finding in patients with disease of the parenchymal cells of the liver. At times it may offer the only conclusive evidence of the existence of liver disease. Changes in urine urobilinogen excretion are likely to occur independently of changes in other tests, and for this reason it is desirable to include it in a battery of such tests.

Two procedures are described for estimation of the urobilinogen excretion in urine. One is the simplified quantitative Ehrlich reaction of Watson, Schwartz, Sborov and Bertie (1944), as modified by Watson and Hawkinson (1947). This method is designed for use as a routine or screening test and for most purposes is capable of supplying the information sought. However, it may be necessary to apply in special circumstances a method yielding more specific data, and for this reason the quantitative method of Schwartz, Sborov and Watson (1944) is also included.

Simplified Quantitative Urine Ehrlich Reaction
(Watson and Hawkinson, 1947)

This method may be applied to random samples of freshly collected urine. The results are more informative when the urine is collected during a timed two hour period. The rate of excretion of urobilinogen in urine varies during the day, and has been found highest between 2 to 4 P.M. Therefore, collections at this time are preferred to random collections or timed collections at other short periods. Although this method may be used for analysis of 24 hour urine collections its authors recommend that the extraction method described below be substituted.

Reagents.—Ehrlich's aldehyde reagent.—Dissolve 0.7 g. paradimethylaminobenzaldehyde in diluted hydrochloric acid. The diluted hydrochloric acid is prepared by adding 150 ml. concentrated hydrochloric acid to 100 ml. of water.

Saturated sodium acetate solution.—Add 100 ml. of water to 150 g. of sodium acetate, anhydrous, reagent quality. Warm gently, cool to room temperature before use. An alternate method is to allow the solution to stand over night. Decant the supernatant.

Procedure.—Freshly voided urine can be analyzed without further treatment. Collections made over longer periods must be treated with ferrous sulfate and sodium hydroxide (as described in the extraction method) to reconvert urobilin to urobilinogen. Twenty-four hour collections are analyzed by the extraction method. Urine specimens must be protected from sun or other sources of intense light. Brown bottles for collection of specimens are recommended.

Transfer 2.5 ml. of urine or filtrate from ferrous hydroxide treatment into each of two photocolorimeter tubes U and B. To tube U add 2.5 ml. of Ehrlich's reagent. Mix well for 15 seconds, then add 5 ml. of sodium acetate solution and mix again.

To tube B add 5 ml. of sodium acetate solution, mix thoroughly, then add 2.5 ml. of Ehrlich's reagent slowly and with constant shaking. This corrects for the color of urine and reagents. Place tube B in the photocolorimeter and adjust the zero. Then with minimum delay measure the absorption of tube U. Readings are made at 565 mu.

Calculation.— $\frac{(U) \times 4 \times \text{urine volume}}{100}$ = Ehrlich units in the sample.

(U) represents the urobilinogen equivalent of the reading as measured from a graph in which the transmittancies of the 10 standard solutions, described in the following table, are plotted against the concentration of urobilinogen represented by each. The results are reported as Ehrlich units. One Ehrlich unit corresponds to 1 mg. of urobilinogen. This designation is preferred because chromogens other than urobilinogen contribute to the color.

Permanent standards.—Urobilinogen is not easily obtained in pure form and furthermore has poor stability. The mixtures of pontacyl carmine and violet described below closely simulate the urobilinogen aldehyde compound.

Stock standard dye solution.—Dissolve 5 mg. pontacyl carmine 2B and 95 mg. pontacyl violet 6R 150%, in 100 ml. of 0.5 per cent acetic acid. This solution keeps indefinitely if preserved with a few drops of chloroform.

Dilute standard.—20.4 ml. of stock solution are diluted to 100 ml. with 0.5 per cent acetic acid solution. This solution corresponds to 0.6 mg. of urobilinogen per 100 ml.

The volume of dilute standard indicated in column 1 of the following table, diluted with the solume of 0.5 per cent acetic acid indicated in column 2, yields a color which is equivalent to the urobilinogen values shown in column 3.

ml. of dilute standard	ml. of 0.5% acetic acid	Urobilinogen in Ehrlich units or mg./100 ml.
20.0	0.0	0.60
16.7	3.3	0.50
13.3	6.7	0.40
10.0	10.0	0.30
8.4	11.6	0.25
6.6	13.4	0.20
5.0	15.0	0.15
3.3	16.7	0.10
1.7	18.3	0.05
0.85	19.15	0.025

Interpretation.—The urine of 95 per cent of a group of healthy individuals was found to contain less than 0.95 Ehrlich units in a 2 hour specimen collected between 2 and 4 P.M. The concentration in a random specimen should not exceed 1.15 Ehrlich units per 100 ml. Values exceeding these limits have been associated with impairment of liver function.

Quantitative Determination of Urobilinogen by the Extraction Method

(Schwartz, Sborov and Watson, 1944)

This method is preferred for measurement of 24 hour outputs of urobilinogen in urine. Urobilin is reconverted into urobilinogen by treatment with ferrous hydroxide. Urobilinogen is extracted from urine by petroleum ether and is then simultaneously converted into the colored aldehyde derivative and extracted from the petroleum ether phase by shaking with Ehrlich's aldehyde reagent.

Reagents.—Ehrlich's aldehyde reagent: as described for the simplified method.

Saturated sodium acetate: as described for the simplified method.

Ferrous sulfate solution, 20 per cent: Crush crystals of $FeSO_4.7H_2O$ to a powder. Dissolve 20 g. in 92 ml. of distilled water. This solution must be made freshly each day.

Sodium hydroxide solution, 10 per cent: This solution is best prepared by diluting a concentrated stock solution.

Petroleum ether: Boiling point 30°-60°C. Reagent quality.

Glacial acetic acid.

Ethyl alcohol, 95 per cent.

Procedure.—Collection of urine is made for 24 hour periods in bottles to each of which has been added 5 g. of sodium carbonate and 50 ml. of petroleum ether.

The bottles should be of brown or other low actinic glass. The bottles containing urine should be refrigerated if possible. Analysis should be started soon after collections are completed. It is highly important that all urine be saved during the 24 hour period. Measure the volume of urine.

1. Reduction of urobilin.—Measure into an Erlenmeyer flask of 125 ml. capacity (low actinic glassware is preferred but not indispensable), 50 ml. of urine and 25 ml. each of ferrous sulfate and sodium hydroxide solutions. Mix well and allow to stand protected from light for one hour. Filter.

2. Preliminary assay.—Measure 2 ml. of filtrate into a tube. Add 2 ml. of Ehrlich's reagent. Mix well and add 4 ml. of sodium acetate solution. Mix and note the intensity of the red color. The amount of filtrate used for the extraction depends on the intensity of the color developed:

Color	Aliquot of filtrate for extraction
Dense red	1 ml.
Medium red	2 ml.
Pale red	10 ml.
Faint pink	25 ml.
No color	50 ml.

3. Extraction of urobilinogen.—This step should follow immediately after the filtration and preliminary assay. Measure into a separatory funnel (pear shape) of about 200 ml. capacity the volume of filtrate specified above. Record this as $V_{filtrate}$. If the quantity is less than 50 ml. add sufficient water to bring it to this volume. Add 30 to 50 ml. of petroleum ether and 5 ml. of acetic acid. Shake vigorously for about one minute, then allow the layers to separate. Draw off the aqueous (lower) layer into a flask and pour the petroleum ether into a 250 ml. Erlenmeyer flask. Return the aqueous layer to the separatory funnel, add 25 to 30 ml. of petroleum ether and shake as before. Again separate the two layers, adding the petroleum ether to the first portion. Return the aqueous layer to the separatory funnel and extract with petroleum ether a third time. Discard the aqueous layer and combine the petroleum ether extract with the two previous portions.

If emulsions form, they can be separated by adding more acetic acid (preferred) or 1 or 2 ml. of alcohol.

4. Washing of the petroleum ether extract.—To the combined petroleum ether extracts in a separatory funnel add 10 to 15 ml. of water. Shake vigorously for a few seconds. Draw off the water and discard. Repeat with two additional portions of water. Leave the petroleum ether extract in the separatory funnel.

5. Extraction of urobilinogen from the petroleum ether.—(a) Add 2 ml. of Ehrlich's reagent to the petroleum ether extract and shake vigorously for one minute. (b) Add 6 ml. of sodium acetate solution and shake an additional minute. Allow layers to separate and draw off the extract into a 50 ml. graduated mixing cylinder. Be sure that none remains in the stem of the funnel. Extract the petroleum ether with additional portions of Ehrlich's reagent and sodium acetate one or more times until further extraction produces only insignificant color. Combine the urobilinogen-aldehyde extracts and discard the petroleum ether. Dilute the extracts with water to a volume that will permit accurate measurement in the photometer. Record this as V_{final}. (Transmittance between 20 and 80 per cent). The dilution should be to an even number e.g. 20 or 30 ml. to simplify calculation.

6. Without delay, measure the density of the color in a photometer at 565 mu. Prepare a blank containing 3 ml. of Ehrlich's reagent and 9 ml. of sodium acetate solution. Use the blank for adjusting the zero setting.

Calculation.— Urobilinogen in mg./24 hrs.

$$= \frac{\text{vol. of urine} + \text{vol. FeOH sol.} + \text{vol. NaOH}}{\text{vol. of urine used}} \times \frac{V_{final} \times V_{24}}{V_{filtrate} \times 100} \times U.$$

Where U is the urobilinogen equivalent of the extract found by referring to the graph of the standard concentrations described on page 00. V_{24} is the 24 hour volume of urine.

Interpretation.—The excretion of urobilinogen in urine by healthy individuals averages 0.64 mg. in 24 hours. Only 2.5 per cent excrete more than 1.56 mg. and only 0.5 per cent more than 2.1 mg. in 24 hours. Patients with disease of the liver may excrete amounts of urobilinogen greatly exceeding these limits. 100 mg. or more may be found in a 24 hour urine collection although values ranging from 2 to 10 mg. are more usual. However, the excretion may fall within normal limits in patients with severe liver damage owing to the small amount of bile pigment reaching the intestine of such patients. Thus high values may be encountered following the onset of jaundice, to be followed by a period in which the output is within normal limits. This may persist for only a day or two or may continue for one or more weeks. It, in turn, is followed by a second period of elevated excretions which gradually return to normal limits as the illness improves.

Urobilinogen (Stercobilinogen) in Feces

The urobilinogen of feces consists of several closely related compounds differing in minor details of structure. The principal one is stercobilinogen which is distinguished from urobilinogen by measurement of optical rotation. Stercobilinogen reacts with Ehrlich's aldehyde reagent and the procedures for its determination are similar to those described for urine. Measurement of stercobilinogen output provides useful information to the clinician concerning (1) the rate of hemoglobin destruction and (2) the completeness of obstruction of the bile ducts. Such information may contribute substantially to the correct evaluation of the patient with jaundice. Knowledge of the rate at which hemoglobin is destroyed is of great value also in study of anemias.

Reagents.— Same as for urine urobilinogen.

Procedure.—Collection of feces should be complete. For estimation of gross changes in output a single day's collection will suffice. If the purpose is to establish the cause of biliary obstruction, a four day collection period is required. Feces are transferred to paper cartons after voiding and stored in a refrigerator until analyzed.

Combine the total amount and weigh. Mix by stirring thoroughly. A mechanical mixer is convenient although not indispensable for this purpose. Weigh approximately 10 g. of the mixed feces. Record the weight to the nearest 0.1 g. Transfer to a mortar of about 250 ml. capacity, or better to a high speed homogenizer such as a Waring Blender or Osterizer. Add 90 ml. of distilled water and thoroughly disperse the feces in the water.

Note: Precautions should be taken, especially if such equipment is used, to protect against finely, dispersed particles that may escape from the mixer with possible hazards of infection by hepatitis virus or other agents. This may be accomplished by placing multiple layers of gauze over the covered homogenizer jar.

Measure 100 ml. of ferrous sulfate solution into a flask of 500 ml. capacity preferably of low actinic glass. Add the entire amount of feces suspension. Rinse the mortar or mixer with 100 ml. of water and add this to the flask. (Omit addition of water if the feces are pale in color). Add 100 ml. of sodium hydroxide. Stopper the flask and mix the contents well. Place it in the dark for one hour. Filter.

The filtrate may be tested either by the simplified quantitative Ehrlich reaction or by the extraction method.

Simplified quantitative Ehrlich reaction.—Dilute 5 ml. of the filtrate to 50 ml. with water. Transfer 2.5 ml. of the diluted filtrate to each of two photocolorimeter tubes (F) and (B).

Add to tube (F) 2.5 ml. Ehrlich's reagent and mix well for 15 seconds. Then add immediately 5 ml. of saturated sodium acetate solution and again mix thoroughly.

To tube (B) add the reagents in reverse order, that is 5 ml. of sodium acetate are added first and thoroughly mixed followed by 2.5 ml. Ehrlich's reagent added slowly enough to permit thorough mixing during its addition.

Measure the transmittance without delay in a photocolorimeter after making a "zero" setting at 100% transmittance by means of tube (B). Obtain the urobilinogen equivalents of the readings as described in the method for urine.

Calculation.—

$$\text{Ehrlich units in sample} = (U) \times \frac{10 \times 10 \times 400 \text{ (or } 300) \times W}{2.5 \times 10 \qquad 100}$$

$$= 16 \text{ (or } 12) \times (U) \times W$$

where (U) represents the stercobilinogen equivalent in Ehrlich units per 100 g. of the readings of tube F. W is the total weight of the feces.

Extraction method for stercobilinogen.—Prepare the sample as directed above. Apply the preliminary color test as described under urine urobilinogen to the diluted filtrate after reduction. Select a suitable aliquot for extraction and continue as directed in the urine method.

Calculation.—

$$\text{Stercobilinogen, mg. in sample} = U \times \frac{10 \times 400 \text{ (or } 300) \times V_{final} \times W}{10 \qquad V_{filtrate} \quad 100}$$

$$= 4 \text{ (or } 3)\ U \times \frac{V_{final} \times W}{V_{filtrate}}$$

Interpretation.—Healthy individuals excrete 100 to 350 Ehrlich units per 100 g. of feces, or 40 to 280 mg. as stercobilinogen. As much as 2000 mg. may be excreted each day by patients suffering from hemolytic anemia. Transfusion of blood often is followed by an increased excretion of stercobilinogen in feces, and may obscure interpretation of the findings.

Obstruction of the bile ducts caused by neoplastic or other growths is characterized by complete or nearly complete interruption of the flow of bile into the intestine. The daily excretion of stercobilinogen by such patients is only 1 or 2 Ehrlich units (or milligrams) and infrequently exceeds 5 Ehrlich units. Obstruction caused by calculi by contrast, interferes less with excretion of bile and the daily output of stercobilinogen in feces will exceed 10 Ehrlich units. It is important to keep in mind, however, that either parenchymal or cholangiolitic disease of the liver, if severe, may suppress secretion of bile or otherwise prevent its entry into the intestine.

As a result, stercobilinogen outputs as low as those found in obstruction due to neoplastic growth may be encountered in hepatitis, cirrhosis, or cholangitis. The low outputs are generally transitory in the latter, in contrast to the persistently low values characteristic of complete mechanical obstruction.

A STABLE SOMOGYI SUBSTRATE FOR THE DETERMINATION OF SERUM AMYLASE

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The procedure of Somogyi (1) is generally accepted as the most reliable method for the determination of serum amylase in the acute pancreatitis. However, the instability of the starch substrate, due to depolymerization by molds, yeast or bacterial action, constitutes a serious drawback. Neidig and Burrell (2) in a compilation report gave references to the merits of esters of para-hydroxybenzoic acid that meet the critical and exacting requirements of pharmaceutic with respect to compatibility and stability.

During the past year use of esters of para-hydroxybenzoic acid, Heyden, was investigated and found to be entirely satisfactory as a preservative for the starch substrate.

METHOD OF PREPARATION

Heat to boiling 600 ml of distilled water in a 1000 ml pyrex flask; thoroughly grind 15 gm of soluble starch, according to Lintner, in a glass mortar with 1.5 gm of methyl para-hydroxybenzoate, 0.3 gm of propyl para-hydroxybenzoate and 50 ml of distilled water; slowly pour the starch paste into the flask of boiling water, meanwhile stirring vigorously; rinse the mortar with 50 ml of distilled water and transfer the washing into the flask; allow to boil for 1 minute with constant stirring; transfer the flask to a hot water bath for 15-30 minutes, covering the mouth of the flask with a small beaker; cool, and add 300 ml of M/15 phosphate buffer at pH 7.2 (17.9 gm $Na_2HPO_4.12H_2O$ and 2.3 gm KH_2PO_4 per liter); dilute to 1 liter with distilled water; transfer the contents of the flask to 500 ml glass-stoppered pyrex bottles and store at room temperature. The pH of this substrate remains at 7.2 over a period of months.

EXPERIMENTAL OBSERVATIONS

Two glass-stoppered flasks of starch substrates were prepared; one containing methyl and propyl para-hydroxybenzoates, the other without para-hydroxybenzoates. Both flasks were exposed to room atmosphere for a period of several hours after which they were stoppered and stored at

A STABLE SOMOGYI SUBSTRATE

TABLE I.

	Starch Substrate with Para-hydroxybenzoate	Starch Substrate without Para-hydroxybenzoate
1st Day		
Appearance	Clear	Clear
Sugar Content	15 mg/100ml	15 mg/100ml
5th Day		
Appearance	Clear	Cloudy..Mold and Bacterial Contamination
27th Day		
Appearance	Clear	Cloudy..Heavy Growth
Sugar Content	15 mg/100ml	430 mg/100ml
101st Day		
Appearance	Clear	Heavy Growth
Sugar Content	15 mg/100ml	.....

room temperature. During the period of 101 days the following observations were made:

As shown in Table II, the starch substrate treated with para-hydroxybenzoate remained stable as indicated by the initial and final sugar content which remained constant for 101 days. The substrate without the preservative showed marked contamination by mold and bacterial growth; the starch underwent marked depolymerization with the resultant sugar content of 430 mg/100ml, an increase of 415 mg over the initial natural sugar content.

In another series of tests simultaneous determinations of amylase activity were made using starch substrate prepared with esters of para-hydroxybenzoic acid and substrate freshly prepared but containing no preservative (3). The results of these determinations are shown in Table II.

From the data presented in Table 2 it can be seen that the reproducibility obtained by the two different starch substrates are in close agreement.

During the experimental period a series of 39 serum amylase determinations were perfromed with starch substrate containing para-hydroxybenzoate. In this series 24 samples showed amylase content between 60-200 Somogyi units. The lowest amylase content was 45 units and the highest was 1695 units. At this time a sample of pancreatic fluid was found to contain over 50,000 units of amylase.

SUMMARY

By incorporating small quantities of methyl and propyl para-hydroxybenzoate in a starch paste, a clear and stable substrate may be prepared for the determination of serum amylase by the method of Somogyi. Para-hydroxybenzoate is found to be compatible with starch and that it does not interfer with the action of amylase on starch or the estimation of reducing sugars by the Folin-Wu copper reagent.

REFERENCES

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(2) Neidig, C. P. and Burrell, H.: The Esters of Para-hydroxybenxoic Acid as Preservatives, Drug and Cosmetic Industry, 54:408 (1944).

(3) Standard Methods of Clinical Chemistry, Vol.1. Academic Press Inc., New York.

Samples of Parasepts (esters of para-hydroxybenzoic acid) were generously supplied by T. R. Aalto, Sales Dept., Heyden Chemical Corporation, Garfield, N.J.

TABLE II.

NAME	DATE	(A) STARCH SUBSTRATE CONTAINING PRESERVATIVE..PREPARED 9/11/53				(B) STARCH SUBSTRATE WITHOUT PRESERVATIVE..FRESHLY PREPARED			
		TOTAL SUGAR 1	STARCH CONTROL 2	SERUM CONTROL 3	AMYLASE ACTIVITY	TOTAL SUGAR 1	STARCH CONTROL 2	SERUM CONTROL 3	AMYLASE ACTIVITY
P.S.	10/21	305	15	105	185	305	15	105	185
M.S. Pan Fluid 1/100	10/23	505	15	0	490	505	15	0	490
E.A.	10/27	330	15	105	210	330	15	105	210
M.K.	10/27	262	15	155	92	280	15	155	110
B.B.	10/27	270	15	125	130	270	15	125	130
K.	10/28	185	15	120	50	190	15	120	55
Van	10/28	195	15	120	60	195	15	120	60
B.B.S.	10/31	1250	15	95	1140	1120	15	95	1010
		Substrate prepared 7/22/53							
Pan. Fluid 1/100	10/31	450	15	0	435	450	15	0	435

REVIEW OF CURRENT LITERATURE

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

FAILURE OF CHOLINE THERAPY TO ALTER SERUM LIPIDS IN PATIENTS WITH CORONARY ARTERY DISEASE. S.U. Greenberg and M. Bruger (Dept. of Medicine, New York University Post-Graduate Medical School, New York City). Proc. Soc. Exp. Biol. & Med. 84: 87-88, 1953.

The oral administration of 4.5 gm. of choline base daily for 1.5 to 8.0 months to 11 patients with coronary insufficiency or myocardial infraction or both, failed to alter the total serum cholesterol, the serum lipid phosphorus and the phospholipid: total cholesterol ratio. E.V.

STEROIDS IN ADRENAL VENOUS BLOOD OF THE DOG. G.L. Farrell and B. Lamus (Dept. of Physiology, Western Reserve University School of Medicine, Cleveland, O. and the Dept. of Pharmacology of Utah, College of Medicine, Salt Lake City). Proc. Soc. Exp. Biol. & Med. 84:89-94, 1953.

Adrenal venous blood of the dog has been quantitatively analyzed by paper chromatography for steroid components. Fourteen fractions have been isolated; 3 have been identified as 17-hydroxycorticosterone, corticosterone and 11-desoxy-17-hydroxycorticosterone; 7 of the unidentified fractions exhibit chemical reactions characteristic of adrenocorticosteroids. E.V.

PERIPHERAL BLOOD CONCENTRATIONS OF STEROIDS IN MAN AFTER ORAL ADMINISTRATION OF 17-HYDROXYCORTICOSTERONE. J.B. Richards and M.L. Sweat (Dept. of Physiology, Western Reserve University School of Medicine, Cleveland, O.). Proc. Soc. Exp. Biol. & Med. 84:125-127, 1953.

Following the oral administration of 50 mgm. of 17-hydroxycorticosterone, the plasma concentration of this hormone was significantly increased in 15 min. The peak concentration occurred between 45 and 60 min., normal values were reached between 4 and 6 hrs. The concentration of corticosterone-like steroids in the plasma were determined simultaneously. No significant change occurred in the concentration of this fraction. E.V.

METHOD OF PAPER ELECTROPHORESIS OF CEREBROSPINAL FLUID. G. Gries, F. W. Aly, and H. F. v. Oldershausen (Univ. Marburg a.d. Lahn, Ger.). Klin. Wochschr. *31*, 644-9 (1953).

A method of concn. and quant. analysis of cerebrospinal fluid by paper electrphoresis is described. The following normal values were found: fore fraction 4.3, albumin 51.3 al- 5.8, α_2 -8.4, β -17.1, γ -6.8, γ -globulin -6.3%. H.A.

A COLORIMETRIC METHOD FOR DETERMINATION OF VITAMIN A AND CAROTENE BY PERCHLORIC ACID. P. Flesch (Dept. of Dermatology, University of Pennsylvania School of Medicine, Philadelphia). Proc. Soc. Exp. Biol. & Med. 84:148-149, 1953.

The method described for the quantitative colorimetric determination of vitamin A and carotene in each other's presence is based on the fact that the purple-red color developed by vitamin A (maximum absorption 525-530 mμ) and the bluish-green color developed by carotene (maximum absorption 725-760 mμ) in the presence of perchloric acid may be stabilized in a solution of amyl acetate. In a mixture of vitamin A and carotene, the latter is estimated directly by determining its absorption at 750 mμ; the amount of vitamin A may be calculated by subtracting from the absorption at 525 mμ, the absorption value which corresponds to carotene at this wave length. E.V.

IMPROVED METHOD FOR DETERMINATION OF PLASMA POLYSACCHARIDES WITH TRYPTOPHAN. J. Badin, C. Jackson and M. Schubert (Dept. of Chemistry and the Study Group of Rheumatic Diseases, New York University College of Medicine, New York City). Proc. Soc. Exp. Biol. & Med. 84:288-291, 1953.

A modification of the tryptophan method for determining polysaccharide concentration in biological fluids is presented utilizing boric acid in the reaction medium. The color intensity is thus increased and the color quality modified to give maximum absorption at a longer wave length where interference by non-specific brown color produced by reagent blanks and proteins is diminished. E.V.

REDUCTION OF HYPERKALEMIA BY CIRCULATING BLOOD THROUGH A CATION EXCHANGE RESIN. B.J. Kessler, J.B. Liebler, J.I. Abrahams and M. Sass (Medical and Surgical Services, Veterans Administration Hospital, Brooklyn, N.Y.). Proc. Soc. Exp. Biol. & Med. 84:508-510, 1953.

A method for removing potassium from uremic dogs by continuous circulation of blood through a cation exchange resin is described. The extracellular potassium concentration was reduced as much as 50% within 4 to 6 hours. Calcium replacement was necessary to prevent tetany. E.V.

LIVER INSUFFICIENCY. H. Kalk (Kassel, Ger.). Die Medizinische *1953*, 1075-80, 1136-9.

The disturbances of liver function, the liver-function tests, and the rapeutic measures are reviewed. 50 references. H.A.

METHODS FOR THE DETERMINATION OF FORMALDEHYDOGENIC STEROIDS BY DIFFUSION AND BY DIRECT REACTION. H. Wilson (Dept. of Chemistry and the Study Group on Rheumatic Diseases, New York University College of Medicine, New York, N.Y.). J. Clin. Endocrinol. 13:1465-1479, 1953.

A method is described for the determination of formaldehydogenic steroids using diffusion of the formaldehyde in Conway units in place of distillation. After oxidation with periodic acid is performed in the inner well of the diffusion unit, and chromotropic-acid reagent is placed in the outer well, the sealed units are left in an oven at 85° for 1 hr. The reaction mixture distills over completely and the color is simultaneously developed. An ether-extraction step has been devised to remove an interfering substance which binds formaldehyde so that both its diffusion and direct reaction with chromotropic acid are inhibited. This procedure results in higher titers for most urine extracts, improved recoveries of added cortisone and better adherence to Beer's Law. A direct procedure without diffusion is described for the assay of uncontaminated corticosteroid material. E.V.

MICROMETHOD FOR THE DETERMINATION OF UREA. Milos Jancic (Sarajevo Univ., Yugoslavia). Bull. soc. chim. repub. pop. Bosnie et Herzegovine *1*, 21-33 (1952) (with French translation)

Into a test tube graduated at 8cc. place 1cc. of substance to be analyzed and add 7cc. of 0.5% dimethylglyoxime in conc. HCl (I). Place in boiling H_2O bath for 80 min., cool and make up to 8cc. with I. Add 0.5 cc. of 0.5% $K_2S_2O_8$ in H_2O. Read in colorimeter after 15 min. Urine should be diluted 1:100 with H_2O, blood made protein free with 20% TCA. H.A.

SERUM IRON, TOTAL IRON-BINDING CAPACITY OF SERUM AND SERUM COPPER IN ACUTE HEPATITIS. P. Brendstrup (Rigs Hosp., Copenhagen). Acta Med. Scand. *146*, 107-13 (1953); cf. C.A. *47*, 11480c.

Acute hepatitis is the only infectious disease associated with an increased serum-Fe level. H.A.

INFLUENCE OF THE BILIRUBIN-PROTEIN INTERACTION UPON THE DIAZO REACTION. Domenico Cora (Civil Hosp., Vicenza, Italy). Acta Med. Scand. *145*, 263-77 (1953).

Bilirubin is bound to the proteins of the blood (mostly albumin) but this does not account for differences in behavior towards diazonium salts. The direct or indirect bilirubin reaction is governed by the equil. $nB - P = B_nP$ (bilirubin-protein complex). H.A.

REVIEW OF CURRENT LITERATURE

THE REASON FOR THE DIRECT DIAZO REACTION OF BILIRUBIN IN SERUM. Clemens Moncke (Med. Univ.-Klin., Rostock, Ger.). Die Medizinische *1953*, 1036-8; cf. C.A. *46*, 7156g.

Paper chromatography of serum bilirubin with $CHCl_3$ shows that the compd. giving the indirect reaction travels just behind the solvent front whereas the one resulting in the direct reaction remains at the origin. This is another proof that 2 different compds. exist. The significances of normal and pathol. bilirubin findings are reviewed. 27 references. H.A.

DETERMINATION OF HORMONES. Heinrich Iselstoger. Subsidia Med. *4*, 153-66, 195-210 (1952); *5*, 1-10, 89-103 (1953).

A crit. discussion of the methods for the quant. detn. of hormones and of the clinical significance of the results. Numerous references. H.A.

HEPATIC COMA. A CLINICAL LABORATORY, AND PATHOLOGICAL STUDY. S. C. Carfagno, R. F. DeHoratius, C. M. Thompson, and H. P. Schwarz (Philadelphia, Pa.). New Engl. J. Med. *249*, 303-9, (1953).

In 11 cases in which clinical observations suggesting advanced hepatic insufficiency were confirmed by autopsy, the comparative results of routine blood studies, blood electrolyte detns., hepatic tests, and studies reflecting carbohydrate, fat, and protein metabolism were recorded. Attention is called to evidence suggesting failure of utilization of carbohydrate through the tricarboxylic acid cycle. H.A.

17-HYDROXYCORTICOSTEROIDS AND 17-KETOSTEROIDS IN URINE OF HUMAN SUBJECTS: CLINICAL APPLICATION OF A METHOD EMPLOYING β-GLUCURONIDASE HYDROLYSIS. A.A. Sandberg, D.H. Nelson, E.M. Glenn, F.H. Tyler, and L.T. Samuels. (Depts. of Medicine and Biochemistry and the Laboratory for the Study of Hereditary and Metabolic Disorders, Univ. of Utah, College of Medicine, Salt Lake City, Utah). J. Clin. Endocrinol. 13: 1445-1464, 1953.

The daily urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroid glucuronides was determined by a method employing β-glucuronidase hydrolysis and chromatography. The daily excretion of 17-hydroxycorticosteroids was more constant than that of 17-ketosteroid glucuronides. Administration of ACTH resulted in marked increases in excretion of 17-hydroxycorticosteroids. The excretion of 17-ketosteroid glucuronides was also increased but to a lesser extent and less regularly. The method presented here probably measures a specific and rather consistent fraction of the steroids produced by the human adrenal cortex. E.V.

CLINICAL SIGNIFICANCE AND TECHNIQUE OF THE HEPARIN TOLERANCE TEST. K. N. v. Kaulla (Frauenspital, Basle, Switz.). Deut. med. Wochschr. *78*, 1075-7 (1953); cf. Souller, et al., C.A. *45*, 5227d.

Details of the technique are given and sources of error are discussed. H.A.

THE THYROIDAL UPTAKE OF STABLE IODINE COMPARED WITH THE SERUM CONCENTRATION OF PROTEIN-BOUND IODINE IN NORMAL SUBJECTS AND IN PATIENTS WITH THYROID DISEASE. B.A. Burrows and J.F. Ross (Radioisotope Unit, Boston Veterans Administration Hospital, the Robert Dawson Evans Memorial, Massachusetts Memorial Hospitals, and the Dept. of Medicine, Boston, Mass.). J. Clin. Endocrinol. 13:1358-1368, 1953.

A comparison of the radioiodine uptake with the protein-bound iodine concentration showed a significant correlation in normal subjects and in patients with thyroid disease who had not received therapy. In patients who had received therapy for hyperthyroidism, the radioiodine uptake values corresponding to each serum PBI level showed greater variation than in the untreated group. The stable iodine uptakes in a small group of patients with high radioiodine uptakes showed a good correlation with the serum PBI concentration. E.V.

ESTRADIOL AND ESTRONE DETERMINATION IN URINE WITH THE BECKMAN QUARTZ SPECTROPHOTOMETER. J. Breitner, A. Eichstadter, and C. H. Brilmayer (Univ. Munich, Ger.). Klin. Wochschr. *31*, 76205, (1953). H.A.

The detn. is based on heating urine exts. with 60% H_2SO_4 and detg. their extinction at 462 mμ and 510 mμ. The concn. of estrone and estradiol is calcd. from Allen's formula (Allen, C.A. *47*, 1763d). H.A.

CONGO RED DETERMINATION IN HEMOLYZED SERUM. S. Kromrey (Hammerstr. *16*, Berlin-Zehlendorf). Munch. med. Wochschr. *95*, 707-8 (1953). H

By deproteinizing the serum prior to the detn. the error due to hemolysis is eliminated. H.A.

CRITIQUE ON THE ICTERUS INDEX DETERMINATION. R. J. Henry, O. J. Golub, S. Berkman and M. Segalove. Am. J. Clin. Path. *23*, 841, (1953). (Bio-Science Labs. California).

Measurements of serum icterus at 420 mμ by direct dilution with 5% sodium citrate were found to be unreliable due to the fact that at this wave length Hb is being measured, not bilirubin. The peak for bilirubin is around 460 mμ and the dilution method at this wave length gives satisfactory results even in the presence of limited amounts of turbidity and hemolysis. Acetone extraction technique gives reliable results at 420 mμ. New potassium dichromate standards are described. C.R.

CITRATE CLOTTING TIME ON ANTICOAGULANT THERAPY. S. Losner, and B. W. Volk. Jewish Sanitarium, Brooklyn, N. Y. Am. J. Clin. Path. *23*, 866, (1953).

Direct correlation was found between the citrate clotting time, as determined by mixing 1 ml of venous blood with 1 ml 0.003M sodium citrate in saline at 37 deg. C., and a) one stage prothrombin time after giving Dicumarol, and b) Lee White clotting time, after administration of heparin. Anticoagulant therapy with hypoprothrombinemic agents was maintained with the citrate clotting time ranging between 30 to 50 min. C.R.

THE CLINICAL SIGNIFICANCE OF AMINO ACIDURIA. Irving B. Brick (Georgetown Univ., Wash., D. C.). New Engl. J. Med. *247*, 635-44 (1952).

A review with 53 references. H.A.

IMPROVEMENTS IN THE RAPID SCREENING METHOD FOR LEAD IN URINE. W. W. Woessner (du Pont de Nemours and Co., Penns Grove, N. J.) and J. Cholak. Arch. Ind. Hyg. Occupational Med. 7, 249-54 (1953).

A rapid and simple screening test for urine lead concn. that can be applied to fresh urine or urine preserved with 10% HNO_3 and thymol. Bi, Tl and Sn interfere. H.A.

DETERMINATION OF CREATINE BY MEANS OF POTASSIUM MERCURIC SULFOCYANIDE AND DITHIZONE. II. Peter Stelgens (Univ. Kinderklinik, Heidelberg, Ger.). Biochem Z. *324*, 228-36 (1953).

The method is applicable to fingertip blood and urine and is claimed to be more accurate than the picrate method. H.A.

A MICRO LIPIDE EXTRACTOR. Peter D. Mitchell (Univ. Cambridge, Engl.). Nature *172*, 124 (1953).

A modification of Reichert's method (C.A. *39*, 1118^2) for pholpholipide detn. on a micro scale is described. A B 14 Quickfit tube is used as a sample holder which can then be used for the methanol treatment as well as for the ether extn., eliminating transfer loss. The total lipide content is reproducible to within the limit of gravimetric error when 5-50 mg. of material contg. 0.3-5 mg. of lipide is used. H.A.

DETERMINATION OF CARBOXYHEMOGLOBIN IN THE BLOOD. A. D. Aruina (Tsentral. Nauch.-Issledovatel. Sanit. Inst. im. Erismana). Gigiena i. Sanit. *1953*, No. 4, 50-1.

Carboxyhemoglobin is determined by diluting blood in 0.4% NH_4OH and reading the absorption maximum at 920 mμ. Concn. of HbCO is read from a previously constructed calibration curve according to the formula $E = E' (70/a)$, where $E' = \log I_o - \log I$, I_o being a water blank and a being the % Hb in the sample. Accuracy is said to be within 2%. H.A.

Biographical Sketches of Proposed New Officers

PRESIDENT

MONROE E. FREEMAN, Chief, Department of Biochemistry Army Medical Service Graduate School, also Chief, Allied Science Section, M.S.C., Office of the Surgeon General, Department of the Army.

Educated at the University of Minnesota, B.S., M.S., Ph.D. 1928-1931 in biochemistry.

Has served as instructor of chemistry at University of Arizona, 1929-30; Assistant Professor Biochemistry, University of Maine 1930-1936; and Professor of Chemistry at University of Massachusetts 1936-1948.

Entered Regular Army in 1948 at the Army Medical Service Graduate School. World War II service as chief of chemistry and toxicology section of the First Medical General Laboratory in the European Theatre of Operations.

Investigative interests in plant virus diseases, hemicelluloses, carbohydrates, clinical chemistry, lipoid hemolysins, hyaluronidase, bacterial antigens.

Doctor Freeman is now serving as Vice-President of the AACC.

VICE-PRESIDENT

OTTO SCHALES, Director of the Biochemistry Laboratory, Alton Ochsner Medical Foundation, New Orleans, La., and Asst. Professor of Biochemistry, Tulane University. He received his doctorate in 1935 from the University of Frankfurt. In 1939 he came to Harvard as a research associate. He has been associated with the Ochsner Foundation since 1944. While at Harvard, Dr. Schales was chemist to the Peter Bent Brigham Hospital.

Dr. Schales is now serving his second three year term as Secretary-Treasurer of the Division of Biological Chemistry of The American Chemical Society. His scientific interests feature work on microanalytical methods, biochemistry of hypertension, iron metabolism, blood pigments, and chemotherapeutics.

SECRETARY

MAX M. FRIEDMAN, Chemist to Lebanon Hospital in New York City, and Consulting Chemist to Medical Arts Center Hospital and the Pack Medical Foundation. He was born in Austria on January 24, 1907 and completed his undergraduate work at the University of Alabama in 1930. After also studying at Columbia and New York University he received his doctorate at the Polytechnic Institute of Brooklyn in 1947. His scientific interest for many years was in body water and extracellular fluid, and also nucleic acids in normal and pathological tissues. He is at present studying blood cholinesterase, arginase and lipase.

TREASURER

LOUIS BASIL DOTTI is Chemist at St. Luke's Hospital in New York City and Lecturer in Physiology and Biochemistry at the New York Medical College. He was born in New York City on August 13, 1903, and graduated from Columbia University in 1929. He also did his post-graduate work at Columbia, receiving his M.A. in 1931 and his Ph.D. in 1936. He has worked extensively on carbohydrate and calcium metabolism, digestive enzymes and liver function tests.

Dr. Dotti has been Treasurer of the AACC since 1948.

MEMBERS OF THE EXECUTIVE COMMITTEE

HUGH J. MCDONALD. Born, Glen Nevis, Ontario, Canada, July 27, 1913; Queen's University, 1930-1932; B.Sc. in Chemistry (with highest honors) McGill University, 1935; M.S., Carnegie Institute of Technology, 1936; D.Sc., 1939. Major work for doctorate in physical chemistry, with minors in organic chemistry, physiological chemistry and physics.

Research fellow, teaching assistant and part time instructor, Carnegie, 1936-1939; instructor in chemistry, Illinois Institute of Technology, 1939-1941; Assistant Professor, 1941-1943; Associate Professor, 1943-1946; Professor, 1946-1948; Professor and Chairman, Department of Biochemistry, Stritch School of Medicine of Loyola University, Chicago, since 1948. Consultant, Argonne National Laboratory, since 1946. Manhattan Project, Columbia University, 1943. Awarded competitive scholarship, Royal Institution for Advancement of Learning, 1933-1934. Sigma Xi research award, 1944; research award, American Academy Arts and Sciences, 1945.

Fellow, A.A.A.S., 1946; Member, American Chemical Society; American Association Clinical Chemists (Chairman Committee on Education); Electrochemical Society; American Association University Professors; Sigma Xip Phi Lambda Upsilon; Alpha Chi Sigma, Chaos Club (Chicago).

Dr. McDonald is the present Vice-President of the AACC, and Chairman of the Committee on Education.

Dr. McDonald is at present President of the AACC.

ROBERT M. HILL, Professor of Biochemistry University of Colorado, received his doctorate in biochemistry from Cambridge 1931 and Copenhagen 1932. Was formerly Asst. Professor of Biochemistry School of Medicine, Loyola University and Assoc. Professor Biochemistry, University of Colorado.

Dr. Hill served with the U.S. Navy and Public Health Service during World War II as a civilian consultant. His scientific interests concern sulphur oxidations, plasma proteins, body temperature control and metabolism of tumors.

MARSCHELLE H. POWER is Professor of Physiological Chemistry in the Mayo Foundation, Graduate School, University of Minnesota, and Head of the Division of Biochemistry at the Mayo Clinic. Dr. Power graduated from the University of Nebraska in 1917 and received his doctorate in organic chemistry from that University in 1923. His publications have included papers relating to the nature of the blood sugar as studied by means of *in vivo* dialysis, carbohydrate metabolism, hyperinsulinism, renal function, acid-base equilibrium in the blood, metabolic abnormalities in Addison's disease and in Cushing's syndrome, the use of radioactive iodine in the study of the thyroid gland and the metabolic effects in man of the administration of adrenocorticotrophic hormone and of various steroid hormones of the adrenal cortex.

MIRIAM REINER, Director of the Chemistry Laboratory at District of Columbia General Hospital, Washington, D.C. Received her B.S. and M.S. degree from Columbia University, 1932, and is a Ph.D. candidate at Georgetown University. She was formerly Senior Assistant Chemist at the Mt. Sinai Hospital, New York City.

Proteins have been her main investigative interest, particularly changes in disease as demonstrated by electrophoresis, as well as studies on "inborn errors of metabolism" (such as pentosuria and levulosuria), and clinical chemical problems of the newborn. Miss Reiner is the author of *Manual of Clinical Chemistry*, Interscience Pub. N.Y. 1941 and Editor of Volume I *Standard Methods of Clinical Chemistry.*

She is Chairman of the Washington, Baltimore, Richmond Section of the AACC.

ALBERT B. SAMPLE, Biochemist to the Laboratory of Clinical Pathology and the John S. Sharpe Research Foundation of the Bryn Mawr Hospital, Bryn Mawr, Pa., received his B.S. degree in chemistry in 1938 from the University of North Carolina. Received his M.S. in biochemistry in 1940, after completion of studies and holding of an assistantship in the Department of Biochemistry, Medical School, University of North Carolina.

From 1941 to 1947 Albert Sample was in charge of the Biochemistry Section of the Research Department of Smith, Kline and French Laboratories. He also headed the Research Analytical Section of the SKF Laboratories during 1947-1948. This new section combined the Research Biochemistry Section and the Organic Microanalytical Laboratory.

Mr. Sample is active in the various national and local scientific organizations. He served as Vice-Chairman of the Philadelphia Section of the AACC 1951-52 and Secretary-Treasurer 1952-1953.

INTERNATIONAL ASSOCIATION OF CLINICAL BIOCHEMISTS

Minutes of 1st Meeting held at Stockholm 31st July, 1st August and 3rd August, 1953.

NATIONAL REPRESENTATIVES

Present:

	E.J. King (Chairman)
France	P. Fleury
Scandinavia	B. Josephson
U. S. A.	A. Sobel
Holland	J.C.M. Verschure
Britain	I.D.P. Wootton (Secretary)

Apologies for absence were received from:

W. Sperry

1. *Minutes.*

The minutes of the first General Meeting of the Association and the Chairman's preliminary report to I.U.P.A.C. were approved.

2. *Matters arising.*

The Chairman reported that it was now becoming clear that the I.A.C.B. was most easily organized as a federation of national societies (as suggested by the American Association of Clinical Chemists). Discussion of this point indicated that there was general agreement, and it was therefore formally approved that the I.A.C.B. should be a federation of societies interested in, and working in the field of, clinical biochemistry, provided that suitable individuals from countries which have not a national society are not excluded. Admission of such individuals shall be at the discretion of the Committee.

The business of the International Association is conducted by its Committee which shall elect the offices of chairman and secretary at intervals of not more than three years. The Chairman pointed out that the Commission consisted, after one year's existence, of two appointed members from I.U.P.A.C. and five elected national representatives. It was envisaged that the Committee should eventually consist entirely of elected national representatives with power to co-opt individuals from countries which have no national societies.

3. *The Third International Congress of Biochemistry.*

The Chairman reported that the Congress would include a Section of Clinical Chemistry and hoped that the local Organizing Committee would accept advice from the International Association on the organization of this Section. The Chairman was asked to suggest that the publications of the Clinical Chemistry Section should carry a title "jointly with the I.A.C.B.", and was requested to arrange collaboration with the local Organizing Committee. It was suggested that the most profitable form of organization was the grouping of similar papers in the form of symposia which might well be opened by specially invited speakers. The following subjects were suggested:

(1) Trace minerals (or serum iron and copper) in Clinical disease.
(2) Physical measurements in clinical chemistry.
(3) Calcium and phosphorus metabolism.
(4) Blood enzymes in disease.
(5) Salt and water metabolism.
(6) Steroid hormones in health and disease.
(7) The chemistry of atheroma.
(8) Muco-polysaccharides.

It was pointed out that each of these subjects can be studied from several aspects: the clinical, the pathological and the analytical. As Congress speaker, wide support was received to the suggestion that Professor D.D. Van Slyke should be invited.

4.(a) *Representation of Belgium and France.*

The Chairman stated that it was originally thought that Belgium and France could well be represented by a single person. This course, however, did not meet with complete agreement in the countries concerned, but the situation had been simplified by the appointment of Professor Fleury as French representative, since he already sat on the Committee by virtue of his membership of the I.U.P.A.C. Commission. A vacancy was therefore left to be filled by a national representative from Belgium. At a meeting in Brussels in April 1953 the three Belgian societies concerned agreed to combine to elect a single representative, but no news of their choice had yet been received. The Committee endorsed the action of the Chairman in this matter and instructed the Secretary to write letters to the secretaries of the three Belgian Societies asking them to expedite their appointment of a Belgian representative.

4.(b) *Further national representatives.*

After discussion it was decided that the Committee had not enough information to warrant inviting further countries to appoint national representatives. P. Fleury and E.J. King were asked to explore the situation in Spain and Italy respectively, and it was decided to consider representation of these countries at the next meeting.

5. *Status of national representatives.*

The present national representatives were not full members of the Section of the Biological Chemistry of I.U.P.A.C. They were entitled to attend Section meetings and take part in discussions, but were unable to vote. It was therefore decided that the Chairman should request the Section to appoint the present national representatives as full members. It was agreed that to avoid administrative difficulties members who ceased to serve as national representatives should resign their membership of the Section.

6. *Regional symposia.*

The Chairman briefly reviewed the proposals for arranging international meetings. He thought that the main meetings should be held as part of the International Congress of Biochemistry, but that it was very desirable at other times to hold regional meetings of a more intimate kind and pointed out that the French Society of Clinical Biology had recently held a most successful Congress in Monaco. J.C.M. Verschure then extended a cordial invitation to the International Association to hold such a regional symposium in Amsterdam during 1954. He suggested that it should be limited in subject matter and hoped that members would attend from most of the countries of Western Europe. It was decided that a four-day symposium in mid-September 1954 would be appropriate and the subjects suggested were "Diagnostic uses of radio-isotopes" and "Physical methods in clinical chemistry". The Chairman was requested to ask for a grant of 200 pounds from the Biological Section of I.U.P.A.C. to help defray the expenses of this symposium.

7. *Membership lists.*

The question of providing a full list of members was discussed, and it was agreed that such a list could not be compiled at the moment. It was therefore decided that the Secretary should collect and keep an up-to-date list of the officers of the national societies involved, and, when available, their membership list. This information would be available to all secretaries of national societies on demand.

8. *Collaborative test of laboratory results.*

The Secretary reported the experience in two such tests conducted in Britain. A.E. Sobel discussed similar experiences which had been obtained in various American surveys. J.G.M. Verschure reported that a test in Holland had shown relatively good results except for Cholesterol and uric acid, and B. Josephson had found good duplication between different hospitals provided they were using the same methods.

Several members emphasized that it was essential to provide stable samples and that the reputation of the test would depend on this. The opinion of the majority favoured the use of human serum if this were technically possible. It was agreed that two dilutions should be supplied and possibly glucose added. A considerable discussion of the analyses which should be requested resulted in a number of different proposals. After voting on these various proposals the majority decided that the laboratories should be asked to analyse samples for the following five constituents: total protein, urea, cholesterol, glucose, chloride, and that, if possible, a further five analyses should be performed,

namely, non-protein nitrogen, sodium, potassium, calcium and inorganic phosphate. It was agreed that the minimum volume of each sample should be 5 ml.

Organization. It was agreed that the collaborative test should be held during the autumn of 1953, although the Scandinavian test could not be carried out until December 1953. The samples were to be numbered before despatching to the individual national secretaries and the latter were to supply the results filled in on a numbered list. Thus anonimity remained in the hands of the national secretaries, who were free to publish their own country's results in any way which they thought fit. An estimate of the number of laboratories taking part was, U.S.A. 27, Scandinavia 25, Holland 100, France 50, U.K. 50; a total of 250. The Secretary was instructed to prosecute inquiries on the preparation of the samples, and the Chairman undertook to request I.U.P.A.C. for a grant of 50 pounds to defray expenses.

9. *Conventions of expressing results.*

A.E. Sobel expressed the American view in favour of substituting weights in place of units when expressing, e.g., enzyme results. It was generally agreed that this change should be made as soon as possible. It was agreed that the expression of ionic components in the collaborative test should be in m.eq./l.

10. *Exchange of technical staff.*

Preliminary arrangements for exchanging technical staff between Sweden and the U.K. were noted with approval.

11. *The Constitution of the Committee.*

The Chairman reported that the Committee was composed of certain appointed members of I.U.P.A.C. (King, Fleury and Sperry) and national representatives (Sobel, Verschure, Josephson and Wootton). The I.U.P.A.C. members would terminate their term of office in 1955 and the Chairman suggested that no alteration in the constitution of the Committee should be made until this time. It was suggested by A.E. Sobel that national representatives should serve for a term of four years, but it was pointed out that the decision as to terms of office was rightly one for each national society to decide. The Chairman then asked the representatives to report to their national societies that while this Commission does not wish to influence them as to the duration of service which they wish their representative to undertake, it would be convenient for continuity of policy and function if each representative served for a fairly long period, e.g., four years. The Chairman moved that in the opinion of this Committee clinical chemistry on the international level would best be served by continuing our association with I.U.P.A.C. for the time being, and that the questions of such continued association be debated by national societies. Their delegates should be instructed accordingly for open discussion of the question at the next general meeting in Brussels in 1955. The Committee felt that most members had not received definite instruction on this question, with the exception of the American representative. The Chairman also indicated that it was desirable that individual national societies should decide whether they favour a small continuous nucleus of semi-permanent members, e.g., appointed by I.U.P.A.C. plus a majority of elected national representatives, or whether they consider that the Committee should be composed entirely of national representatives. It was the opinion of a majority of the Committee that eventually the Committee should consist entirely of national representatives with power to elect or co-opt its officers from amongst itself or from outside, as it sees fit.

12. *Name of Association.*

After preliminary discussion the Chairman ruled that this Committee was not competent to change the name of the Association which had been decided at an open meeting in Paris in 1952. The Committee, however, could recommend the change to the next meeting in Brussels. B. Josephson favoured the word "federation" as indicating a federal organization of national societies, and this proposal received general support. There was considerable discussion between the alternative of "clinical chemistry" and "clinical biochemistry". It was the opinion of the delegates from U.S.A., Holland, France and Scandinavia that "clinical chemistry" was preferable, with the U.K. delegate dissenting. The Committee, therefore, recommended that the title of the Association should be changed to "The International Federation of Clinical Chemistry".

FIRST EUROPEAN CONGRESS ON CLINICAL CHEMISTRY

Amsterdam, September 23-28, 1954

The First European Congress on Clinical Chemistry will be held in the Netherlands under the auspices of the "Commission on Clinical Chemistry" of the International Union of Pure and Applied Chemistry and will be organized by the Netherlands Society for Clinical Chemistry.

The Congress will take place in Amsterdam from Thursday, September 23rd until Tuesday, 28th 1954 in the Royal Institute for the Tropics, 63, Mauritskade, Amsterdam-O.

The two leading subjects will be:

1. Isotopes in the Clinico-Chemical Laboratory.
2. The physical methods of measurement in connection with clinico-chemical problems.

These subjects will be presented in symposia, consisting of several lectures for which qualified speakers will be invited, and papers on original research related to the above subjects. A maximum of 15 minutes will be allowed for individual papers.

Members who intend to present a communication to the Congress are urgently requested to send their papers to the Secretary: *Ir O. Meulemans, Racinelaan 17, Utrecht, Netherlands,* before April 1, 1954. *After this date no further papers will be accepted.* The papers should be accompanied by an abstract of 100-150 words, which will be printed in the final program.

Excursions to various institutes and laboratories and also to scenic parts of the Netherlands are being planned. Provision will be made by a special Ladies-Committee to entertain wives and guests accompanying the delegates. Special excursions, which do not coincide with the scientific trips, will be organized for this group.

REGISTRATION FEES

The amount of the Registration fees for membership of the Congress will be:

Full Members per person: *f* 25.— Neth. currency.

Accompanying members (wives and other relatives of the full members): *f* 10.— Neth. currency.

Approximately: 1 £ = 10.70 Netherl. guilders; 1 $ = 3.80 Netherl. guilders.

GENERAL INFORMATION

Hotel Accommodation in Amsterdam.

I. *Prices in Hotels* vary from (approximately) *f* 7.— to *f* 17.—, without bath, breakfast included, and from *f* 10.25 to *f* 20.—, with bath, breakfast included, according to the class of hotel and per day, per person.

II. *Family Hotels:* *f* 10.— to *f* 12.—, breakfast and one meal included, per person, per day.

III. *Simple accommodation for men and and women in Student Home* (running water and showers):

(Continued on Page 18)

BOX 123

Letters From Members

Gentlemen:

During the last few weeks I have been busy gathering statistics of the past year along with all the other clinical chemists across the country. I've been wondering what is the best and most representative method to show how much work has been done. The usual method of the addition of the number of *specimens* handled, or even the number of *tests* does not give a proper evaluation of the work-load.

Does anyone know of any sort of time-study that has been done where the individual tests have been clocked? For example, should a cholesterol and ester be worth 10 points or units in comparison to a blood sugar or urea nitrogen worth one unit.

In smaller hospitals, such an evaluation might not be critical since one can explain things personally to the Superintendent or Board of Trustees, but in large government hospitals where the budget—personnel as well as monetary—must go through a series of committees, it is important to show a true picture which needs no extra explanation.

Has the amount of work expected from a technician in a routine clinical chemistry laboratory ever been decided upon? This is not meant facetiously but would assist in calculating how many technicians would be necessary in running a laboratory for a hospital of designated size.

Another fact to be taken into account is the amount of leave—sick and annual (vacation). In the government, vacations or leave are taken throughout the year. Consequently in a laboratory with eight people, the leave amounts to about one year's absence. I am *not* advocating less leave, I am only advocating enough help to cover this shortage.

It will be interesting to hear how our colleagues are solving this problem. Shall we try to standardize our work-load along with our methods?

Sincerely yours,
Miriam Reiner
Director of the Chemistry Laboratory
D.C. General Hospital

Washington, D.C.

SITUATION WANTED

CLINICAL CHEMIST, M.S. with more than 15 years experience in the operation and administration of biochemistry departments of hospital laboratories. Desires position, salary, commensurate with abilities and experience. Will consider any area in the U.S. Write: Box 123, Lenox Hill Station, N.Y. 21, N.Y.

INSTRUMENT NEWS

Barnstead Still & Sterilizer Co., of Boston, has announced the publication of a new 20-page catalog which describes the firm's entire line of mixed-bed, two-bed, and four-bed demineralizers for laboratory, hospital, and industrial use.

The new publication, which contains many photographs, drawings, and diagrams, explains in detail the principles, construction, and operation of both mixed-bed and multi-bed demineralizers, ranging in capacity from 5-gallon-per-hour laboratory units to industrial installations producing 2500 gallons per hour. It gives dimensional and performance data on all models and explains the difference between distilled and demineralized water, considerations affecting selection of proper equipment for a given job, and the actual installation and operation of Barnstead Demineralizers.

The new catalog, #127, may be obtained without charge by writing to Barnstead Still & Sterilizer Co., 247 Lanesville Terrace, Forest Hills, Boston 31, Mass.

* * * * *

A 36 page catalog of Hydrometers and Thermometers has just been published by the Emil Greiner Co., 20-26 North Moore St., N.Y. 13, N.Y.

This catalog, which may be obtained free of charge, features nearly 1,000 models all made in accordance with requirements of the National Bureau of Standards, A.S.T.M., A.P.I. and other official sources of standard specifications.

* * * * * * *

CORRECTION

The following paragraph was omitted from the method for glucose (Nelson-Somogyi) in Standard Methods of Clinical Chemistry Volume I, and should be inserted following paragraph 2, on page sixty five: "3. Working solutions of zinc sulfate and barium hydroxide are prepared from the preceding by addition of 7500 ml. of water to each."

BOOK REVIEWS

ADVANCES IN ENZYMOLOGY, Volumes XIII and XIV. Edited by F. F. Nord. XIII - 413 pages, $8.50 — XIV - 470 pages, $9.25. Interscience Publishers, Inc., 250 Fifth Ave., New York 1, N.Y.

Reviewed by B. N. LaDu, Jr., National Heart Institute, Bethesda, Md.

Volume 13 in this series contains papers on:

- Localization of Enzymes in Cytoplasm by Holter
- Some Aspects of the Application of Tracers in Permeability Studies by Hans Ussing
- La Biosynthese Induite des Enzymes (Adaptation Enzymatique) by J. Monod and H. Cohn
- Adenosine Triphosphate and the Structural Proteins in Relation to Muscle Contraction by D. M. Needham
- Genetic Formulation of Gene Structure and Gene Action by G. Pontecorvo
- Hyaluronidases by Karl Meyer and Maurice Rapport
- Certain Aspects of the Intermediary Metabolism of Glutamine, Asparagine and Glutathione by Heinrich Waelsch
- Stoichiometric Inhibition of Chromotrypsin by A.K. Balls and E.F. Jansen
- The Comparative Biochemistry of Nitrogen Fixation by P.W. Wilson

Volume 14 contains chapters on:

- Probleme des Energietransports innerhalb lebender Zellen, by Theodore Buecher
- Pantethine and Related Forms of the Lactobacillus bulgaricus Factor (LBF), by Esmond E. Snell and Gene M. Brown
- The Metabolism of Phenylalanine and Tyrosine, by A.B. Lerner
- Oxidation of Proteins by Tyrosinase and Peroxidase, by I.W. Sizer
- Chemismus der organischen Katalyse, by Wolfgang Langenbeck
- Enzymic Isomerization and Related Processes, by L.F. Lelair
- Suggestions for a More Rational Classification and Nomenclature of Enzymes, by O. Hoffmann-Ostenhof
- Quelques Techniques Nouvelles for l'Etude de la Structure des Proteins, by Pierre Desnuelle
- Adsorption Studies of Enzymes and Other Proteins by C.A. Zittle
- Principles and Procedures in the Isolation of Enzymes by Sigmund Schwimmer and A.P. Pardee

These volumes include a wide variety of topics, as the above titles indicate. Space limitations prevent a detailed review of the individual papers, but this series is so well known that it is enough to say that these volumes continue to meet the high standards set by the preceding members of this series.

Without doubt, many of the topics

BOOK REVIEW (cont'd)

selected will be of most interest to specialists in the respective fields of research; but as a group of critical reviews of current topics in biochemistry, these volumes will be indispensable to enzymologists and of great value to all of those interested in biochemistry.

NEW YORK SECTION

A meeting of the Metropolitan-New York Section of the AACC will be held Tuesday evening, March 23, at the New York Academy of Sciences.

The scientific session will feature a symposium on "The Lipoproteins". Dr. John L. Oncley of Harvard University Medical School will speak on "The Chemical and Physical Aspects of the Lipoproteins", and Dr. David P. Barr of Cornell University Medical College will discuss "The Clinical Implications of the Lipoproteins".

Dr. I. J. Greenblatt will preside at the scientific session.

MIDWEST SECTION

A group of members of the AACC met at Iowa City, Iowa on January 21, 1954 for the purpose of organizing a section of the AACC. Joseph I. Routh, University Hospitals, was elected temporary chairman, and Lawrence C. Kier, temporary secretary.

A request for official sanction as a section, accompanied by a list of thirty charter members, was submitted to the National Executive Committee. This was approved, and the Midwest Section was organized to consist of Iowa as a nucleus, and will include the States of Wisconsin, Minnesota, South Dakota, Nebraska, Missouri and western Illinois. It is hoped that in the near future each of the above states will be able to support its own independent section.

The Midwest Section now becomes the seventh local section of the AACC. The others, in the order of their organization charters are: Metropolitan-New York, Boston, Philadelphia, Southern California, Chicago, and Washington-Baltimore-Richmond.

SOUTHERN CALIFORNIA SECTION

Robert L. Pecsok, Ph.D., Assistant Professor of Chemistry, University of California at Los Angeles, discussed the "Polarograph and Its Application to Clinical Problems" January 5 at the Veterans Administration Center, Los Angeles.

Harvey A. Itano, M.D., Ph.D., Senior Research Fellow in Chemistry, California Institute of Technology, discussed the "Detection of Abnormal Hemoglobins" February 2, at the Cedars of Lebanon Hospital, Los Angeles. Dr. Itano's appearance before the local section at this time was a special event in that he has just recently been announced as the 1954 recipient of the Eli Lilly and Co. Award in Biological Chemistry, administered by the American Chemical Society, in recognition of his pioneering work in hemoglobin chemistry. (See *Chem. and Eng. News*, **32**, 408 (1954)).

Dr. Pecsok, who specializes in polarography, commented that this electrochemical technique has yet to be fully appreciated by the clinical chemist. He pointed out that it is possible, in a favorable case, to determine within 15 minutes six or more individual components present together at very low concentrations in a mixture. Accuracies of one to five percent or better are obtainable. The clinical chemist should consider this technique for determining heavy metals, various reducible or oxidizable organic compounds, and other substances. He compared several commercial instruments, but reminded that simple laboratory setups can give useful results.

Dr. Itano described the tests which he has employed to separate and identify the various hemoglobins: alkali denaturation test, sickling test, solubility test with phosphate buffers, and electrophoresis. From methodology he turned to a discussion of the various combinations of A, S, C, D and F hemoglobins that have been observed in particular patients, correlating these combinations with underlying genetic factors.

* * * * *

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FIRST EUROPEAN CONGRESS

(continued from page 16)

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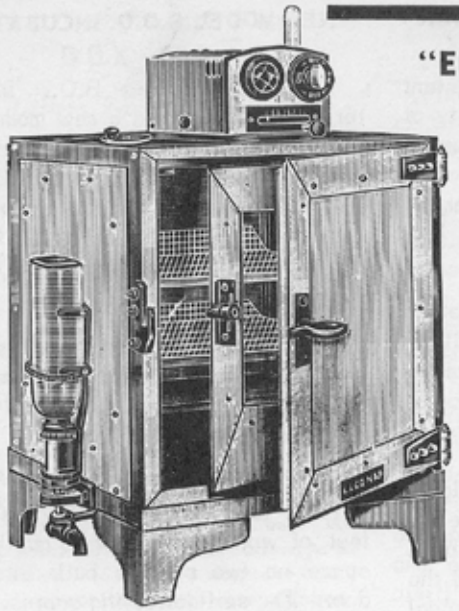
N.B. Hotel Directors will appreciate if guests will take one meal (either lunch or dinner) in their hotel. Additional 15% will be added for service on all prices.

All those who may possibly want to attend the Congress are invited to send a postcard as soon as possible to the Secretary General Ir O. Meulemans, Racinelaan 17, Utrecht, Netherlands. Those interested will receive further details.



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