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Official Publication
of
AMERICAN ASSOCIATION
of
CLINICAL CHEMISTS,
INC.

Committee On Laboratory Standards and Personnel Survey
ORIGIN OF THE MIDWEST SECTION

The first meeting of the Midwest Section was held at the Iowa Methodist and Blank Memorial Hospitals in Des Moines, March 5th. Kurt Dubowski and Mary Doris Sandin arranged a tour of the hospitals and the laboratories. The tour was concluded by a discussion of the problems, the activities and the plans for clinical chemistry in the two hospitals by Dr. Dubowski. Nineteen members of the section were joined for lunch in the Doctors Dining Room by Dr. Dunn, the hospital pathologist. A business meeting followed the lunch. The group worked on by-laws for the section and discussed officers, election of officers, formation of committees, etc. Future programs were considered and plans were made to visit several laboratories in the section.

J. I. Houth

NAACL CONVENTION

The National Association Of Clinical Laboratories held their 1954 National Convention at the Hotel Holland, Cleveland, Ohio, May 14-16.

This Sixth Annual Convention featured as part of the scientific sessions, "A Symposium On Recent Improvements In Laboratory Procedures". The speakers were: Charles C. Croft, John D. Potterfield, Roger W. Marnet, Nelson F. Young, S. W. Eisenberg.

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.
AACC

LABORATORY SURVEY

Enclosed with this number of The Clinical Chemist is a letter, questionnaire and stamped envelope from the AACC Committee on Laboratory Standards and Personnel. The committee survey was authorized by the National Executive Committee in 1953, after a number of members held some informal discussions of mutual problems. It soon became apparent that the conditions under which many Clinical Chemists work are widely different. It was felt that if the information exchanged by the few then present could be obtained from a larger group the result might serve as a picture of the status currently enjoyed by the profession.

Formal proposal was then made to the Executive Committee for a survey of the entire membership. A group of four members was chosen to design an appropriate list of questions, and after considerable deliberation the enclosed form resulted.

No attempt at standardization is implied or intended, but on the assumption that Clinical Chemists operate good laboratories which do good work the survey will point out the generally accepted requirements which insure such work. Other laboratories can use the survey as a data to improve their own situation as to personnel and facilities.

There has been considerable adverse criticism lately published regarding the quality of work performed by clinical laboratories. Perhaps the present effort will help convince hospital administrators and pathologists that reliable data can be obtained by reliable individuals, namely, clinical chemists.

The success or failure of surveys is always conditioned by the sampling and the response. Sampling errors have been avoided by sampling the entire population, but the hazard of response rests with the individuals polled. Each of you is urged to assist in the compilation of valid data by use of the enclosed envelope, prestamped and addressed for your convenience. Complete and return the forms as promptly as possible, please.

A.H.T. CO. SPECIFICATION

DENSIGRAPH
(RECORDING DENSITOMETER)

4937X.

- A manually operated Recorder which provides a continuous ink tracing indicating percentage light transmission
- For convenient and rapid photometric analysis of light absorbing materials on dry paper strips obtained in electrophoresis and chromatography

DENSIGRAPH (Recording Densitometer), A.H.T. Co. Specification, (Patent Applied For), manually operated. For convenient and rapid photometric analysis of light absorbing materials by determining light transmission and linear separation of stained areas on dry strips of paper as obtained in electrophoresis and chromatography.

Simultaneously combines the usual operations of scanning, indicating and recording to produce a continuous inked tracing of the output of a photoelectric cell on graph paper marked in millimeter squares to indicate percentage light transmission. Takes paper strips up to 40 mm wide and treatment of the paper to make it translucent is not required.

Consisting of a modified microamperometer with extra manually controlled pointer, photocell, 5-volt lamp, adjustable slit, constant voltage transformer, to operate the lamp, and a pen which traces a curve when the pointer on the microampermeter is followed closely by the manually controlled pointer which is operated from the front of the cabinet by means of a mechanically linked lever.

In use, stained paper strips are attached by adhesive tape to the right edge of the graph paper, below the adjustable slit, and advanced beneath the photocell housing by the hand wheel at the left of the cabinet. Lateral movement of the lever with the right hand makes it possible to align the manually controlled pointer continuously with the indicating pointer of the meter and, as the rate of travel of the graph paper is under the control of the operator's left hand, the fidelity of the resulting curve depends upon the manipulative skill of the operator. A continuous record of an electrophorogram can be completed in approximately 5 minutes and portions of the curve can be rechecked by simple roll-back of the graph paper.

4937X. Densigraph (Recording Densitometer), A.H.T. Co. Specification, (Patent Applied For), as above described, complete with constant voltage transformer, 50 ft. roll of graph paper, ink writing pen, 4 oz. bottle of ink, roll of adhesive tape with dispenser, carrier for 2 x 2-inch glass filaments, 6 ft. three-wire cord with two-prong plug with grounding lug, and directions for use. Power consumption 25 watts. For use on 115 volts, 50 cycles, a.c. only. ................. 475.00

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-20-
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 III

(I) Measurement Of Excretory Capacity Of The Liver

(II) Miscellaneous Tests

(J) Selection Of Procedures

(K) Addenda

(L) References

Measurement of excretory capacity of the liver—Bile acids and various other substances, among them a number of dyes, are taken up by the liver and secreted into the bile with great rapidity. It has been found that in liver disease the rate of excretion may be lowered, and because of this the use of certain of these substances has been of great value in measurement of liver function. Disodium phenyltetrazolium chloride sulfate (sulfobromophthalein, sodium bromosulfalein), introduced by Rosenthal and White (1925), has proven to be superior to numerous other substances tested for this purpose.

Technique of the Bromsulfalein Test

(Reinhold and Hutchinson, 1951)

Principle.—A measured amount of bromsulfalein is injected intravenously. The liver rapidly removes the dye from its combination with plasma proteins and excretes it into the bile. If liver function is impaired, excretion is delayed and a larger proportion of the dye remains in the circulation. The dye concentration in serum is measured after addition of alcohol to convert it to its intensely colored sodium salt. Corrections are made for interference by turbidity and pigmentation of the serum. Solutions.—Sodium hydroxide solutions. Approximately 0.5N, and 0.05N. The 0.05N solution is prepared from 0.5N by diluting tenfold with water. Phenol tetrazolium bromsulfalein (sulfonate, sodium bromsulfalein). Standard solution.—Transmit 50 mg. to a 500 ml. volumetric flask. Add 250 ml. of the 0.05N sodium hydroxide solution and dilute to volume with water. (If crystalline bromsulfalein is not available, substitute 1 ml. of 5% solution from an ampoule.) The standard solution so prepared contains 10 mg. in 100 ml. and is equivalent to 100 per cent retention of dye when 5 mg. per kg. are injected. (It is assumed that plasma volume represents 5 per cent of the body weight.) This standard is further diluted by measuring 10, 25, and 50 ml. aliquots and diluting them to 100 ml. with 0.05N sodium hydroxide. The resulting standard solutions are equivalent to 1.0, 2.5, and 5.0 mg. per 100 ml. and represent 10, 25, and 50 per cent of the initial dye concentration. The standards remain unchanged over long periods. For actual measurement, 0.5 ml. of each working standard is diluted to 6 ml. with 0.05N sodium hydroxide.

Apparatus.—A photoelectric photometer is preferred to enable accurate measurement of solution concentrations and to enable correction to be made for interference from hemoglobin. However, approximate measurements can be made by means of visual comparators. When the latter are used additional standards are prepared so as to give concentration intervals of 0.5 mg. up to 3.0 mg., and at 1.0 mg. intervals above this.

Method.—Preparation of patients, injection of dye, and collection of blood:

The test is best done in the morning. The evening meal on the previous day should not have included foods rich in fat. A breakfast of uncrushed toast and coffee, tea, (without cream) or fruit juice is permissible after which the patient should not eat until the test has been completed.

The quantity of bromsulfalein required is calculated according to the patient’s ideal weight. See Table I. For children, divide the body weight in pounds by 22 to obtain the necessary volume in ml. This volume is introduced into a sterile syringe which is put aside ready for the injection. A vein is entered and about 5 ml. of blood withdrawn. This blood is used as a control specimen if necessary. The syringe containing dye is then attached to the needle and the dye injected slowly over a two minute period. Care should be taken that the dye does not escape into the tissues. 45 minutes later about 5 ml. of blood are collected from the vein of the opposite arm by means of a different syringe and needle. The exact time of completing the injection and of collecting the blood should be noted. The blood is allowed to clot and serum removed for analysis. Care is used to avoid hemolysis. Needle and syringe should be dry and the blood transferred from syringe to tube gently and without production of foam.

Measurement of dye concentration.—Measure 0.5 ml. of the dye containing serum into a cuvette containing 5.0 ml. of water. Add 0.5 ml. of 0.05N sodium hydroxide solution. Mix well and measure the absorbancy at wave length settings of 660, 655, and 430 millimicrons, using water for the zero settings at each wave length. Maximum absorption occurs at 580 millimicrons and this wave length setting is theoretically to be preferred, however, interference from hemoglobin is less marked at 565 millimicrons. If serum bilirubin is elevated, the control sample is diluted and absorbancy measured as described for the dye sample. The figures so obtained are subtracted from those of the bromsulfalein containing serum. Standards can be dispensed with once f has been determined, save for occasional treatment checks. The readings follow Beer’s Law.

Calculation.—Bromsulfalein mg./100 ml. = \( \frac{f_1 - 1.28\times f_{655} - 0.15\times f_{430} - 1.85\times f_{580}}{S_{580}} \)

Where \( f_{655} \), \( f_{680} \) and \( f_{430} \) represent absorbancies of the diluted serum at the wave lengths of the subscripts, and \( S_{580} \) the corresponding absorbancy of a standard. \( C \) is the concentration of the standard measured. \( f \) is calculated for each standard and since Beer’s law is obeyed the average of the several values obtained may be used.

Per cent retention = mg./100 ml. x 10.

Since hemoglobin even at a wave length of 565 mm may cause falsely high readings, a correction is applied in the method described to compensate for the presence of hemolysis.

The effect of haeemolysis in serum is eliminated by making a third absorbancy measurement on 640 millimicrons where neither bromsulfalein or hemoglobin absorb light*. The effects of bilirubin in serum may be overcome by use of a control sample collected before injection of dye. Zieve, Hansen and Hill (1951) have established a correction which may be used when the test is applied to jaundiced patients.

The use of actual instead of ideal weight in calculating dosages may cause erroneous results especially in the presence of ascites, edema, or oedema.

Rosenthal and White (1925) used 2.5 mg. per kg. of body weight. The 5 mg. per kg. dosage, however, has been adopted widely and provides a more sensitive and more precise test. The existence of an enterohepatic circulation of bromsulfalein (Lother and Shroy, 1952) does not appear to influence the results sufficiently to necessitate correction.

Note: It is advisable to question the patient concerning sensitivities to drugs. Reactions to bromsulfalein are rare but a few individuals do not tolerate it, and it may have effects so severe as to constitute a medical emergency. A preliminary skin test may be done. Obviously, the injection of bromsulfalein should be done only by a physician or under his immediate supervision.
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The time at which the post-injection blood specimen is collected is not of great importance, provided that appropriate standards of normal for that time are used. Motez, et al. (1940) introduced the 45 minute sampling time and it has proved to be satisfactory. Calculation of the rate of disappearance of bromsulfolaine by means of multiple specimens collected at 5 to 15 minute intervals after the injection has been advocated. The liver removes a constant fraction of the bromsulfolaine remaining in the circulating blood during any time interval (Bradley, 1940) and by collecting a series of blood specimens the accuracy of the estimate of the bromsulfolaine retained is improved. For a description of “clearance” studies of patients the reader is referred to papers by Lewie (1949), Lavoie et al. (1949) and Goodman (1952). Norcross, White and Bradley (1951), report that up to 9.8 per cent of injected bromsulfolaine may be lost in the urine within 45 minutes in patients with marked retention of dye. With lower concentrations of dye in serum the losses in the urine are much smaller.

Interpretation.–Healthy individuals after injection of 5 mg. of bromsulfolaine per kg. of body weight retain loss than 10 per cent at 30 minutes and 7.0 per cent at 45 minutes. At 60 minutes, no dye is retained.

Bromsulfolaine retention is generally accepted as the most sensitive and dependable among the laboratory procedures currently used to demonstrate involvement of the liver. It is especially helpful for evaluating suspect positive or positive results obtained by means of injection tests in the absence of hyperbilirubinemia. The bromsulfolaine test outscores all others in the proportion of positive tests found in Loevner’s cirrhosis. It has been among the most useful for following recovery from viral hepatitis and for detecting residual liver damage from this disease. It is probably the only procedure capable of detecting fatty liver, although it cannot be depended upon to do so consistently. For the study of the jaundiced patient the bromsulfolaine test has little to offer. Maximal retention occurs in the presence of severe liver damage, and further deterioration of the liver function can have no additional effect on dye retention. Thus it is rarely used when hyperbilirubinemia or clinical jaundice are present.

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Liver damage is not consistently accompanied by significantly lowered cholinesterase activity. Serial measurement of cholinesterase activity appears to provide a useful method for following the course of liver disease. It is of special value for study of the liver damage incident to deficient diets. Current articles dealing with the application of the study of liver disease include those of Mann et al. (1952), Fremont-Smith et al. (1952), Wilson et al. (1952) and Vorhaus and Kirk (1953).

Serum cholinesterase may be measured conveniently by the method of Michel (1940) or by a photometric adaptation of it (Reinhold et al., 1953).

Coproporphyrin in urine.—The output of coproporphyrin in urine rises markedly in patients suffering from various types of liver disease (Watson, Hawkinson et al. 1954; Watson, Sutherland, and Hawkinson, 1951). Measurement of coproporphyrin excretion provides a sensitive and valuable method for detecting liver damage that may escape detection by other methods. The excretion of coproporphyrin generally is within normal limits in persons who have biliary tract lesions but high values occur with sufficient frequency to impair its usefulness for differential diagnosis. Care must be used also to exclude other causes of increased porphyrin excretion, e.g. various drugs and intoxicants. An idiosyncratic coproporphyrinuria has been described (Watson, Schwartz, et al., 1949).

A simplified technique for determination of coproporphyrin has been described recently (Schwartz, Zieve, and Watson, 1951). The small amount of coproporphyrin in many normal samples of urine require the use of a highly sensitive fluorimeter for dependable measurements. The high cost of such instruments has been a deterrent to the wider use of porphyrin analysis. The improved method now available gives results that are more reproducible and reliable than those formerly used mainly because of the detection of porphyrin precursor in urine and their inclusion in the assay (Watson, de Mello, et al., 1951).

Coproporphyrin and his associates (see references above) have shown that the type of coproporphyrin excreted is related to the cause of liver disease. Thus Type I coproporphyrin predominates in the urine of patients ill with viral hepatitis, whereas the Type III predominates in cirrhosis of “alcoholic” origin. Analytically dependable separation of the isomers is difficult to accomplish and a more extensive application of this interesting finding will await improved and simplified methods.

Combined intravenous bromsulfolaine-kupurie acid-galactoside test.—Zieve, Hill, and Nashitt (1950) have devised a procedure for administration of the three substances simultaneously. They find that the simultaneous administration does not alter the behavior of the test substances.

Pepidase activity of human serum.—Fleisher and Boit (1953) have found tripeptidase activity to be increased in serum of patients with liver disease or with ob-
CHEMICAL EVALUATION OF THE FUNCTION OF THE LIVER - PART III

struction of the bile ducts. Bile shows marked tripeptidase activity. Hydrolysis of several digestives is decreased in liver disease, according to these authors.

Selection of procedures.-The choice of methods to be applied in the study of a patient with disease of the liver or biliary tract will vary according to the type of information sought. The following outline shows the principal purposes for which chemical methods are applied together with a list of the procedures likely to prove useful.

1. Detection of liver damage in absence of jaundice, e.g. early or subclinical hepatitis; urine bilirubin, bromsulphalein retention, direct and total bilirubin, flocculation tests (e.g. cephalin cholesterol flocculation, thymol turbidity and flocculation, zinc turbidity), urine urobilinogen.

2. Detection of residual liver damage, "recovery" stages of hepatitis, chronic persistent or chronic active hepatitis; bromsulphalein, direct and total bilirubin, flocculation tests, serum albumin and globulin, prothrombin, serum cholesterol, urine urobilinogen and coproporphyrin.

3. Following the course of the jaundiced patient suffering from parenchymatous diseases; serum direct and total bilirubin, flocculation tests, serum albumin and globulin, prothrombin. In addition, if severe, serum esterified cholesterol or cholesterol ester (Blood urea N or NPN, glucose, and serum electrolytes, also are important).

4. Differentiation of jaundice due to bilirinary disease from that due to parenchymatous diseases; serum alkaline phosphatase, cephalin-cholesterol flocculation and thymol turbidity, g-Glutathione tolerance, prothrombin response, repeated fecal urobilinogen tests.

5. Differentiation of extra hepatic biliary obstruction due to calculi from that due to neoplasms, stricture, etc. feces urobilinogen.

6. Follow the course of the surgical patient with disease of the biliary tract; plasma prothrombin, phosphatase, serum direct and total bilirubin, albumin and globulin, electrophoresis, blood urea N or NPN, and serum lipids.

7. Differentiation of hemolytic jaundice: serum direct and total bilirubin, feces urobilinogen, erythrocyte fragility, reticulocyte count.

A decision regarding the number of tests to be used required experience and judgment. The information gained increases as the number of tests increases but with rapidly diminishing returns beyond an optimum that will vary in different patients. Usually, the tests in italics will suffice for an initial study, with additional requests to be made if further information is needed. To apply simultaneously the entire group of tests listed in any of the categories would seldom be justified.

Comparatively little has been done to evaluate the advantage gained by the use of two or more tests in combination. Gutman and Hanger (1941) found that serum phosphatase and cephalin cholesterol flocculation supplemented each other for the diagnosis of common duct disease and that combining the findings of the two tests substantially improved the accuracy. MacLagan (1947) compared a number of tests singly and in pairs. Thymol turbidity alone gave correct diagnoses in 22 per cent of cases of liver disease that could be unequivocally classified as either obstruction or parenchymal. Serum alkaline phosphatase was correct in 45 per cent. However, a combination of thymol turbidity and alkaline phosphatase gave correct diagnoses in 79 per cent.

Wootton, King and MacLagan Smith (1951) cite a statistical analysis by R. A. Fisher in which he used the method of discriminant functions to compare the effectiveness of four tests applied to jaundiced patients with that of two tests. The four test group yielded only slightly more reliable information than a properly selected pair of tests. It is possible, therefore, to eliminate some procedures.

The practice in clinics where interest is centered on the study of liver disease, varies with respect to selection of the flocculation tests that are regularly used. Reliance on a single test is infrequent. Usually two or three tests are used. Probably the most frequent combination is the thymol test (MacLagan, 1944) and cephalin-cholesterol flocculation (Hanger, 1949). Often the zinc turbidity (Kunkel, 1947) or the ammonium sulfate turbidity (Hueper and Popper, 1949) are included. The colloidal red test (Ducci, 1947) gives results resembling closely those of the colloidal gold test applied to serum. The latter, according to MacLagan (1951) largely duplicates theampler tests and thus no longer performs a useful service. Neese and et al (1950) found no need for routine use of the colloidal red test in the study of viral hepatitis. A few clinics routinely use five or six tests, but this is done largely for the sake of comparison and evaluation of the tests.

A comparison of a group of tests with respect to their behavior during the onset of viral hepatitis induced in volunteer subjects has been made by Neese and Reinhold (1948). Seven tests were abnormal in 95 per cent or more of the patients. These were the one minute direct bilirubin, urine bilirubin by the Harrison spot method, cephalin cholesterol flocculation, thymol turbidity, thymol flocculation, colloidal gold and bromsulphalein retention. Thus any of the seven might have served. However, the study showed that these tests differed distinctly in the times at which they first became positive. The bromsulphalein test showed a striking advantage in this respect and urine bilirubin ranked second. Had the reliance been placed only upon the thymol test, the hepatitis of some of the individuals might have escaped detection.

The ranking of the same group of tests in the same subjects was distinctly different when they were evaluated according to their ability to disclose persistence of the disease or its sequelae. In general the order is now reversed with the tests depending on changes in serum proteins showing a greater tendency to persist than those based on excretory function. Again dependence on a single test of either group would have meant failure to detect change in some patients. Furthermore, bromsulfalein retention persisted in some patients long after flocculation tests had become normal so that dependence on the latter alone may fail to demonstrate liver damage in these circumstances. It appears indisputable therefore to chart too severely the number of procedures used, particularly when a wide variety of clinical material is being studied.

Quite frequently patients are encountered with equivocal signs of involvement of the liver which cannot be conclusively demonstrated either by clinical examination or laboratory studies. In such circumstances biopsy of the liver done either in the course of surgical exploration or by needle is considered essential. Besides providing helpful information about such individuals, the widespread use of liver biopsy has greatly increased the understanding of liver disease. It has also enabled correlations to be made between the results of laboratory studies and the appearance of the liver under the microscope. A number of such studies have shown that there is a general correlation between the severity of the changes in the liver and the degree of change in the chemical tests used.

Popper (1951) found that the cephalin-cholesterol flocculation, thymol turbidity and serum albumin and globulin concentrations showed a statistically significant correlation between degree of liver cell destruction and degree of chemical abnormality. No significant correlation was found with phosphatase, prothrombin activity, urine urobilinogen, serum total cholesterol, or blood sedimentation rate.

At times the results of chemical studies will be within normal limits despite the existence of pathologic changes in the sample of liver tissue. Frequently however the results of the chemical studies will be abnormal in the absence of conclusive indications of abnormality in the liver sections. There are a number of explanations for such discrepancies, among them the possibility of sampling errors in removing the 10 mg. (or less) sample of liver that a needle biopsy yields. It is probable also that methods of staining and examining sections of liver fail to demonstrate changes in cell content or structure. The present utility of each method for the study of liver disease will be realized if liver biopsy and chemical studies are coordinat-
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<td>Viral hepatitis without jaundice</td>
<td>May be present in urine and may increase slightly in serum. Urobilinogen may increase in urine.</td>
<td>BSF usually abnormal, CCF, TT, TF, ZT may be abnormal</td>
<td>Normal</td>
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<td>Viral hepatitis with jaundice</td>
<td>Increased in serum and urine. Urobilinogen generally increased in urine and feces but may be absent.</td>
<td>Abnormal</td>
<td>Mainly normal but may be abnormal</td>
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<td>&quot;Toxic&quot; hepatitis: cholangiitic jaundice</td>
<td>Increased in serum and urine. Urobilinogen variable.</td>
<td>CCF, TTT, TF, ZT may be normal or abnormal A/G usually normal.</td>
<td>Increased serum phosphatase, lipids. Similar to extrahepatic obstruction.</td>
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<td>Laennec's cirrhosis</td>
<td>May or may not be abnormal in serum and urine. Urobilinogen variable.</td>
<td>BSF abnormal, CCF, TT, TF or ZT abnormal in about 3/4, A/G abnormal.</td>
<td>Variable. Phosphatase may be high.</td>
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<td>Extrahepatic obstruction, Partial.</td>
<td>Variable. Intermittent or continuous elevation.</td>
<td>Generally normal, but liver parenchyma may become injured and tests positive.</td>
<td>Variable but with elevated serum phosphatase and lipids. Good response to vitamin K.</td>
</tr>
<tr>
<td>Biliary cirrhosis</td>
<td>Bilirubin elevated. Urobilinogen variable.</td>
<td>BSF increased, A/G abnormal. TT increased. CCF about 1/2 increased.</td>
<td>Elevated phosphatase. Marked elevation of serum lipid especially phospholipid.</td>
</tr>
<tr>
<td>Hemolytic jaundice</td>
<td>Total serum bilirubin moderately elevated; 1 minute bilirubin normal or slightly increased. Urine bilirubin negative. Feces urobilinogen increased urine urobilinogen often normal.</td>
<td>Seldom abnormal but may become so due to hypoxia, or other complications.</td>
<td>Seldom abnormal. Pigment stones may cause biliary obstruction.</td>
</tr>
</tbody>
</table>

Abbreviations: BSF, bromosulfalein; A, serum albumin; G, serum globulin; CCF, cephalin-cholesterol flocculation; TT, thymol turbidity; TF, thymol flocculation; ZT, zinc turbidity.

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**ADDENDA**

*Standardization of thymol and other turbidity tests.*** Shortly after the first section of this paper was sent to the printer, it was discovered that the turbidity produced by reaction of the thymol reagent with serum rich in lipid differed in its optical properties from that produced by reaction with the protein components. The value of 6.5 units assigned to the glass suspension having an absorbancy of 0.10 corresponded more nearly with the turbidity caused by lipid. The preparation of the glass standard has been altered to give a particle size distribution that represents more nearly a median of the particle sizes produced in the two types of turbidity-producing reactions. This is done by allowing the suspended glass to sediment only three days instead of fourteen days and taking the upper 300 ml instead of the upper 500 ml. An absorbancy of 0.10 then corresponds to approximately 4.7 Shamb-Hougaard units, but the final factor assigned will be derived from a survey now in progress. A report describing the characteristics of colloidal glass suspensions including these recent observations is to be submitted to Analytical Chemistry for...
publication. The results of surveys of various laboratories made for the purpose of assigning a value to the glass standard will be submitted to the American Journal of Clinical Pathology.

Mechanism of the thymol test: Dr. Margaret Kaser has pointed out to the writer that the rule of beta globulins in the thymol test is not specifically mentioned. The work of Recent, Chorafos, and Hanger (Proc. Soc. Expt. Biol. and Med. 60:245 (1945), of Cohen and Thompson (J. Lab. Clin. Med. 32:475 (1947)), and of Kunkel and Horgan, (J. Clin. Invest., 27:1000 (1947)) indicates that beta globulins are fluid associated with beta globulins contribute to the turbidity. There is, however, an impressive amount of information, both clinical and experimental, indicating that gamma globulin is an important reactant. In view of the recent comprehensive review of the mechanism of the flocculation test by Saifer, the author presumably refers to detailed discussion of this aspect.

Amino acids in blood in liver disease: The failure of Murphy and associates to find elevated concentrations of amino acids in blood in patients with hepatic coma (N. Eng. J. Med. 239:703 (1949)) should have been mentioned on page 23. This observation has been confirmed by others, and it appears that elevation of amino acids in blood is by no means a consistent occurrence in patients with severely damaged livers.

Morphology of the diseased liver: An outstanding review of this subject by Dr. Hans Popper has recently appeared in Am. J. Med. 16:98 (1954).

Acknowledgement

This article is an amplification of similar articles on the same subject prepared by the author for "Practical Physiological Chemistry, 13th Edition" by Chave, Oser and Sumnerman and for "Laboratory Methods: Clinical and General" by Simmon and Gentzow. Their courtesy in consenting to its separate publication is greatly appreciated.

The author acknowledges with thanks the assistance of Miss C. A. J. von Frijtag Drabbe with the manuscript and of Dr. David Seligson for a number of helpful suggestions.

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PLASMA PROTEINS AND PLASMA SUBSTITUTES

By Kurt G. Stem

Blood plasma represents a polymeric system of proteins ranging in molecular weight from 70,000 (serum albumin) to 1,200,000 (lipoalbumin) in shape from spheres (beta lipoprotein) to elongated rods (fibrinogen). Serum albumin, owing to its relatively low molecular weight and high concentration, is largely responsible for the maintenance of the normal colloidal-osmotic pressure and, hence, of the volume of blood plasma.

Plasma substitutes are designed to replace temporarily loss of plasma proteins due to hemorrhage or leakage through the kidneys. They combat shock through the maintenance or restoration of the normal plasma volume. A satisfactory plasma substitute or plasma expander should be compatible with the plasma proteins as well as the formed elements of blood and should remain in the circulation for a reasonable period of time following administration and subsequently be either excreted or metabolized. Its viscosity should be closer to that of plasma and its osmotic pressure should approach that of serum albumin. It should not be deposited in the tissues of the rat and be used in the rat to determine whether it should not give rise to allergic or anaphylactic reactions.

Of the considerable number of colloids which have been proposed as plasma expanders (gum arabic, gelatin, starches, bovine plasma albumin, polyethylene glycol, etc.; dextran and polysulfonblastics (P/S) have been the most widely accepted substances.

Dextran is prepared for clinical use by controlled degradation and fractionation of a group of high-polymer polysaccharides which are produced from sucrose by certain strains of the lactic bacillus (Leuconostoc mesenteroides) on suitable culture media. An enzyme system, called dextran-sucrase, splits the sucrose into glucose and fructose and then incorporates the glucose moiety into dextran chains containing 1.6 linkages, with branches attached to the main chain through 1.4 linkages. This synthesis takes place, both in the presence of viable microorganisms (whole culture methods) and in cell-free extracts containing purified enzyme. Free glucose is not utilized for dextran formation while sucrose is quantitatively converted into the polymer of many millions molecular weight. The latter may be controlled, within certain limits, through the addition of suitable glucosyl acceptors to the enzyme-substrate system. High-polymer dextran may be depolymerized by dilute acid at elevated temperatures, thermal degradation, sonic and ultrasonic oscillations, and by an enzyme, called dextranase, produced by certain molds. The dextran hydrolyzates are then fractionated by means of acetone, methanol, or ethanol under carefully controlled conditions. The fraction possessing an average molecular weight of 75,000 (25,000) and an intrinsic viscosity of 0.23 at 0.05 % is employed as a plasma expander in the form of 6 per cent solutions in physiological saline. It is considered undesirable to have appreciable amounts of particles of over 200,000 and under 25,000 molecular weight present in these clinical preparations.

The results of physical-chemical studies on high-polymer and partially depolymerized dextran preparations, performed with the aid of the analytical ultracentrifuge, diffusion and electrophoresis apparatus, the electron microscope and light-scattering photometer were discussed in relation to the chemical structure of this group of polymers.

FEDERATION DINNER

The impromptu Federation Dinner meetings of AACC members attending the Federation Meetings once again showed that the AACC membership has become a strong fraternity. More than fifty members attended the dinner held at the Hotel Jefferson, Atlantic City, on the evening of April 14. The dinner featured two speakers. Walter R. Bloom, invited the members to attend the Federation meeting in 1955 which will be held in California, and told of his experiences in crossing the country to California in 1916 by automobile. He invited the members to do the same now that in 1955 the facilities are better. Arthur Knudson, Albany Medical College, then recounted his experiences in Thailand. Dr. Knudson recently returned from the Far East after spending more than two years with an American medical group sent to that country to train and install modern medical teaching facilities in that country.
PONTACYL STANDARDS FOR THE BIURET METHOD FOR TOTAL SERUM PROTEIN

Daniel Sanshuk and Monroe E. Freeman
(from the Department of Biochemistry, Walter Reed Army Medical Center,
Army Medical Service Graduate School, Washington 12, D. C.)

Presented before the Division of Biological Chemistry at the 124th Meeting of the American Chemical Society, Chicago, Ill.

The general acceptance of the biuret procedure, (1-4) for total serum protein, has suggested the need for a standard that is more convenient, reproducible, and permanent than the "normal" pooled sera commonly used. Several alternatives have been tested in this laboratory, including: concentrated normal human serum albumin, salt poor; biuret procured from Delta Chemical Co., New York; p-nitrobenzene diazo reagent; and mixtures of pontacyl carmine 2B and pontacyl violet 6R. The pontacyl dyes have proved most satisfactory when employed in the following manner:

EXPERIMENTAL. 88 mg. of pontacyl violet 6R, concentration 150 per cent and 15 mg. of pontacyl carmine 2B3 were dissolved in distilled water, diluted to 1000 ml., stored as a stock solution for all subsequent working standard solutions.

In Table 1 are the absorption curves for a pontacyl dye standard and a 5 per cent albumin-biuret solution as determined in a Beckman DU Spectrophotometer. The pontacyl standard was prepared by diluting 2 ml. of the stock solution with 8 ml. of distilled water and read against a blank of distilled water. The albumin-biuret solution was prepared from a 5 per cent albumin solution and alkaline copper sulfate in the usual manner and read against a reagent blank of distilled water and alkaline copper sulfate. On both curves the well-defined maxima at 549 μ and minima at 440-450 μ demonstrate the suitable absorption characteristics of this mixture of pontacyl dyes.

![Absorption curve for pontacyl dye standard solution and 5% albumin-biuret solution as determined in Beckman DU Spectrophotometer.](image)

**TABLE 1**

Concentrations and Optical Density of Standard Pontacyl

<table>
<thead>
<tr>
<th>Pontacyl Standard</th>
<th>Protein-biuret Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilutions 1</td>
<td>2</td>
</tr>
<tr>
<td>Pontacyl conc. mg. per ml.</td>
<td>Optical Density</td>
</tr>
<tr>
<td>Optical Density</td>
<td>Optical Density</td>
</tr>
<tr>
<td>0.5 ml. diluted to 10</td>
<td>.0015 mg.</td>
</tr>
<tr>
<td>1.0 ml. diluted to 10</td>
<td>.0030 mg.</td>
</tr>
<tr>
<td>1.5 ml. diluted to 10</td>
<td>.0045 mg.</td>
</tr>
<tr>
<td>2.0 ml. diluted to 10</td>
<td>.0060 mg.</td>
</tr>
<tr>
<td>2.5 ml. diluted to 10</td>
<td>.0075 mg.</td>
</tr>
<tr>
<td>3.0 ml. diluted to 10</td>
<td>.0090 mg.</td>
</tr>
<tr>
<td>3.5 ml. diluted to 10</td>
<td>.0105 mg.</td>
</tr>
<tr>
<td>4.0 ml. diluted to 10</td>
<td>.0120 mg.</td>
</tr>
<tr>
<td>4.5 ml. diluted to 10</td>
<td>.0135 mg.</td>
</tr>
<tr>
<td>5.0 ml. diluted to 10</td>
<td>.0150 mg.</td>
</tr>
</tbody>
</table>

1. 39 ml. stock solution diluted to 100 ml., then diluted as indicated with distilled water.

2. Average of numerous samples of normal pooled sera and albumin solutions.

3. Protein or pooled "normal" sera standardized by Kjeldahl determination and treated with biuret reagent in usual manner.

The conformance of appropriate dilutions to Beer's law and close agreement with the protein concentration of biuret samples is shown in Table 1 and Figure 2. The pontacyl standards were prepared by diluting 30 ml. of stock solution to 100 ml. with distilled water followed by the dilutions indicated in Table 1. The optical density and concentrations of the protein-biuret samples in this table are the averages of numerous samples of pooled sera and albumin solutions standardized by Kjeldahl analysis. In Figure 2, optical density of samples in 19 mm x 150 mm cuvettes in a Coleman Jr. Spectrophotometer No. 6 at wavelength 540 μ has been plotted against the protein concentration of the biuret samples and against the pontacyl concentration.

These successive batches of these dyes have been secured at intervals over a period of two years with only minor differences noted among these lots.

The recommended procedure for serum protein determination with the pontacyl standards is to prepare standards as indicated above and verify appropriate dilution schedule against a series of standard protein solutions as shown in Table 1. In our experience, 0.105 mg. of the dye mixture in 10 ml. of distilled water has the same optical density as 7 per cent serum protein, treated with biuret reagent in the usual manner. The pontacyl solutions, once standardized, against proteins-biuret solutions of known concentration can be sealed in curvettes and kept for long periods of time without change in optical density.

Results may be calculated from standard curves, Figure 2, or by usual procedures such as:

**Optical Density of Unknown × g per cm. per 100 ml.**

**Optical Density of Standard × 7 g per cm.**

**Summary.** Standard solutions of pontacyl dyes have been tested as spectrophotometric standards for the determination of
PONTACYL STANDARDS

serum proteins, by the bluer procedures with good success. Absorption characteristics over the critical wavelength range for this determination were satisfactory. A linear relation was found between dye concentrations and protein concentration (bluer) from 1 to 10 per cent. The particular advantage of the pontacyl standards is the definite composition of the standard solutions as compared to the variability and uncertainty of pooled "normal" sera, and the stability and permanence of pontacyl standards as compared to the perishable nature of the pooled sera or protein solutions used as primary standards.

Using this procedure it would be unnecessary to determine proteins by the Kjeldahl method in order to set up a curve as is done at present.

REFERENCES


REVIEW OF CURRENT LITERATURE

ELLENMAE VIERGIVER — EDITOR
CECILIA RIEGEL, C. VON FRUITAGE DRABBE, HARRY G. ANRODE


In rats and in humans, the serum phosphatase levels were significantly lowered by fasting. This drop in phosphatase activity could be observed in both sera and urine, but not by feeding either carbohydrates or proteins. This study indicates that the serum alkaline phosphatase activity is associated with enzyme activity of other organs as well as bone.

A SIMPLE PROCEDURE FOR SEPARATION OF PROTHROMBIN AND ACCELERATOR GLOBULIN FROM CIRCUITATED HUMAN PLASMA. M. L. Lewis, C. V. G. Ware (Dept. of Biochemistry & Nutrition, School of Medicine, Univ. of Southern California and the Los Angeles County Hospital, Los Angeles). Proc. Soc. Exp. Biol. & Med. 84: 639-646, 1953.

A method is described for the preparation of purified prothrombin and of partially purified accelerator globulin from serum samples of 10-100 cc. of fresh citrated plasma. The procedure is easily completed in one day.

PYRUVATE ACCUMULATION IN PRESERVED BLOOD. Marcel C. Blaschko and S. L. Baldwin (Univ. Manitoba, Winnipeg, Can.). J. Appl. Physiol. 6: 3-14, 1953.

A rise in pyruvate occurs in stored blood kept at 5°C in an acid citrate-dextrose solution. An initial rapid rise in pyruvate is followed by the rapid disappearance of most of the 2,3-diphosphoglycerate from the erythrocytes and is followed by a sustained rise in pyruvate which could be partially inhibited by pyruvate oxidase. The blood was stored in an unstirred hemoglobin incubation.

A second increase was observed in erythrocyte pyruvate kinase activity and could be inhibited partially by pyruvate oxidase.


A method is presented which is specific for pyruvate kinase activity.


A method is presented which is specific for pyruvate kinase activity.


Alkaline phosphatase from dog intestine was prepared by fractional precipitation with acetone and digestion with trypsin.

Antibodies were obtained by injection of the antigen in white male rabbits. Tests for homogeneity of immune serum antibodies were performed by the agar diffusion technique. Both enzymes and antibodies showed considerable heterogeneity. The antigen appears to be a mixture of phosphatases.

The catalytic site of the major phosphatase component is not involved or blocked by combination with its antibody. The minor phosphatase component however, loses its activity in the reaction with its antibody.

C. V. G. W.


Intestinal, kidney and liver phosphatase was prepared by fractional precipitation with alcohol.

Study of the response to inhibition showed that kidney and liver phosphatase are inhibited approximately 50 per cent by taurocholate, whereas intestinal phosphatase is not. There is little difference in behavior of the three phosphatases in other respects.

Immunohemolytic differences as tested by the precipitin reaction with anti-intestinal phosphatase serum showed complete precipitation of intestinal phosphatase and no precipitation of kidney and liver phosphatase.

C. V. G. W.

A cleavage procedure in which acridine is used as described. If hydrochloric acid is used, maximal cleavage of the different heme proteins occurs at a pH which is different for each protein. This property may be used to characterize and to judge the purity of heme proteins. The latter in cases where the chromatoprotein contains one heme per molecule.

C. V. F. D.


Experiments, in which deuterium was used as a tracer, indicate that reduction of DPN occurs in the porin position rather than in the ethyl position as previously assumed.

The conclusions of previous investigations, concerning the mechanism and stereospecificity of DPN reduction, have been confirmed.

C. V. F. D.


Azo dye T-1824 is readily reducible at the dropping mercury cathode. The 2 azo groups are reduced to hydrazo groups. The half wave potential shifts to more negative values as the pH increases.

Free T-1824 gives a distinct polarographic reduction curve. The protein-bound dye is inactive.

C. V. F. D.


Four enzyme systems were used for parallel assays of organic pyrophosphates in human blood: potato asparagine, yeast hexokinase, myokinase and 5-adenylic acid deaminase.

The organic pyrophosphates are composed mainly of ATP, and traces of AMP and ADP included in an unidentified fraction.

The concentrations of the various organic pyrophosphates were determined by measuring the inorganic P released from the compounds by the action of the specific enzymes described above. The organic pyrophosphate fraction was also determined by the Lohmann method of acid hydrolysis.

C. V. F. D.

BODY COMPARTMENTS, THEIR MEASUREMENT AND APPLICATION TO CLINICAL MEDICINE. George J. Hanburg and Stuart Uralch (Ohio State Univ., Columbus). Metabolism 2, 391-433 (1953).

Measurements were made, on 39 patients, of body water (Total and extracellular), total cell mass, units of fat and minerals, and lean body mass. Variations in body composition, results of starvation, obesity, diabetes, or old age are described.

H.A.


The aldolase in normal adults and children was found to be 0.30 and 0.57 Meyerhof units, resp., as analyzed by Sibley-Lehninger method (C.A. 43, 7541). In 92% of myopathic patients these values were found to be 10 to 30 times as high as in the controls.

H.A.


A simple dialysis technique is presented for removing interfering chromogenic material without loss of uropepsin. The uropepsin is then determined using the hemoglobin substrate procedure.

E.V.


The authors found that with increasing age there is a statistically significant decrease in the titers of androgenic activity and in the livers obtained by colorimetric assay. It would appear that the major portion of the decrease in testicular secretion since most of the decrease is in the alpha fraction, which contains the chief metabolites of testicular secretion.

E.V.


Various alcohol-water mixtures were used as solvent systems. Sorb techniques were employed to detect phosphate, choline ester and unsaturated groupings.
REVIEW OF CURRENT LITERATURE


A procedure is described for hyaluronidase determination in crude extracts of rat testis. To different concentrations of enzyme, substrate is added (prepared from human umbilical cords), followed by addition of color reagent. The color reagent is composed of plasmas, citrate-phosphate buffer, bromosulfalein, and urea.

An improved technique for extraction of testicular homogenate is described, resulting in a much higher yield of enzyme.

C. V. F. D.

THE BINDING OF STEROIDS TO PROTEIN. 1. SOLUBILITY DETERMINA-

Reversible combination occurs between cholesterol and a large group of steroids studied (except cholesteryl). The strength of the binding bears a inverse relationship to the number of polar groups.

The solubilities of testosterone in several plasma substitutes are reported. Only modified beef globin showed an appreciable affinity for testosterone.

The binding of steroid hormones with albumin explains their low concentrations in urine, while the poor binding and high aqueous solubility of the conjugates which the metabolic products form with glucuronic and sulfuric acid explain their rapid excretion.

C. V. F. D.


The tablet containing sodium sulfanilic and sodium acetate is substituted for the usual sulfanilic acid-sodium acetate solution.

C. H. R.


Significant differences in the purine and pyrimidine contents of DNA and RNA were observed in the uterus, liver, spleen of rats who were maintained on varying levels of steroid sex hormones.

Significant variations were observed in the proportions of adenine and guanine in RNA of human endometrium in various phases of the menstrual cycle.

C. V. F. D.


Lysine and tryptophan are indispensable dietary components. Deficiency in these amino acids is promptly followed by a pronounced negative nitrogen balance.

Arginine and histidine are nonessential.

Final classification of the amino acids with respect to their role in nitrogen equilibrium in adult man is summarized.

C. V. F. D.

MICRO FLAME PHOTOMETRIC DETERMINATION OF BOD t, POTASSIUM, CALCIUM IN SERUM WITH ORGANIC SOLVENTS. G. R. Kingsley & R. A. Schuette (Dept. of Physiological Chemistry School of Medicine, University of California at Los Angeles, and the Clinical Biochemistry Laboratory, Veterans Administration Hospital, Los Angeles, Calif.). J. Biol. Chem. 206:607-815, 1954.

The use of a solvent mixture of acetone, glacial acetic acid and an aqueous solution of Sterox (non-ionic wetting agent) greatly enhanced the emission spectra of sodium, potassium and calcium. Flame photometers used were the Beckman Model DU and Model B.

E. V.


The precipitation of protein by tungstic acid is maximal and filtrates of serum, plasma or blood are clear when the pH of the filtrate is 5.1 or less. The pH of random samples of blood and serum ranged from 3.2 to 4.7 with a mean of 4.1. Although pH of the filtrates increased with increase in protein concentration of the blood, protein precipitation was maximal and filtrates were clear over the range of protein encountered clinically.

E. V.


This method for determination of isonicotinic acid hydrazide (isoniazid) is based upon the reaction resulting from condensation of isoniazid with glucosamine aldehyde formed by alkaline hydrolysis of 4-pyridylpyridinium dichloride. The color intensity conforms to Lambert-Beer's law and is reproducible and sensitive.

E. V.


Deposition of fat in the livers of weanling rats may be reduced by adding protein or threonine to a diet low in casein and containing choline.

Glycine, serine and histidine have an effect which is less marked but similar to that of threonine.

C. V. F. D.

THE REACTION OF PYRININE NUCLEOTIDES WITH CARBONYLYL COM-
Pounds. R. M. Burton and N. O. Krap-
lan (McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Maryland). J. Biol. Chem. 206:283, 1954.

Cytocytoxycyclase (DHA) and similar alpha-
ketol-carbonyl compounds react with diphosphorredy nucleotide (and related N-incarboxyl derivatives) to form products closely resembling DPNL. The product formed, fluoresce, is acid-labile, and has a spectrum which is identical to that of DPNL. It differs from DPNH in enzyme activity.

Acid decomposition results in amino diphosphate ribose and a nicotinamide derivative with an absorption maximum at 290 m.

The DPN-DHA complex is believed to be an addition product with the carbonyl base added to carbon 4 of DPN.

C. V. F. D.

FLAME ANALYSIS OF SODIUM AND POTASSIUM, IN SMALL VOLUMES OF SERUM, HEPARINIZED PLASMA AND CEREBROSPINAL FLUID. R. F. Bor-

50 and 20 cu. mm. samples (in silicones calibrated Suhl pipettes) were used with a reproducibility of ±1.7% for Na and ±3.2% for K.

C. R.

INVESTIGATION OF SERUM PROTEIN PATTERNS IN PATIENTS UNDERGO-
ING OPERATION. C. Hoch-Ligeti, K. Irvine and E. P. Sprinkle (Dept. of Pathology, School of Medicine, University of Virginia, Charlottesville, Va.). Proc. Soc. Exp. Biol. & Med. 84:707-710, 1953.

Serum protein patterns were determined by paper strip electrophoresis in 45 patients undergoing surgery. There was a significant decrease in the albumin and an increase in the alpha 1 and alpha 2 globulin fractions within 24 hours in one third of the cases, and in 80% of all cases within 4 days. The beta globulins remained unchanged in 82% and the gamma globulins in 61% of all cases. Total proteins did not change significantly. All components thereafter tended to return to preoperative levels.

E. V.
LOCAL SECTIONS

BOSTON SECTION

The Boston Section held its fifth meeting of the current season on the evening of February 17th, at the New England Medical Center. The speaker of the evening, Mr. William Reddy of Dr. Thorn’s Laboratory at the Peter Bent Brigham Hospital, spoke on “An Evolution of Methods for 17-Ketosteroids.”

Mr. Reddy prefaced his talk with an excellent review of the biological origin of the ketosteroids, and their nomenclature.

Of the sixty different steroids isolated from urine, androstosterone and etiocholanolone constitute about fifty percent. These have their origin in both the testicle and adrenal cortex, whereas other sources may exist, the speaker added. Detoxification by the liver causes excretion of these steroids as both sulfate and glucuronic acid conjugates, in which forms they exist in the urine.

In the methodology of the ketosteroids, it is the splitting of these conjugates which has caused great difficulty. Hot acid hydrolysis, the most common method, unfortunately produces structural alterations, which according to the speaker introduces an error which may be 30–40 percent lower. Milder hydrolytic agents as barium hydroxide and enzymes have been used.

Following hydrolysis, extraction is ideally done with peroxide-free ether. Caution should be used when evaporation of the ether extract to dryness, since the ketosteroids are sensitive to heat. Reduced temperature should be used; some workers even advocate exclusion of oxygen.

For the ultimate color reaction; Zimmerman’s Reaction is usually done, although Girard’s reagent which reacts with keto groups in the three and seventeen position may be used. Diphenylboric acid may be used to separate alpha and beta ketosteroids, although this affords no real clinical significance Mr. Reddy said.

Final evaluation of 17-ketosteroid output by the clinician is difficult however, since their chief source is both the testicle and the adrenal, and it is therefore conceivable that a normal value could result if the testicular output were depressed and the adrenal increased. For this reason, the clinician is more likely to be interested in a significant departure from normal.

Some differentiation is possible by determining dehydroepiandrosterone which is an index of adrenal activity. This may be measured by using the Pettitkofer reaction.

WASHINGTON – BALTIMORE RICHMOND SECTION

The first meeting of the 1953–54 year of the Washington-Baltimore-Richmond Section was held October 29, 1953 at the Clinical Center, National Institutes of Health, Bethesda, Md. Dr. Kurt Stern of Brooklyn Polytechnic Institute, Brooklyn, N.Y., discussed plasma proteins and plasma substitutes. An abstract of his talk is published on page 27 of this issue.

The second meeting was held January 21 at Georgetown University, Washington, D.C. The program was conducted by Dr. Ralph E. Peterson and Dr. Erich Heintzmann of the National Institutes of Health who spoke on newer methods in microanalysis of the 17-ketosteroids and corticosteroids in biological fluids. Dr. Peterson described a new modification of the aequous-alkaline Zimmermann urinary 17-ketosteroid method. This method utilizes a more selective extracting solvent for removal of the 17-ketosteroids from acid hydrolyzed urine, and improves the specificity of the m-dinitrobenzene reaction by a partitioning of the final reaction product between 50% ethanol and methylene chloride. This results in a method of improved selectivity for the determination of the hydroxylated C 19 17-ketosteroids in the urine. An improved and simple procedure for the determination of the plasma corticosteroids was presented. This is a modification of a new and unpublished phthalhydrin method of Silber, which employs a sulfuric acid-ethanol extraction of the corticosteroids from methylene chloride extract of plasma.

Dr. Heintzmann described a new method for inactivating the acid hydrolyzed urinary corticosteroids with column chromatography. This procedure employs adsorption of the steroids on silica gel, and utilizes the principle of ‘gradient elution’ of the adsorbed compounds.

The third meeting was held March 18 at the Army Medical Center, Washington, D.C. Lt. Col. Emmet L. Durrum, Army Medical Service Graduate School, discussed newer techniques in paper electrophoresis. He traced the development of this field from its inception in 1937, at which time its value was obscured by Tiselius's more spectacular moving boundary electrophoretic technique, to its present day recognition. The different types of apparatus and densitometers for analyzing the patterns were mentioned. Following the formal talk a long discussion ensued in which many technical points were covered. Refreshments were then served.

CHICAGO SECTION

The first meeting of the Chicago Section of the American Association of Clinical Chemists for the 1953–54 season was held on November 10, 1953 at St. Luke’s Hospital. Dr. Samuel Natelson of Rockford Memorial Hospital discussed and demonstrated various microtechniques in clinical chemistry. Most of the apparatus demonstrated was obtained through the courtesy of the Scientific Products Division of the American Hospital Supply Corp., Inc.

A short business meeting was held following Dr. Natelson’s talk. The results of the election of officers were as follows:

President: Harry F. Weisberg
Vice-President: Alvin Dubin
Secretary: Robert S. Meilville
Treasurer: Alex Kaplan

The second meeting of the current year was held on January 15, 1954 at the Sarah Morris Hospital for Children at Michael Reese Hospital. Dr. Harold Persky, Chief, Biochemical Division, Institute for Psychiatric and Psychosomatic Training spoke on the subject: Chemical Indices of Psychological Stress.

The third meeting was held at Northwestern University Medical School on February 26, 1954. Dr. John A. D. Cooper, Director of the Radioisotope Unit and Associate Professor of Biochemistry presented a very comprehensive review of the subject “Radioisotopes in Clinical Medicine.”
LOCAL SECTIONS (cont'd)

NEW YORK SECTION

Abraham Saffer, Jewish Sanitarium and Hospital for Chronic Diseases, and Secretary-Treasurer of the Metropolitan New York Section of the AACC has announced the results of the recent election for officers of the section.

Chairman: Bernard Klein, Bronx V.A. Hospital.
Vice Chairman: Julius Carr, Mt. Sinai Hospital.
Secretary-Treasurer: Abraham Saffer.
Executive Committee: Kurt G. Stern, Israel Klein, Gerta Mayer, Mary E. McKenna, Emil Baumann, and Gerald Dobkin.

The last meeting of the section was held on March 23, at the New York Academy of Sciences Building. It featured a symposium on "Lipoproteins," which drew a capacity audience of both members and visitors.

John L. Oncley, Harvard University Medical School, spoke on "Physical and Chemical Aspects of Lipoproteins" and David P. Barr, Cornell University Medical School, spoke on "Clinical Implications of Lipoproteins." Irving J. Greenblatt, Beth-El Hospital was Chairman.

Dr. Oncley described the work of his laboratory in the separation and characterization of the lipoprotein fractions with both the ultra centrifuge and electrophoresis. Dr. Barr held the interest of all with his presentation of data on the lipoprotein patterns of men and women in various age groups and in various disease states. He emphasized that he was only presenting data and did not present conclusions nor advance any theories as to the observed changes in lipoprotein patterns in advancing age or the changes observed during hormone therapy.

A discussion period followed the speakers.

ENDEAVOUR PRIZES

As a contribution to the meeting of the British Association for the Advancement of Science to be held in Oxford on 1st-8th September, 1954, Imperial Chemical Industries Limited, publishers of the quarterly scientific review ENDEAVOUR, have offered the sum of 100 guineas to be awarded as prizes for essays submitted on a scientific subject. As the primary purpose of these awards is to stimulate younger scientists to take an interest in the work of the British Association and to raise the literary standard of scientific writing, the competition is restricted to those whose twenty-fifth birthday falls on or after 1st June, 1954.

Five prizes will be awarded—
a first prize of 50 guineas, a second prize of 25 guineas, a third prize of 15 guineas—two special prizes of 5 guineas for competitors who have not passed their eighteenth birthday on 1st June, 1954.

The subjects for the essays are as follows:
1. The upper atmosphere
2. Heat of the earth
3. Coal as a raw material
4. Water supply
5. The span of life
6. Colour photography

The essays, which must be in English and typewritten, should not exceed 4000 words in length, and only one entry is permitted from each competitor.

All entries should be addressed to: The Assistant Secretary, British Association for the Advancement of Science, Burlington House, Piccadilly, London, W. I., and the envelope should be clearly marked 'ENDEAVOUR Prize Essay.' The latest date for receipt of entries is 1st June, 1954. The essays will be judged by the editors of ENDEAVOUR in consultation with representatives of the British Association. The successful competitors will be invited to attend the whole of the Oxford meeting, at which the prizes will be presented, and their expenses within the United Kingdom will be paid. The judges' decision is final, and they reserve the right to withhold all or any of the prizes should no entries of sufficient merit be received.

The essays should be submitted without signature. The competitor's full name and address and date of birth should be enclosed in a sealed covering letter attached to the essay and addressed to the Assistant Secretary of the British Association, who will acknowledge all entries. The names will not be disclosed to the judges until after the prize-winning essays have been selected.

BOOK REVIEWS


Reviewed by Harold D. Appleton, Chemistry Department, Metropolitan Hospital, New York, N.Y.

King's College Hospital Medical School, London, has recognized the overlap of medical physiology, clinical pathology, and clinical chemistry and has advanced the study into a separate discipline. Thus, Dr. C.H. Gray has the unique honor of being the first to hold a chair as Professor of Chemical Pathology, University of London and also to be designated, Clinical Pathologist, at King's College Hospital.

Professor Gray has based this small text on his lecture series. As he states in his preface, "It does not pretend to be comprehensive, but presents those features of the subject which seem to the author to be of particular value in assisting the medical student to appreciate some of the chemical aspects of disease." The author also believes that others would appreciate knowing the value and limitations of the clinical analysis they request and are called upon to perform.


Some may wonder how the above subjects can all be covered adequately in 138 pages, until the realization that the author has at his disposal excellent commend, of language and an ability to present the subject matter with an economy of verbiage. This is not intended to be a test for the specialists in the various fields, but does the job excellently to bring the subject matter into clear focus for the medical student, laboratory worker and the practicing physician. The latter sometimes being at a loss just how to make good use of modern laboratory facilities.

Clinical Chemists will find the book excellent as required reading in the training of assistants in the laboratory, as easy to come to with only chemical training and very little, if any, background in physiology. This book is a worthwhile addition to both laboratory and personal bookshelves.

In judging the results special attention will be paid to the originality of the approach to the subject, and great importance will be attached to literary style. The competitor's age will also be taken into account. The essay winning the first prize will be published in Advancement of Science, journal of the British Association.
NEW APPARATUS

REDESIGNED "TIME-IT" ELECTRIC STOPWATCH

The Precision Scientific Company has redesigned the "Time-It" Electric Stopwatch to provide even greater accuracy in laboratory work where precise, split-second timing is a must.

The new design with its double-strength motor gives the extra durability needed to withstand rough treatment and continuous service, assuring years of reliable, trouble-free operation.

The "Time-It" is offered in two models, one of which registers up to 1,000 minutes in 1/100 min. intervals, the other up to 10,000 seconds in 1/10 sec. intervals. Both models have a clear indicating counter, with large, legible numbers. Other features include a dust-tight, enamelled aluminum housing and location of the resetting knob on the left side, to free the right hand of the user for writing. A free copy of descriptive "Bulletin #519" will be mailed upon request. Precision Scientific Company, 3733 W. Cortland St., Chicago 47, Illinois.

"PRECISION" JUNIOR IONOGRAPH

Precision Scientific Company now offer the Junior Ionograph, a low cost research instrument, ionography is electrophoresis on wet filter paper and depends upon movement of charged particles in an electrical field. The ionograph separates and identifies mixtures of biological materials such as amino acids and proteins, and is used in analyses of pharmaceutical products and other investigations.

The Junior Ionograph is a modification of the Precision Ionograph announced two years ago. While not as versatile, the Junior Ionograph provides an inexpensive means of performing many investigations. It accommodates seven filter paper strips or a single sheet for two-dimensional electro-migrations. The range of potential is 150 to 500 volts of regulated current. The apparatus consists of a single-walled, non-conducting cabinet which holds the paper strip frame and electrode vessels, and a separate power supply system containing the voltmeter, ammeter and controls. Safety switches prevent shock hazards.

The Ionograph may be operated by the average technician. Micro quantities of test materials are used - from 10-20 mg to 0.5 mg. Test results may be obtained in a few hours, rather than the day or more consumed by chemical techniques. The instrument is portable, measuring 36" wide, 12" deep and 7" high, and may be placed in an incubator or low temperature chamber.

The Junior Ionograph is immediately available. An illustrated folder, Bulletin #593, with a reprint: "Electromigration of Stabilized Electrolytes," will be sent upon request. Precision Scientific Company, 3733 W. Cortland St., Chicago 47, Illinois.

PUBLISHERS' CORNER

"The Biochemistry of the Nucleic Acids" by J. N. Davidson is now available in a second edition. Published by John Wiley & Sons in February, the book is one of Netherton Monographs on Biochemical Subjects.

The new edition, which provides an out, line of the main features of the nucleic acids and nucleoproteins, incorporates later discoveries in this rapidly changing field. Among the chapters containing new material are those on the hydrolysis products of the nucleic acids, the structure and properties of the polynucleotides, the cell nucleus, and the biosynthesis of the nucleic acids. The author also discusses chronography applied to the nucleic acids, nucleotides and related enzymes, ultraviolet absorption, histochemical tests, and chemical methods for nucleic acid estimation.

Other chapters deal with the nucleic acid content of tissues, nucleic acids in the cell cytoplasm, metabolism, biological activity, and the nucleic acids in microorganisms.

Dr. Davidson is Gardner professor of physiological chemistry in the University of Glasgow. Also available in the biochemical series are Albert's: "Selective Toxicity" and Gray's "The Bile Pigments," published in this country by Wiley in 1901 and 1928 respectively.

"The Biochemistry of the Nucleic Acids" contains 200 pages and is priced at $2.25.

A summary and correlation of the field, "Instrumental Analysis" by John H. Harley and Stephen E. Wheeler was published in March by John Wiley & Sons.

The authors recognize the fact that the analytical chemist generally uses commercial instruments for the determination of various elements or compounds. Therefore, instead of stressing the particular system being measured, Harley and Wheeler emphasize the utility of various instruments, and provide a broad picture of the field of commercial instruments.

A discussion of theory lays the groundwork for the book. This is followed by an examination of the components of each instrument. The systematic arrangement then continues with a consideration of actual commercial instruments and finally, the authors cover typical analytical procedures and applications. Completely up to date, the book includes such new techniques as atomic absorption spectroscopy, and devotes individual chapters to flame photometry and high frequency titration methods.

Dr. Harley is with the U. S. Atomic Energy Commission in New York, where he is chief of the analytical branch of the Health and Safety Division. Dr. Wheeler is assistant professor of analytical chemistry at Rensselaer Polytechnic Institute.

Dear Sir:

The February issue of "Clinical Chemists" carried an article, "Stable Somogyi Substrate for the Determination of Serum Amylase", by Roy G. Wenger, showing that starch solutions may be preserved by the use of certain esters of para hydroxybenzoic acid.

It may be of interest to your readers to know that at Abington Memorial Hospital we have been using 0.5 percent phenol with success. Solutions have been set aside and have shown no deterioration even at two years.

The use of phenol suggested itself to us from an article by J. M. Waldron, in "Journal of Laboratory and Clinical Medicine", 36, 148 (1951), in which the use of pine oil is recommended as a preservative for starch suspensions.

Sincerely yours,
Peace Tashinsky
Abington Memorial Hospital
Abington, Pa.

QUID HUNCUS

The Williams and Wilkins Co., Baltimore, Md., announces the publication of a new book "Water, Electrolyte, and Acid-Base Balance", by HARRY F. WEISBERG, M.D., Clinical Chemist to the Mount Sinai Hospital, Chicago. Dr. Weisberg is also the newly elected president of the Chicago Section of the AACC.

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HARRY SOBOTKA, Director of Chemistry Laboratories, Mount Sinai Hospital, and Adjunct Professor, Department of Chemistry, Polytechnic Institute of Brooklyn, addressed the Annual Convention of the Empire State Association of Medical Technologists on April 24 on "Modern Developments in Clinical Chemistry". Subsequently, sixty of their members participated in a tour of the chemistry laboratories and animal facilities in the new Atran Building of the Mount Sinai Hospital.

CHEMISTS ORGANIZE COMMITTEE FOR 1954 UNITED JEWISH APPEAL CAMPAIGN

Dr. Kurt G. Stern, of the Polytechnic Institute of Brooklyn, chairman of the newly formed Chemists' Division of the United Jewish Appeal of Greater New York, has announced the formation of a division committee to guide the profession's campaign in behalf of UJA. Dr. Abraham Mazur, of the Department of Chemistry of the City College of New York, has been named as co-chairman. The division secretary will be Dr. Morris R. Jacobs, of the New York City department of Air Pollution Control.

Included on the division's executive committee are Norman Appleyezeig; Dr. Ernest Becker, Polytechnic Institute of Brooklyn; Miss Rose L. Berman, Berman Clinical Laboratory; Dr. Paul Fodor, of the Department of Bio-Chemistry of New York Medical College; Dr. Roger Gilmont; William Gruen, Beeber Co.; Dr. Walter P. Hocheinstein, of the Polytechnic Institute of Brooklyn; Dr. Theodore Shedlovsky, of the Rockefeller Institute for Medical Research. Dr. Albert E. Sobel, of the Jewish Hospital of Brooklyn; Dr. Nathan Weiner, Endo Products; and Dr. Norman Weissman, Chemist to the Medical Services of Maimonides Hospital.

The Inaugural Meeting was held on May 19 at the Hotel Park Sheraton. Professor Raymond Fuses was the principal speaker. His topic "Science in Israel" showed how the principles of modern science applied to the establishment of a new country has greatly improved the development of all phases of economy, as well as decreasing a large extent the time element.

The guests of honor at the Dinner-Meeting were Prof. Benjamin Harrow of the College of the City of New York, Dean Raymond E. Kirk of Brooklyn Polytechnic Institute, Prof. Herman F. Mark and Prof. Severo Ochoa, newly appointed head of the Department of Biochemistry, New York University School of Medicine.

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HAROLD D. APPLETON has been appointed to head the Clinical Chemistry Department of Metropolitan Hospital, Welfare Island, N.Y. He was formerly associated with the New York University Research Service, Goldwater Memorial Hospital, New York.

SITUATION WANTED

Biochemist with Ph.D. and two years experience in Clinical Biochemistry Desires position as Clinical Biochemist, or teaching Biochemistry. Write Box 123, New York 21, N.Y.
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A compact, portable, versatile "Black-Light" fluorescent viewing unit. Eliminates the necessity of a dark room. Gives full protection to the viewer from direct or reflected ultraviolet rays. Ideal for the Diagnex Test for Aclorhydria. Can be used as a shadow box unit or can be easily disassembled and the lamp unit utilized alone as a microscope lamp or illuminator. The handsome grey hammeroid and chrome case is furnished on a tilting leg stand.

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

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

VIEWING CHAMBER

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

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The "Eben-Scope" measures 10 1/2" wide, 9 1/2" high by 8" deep, supplied complete with 6 "Pyrex Brand" culture tubes 13 x 130 mm and rack. Operates on 110/115 volts, 60 cycle A.C.