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Survey

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THE SECRETARY REPORTS

Plans are now in progress for the Sixth Annual Meetings of the AACC. These will be held in New York City during September 13-16, 1954 in conjunction with the 126th National Meeting of the American Chemical Society. The many interesting features of our past meetings will again be carried through, including the Ernst Bischoff Award and Annual Dinner.

Some of the newer members in the Association may not be aware of the fact that membership certificates are available to them. These engraved certificates are suitable for framing and may be obtained by sending a check to the amount of four dollars to the National Treasurer and indicating the name to be inscribed as well as degrees if one so wishes.

Negotiations are now being carried on with an outstanding publishing house for a journal of clinical chemistry. It is hoped that a complete report on this matter will be ready for the next issue of THE CLINICAL CHEMIST.

The first number of a newsletter by the British Association of Clinical Biochemists reached us a short time ago. From our own early experience with THE CLINICAL CHEMIST we can well imagine the thrill that this first effort must have given to our British colleagues. We most certainly extend to them our best wishes in this editorial venture.

This might also be a good time for a reminder that the First European Congress on Clinical Chemistry is to meet in Amsterdam from September 23-28, 1954. This Congress has been organized by the Netherlands Society for Clinical Chemistry and extends its cordial invitation to American participants.

Max M. Friedman, National Secretary

ORIGIN OF THE MIDWEST SECTION

The need for increased participation in the activities of the national association at the Section level was stressed at the Chicago meeting last September (see also Clinical Chemist, page 80, Dec. 1953). The Executive Committee and National Officers expressed a desire for the formation of more local sections.

Following the meetings in Chicago, a survey of all members of the Association in Iowa and nearby areas was made. Letters were sent to each member proposing the formation of a Midwest Section, outlining the activities of such a section, and requesting ideas and interest in its formation. Encouraging replies were returned from several members in Iowa, Nebraska, and western Illinois.

The favorable response to this first survey encouraged us to further our efforts to obtain new members in the area and to make definite plans for the formation of a Midwest Section. Within a short time, the group in Iowa City grew from two to fifteen and more members were obtained in the area.

On January 14, an organizational meeting was held at the University Hospital in Iowa City. A temporary Chairman (J. I. Routh) and a temporary Secretary (L. C. Kier) were elected and requested to petition the Executive Committee for the formation of the Midwest Section. Iowa was to serve as a nucleus of the area including Wisconsin, Minnesota, South Dakota, Nebraska, Missouri, and western Illinois. It was felt that the members in this relatively large area could profit by participation in section activities until such time as new local sections were formed in the member states. The petition for official recognition of the Midwest Section contained thirty charter members.

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 an plans were made to visit several laboratories in the section.

J. I. Routh

NACL CONVENTION

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

This Sixtn 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. Porterfield, Roger W. Marsters, 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 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.

CHEMICAL EVALUATION OF THE FUNCTIONS OF THE LIVER

by

John G. Reinhold

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PART III

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 phenoltetrabromophthalein sulfonate (sulfobromophthalein, sodium; bromsulfalein), 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 alkali 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 tetrabromsulfonphathalein (sulfonate, sodium bromsulfalein). *Standard solution.*—Transfer 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 border line retention 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 standard solutions 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 unbuttered toast and coffee, tea, (without cream) or fruit juice is permissable 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 1. 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 three 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 a 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 0.5N sodium hydroxide solution. Mix well and measure the absorbancy at wave length settings of 660, 565 and 420 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 dyed 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 instrument checks. The readings follow Beer's law.

Calculation.—Bromsulfalein, mg/100 ml = $f(U_{565} - 1.28\,U_{660}) - 0.15\,(U_{420} - 1.95\,U_{660})$

$$f = \frac{C}{S_{565}}$$

Where U_{565}, U_{660}, and U_{420} represent absorbancies of the diluted serum at the wave lengths of the subscripts, and S_{565} 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. × 10.

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

The effect of lactescence in serum is eliminated by making a third absorbancy measurement at 660 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 adiposity.

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 (Lorber and Shay, 1952) does not appear to influence the results sufficiently to neces-

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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.

TABLE 1

Volume of bromsulfalein solution (5%) required for patients according to stature, build, and sex, calculated to provide 5 mg. per kg. of ideal weights.

MEN

Height	Small frame Avg.wt.	ml.	Medium	ml.	Large	ml.
5 ft. 2	120	5.4	128	5.8	136	6.2
3	123	5.6	131	5.9	138	6.3
4	127	5.8	135	6.1	142	6.4
5	131	5.9	139	6.3	146	6.6
6	134	6.1	142	6.4	150	6.8
7	138	6.3	146	6.6	155	7.0
8	141	6.4	150	6.8	159	7.2
9	145	6.6	154	7.0	163	7.4
10	149	6.8	158	7.2	167	7.6
11	153	6.9	162	7.3	172	7.8
6 ft. 0	158	7.2	167	7.6	176	8.0
1	163	7.4	171	7.8	181	8.2
2	169	7.7	177	8.0	186	8.4
3	174	7.9	182	8.3	191	8.7

WOMEN

Height	Small frame Avg.wt.	ml.	Medium	ml.	Large	ml.
4 ft. 11	107	4.8	114	5.2	122	5.5
5 ft. 0	108	4.9	116	5.3	124	5.6
1	110	5.0	118	5.4	126	5.7
2	113	5.1	121	5.5	129	5.8
3	116	5.3	124	5.6	132	6.0
4	119	5.4	128	5.8	136	6.2
5	122	5.5	131	5.9	139	6.3
6	126	5.7	134	6.1	143	6.5
7	129	5.8	138	6.3	147	6.7
8	132	6.0	141	6.4	151	6.8
9	136	6.2	145	6.6	155	7.0
10	139	6.3	149	6.8	158	7.2
11	142	6.4	152	6.9	161	7.3

sitate a return to the original dosage (Owen, 1951).

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. Mateer, et al (1943) introduced the 45 minute sampling time and it has proved to be satisfactory. Calculation of the rate of disappearance of bromsulfalein 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 bromsulfalein remaining in the circulating blood during any time interval (Bradley, 1949) and by collecting a series of blood specimens the accuracy of the estimate of the bromsulfalein retained is improved. For a description of "clearance" studies of patients the reader is referred to papers by Lewis (1948), Lavers et al (1949) and Goodman (1952). Norcross, White and Bradley (1951), report that up to 9.8 per cent of injected bromsulfalein may be lost in the urine within 40 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 bromsulfalein per kg. of body weight retain less than 10 per cent at 30 minutes and 7.0 per cent at 45 minutes. At 60 minutes, no dye is retained.

Bromsulfalein 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 suspicious or positive results obtained by means of flocculation tests in the absence of hyperbilirubinemia. The bromsulfalein test outscores all others in the proportion of positive tests found in Laennec'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 bromsulfalein 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 hyperbiliruminemia or clincal jaundice are present.

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*The correction factors given in the calculation were calculated from readings made using an Evelyn (1936) photocolorimeter. They may be applied to readings made with other photometers provided the transmittancies at the wave lengths used do not greatly differ from those of the Evelyn. If desired, the factors may be calculated by measuring the absorbancy of a dilute solution of hemoglobin containing roughly 10 to 20 mg. of hemoglobin per 100 ml. at 420 and 565 uu. The correction for hemolysis is calculated by means of the ratio A 565/A 420 obtained from the readings of the hemoglobin solution. This represents the factor 0.15 in the calculation. The turbidity correction is based on readings of a colloidal glass suspension similar to that described under thymol turbidity, made at 420, 565, and 660 mu. Although turbid solutions have no absorption bands, their absorbancy increases progressively from the red to the blue regions of the spectrum. Therefore, the ratio of A 565/A 660 of the glass suspension represents the factor by which any measurements made at 660 uu must be multiplied, and A 420/A 660 the corresponding factor for the reading at 420 uu in order to correct for turbidity. In the calculation above, these ratios are 1.28 and 1.95 respectively.

No corrections need by applied to the standards. The turbidity correction will vary with particle size and therefore represents an approximation.

Miscellaneous tests.—Serum cholinesterase activity is usually lowered in the presence of atrophy of or damage to the liver parenchyma. Similar changes have been reported for serum esterase and lipase. Depression of serum cholinesterase activity closely follows changes in serum albumin concentrations. However, severe 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 associated with deficient diets. Current articles dealing with its application to the study of liver disease include those of Mann et al (1952), Fremont-Smith et al (1952), Wilson et al (1952) and Vorhaus and Kark (1953).

Serum cholinesterase may be measured conveniently by the method of Michel (1949) 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, 1949; 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 patients 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 idiopathic 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 amounts of coproporphyrin in many 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 methods now available give much higher results than those formerly used mainly because of the detection of porphyrin precursors in urine and their inclusion in the assay (Watson, de Mello, et al, 1951).

Watson and his associates (see references above) have shown that the type of coproporphyrin excreted is related to the cause of liver damage. 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 bromsulfalein-hippuric acid-galactose test.—Zieve, Hill, and Nesbitt (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.

Peptidase activity of human serum.—Fleisher and Butt (1953) have found tripeptidase activity to be increased in serum of patients with liver disease or with ob-

struction of the bile ducts. Bile shows marked tripeptidase activity. Hydrolysis of several dipeptides 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, bromsulfalein 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 passive congestion, portal cirrhosis: *bromsulfalein, direct and total bilirubin, flocculation tests, serum albumin and globulin,* prothrombin, serum cholinesterase, urine urobilinogen and coproporphyrin.

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

4. Differentiation of jaundice due to biliary disease from that due to parenchymatous disease: *serum alkaline phosphatase, cephalin-cholesterol flocculation and thymol turbidity,* galactose tolerance, prothrombin response, repeated feces urobilinogen tests.

5. Differentiation of extra hepatic biliary obstruction due to calculus from that due to neoplasm, stricture, etc. *feces urobilinogen.*

6. For following the course of the surgical patient with disease of the biliary tract: *plasma prothrombin, phosphatase, serum direct and total bilirubin, albumin and globulin, electrolytes, 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 Hänger (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 46 per cent. However, a combination of thymol turbidity and alkaline phosphatase gave correct diagnoses in 79 per cent.

Wootton, King and Maclean 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, 1939). Often the zinc turbidity (Kunkel, 1947) or the ammonium sulfate turbidity (Huerga 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 the results of simpler tests and thus no longer performs a useful service. Neefe 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 purpose 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 Neefe and Reinhold (1946). 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 bromsulfalein 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 bromsulfalein 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 do 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 inadvisable therefore, to curtail 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 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 damage 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 pathological 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 function. The greatest utility of each method for the study of liver disease will be realized if liver biopsy and chemical studies are coordinat-

TABLE 4

SYNDROME	BILIRUBIN	TESTS FOR PARENCHYMAL INVOLVEMENT	TESTS FOR BILIARY TRACT INVOLVEMENT
Viral hepatitis without jaundice	May be present in urine and may increase slightly in serum. Urobilinogen may increase in urine.	BSF usually abnormal, CCF, TT, TF, ZT one or more may be abnormal	Normal
Viral hepatitis with jaundice	Increased in serum and urine. Urobilinogen generally increased in urine and feces but may be absent.	Abnormal	Mainly normal but may be abnormal.
"Toxic" hepatitis: cholangiolitic jaundice	Increased in serum and urine.	CCF, TTT, TF, ZT may be normal or abnormal A/G usually normal.	Increased serum phosphatase, lipid. Similar to extrahepatic obstruction.
Laennec's cirrhosis	May or may not be abnormal in serum and urine. Urobilinogen variable.	BSF abnormal. CCF, TT, TF or ZT abnormal in about 3/4. A/G abnormal.	Variable. Phosphatase may be high.
Extrahepatic obstruction. Partial.	Variable. Intermittent or continuous elevation.	Generally normal, but liver parenchyma may become injured and tests positive.	Variable but with elevated serum phosphatase and lipid prevalent. Good response to vitamin K.
Extrahepatic obstruction. Complete.	Extreme elevation. Very low fecal urobilinogen.	Same as preceding.	Elevated serum phosphatase and lipid. Good response to vitamin K.
Biliary cirrhosis	Bilirubin elevated. Urobilinogen variable.	BSF increased. A/G abnormal. TT increased. CCF about 1/2 increased.	Elevated phosphatase. Marked elevation of serum lipid especially phospholipid.
Hemolytic jaundice	Total serum bilirubin moderately elevated; 1 minute bilirubin normal or slighly increased. Urine bilirubin negative. Feces urobilinogen increased urine urobilinogen often normal.	Seldom abnormal but may become so due to hypoxia, or other complications.	Seldom abnormal. Pigment stones may cause biliary obstruction.

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

ed. In such a plan, chemical studies will serve for screening and evaluation of the patients, and for following the clinical course. Liver biopsy will supplement such studies particularly in patients who present diagnostic problems. The coordinated use of liver biopsy and biochemical studies is discussed by Popper and Schaffer (1952).

Table II summarizes the prevailing response obtained when tests for the bile pigments and other procedures commonly used are applied in study of diseases of the liver parenchyma and the contrast in the response in disease of the biliary tract.

ADDENDA

Standardization of thymol and other turbidity tests. Shortly after the first section of this review was sent to the printer, it was discovered that the turbidity produced by reaction of the thymol reagent with sera 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 Shank-Hoagland 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 role of beta globulins in the thymol test is not specifically mentioned. The work of Recant, Chargaff, and Hanger (Proc. Soc. Exp. Biol. and Med. 60:245 (1945), of Cohen and Thompson (J. Lab. Clin. Med. 32:475 (1947), and of Kunkel and Hoagland, (J. Clin. Invest, 26:1060 (1947) indicates that beta globulin or lipid associated with beta globulin 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 mechanisms of the flocculation test by Saifer, the author purposely refrained from detailed discussion of this aspect.

Amino acids of blood in liver disease: The failure of Murphy and associates to find elevated concentrations of amino acids in blood in patients in hepatic coma (N. Eng. J. Med. 239:605 (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).

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CHEMICAL EVALUATION OF THE FUNCTION OF THE LIVER – PART III

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PLASMA PROTEINS AND PLASMA SUBSTITUTES

By Kurt G. Stern

Blood plasma represents a polymolecular system of proteins ranging in molecular weight from 70,000 (serum albumin) to 1,300,000 (beta lipoprotein) and 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 colloid-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 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, it should remain in the circulation for a reasonable period of time following administration and subsequently be either excreted or metabolized. Its viscosity should be similar to that of plasma and its osmotic pressure should approach that of serum albumin. It should not be deposited in the tissues of the reticule-endothelial system and 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, isinglass, bovine plasma albumin, polyglucose, etc.), dextran and polyvinylpyrrolidone (PVP) have been the most widely accepted substances.

Abstract of talk given by Kurt G. Stern of the Polytechnic Institute of Brooklyn, Brooklyn, N.Y. Oct. 29, 1953 at a meeting of the Washington-Baltimore-Richmond section of the American Association of Clinical Chemists, Inc.

Detran 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 lactobacillus, *Leuconostoc mesenteroids*, 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 method) 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 hydrolysates 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 ±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 Federations 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. Bloor, 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

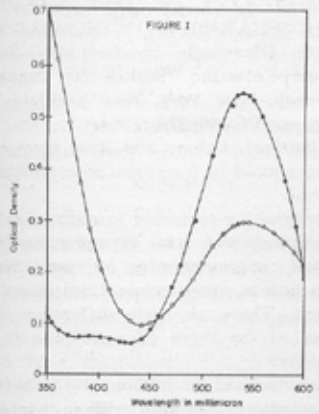
(from the Department of Biochemistry, Walter Reed Army Medical Center, Army Medical Service Graduate School, Washington 12, D. C.)

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-azo resorcinol; and mixtures of pontacyl carmine 2B and pontacyl violet 6R. The pontacyl dyes have proved most satisfactory when employed in the following manner:

Experimental—85 mg. of pontacyl violet 6R, concentration 150 per cent and 15 mg. of pontacyl carmine 2B[1] were dissolved in distilled water, diluted to 1000 ml., stored as a stock solution for all subsequent working standard solutions.

In Figure 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 distilled water blank. 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 540 μ and minima at 440-430 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. ● Pontacyl dye standards; o Protein-Biuret standards.

TABLE I

Concentrations and Optical Density of Standard Pontacyl And Standard Protein (Biuret) Solutions

	Pontacyl Standard		Protein-biuret Standard[2]	
Dilutions[1]	Pontacyl conc. mg. per ml.	Optical Density	Optical Density	Protein[3] conc. per cent
0.5 ml. diluted to 10	.0015 mg.	.061	.058	1
1.0 ml. diluted to 10	.0030 mg.	.119	.119	2
1.5 ml. diluted to 10	.0045 mg.	.171	.174	3
2.0 ml. diluted to 10	.0060 mg.	.233	.229	4
2.5 ml. diluted to 10	.0075 mg.	.292	.288	5
3.0 ml. diluted to 10	.0090 mg.	.347	.342	6
3.5 ml. diluted to 10	.0105 mg.	.392	.398	7
4.0 ml. diluted to 10	.0120 mg.	.450	.456	8
4.5 ml. diluted to 10	.0135 mg.	.502	.509	9
5.0 ml. diluted to 10	.0150 mg.	.553	.561	10

1. *30 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 I 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 I. 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 curvettes in a Coleman Jr. Spectrophotometer No. 6 at wavelength 540 mu has been plotted against the protein concentration of the biuret samples and against the pontacyl concentration.

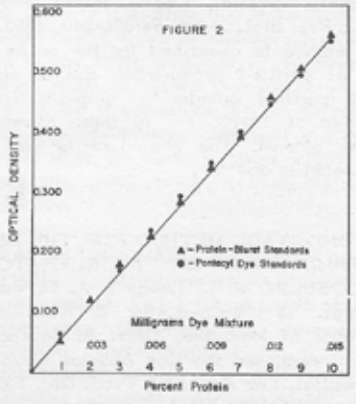
Three successive batches of these dyes have been secured at intervals over a period of two years with only minor differences noted among these lots.

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 II. 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 protein-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:

$$\frac{\text{Optical Density of Unknown}}{\text{Optical Density of Standard}} \times 7 = \text{gm. per cent protein}$$

Summary—Standard solutions of pontacyl dyes have been tested as spectrophotometric standards for the determination of



Calibration curves of pontacyl dye standards and biuret-protein standards as determined in Coleman Jr. Spectrophotometer No. 6.

serum proteins, by the biuret 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 (biuret) from 1 to 10 per cent. The particular advantage of the pontacyl standards are 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.

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3. Weichselbaum, T. E.: An accurate and rapid method for the determination of proteins in small amounts of blood serums and plasma. Am. J. Clin. Path., *16:* 40-49, 1946.
4. Wolfson, W. Q., Cohn, C., Calvary, E., and Thomas, E. M.: Studies in serum proteins. Am. J. Clin. Path., *18:* 723-33, 1948.

[1] *These dyes were kindly supplied by the E. I. Dupont DeNemours & Co., Wilmington, Delaware.*

REVIEW OF CURRENT LITERATURE

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

INFLUENCE OF DIET ON SERUM ALKALINE PHOSPHATASE IN RATS AND MEN. M. Sukumaran & W. L. Bloom (Depts. of Medicine & Biochemistry, Emory University, Ga.). Proc. Soc. Exp. Biol. & Med. *84*:631-634, 1953.

In rats and in humans the serum phosphatase level was significantly lowered by fasting. This drop in phosphatase activity could be overcome by feeding fats, but not by feeding either carbohydrates or proteins. The authors feel that the data indicate that the serum alkaline phosphatase activity is associated with enzyme activity of other organs as well as of bone.

E.V.

A SIMPLE PROCEDURE FOR SEPARATION OF PROTHROMBIN AND ACCELERATOR GLOBULIN FROM CITRATED HUMAN PLASMA. M. L. Lewis & A. 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*:636-640, 1953.

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

E.V.

A ONE-STAGE METHOD FOR THE DETERMINATION OF ACCELERATOR GLOBULIN. M. L. Lewis & A. 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*:640-643, 1953.

A method is presented which is specific for accelerator-globulin activity.

E.V.

PYRUVATE ACCUMULATION IN PRESERVED BLOOD. Marcel C. Blanchaer and S. L. Baldwin (Univ. Manitoba, Winnipeg, Can.). J. Appl. Physiol. *6*, 8-14 (1953).

A biphasic rise in pyruvate occured in sterile blood kept at 5° C in an acid citrate-dextrose soln. An initial transient pyruvate increase coincided with the rapid disappearance of most of the 2,3-diphosphoglycerate from the erythrocytes and was followed by a sustained rise in pyruvate which could be partially inhibited by cyanide or deoxygenation of the blood. This second increase was absent in congenital methemoglobinemic blood. It is concluded that a portion of the terminal pyruvate increase in preserved blood was caused by a partial aerobic coupling of 3-phosphoglyceraldehyde (I) oxidation through diphosphopyridine nucleotide, a flavoprotein, and a hemeprotein. A portion of the pyruvate formed from the oxidation products of I was not reduced to lactate and accumulated. H. A.

EFFECT OF HEAT AND pH ON HYALURONIDASE. M. B. Mathews and A. Dorfman (Department of Pediatrics, University of Chicago, Chicago, Illinois). J. Biol. Chem. *206*:143-149, 1954.

Partially purified bovine testicular hyaluronidase retains 10 per cent of its original activity after heating 10 minutes at 100°C at low pH (0-3.5). Heating at neutral or alkaline pH results in complete loss of activity.

The residual activity is effective in depolymerizing chondroitinsulfuric acid as well as hyaluronic acid in the same ratio of effectiveness toward the substrates as was shown by the untreated enzyme. From these experiments it may be concluded that both activities are functions of one and the same enzyme.

C. vF. D.

PRODUCTION OF ANTIBODIES AGAINST DOG INTESTINAL PHOSPHATASE. M. Schlamowitz (Research Biochemistry Section, Sloan-Kettering Institute for Cancer Research, New York, New York). J. Biol. Chem. *206*:361-367, 1954.

Alkaline phosphatase from dog intestine was prepared by fractionation 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 enzyme and antibodies showed considerable heterogeneity. The enzyme 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. vF. D.

SPECIFICITY OF DOG INTESTINAL PHOSPHATASE ANTISERUM. M. Schlamowitz (Research Biochemistry Section, Sloan-Kettering Institute for Cancer Research, New York, New York). J. Biol. Chem. *206*:369-374, 1954.

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

Study of the response to inhibitors 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.

Immunochemical 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 phosphastase.

C. vF. D.

PROTEIN METABOLISM IN THE CHOLINE-DEFICIENT RAT. II. EFFECTS OF AGE AND SEX ON SERUM PROTEINS. M. A. Fischer and G. C. Garrity (Biochemistry Department, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania). J. Biol. Chem. *206*:345-352, 1954.

In choline deficient rats of both sexes, increased albumin synthesis precipitates choline depletion.

Rise in alpha globulins ($alpha_1$ and $alpha_2$) correlates with kidney damage and starts one day earlier in males than in females.

In young animals the use of amino acids for albumin synthesis takes precedence over their use for choline synthesis, when fed a choline deficient diet. When deficiency becomes severe, $alpha_1$, $alpha_2$ and $beta_1$ globulins double in value, as measured from the surface area in electrophoretic patterns.

Gamma globulin values and albumin values fluctuate in similar manner.

C. vF. D.

ISOLATION OF A SULFATE ESTER OF HYALURONIC ACID FROM HEART VALVES. W. P. Deiss and A. S. Leon (Department of Medicine, Medical School, University of Wisconsin, Madison, Wisconsin). J. Biol. Chem. *206*:375-380, 1954.

From a dilute 0.01 N NaOH extract of fresh bovine heart valves an acid mucopolysaccharide, unlike those previously isolated from this tissue, was isolated. It has equimolar quantities of hexosamine, hexuronic acid, acetyl and sulfate.

It was tentatively classified as a sulfate ester of hyaluronic acid, based on its optical rotation and its enzymatic hydrolysis by testicular and pneumococcal hyaluronidases.

C. vF. D.

THE INCORPORATION OF THE CARBOXYL CARBON FROM ACETATE INTO CHOLESTEROL BY RAT LIVER HOMOGENATES. I. D. Frantz and N. L. R. Bucher (Cardiovascular Research Laboratory, Department of Medicine, Harvard Medical School, Boston, Massachusetts). J. Biol. Chem. *206*:471-481, 1954.

Experiments were carried out with acetate-1-C^{14}. The radioactive cholesterol formed was recovered as the digitonide.

C. vF. D.

A SEMIAUTOMATIC RECORDING DENSITOMETER FOR USE AFTER PAPER-STRIP ELECTROPHORESIS. A.L. Latner, L. Molyneux and J. D. Rose (Depts of Pathology, Anesthetics and Surgery, Medical School, King's College, Newcastle upon Tyne, England). J. Lab. & Clin. Med. *43*:157-164, 1954.

A densitometer is described which makes use of either transmitted or reflected light. Using reflected light the instrument was employed for scanning paper strips after electrophoresis of serum proteins.

E.V.

ACID CLEAVAGE OF HEME PROTEINS. U. J. Lewis (Biochemical Department, Medical Nobel Institute, Stockholm, Sweden). J. Biol. Chem. *206*:109-120, 1954.

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

C. vF. D.

ON THE STRUCTURE OF REDUCED DIPHOSPHOPYRIDINE NUCLEOTIDE. M. E. Pullman, A. San Pietro and S. P. Colowick (McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Maryland). J. Biol. Chem. *206*:129-141, 1954.

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

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

C. vF. D.

A POLAROGRAPHIC STUDY OF EVANS BLUE AND ITS COMBINATION WITH PLASMA PROTEINS. G. Markus and J. P. Baumberger. (Department of Physiology, Stanford University School of Medicine, Stanford, California). J. Biol. Chem. *206*:59-65, 1954.

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. vF. D.

ENZYMATIC FRACTIONATION OF ADENOSINE PYROPHOSPHATES IN HUMAN BLOOD. B. Mackler, P. Foris and G. M. Guest. (Children's Hospital Research Foundation, University of Cincinnati, Cincinnati, Ohio). J. Biol. Chem. *206*:77-82, 1954.

Four enzyme systems were used for parallel assays of organic pyrophosphates in human blood: potato apyrase, 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. vF. D.

BODY COMPARTMENTS. THEIR MEASUREMENT AND APPLICATION TO CLINICAL MEDICINE. George J. Hamwi and Struat Urbach (Ohio State Univ., Columbus.). Metabolism *2*, 391-403 (1953).

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

H.A.

A BIOCHEMICAL TEST OF MYOPATHIA: THE INCREASE OF SERUM ALDOLASE. Georges Schapira, Jean Claude Dreyfus, and Fanny Schapira (Hop. Enfants-Malades, Paris). Sem.hop, Paris *29*, 1917-20, (1953).

The aldolase in normal adults and children was found to be 0.30 and 0.575 Meyerhof units, resp., as analyzed by Sibley-Lehninger method (C.A. *43*, 6724i). In 92% of myopathic patients these values were found to be 10 to 30 times as high as in the controls.

H.A.

AN IMPROVED TECHNIQUE FOR UROPEPSIN ASSAY. J. D. Duffin and K. Kowalewski (Laboratories, Colonel Belcher Hospital, Dept. of Veteran Affairs, Calgary, Alberta). J. Lab. & Clin. Med. *43*:165-168, 1954.

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.

AGING IN APPARENTLY NORMAL MEN. II. ANDROGENIC ACTIVITY OF URINARY KETOSTEROIDS AND OF THEIR ALPHA AND BETA FRACTIONS; THE RELATIONSHIP OF ANDROGENIC TITERS TO VALUES OBTAINED BY COLORIMETER ASSAY. J. B. Hamilton, H. B. Hamilton and G. E. Mestler (Dept. of Anatomy, College of Medicine, State Univ. of New York Medical Center at New York City, Brooklyn, N.Y.). J. Clin. Endocrin. & Metab. *14*:139-153, 1954.

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

E.V.

PAPER CHROMATOGRAPHY OF LECITHINS. F. M. Huennekens, D. J. Hanahan, and M. Uziel (Department of Biochemistry, University of Washington, Seattle, Washington). J. Biol. Chem. *206*:443-447, 1954.

Various alcohol-water mixtures were used as solvent systems. Spray techniques were employed to detect phosphate, choline ester and unsaturated groupings.

C. vF. D.

REVIEW OF CURRENT LITERATURE

EXTRACTION AND COLORIMETRIC MEASUREMENT OF RAT TESTICULAR HYALURONIDASE. R. L. Greif (Hospital of the Rockefeller Institute for Medical Research, New York, New York). J. Biol. Chem. *206*:381-390, 1954.

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 plasma, citrate-phosphate buffer, bromosulfalein and urea.

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

C. vF. D.

THE BINDING OF STEROIDS TO PROTEIN. I. SOLUBILITY DETERMINATIONS. K. Eik-Nes, J. A. Schellman, R. Lumry, L. T. Samuels (Department of Biological Chemistry, University of Utah College of Medicine, Salt Lake City, Utah). J. Biol. Chem *206*:411-419, 1954.

Reversible combination occurs between albumin and a large group of steroids studied (except cholesterol). The strength of the binding bears an 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. vF. D.

A DIAZO REAGENT TABLET FOR SERUM BILIRUBIN. L. Sherman and B. Zak. (Wayne Univ. College of Medicine, Detroit). Am. J. Clin. Path. 23:946-947, 191953.

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

C.R.

THE COMPOSITION OF UTERINE AND ENDOMETRIAL NUCLEIC ACID. N. I. Gold and S. H. Sturgis (Department of Surgery, Peter Brent Brigham Hospital, Boston, Mass.). J. Biol. Chem. *206*:51-58, 1954.

Significant differences in the purine and pyrimidine contents of DNA and PNA were observed in the uterus, liver and 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 the PNA of human endometrium in various phases of the menstrual cycle.

C. vF. D.

THE AMINO ACID REQUIREMENTS OF MAN. V. THE ROLE OF LYSINE, ARGININE AND TRYPTOPHAN. W. C. Rose, W. J. Haines and D. T. Warner (Division of Biochemistry, Noyes Laboratory of Chemistry, University of Illinois). J. Biol. Chem. *206*:421-430, 1954.

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

Arginine and histidine are non-essential. Final classification of the amino acids with respect to their role in nitrogen equilibrium in adult man is summarized.

C. VF. D.

MICRO FLAME PHOTOMETRIC DETERMINATION OF SODIUM, POTASSIUM, CALCIUM IN SERUM WITH ORGANIC SOLVENTS. G. R. Kingsley & R. R. Schaffert (Dept. of Physiological Chemistry School of Medicine, Univ. of California at Los Angeles, and the Clinical Biochemistry Laboratory, Veterans Administration Center, Los Angeles, Calif.). J. Biol. Chem. *206:* 807-815, 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. The flame photometers used were the Beckman Model DU and Model B.

E.V.

TUNGSTIC ACID PRECIPITATION OF BLOOD PROTEINS. S. Berkman, R. J. Henry, O. J. Golub and M. Segalove (BioScience Laboratories, Beverly Hills, Calif.). J. Biol. Chem. *206*:937-943, 1954.

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.

RAPID COLORIMETRIC METHOD FOR THE DETERMINATION OF ISONICOTINIC ACID HYDRAZIDE IN BLOOD PLASMA. B. Prescott, G. Kauffmann and W. D. James (U.S. Dept. of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Bethesda, Md.). Proc. Soc. Exp. Biol. & Med. *84*:704-706, 1953.

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

E.V.

FACTORS OTHER THAN CHOLINE WHICH AFFECT THE DEPOSITION OF LIVER FAT. A. E. Harper, W. J. Monson, D. A. Benton, M. E. Winje and C. A. Elvehjem (Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wisconsin). J. Biol. Chem. *206*:151-158, 1954.

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 betaine have an effect which is less marked but similar to that of threonine.

C. vF. D.

THE REACTION OF PYRIDINE NUCLEOTIDES WITH CARBONYL COMPOUNDS. R. M. Burton and N. O. Kaplan (McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Maryland). J. Biol. Chem. *206*:283, 1954.

Dihydroxyacetone (DHA) and similar alpha-hydroxy carbonyl compounds react with diphosphopyridine nucleotide (and related N^1-nicotinamide derivatives) to form products closely resembling DPNH. The product formed fluoresces, is acid-labile, and has a spectrum which is identical to that of DPNH. It differs from DPNH in enzyme activity.

Acid decomposition results in adenosine diphosate ribose and a nicotinamide derivative with an absorption maximum at 290 mu.

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

C. vF. D.

FLAME ANALYSIS OF SODIUM AND POTASSIUM. IN SMALL VOLUMES OF SERUM, HEPARINIZED PLASMA AND CEREBROSPINAL FLUID. R. E. Bernstein (Univ. of Witwatersrand, Johannesburg, S. Africa). Am. J. Clin. Path. 23:933-937, 1953.

50 and 20 cu. mm. samples (in siliconed calibrated Sahli pipettes) were used with a reproducibility of ±1.7% for Na and ±2.2% for K.

C.R.

INVESTIGATION OF SERUM PROTEIN PATTERNS IN PATIENTS UNDERGOING 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 in 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 globulin in 61% of all cases. Total proteins did not change significantly. All components thereafter tended to return to preoperative levels.

E.V.

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 Evaluation 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, androsterone and etiocholanolone constitute about fifty percent. These have their origin in both the testicle and adrenal cortex, although 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 evaporating 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. Digitonin 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 Pettenkofer 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 Heftmann 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 aqueous-alkali Zimmerman 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 phenylhydrazine method of Silber, which employs a sulfuric acid-ethanol extraction of the corticosteroids from methylene chloride extract of plasma.

Dr. Heftmann described a new method for fractioning the acid hydrolyzed urinary corticosteroids with column chromatography. This procedure employes 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 period 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 microtechnics 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. Melville
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 Saifer, 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 Saifer

Executive Committee: Kurt G. Stern, Isreal Kleiner, 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 ultracentrifuge and electrophorisis. 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. 1,* 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 disclosed 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.

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.

BOOK REVIEWS

CLINICAL CHEMICAL PATHOLOGY, by C.H. Gray. 138 pages, $3.00. The Williams and Wilkens Co., Baltimore 2, Md.

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, Chemical Pathologist to 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 chemical analysis they request and are called upon to perform.

The book is divided into 12 chapters: Renal Function, Acid-Base Balance, Fluid Balance-Edema, Fluid Balance-Salt Deficiency, and Water Deficiency, Liver Function, Laboratory Tests in Liver Disease, Blood Sugar, Calcification, Clinical Aspects of The Digestion and Absorption of Fats, The Chemical Pathology of the Alimentary Tract, Biochemical Tests in Endocrine Disease.

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 command of language and an ability to present the subject matter with an economy of wordage. This is not intended to be a text for the specialists in the various fields, it 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 many come to us with only chemical training and very little, if any, background in physiology. This book is a worthwhile addition to both laboratory and personal bookshelves.

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.

Timing as accurate as the cycle frequency of your electric current makes this the ideal timer for radioactivity counting systems, as well as for ordinary laboratory uses.

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, enameled aluminum housing and location of the re-setting knob on the left side, to free the right hand of the user for writing. A free copy of descriptive "Bulletin #646" will be mailed on request. Precision Scientific Company, 3737 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 hazard.

The Ionograph may be operated by the average technician. Micro quantities of test materials are used — from 10 gamma 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 #690, with a reprint "Electromigration of Stabilized Electrolytes," will be sent upon request. Precision Scientific Company, 3737 W. Cortland St., Chicago 47, Illinois.

To answer the demand for cartridge type demineralizer that will deliver pure demineralized water *under pressure* so that it can be piped to any desired point, employed in pilot plant operations, used as feedwater to other devices, etc., Barnstead Still & Sterilizer Co., announces its new Model BD-2 Pressure Bantam Demineralizer.

The Barnstead Pressure Bantam is a compact wall mounted Demineralizer that connects directly into any water line as simply as a filter. It is a non-regenerative unit that does not require acid and caustic regenerant solutions but employs a replaceable resin cartridge exactly like the one provided in Barnstead's BD-1 Non-Pressure Bantam Demineralizers.

The Barnstead Pressure Bantam delivers demineralized water in continuous flow under pressure from 5 to 25 gallons per hour. No heat or cooling water is required. It requires, states the manufacturer, no operating attention other than to change cartridge when Pura-Lite goes out. Full particulars may be had by writing to Barnstead Still & Sterilizer Co., 247 Lanesville Terrace, Forest Hills, Boston 31, Mass.

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 Methuen's Monographs on Biochemical Subjects.

The new edition, which provides an outline of the main features of the nucleic acids and nucleoproteins, incorporates late discoveries in this rapidly changing field. Among the chapters containing new material are those on 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 chromatography applied to the nucleic acids, nucleases 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 micro-organisms.

Dr. Davidson is Gardiner 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 1951 and 1953 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. Wiberley 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 Wiberley 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 echelle 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. Wiberley is assistant professor of analytical chemistry at Rensselaer Polytechnic Institute.

A 440-page book, "Instrumental Analysis" is priced at $6.50.

BOX 123

Letters From Members

Dear Sir:

The February issue of "Clinical Chemists" carried an article, "Stable Somogyi Substrate for the Determination of Serum Amylase", by Ray 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", *38*, 148 (1951), in which the use of pine oil is recommended as a preservative for starch suspensions.

Sincerely yours,
Peace Paubionsky
Abington Memorial Hospital

Abington, Pa.

QUID NUNCS

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.

UJA CHEMISTS COMMITTEE

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 B. Jacobs, of the New York City department of Air Polution Control.

Included on the division's executive committee are Norman Applezweig; 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. Hohenstein, 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 Inauguration Meeting was held on May 19 at the Hotel Park Sheraton. Professor Raymond Fuoss 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 to 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 Polytecnnic Institute, Prof. Herman F. Mark and Prof. Severo Ochoa, newly appointed head of the Department of Biocnemistry, 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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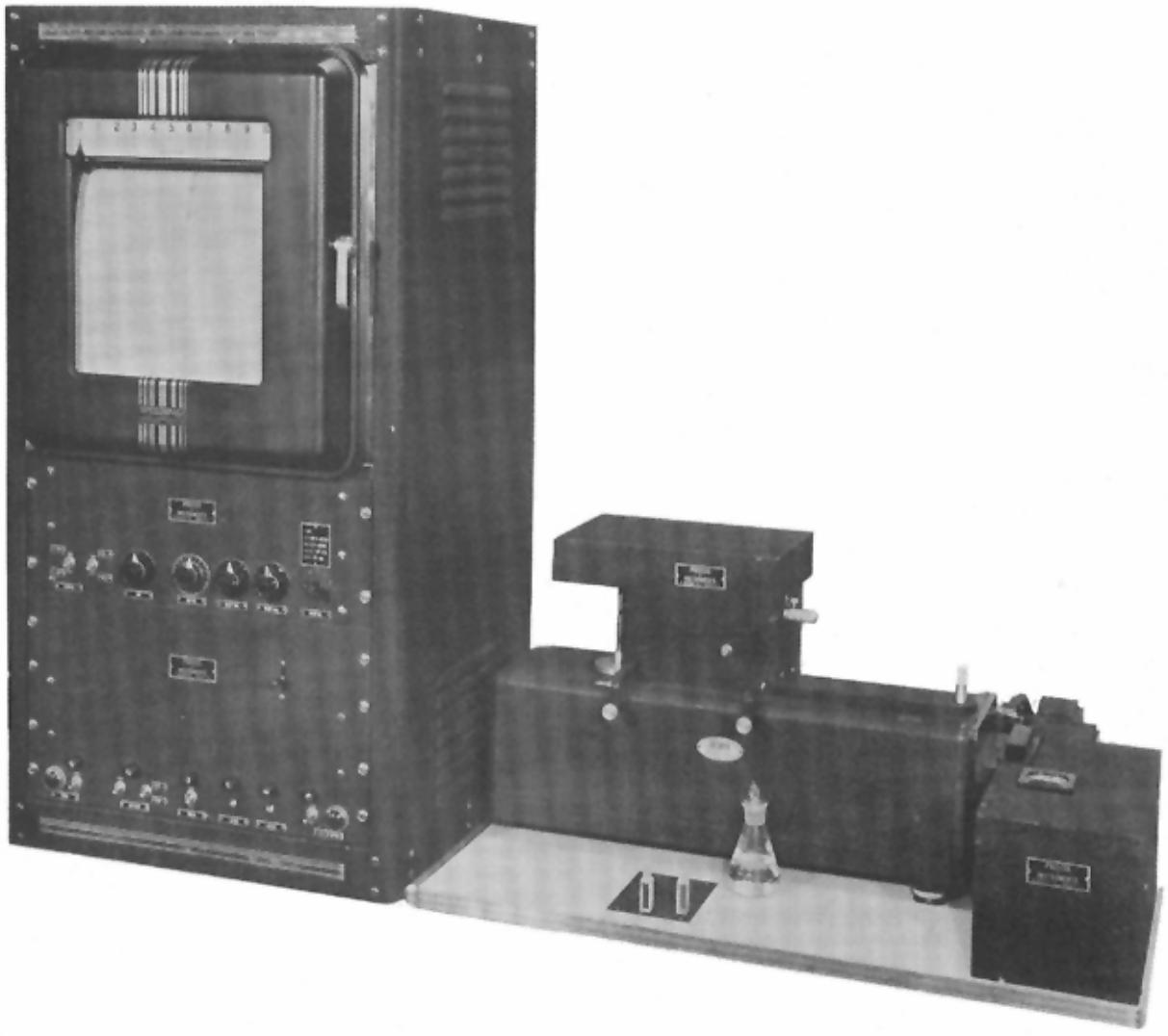

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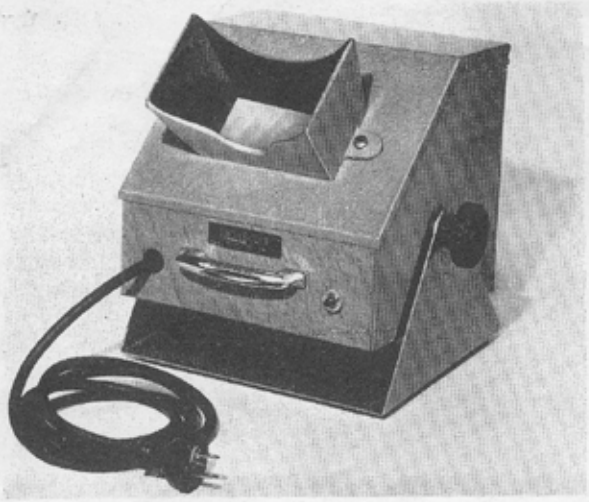

A compact, portable, versatile "Black-Light" fluorescent viewing unit. Eliminates the necessity of a dark room. Gives full protection to the viewer from direct or reflected ultraviolet rays. Ideal for the Diagnex* Test for Achlorhydria. Can be used as a shadow box unit or can be easily disassembled and the lamp unit utilized alone as a microscope lamp or illuminator. The handsome grey hamerloid and chrome case is furnished on a tilting leg stand.

CAN BE USED FOR THE

1. Detections of Quinine Compounds in the latest tests for Achlorhydria, (Squibb "DIAGNEX*" assay procedure) eliminating the necessity for intubation.
2. Examination for the presence of Porphyrins in Organic and Clinical chemistry.
3. Detection of Milkstone and impurities in the Dairy and Food industries.
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6. Examination for fluorescent tracer materials in Criminology and Toxicology.
7. Clinical determination of Porphyrin.
8. Has application in Minerology and Oil analysis.
9. Can be applied to the activation of an extensive group of fluorescent materials, including many organic materials, dyes and inks, used in fabric, paper, plastics, lacquers and paints.

The "Ebon-Scope" measures 10¼" wide, 9¾" high by 8" deep, supplied complete with 6 "Pyrex Brand" culture tubes 18x150 mm and rack. Operates on 110/115 volts, 60 cycle A.C.

DETACHABLE LAMP UNIT

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 longwave 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 Ebon-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 Ebon-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 Ebon-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 "Ebon-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 cast 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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