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# The CLINICAL Chemist

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The CLINICAL Chemist

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## THE SECRETARY REPORTS

At a time when enthusiasm is running high in the anticipation of our new journal, *CLINICAL CHEMISTRY*, may we be permitted to make a few observations about the evolution of the present publication *The CLINICAL CHEMIST*, which will cease publication with the completion of the current volume.

In the matter of only a few weeks after the Association was organized in December, 1948 an editor and assistant editor were appointed with instructions to prepare a bimonthly newsletter that could be sent to the members. The funds at that time were quite limited and this editorial venture had to be confined to a small typewritten and offset product. Yet the oldtimers (those who were members five years ago) might well recall the thrill upon receiving this little pamphlet.

A short time after that the Executive Committee decided to expand this newsletter and adopted the present format while adding several new features, including reproduction of photographs and making the newsletter available to advertisers. Although it still remained a professional and what might be called a "trade paper" yet various members began to submit short scientific notes that proved most interesting. It was this latter development that prompted the Executive Committee meeting in Milwaukee in September, 1952 to recommend to the membership that a moderate increase in dues would permit funds to expand the newsletter into a journal with original scientific contributions. This recommendation was favorably received and the issues of *The CLINICAL CHEMIST* of the past two years are the product thereof.

But this more elaborate effort has also proved inadequate for the needs and the Association has now decided to move into the "major league" with a regular journal and with all the benefits and risks appertaining thereto.

For those working on this new editorial project the excitement can be no greater than it was at the time of the first typewritten newsletter. An important difference is that the support of the membership is needed now more than before. *CLINICAL CHEMISTRY* must be more than the official publication of the American Association of Clinical Chemists. It must evoke the the personal pride of each and every member.

*Max M. Friedman, National Secretary*

## ABCC ELECTS NEW MEMBERS

The American Board of Clinical Chemistry, Inc., held its annual meeting in Philadelphia, April 16-17, 1954. The following officers were reelected: Otto A. Bessey, President; A. E. Osterberg, Vice-President; O. H. Gaebler, Secretary-Treasurer. Other present members of the Board are: Joseph W. E. Harrisson, C. W. Muehlberger, M. H. Power, W. A. Wolff, and W. M. Sperry. Resignations of Michael Somogyi and D. D. Van Slyke were accepted with regret. The term of H. H. Bunzell expired at the end of this meeting. Harry Sobotka, Robert Hill, and Albert Chaney were elected to membership. Their terms of office will begin at the termination of the 1955 annual meeting.

Six clinical chemists were certified: Clara M. Ambrus, Julian L. Ambrus, Julius J. Carr, Max E. Chilcote, Smith Freeman, and Robert P. MacFate. The total number of certified clinical chemists is now 237.

Information concerning requirements for certification is available without cost from the Secretary, Dr. O. H. Gaebler, Henry Ford Hospital, Detroit 2, Michigan.

## ERNST BISCHOFF AWARD TO JOHN G. REINHOLD

John G. Reinhold, Pepper Laboratory of Clinical Medicine, University of Pennsylvania, was awarded the 1954 Ernst Bischoff Award in Clinical Chemistry. The award was made at the Annual Meeting Dinner of the American Association of Clinical Chemists at the Hotel McAlpin, New York, September 16. Lt. Col. Monroe Freeman, President of the AACC, made the presentation.

Dr. Reinhold was honored for his work and researches on chemical liver function tests and for his studies of hepatitis. He was also cited for his work on behalf of the AACC and his efforts to secure professional recognition for the clinical chemist. Besides his work for the Association, Dr. Reinhold is chairman of the Committee on Clinical Chemistry of the American Chemical Society.

John Reinhold is the third recipient of the clinical chemistry award, which is given by the Ernst Bischoff Company of Ivoryton, Conn. and administered by the AACC. The award consists of a bronze medal, scroll and honorarium of five hundred dollars.

In his award address, the third Ernst Bischoff Lecture, Dr. Reinhold discussed the work of his group at the University of Pennsylvania and a team at the National Institutes of Health in developing chemical procedures for the detection of carriers of viral hepatitis among blood doners. He showed the methodology that has been sucessful in good percentage of cases in eliminating the blood from these carrier doners from the blood bank pool. The blood from these carrier doners are not lost to the blood bank as they can be used in the preparation of human serum albumin and other blood products, where the processing eliminates the virus.

Dr. Reinhold's complete paper will be published in the first issue of the Association's new journal, *CLINICAL CHEMISTRY*.

***Support Your***

***New Journal***

## CORRECTION

Under "The Secretary Reports" in our June issue the paragraph on the price to members of the new journal, *CLINICAL CHEMISTRY*, may have given an erroneous impression. *CLINICAL CHEMISTRY* will be an $8.00 journal. A subscription will be entered automatically for each member by the Treasurer and paid for out of dues. As the present dues will not cover the entire price of the new journal, dues will be increased by $3.50 beginning with 1955, to help make up the difference.

This increase in the dues has been passed unanimously by the members present at the Annual Meeting, September 16, 1954.

## GENERAL LABORATORY STANDARDS UNDER INVESTIGATION

The standardization committee of the New York Section is seeking suitable preparations from any interested manufacturer to be used as a general laboratory standard for clinical analysis. One such preparation, bovine serum ultrafiltrate, is presently undergoing analysis for such a purpose. Any interested manufacturer or individual who knows of such an item is invited to communicate with the chairman of the standardization committee (Abraham Saifer, Biochemist, Jewish Chronic Disease Hospital, Brooklyn 3, New York).

## INVITE MANUSCRIPTS

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

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

# LIPOPROTEINS

By

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The transportation of absorbed triglycerides does not appear to be a particularly mysterious affair. Postprandial plasma is more or less turbid, and has long been known to contain emulsified fat. It has been shown that plasma proteins will readily stabilize emulsions of this type, with an average particle size of about 0.5 $\mu$. Indeed, serum albumin will also stabilize such emulsions, though with a somewhat greater particle size (1 $\mu$) (1). Electrophoretic studies suggest, however, that it is a $\beta$-globulin which is primarily responsible for stabilizing the chylomicron in plasma.

Of course the formation, transportation, and disposal of chylomicrons involves more complexities than are suggested here, but it seems to be a less striking problem than that of the lipid in post absorptive plasma. Despite the fact that plasma contains some 7 to 8 gm. of lipid per liter, normal, fasting plasma is clear by transmitted light, and cannot contain any appreciable quantity of lipid particles approaching the size of chylomicrons. The idea, that this may be due to the existence of protein-lipid complexes is not new. In 1913, Haslam (2) found that 8 - 10% of a "fatty, lecithin-like body" was associated with plasma euglobulin, and in 1929, Macheboeuf isolated an apparently homogeneous component of horse plasma which contained 59% protein, 23% lecithin, and 18% cholesterol esters (3). It has also been known for many years that the lipids of fasting plasma are not extracted at 37°, or room temperature, with ether and hence appear to be combined in some way with protein. With the development of electrophoresis, it also was possible to show that both phospholipid and cholesterol migrated with plasma proteins, — particularly the $\alpha$- and $\beta$-globulins (4). Ultracentrifugal studies of Pedersen (5), extended by Goffman and co-workers in recent years, have also indicated the existence of protein-lipid complexes containing large and various proportions of lipid.

Although some interaction between lipid and protein clearly exists which gives rise to small, relatively stable units, the precise character of the units in human plasma still requires elucidation. There would, rather obviously, be some considerable difference between particles stabilized by a particular plasma protein, but having variable sizes and proportions of lipids, and what might properly be called lipoprotein molecules with definite sizes and composition. Although both may be imagined to result from some interaction of pre-existing protein and lipid particles, the latter might more likely arise by some specific, biosynthetic process. Unfortunately, many of the criteria for the existence of protein-lipid complexes would not distinguish between the two possibilities. Whatever forces are involved must be weak, as indicated by the fact that a large proportion of the lipid can be extracted after freezing at -15° C, or by denaturing the protein. A decision regarding the nature of any lipid-protein complex can be made adequately only after its isolation and a careful study of its size, composition and other properties. Since there have been a variety of methods used for the study of lipid-protein complexes, and since they each yield somewhat different sorts of information, some of the evidence obtained by the application of each method will be considered in an effort to evaluate the present status of knowledge about the state of lipids in fasting, human plasma.

## Isolation Studies

In 1943, Adair and Adair (6) reported the isolation of a very nearly homogeneous lipoprotein by ammonium sulfate fractionation. This was precipitated at pH 7, between 50 and 60% saturated $(NH_4)_2SO_4$ and purified by repeated re-precipitation. It was found to have a density of 1.10, and contained 8.5% phospholipid, 16.4% cholesterol, and 20.4% esterfied fatty acids.

Cohn and co-workers (7) have isolated two lipoproteins by low temperature alcohol fractionation, an $\alpha$-globulin from Fraction IV, and a $\beta$-globulin from Fraction III. The $\alpha$-globulin is less well characterized, but contains 65% protein and 35% lipid, resembling the lipoprotein of Adair and Adair. It is estimated to have a molecular weight of 200,000, and to have dimensions of about 50 x 300 A°. The solubility is that of a pseudoglobulin.

The $\beta$-lipoprotein is estimated to account for about 5% of the total protein (about 3.5 gm. per liter), and to contain about 3/4 of the lipids of plasma (about 6 gm. per liter). It is a euglobulin with a solubility minimum at around pH 5.4. This lipoprotein has the rather surprising composition of 25% protein, 30% phospholipid, and 45% of cholesterol and cholesterol esters. As in whole plasma, the cholesterol is about 75% in the ester form. The shape of the molecule is approximately spherical with a hydrated radius of about 165 A°, an anhydrous molecular weight of 1,300,000 with hydration of 0.6 gm per gm protein, and an anhydrous specific volume of 0.95. (See also (8), (9).

## Electrophoresis

A number of early studies had indicated that the lipids of human plasma are associated with globulins, particularly $\alpha$- and $\beta$-globulins (4, 10). Association of lipids with the $\beta$-globulins was also indicated by the disappearance of the $\beta$-anomaly after low temperature ether extraction (11), and by the fact that the greatly increased $\beta$-globulin peak of obstructive jaundice plasma, which is also clear, is reduced by ether extraction.

The moving boundary method of electrophoresis has serious deficiencies when one wishes to determine the composition of materials associated with a particular protein component. Only a portion of the most rapidly moving and slowest components can be isolated from the electrophoresis cell. Other components can only be obtained admixed with one or more additional proteins. So-called "zone electrophoresis" on filter paper or in a supporting slab of starch offers better possibilities for the study of the chemical composition of particular electrophoretic fractions. With this method, the material isolated as a separate electrophoretic zone may be removed from the paper or starch and analyzed for its various components. Alternatively, on paper, the whole strip may be treated with a suitable reagent which will develop a color with lipid, protein, cholesterol, etc. Studies on filter paper, using Sudan IV or Oil Red O to stain lipids and the Shultz reaction for cholesterol, have shown that the largest part of the lipid and cholesterol are present in the $\beta$-globulin, and a smaller amount is present in a fast $\alpha_1$-globulin (12, 13, 14). The results are somewhat complicated by the tendency of lipoprotein or lipid to be adsorbed on the paper, which leads to trailing of the adsorbed material behind the moving zone. Much more elegant results are provided by the results of zone electrophoresis in starch (15) with analyses for cholesterol and phospholipid. These studies show that at pH 8.5 the $\beta$-globulin contains most of the cholesterol, with a phospholipid/cholesterol ratio of about 0.8. The fast $\alpha_1$-globulin contains about 1/3 as much cholesterol as the $\beta$- and about 2/3 as much phospholipid, so that the phospholipid/cholesterol ratio is about 2. The unesterified cholesterol of both fractions is about 25% of the total.

It might be well to note, here, one of the limitations of electrophoresis. The separation attained is a function of charge, and two or more proteins may have such a charge that they have essentially equal mobilities at a particular pH. The results of Kunkel and Slater, for example, indicate that at lower values of pH and ionic strength the α-lipoprotein may be resolved into 2 to 4 components, and the β-lipóprotein may show 2 to 3. Consequently, it seems quite probable that we should not think of one α- or one β-lipoprotein, — but of the likelihood that there may be more than one of each. It should also be emphasized that two particles of very different size, but having a surface coating of the same protein would have essentially the same mobility. This is most clearly seen from the experiments of Abramson and Moyer (16), which showed that small particles of quartz, etc. with an adsorbed layer of protein, have a mobility which is very nearly the same as that of the molecules of the adsorbed protein.

**Ultracentrifuge**

Early studies of undiluted or slightly diluted serum or plasma in the ultracentrifuge had shown the apparent proportion of albumin to be in the neighborhood of 85%, rather than approximately 55% as indicated by isolation, or by ultracentrifugation or electrophoresis of diluted serum or plasma (17). Studies of this phenomenon lead Pedersen to conclude that there was an X-protein present, a complex of albumin and lipoprotein with a sufficiently low density to reduce its rate of sedimentation to that of albumin. He was also able to show that the rate of sedimentation of this component was markedly influenced by the density of the medium, and that the X-protein could be isolated by flotation in concentrated salt solution (18). The protein of the X-protein could be inferentially related to β-globulin, since electrophoretically isolated β- and γ-globulin contained a low-density lipoprotein (1.03), similar in this respect to the X-protein, whereas pure γ-globulin did not. There are still some problems presented by the apparent concentration of X-protein in undiluted plasma, since it is doubtful that lipoproteins involved could amount to more than 10% of the total serum proteins, and Johnston and Ogston (19) have considered the role of boundary anomalies in this phenomenon. There is no question, however, of the presence of low-density materials containing lipid and protein, which can be isolated from plasma by flotation in a medium of elevated density. The study of these materials has been principally pursued in this country by Gofman, Lindgren, Elliott, and other collaborators who have generally followed the practice of separating floating components in a medium of density 1.063 (not including the contribution of proteins to density) by prolonged centrifugation at about 81,000 x g. They also showed that other materials floats between a density of 1.063 and 1.24, and that material containing 95% of the total cholesterol may be removed at a density of 1.24 (20). An attempt has been made to bring together the results obtained by this group of workers in Table I. (See also 21, 22) The $S_f$ values refer to flotation rates in a medium of density 1.063 at 27° C. and the densities are obtained from measurements of $S_f$ in media of two or more different densities.

**TABLE I.**

| $S_f$ | Density | Molecular Weight | Lipid Components | Remarks |
|---|---|---|---|---|
| $10^4$ | 0.95 | Chylomicrons | Triglycerides, 1% of total cholesterol | Present in alimentary lipemia |
| 40 | 0.958 | 5,900,000 | All contain cholesterol and phospholipid. $S_f$10-20 contain about 30% cholesterol. | Major fraction in alimentary lipemia. |
| 27 | 0.988 | | | |
| 18 | 0.988 | | | Normal value 45 ± 25 mg % |
| 12 | 1.016 | 3,400,000 | | |
| 9.7 | 1.022 | | | Total $S_f$ fraction in $\sigma < 1.063$ is about 5% of total protein. = ca 350 mg % |
| 6.1 | 1.035 | 2,500,000 | | |
| 4.1 | 1.042 | 1,700,000 | | |
| 2 | 1.051 | 1,500,000 | | |
| | 1.072 | 250,000 | | α-globulins |
| | 1.125 | | | |

If the material floating in a density of 1.063 contains about 5% of the plasma protein, and since it must contain over 75% lipid (density less than 1.06), it should amount to about 10 gm per liter. It seems more likely that Gofman *et al.* mean that it contains 5% of the material contributing to the refractive index gradient, or about 3.5 gm per liter. In any event, the material with $S_f$ 2-4 (probably 2 only) must correspond to the β-lipoprotein of Gurd *et al.* (7). Whether the protein associated with larger particles is the same cannot be stated at present, nor can the degree of homogeneity of these components be precisely evaluated. Since their density is less than that of β-lipoprotein (using this term only for the component isolated by alcohol fractionation) they cannot be aggregates of β-lipoprotein. The lower densities must be interpreted as meaning that they contain less protein ($\rho$ ca 1.3), less cholesterol ($\rho$ ca 1.06) or a larger proportion of fatty acids ($\rho$ ca 0.92) as phospholipid, triglyceride, or cholesterol esters.

Another ultracentrifugal approach has been that of Turner, Snavely, Goldwater, Randolph, Sprague, and Unglaub (23). Serum is centrifuged at 130,000 x g for 4 hours at 28° C. A period of 75 - 110 minutes is allowed for deceleration, so that the stratification produced by centrifuging will not be disturbed, and the tubes are cut with a special knife arrangement which seals off the section of the tube between cuts. Typical data for a tube divided into 10 sections, and numbered from top to bottom, are given. An experiment such as this is difficult to interpret, since both sedimentation and flotation must be taking place, and although an equilibrium may be approached, it can hardly have been reached. However, it is interesting to note that the lightest layer, which is richest in triglyceride, has a relatively low albumin (as well as total protein), a relatively high ratio of phospholipid to cholesterol compared with other fractions, and a relatively high proportion of free cholesterol. The region around section 4 contains the largest proportion of cholesterol, and would be presumed to correspond to β-globulin, but the phospholipid/cholesterol ratio is even lower than that of β-lipoprotein (0.6 as compared with about 0.8). The lower levels, around 8, have phospholipid/cholesterol ratios of about 2, like the α-lipoprotein, but the most remarkable fact (in relation to the other lines of evidence previously discussed) is the finding of relatively high phospholipid and triglyceride, with low cholesterol, in section 10. This would indicate that although the cholesterol containing fractions may be essentially entirely removed by flotation in the ultracentrifuge, there is a component (or components) of fairly high density with little or no cholesterol, but containing appreciable amounts of phospholipid and triglyceride. This might well be of more importance in the transport of

non particulate triglycerides than the β-lipoprotein.

### Extraction of Lipids from Serum

The inability to extract lipids with fat solvents at ordinary temperatures has been recognized for many years, and has been taken to be due to binding of lipids by some component of serum or plasma. Although treatment with alcohol, at room temperature, makes the lipids extractable, it also denatures the protein. However, when protein is extracted at -15° C with absolute ethanol, and then with ether, a large part of the lipid may be extracted without denaturing at least much of the protein. Macheboeuf has applied this technique to his lipoprotein from horse plasma (cenapses acidique), and found the electrophoretic mobility unchanged and the solubility properties but little altered (3). The study of lipid extraction with ether as influenced by alcohol or pH have been studied (24, 25). In 1942, Adair showed the extraction of lipids can be accomplished with ether, alone, if the mixture is frozen at a low temperature (26). In keeping with this evidence for the disruption of lipid-protein complexes by freezing, Cohn and co-workers have reported that their β-lipoprotein will not withstand freezing. In view of these facts, it is perhaps surprising that only a small fraction of the cholesterol of normal, lyophilized serum is extracted with cold chloroform (27, 28).

On the other side of the picture, indicating even less firm binding with protein is the report that after complete precipitation of dog plasma proteins with $Ag_2SO_4$ or acetic acid, 80 to 90% of the cholesterol remains in the supernatant (29). The cholesterol is only partly precipitated from protein-free plasma at 0.5 sat. $(NH_4)_2SO_4$, but is completely precipitated by 0.7 sat. It is also reported by Koehler (30) that about 90% of the cholesterol of human serum can be extracted with ether at 0° C. This is surprising, in view of the previous experience of others, and in view of the report that when horse serum is extracted with ether containing 6 - 10% ethanol at 0° C, it does not extract lipids, as it does at room temperature, but causes a precipitate of protein which is rich in lipids. The precipitate is soluble in serum at 15° or above, but not in $H_2O$ or saline (31). Another experiment indicating that the free cholesterol of plasma is relatively mobile involved the labeling of dog plasma and red cell cholesterol with $C^{14}$ acetate (32). When the labeled cells, or plasma, were separated and equilibrated with their unlabeled counterparts, rapid exchange of free cholesterol was found to take place, with a half time for equipartition of about 1.4 hours. Human cells were also shown to enter into this same equilibrium with dog plasma. Since there is no appreciable esterfied cholesterol in the red cell, the experiments yield no information regarding this fraction. With another type of cell, uptake of lipoprotein might have had to be considered seriously, but in this case it must mean that the free cholesterol of both the red cell and the plasma are readily able to leave any combinations in which they normally exist.

### Factors Involved in Producing Clear Lipid-Containing Systems

Although a number of studies have shown that many proteins, serum albumin being a notable example, will combine strongly with organic anions (see for example, 33), even when the protein bears a net negative charge, these studies do not appear to contribute greatly to our understanding of the normal lipid-protein complexes of plasma. They do, however, make it clear that an increasingly large, nonpolar group may increase the strength of binding, although a negative charge appears to be necessary, since uncharged molecules or positively charged ions do not appear to be bound. Actually, the fact that albumin will bind only some 6 to 7% of fatty acid ($C_{12}$) constitutes no real objection, since another protein might bind more by a similar mechanism. If, for example, the negative charge on lecithin were involved, and if one of the fatty acid residues were to be involved, by van der Waal's forces, with holding cholesterol in the complex, a rather large proportion of lipid might be bound. There does not appear, however, to be any good evidence regarding the binding of lecithin by proteins.

The implication of phospholipid comes from a number of sources. In the first place, all of the lipid-protein complexes except the relatively large ones contain phospholipid. One of the characteristic features of obstructive jaundice is a hyperlipemia without turbidity, and a high phospholipid content with a great increase in β-globulins (34). The release of lipid from the X-globulin by lecithinase was studied by Petermann (35), and Ahrens and Kunkel (36) showed that the clear, hyperlipemic sera of obstructive jaundice become turbid when treated with lecithinase. They were, on the other hand, unable to clear turbid serum by mixing it with clear, obstructive jaundice serum. McFarlane (37) has also attempted to disperse cholesterol, cholesterol oleate, or triolein in rabbit serum with the aid of an ultrasonic generator. He was unable, however, to obtain clear suspensions. The addition of brain lecithin or of an egg yolk lecithin and bile salt emulsion to rabbit serum was also studied. It was found that about 15% of the lecithin dialyzed out during the preparation of samples for electrophoresis (2 1/2 days), whether in the presence of absence of serum, and that neither the mobility or proportion of lipid phosphorus in the plasma globulins was changed by the addition of lecithin. To quote "It would appear that the body has some mechanism which we have not been able to imitate by which it assembles lipids in proper proportions, possibly including traces of still unrecognized material and assembles the whole inside an α- or β-globulin envelope which effectively isolates the micelles".

A somewhat different approach is provided by studies of the "clearing factor" formed as the result of injecting heparin. Following the report in 1943 by P.F. Hahn that alimentary lipemia was abolished by the injection of heparin (38), Graham *et al.* (39) found that injected heparin promotes a shift from high to low $S_f$ values in the large lipoprotein components, as well as disappearance of chylomicrons. Studies of the mechanisms involved have been carried on by Anfinsen and co-workers (40, 41), and it has been found that heparin promotes (in viva) the conversion of a plasma protein present in Fraction IV-1 to "clearing factor", which appears in Fraction III-1,2,3. "Clearing factor" may act catalytically, producing a change in which "co-protein" (present in Fraction III-0) and large lipoproteins form smaller lipoprotein units. The interesting aspect of this reaction is not simply that cleavage into smaller units takes place, but that components are formed which have the properties of a lipoproteins. This is indicated by ultracentrifugal evidence (41), as well as by the earlier studies of Rosenberg (12) of the behavior in filter paper electrophoresis.

An additional observation, which as yet has to be evaluated in terms of its physiological significance, is based upon the recognized instability of the lipoproteins to procedures such as dialysis. It has been reported that this may be prevented by a material isolated from lyophilized beef plasma (42). Since the initial extraction is carried out with glacial acetic acid, and since the substance is precipitated from ether solution by acidification, and subsequently taken up in aqueous solution to give a yield of 3 gm per liter of plasma, it is certainly difficult to relate it to any substance known to pre-exist in plasma.

### Discussion

On the basis of isolation studies, primarily, there are two types of lipoprotein which account for the major portion of the lipid present in post-absorptive plasma. The behavior of both of these suggests that the surface is predominantly protein, and it would certainly be unreasonable to postulate a structure in which the surface was lipid in nature. As a matter of fact, the size and shape assigned to the α-globulin by Cohn and co-workers would correspond nicely with a lipid core covered by a monolayer of protein. In the case of the β-globulin, however, there is only half enough protein to cover the surface of a sphere containing the lipid. We would have to assume a patchy surface, in which

it would be most reasonable to assume that the balance of the surface would be made up by the polar portion of the phospholipid present. In the case of the β-globulin in particular it is difficult to see how the molecular size would be controlled unless the molecule is synthesized by some rather specific biosynthetic mechanism. It does not seem too likely that a spontaneous physical process could give rise to the assembly of the proper amount of cholesterol and phospholipid. The implication of the work on the clearing factor, on the other hand, is that the smaller lipoproteins arise from some sort of rearrangement in the presence of the proper co-protein. However, the identity of the material formed under the influence of clearing factor with those present in postabsorptive plasma has not been demonstrated, — and it seems unlikely that such identity can be demonstrated until the normal lipoproteins are adequately characterized.

It is evident from the work of Kunkel and Slater that there are probably several α-lipoproteins and several β-lipoproteins, — leaving out of consideration the lighter and larger complexes in which Gofman and co-workers have been interested. This view has been confirmed by the work of Gitlin (43), who has found several immunological components in the β-lipoprotein of Fraction III-O. He also reports that Oncley has found that the β-lipoprotein, which was previously considered to be homogeneous, can be separated into three components by centrifugation in a medium of high density. It seems evident, therefore, that a further investigation of these lipoproteins, using the mildest possible methods for their separation, and with more attention to the precise nature of the lipids present, would be in order.

There are also a number of problems relating to the absorption and transportation of cholesterol and of carotenoids which must be considered in dealing with the general problem of lipoproteins. Species differences in susceptibility to dietary hypercholesterolemia appear to be related to differences in the plasma proteins (44). We have been interested, in this laboratory, in the possibility that species differences in the absorption of carotenoids might be related to the presence or absence of suitable, specific transport proteins (45, 46). Although carotene and vitamin A have been reported to be present in β-lipoprotein, the molar amounts in the purified protein are small and may be adventitious. If the vitamins are transported specifically, it would not be likely to be by virtue of being dissolved in the relatively large amount of lipid in β-lipoprotein. These questions, again, cannot be answered until we have a more precise understanding of the nature of the lipoproteins of normal, postabsorptive plasma.

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*Presented before the Southern California Section of the American Association of Clinical Chemists, Inc.*

## SCIENTIFIC SESSION PROGRAM

**THURSDAY AFTERNOON, SEPT. 16**
Hotel Park Sheraton, Ballroom

### SYMPOSIUM ON LIPIDES AND LIPOPROTEINS

**Arthur Knudson, *Presiding***

2:00 Arthur Knudson. Introductory Remarks.
2:05 Ray K. Brown. Chemical and Enzymatic Studies of Serum Lipoproteins.
2:35 Harry Sobotka and David Adlersberg. Electrophoresis and Monomolecular Studies with Serum Lipoproteins.
3:05 Raymond Reiser. Recent Studies on Fat Digestion and Absorption.
3:35 David E. Green. Oxidation of Fatty Acids.
4:05 Ancel Keys. The Relationship of Blood Lipides in Man to the Diet.
4:45 Business Meeting, AACC.

**FRIDAY MORNING, SEPT. 17**
Hotel Park Sheraton, Tropical Room

### CLINICAL CHEMISTRY AND GENERAL

**Max M. Friedman, *Presiding***

9:00 Max M. Friedman. Introductory Remarks.
9:05 Allen F. Reid, Jack K. Jeanes, Richard C. Gilmore, Jr., and Margaret C. Robbins. Studies on the Mechanism of Inhibition of Inorganic Phosphate Loss from Erythrocytes.
9:20 T. B. Van Itallie, W. B. A. Bentley, Mary C. Morgan, and L. B. Dotti. Glucagon-Induced Hyperglycemia as an Index of Liver Function.
9:35 Paul Numerof, Maxwell Gordon, and Jacques M. Kelly. The Metabolism in the Mouse of 3,4,5-Trimethoxybenzoyl (Carboxyl-$C^{14}$) Methyl Reserpate (Reserpine).
9:50 Raymond B. Poet and Jacques M. Kelly. The Estimation of Submicrogram Quantities of Reserpine in Biological Media.

*Cont. p. 63*

# DIFFERENTIAL DIAGNOSIS IN ELECTROLYTE DISTURBANCES*

**Telfer B. Reynolds,**
**Assistant Professor of Medicine**
***University of Southern California School of Medicine, Los Angeles, California***

Physicians often utilize blood levels of electrolytes more or less as screening tests. An abnormal result then requires consideration of certain possible causes. In the following discussion an attempt has been made to list the commonest causes of abnormalities in blood electrolyte values according to the frequency with which they are seen in hospital practice and to indicate some simple biochemical points in differential diagnosis. This is a practical if not a physiologic viewpoint.

**I. Blood non-protein nitrogen (or urea) level**

Though not an electrolyte, this blood constituent is intimately associated with electrolyte problems, and the determination is often used as a screening test. The NPN level in the blood is dependent both on protein breakdown and on renal excretion. However, the second factor is of such paramount importance as to warrant the generalization that the NPN level is inversely proportional to glomerular filtration rate (GFR) when the latter is reduced beyond 50%.

A. *Causes of an elevated NPN*

1. *Chronic renal disease.* GFR is reduced because of reduction in functioning nephrons. The elevated NPN is accompanied by low serum bicarbonate and pH, hyperphosphatemia, albuminuria, isosthenuria and anemia.

2. *Circulatory insufficiency due to shock* (hemorrhage or myocardial infarction). No characteristic biochemical disturbance.

3. *Circulatory insufficiency due to dehydration.* (Loss of gastrointestinal secretions from vomiting, diarrhea or fistulae; exudation from burns). The urine is concentrated; the hematocrit, plasma protein level, and plasma specific gravity are high. There is loss of skin turgor, and there may be acidosis or alkalosis.

4. *Acute tubular necrosis.* There is oliguria with a low specific gravity, an elevated serum potassium and usually some evident etiology for the tubular damage.

5. *Urinary obstruction.* (Prostatism or carcinomatous ureteral obstruction). Differentiation from acute tubular necrosis is by bladder or ureteral catheterization.

6. *Acute nephritis.* The findings are similar to those in chronic nephritis with the addition of edema and microscopic or gross hematuria.

* Abstract of talk given April 7, 1953 before Southern California Section of the American Association of Clinical Chemists.

**II. The serum bicarbonate level is second only to the NPN in value as a screening. test.**

A. *Causes of an elevated serum bicarbonate*

1. *Vomiting of stomach juices* (peptic ulcer of the pylorus, alcoholic pyloric gastritis, carcinoma of the pylorus). The characteristic findings are evidences of dehydration, a high NPN and bicarbonate level, and a low serum $Cl^-$ and $K^+$.

2. *Postoperative gastric suction.* Similar findings, but the azotemia is usually minimal.

3. *Mercurial diuresis.* The serum $Cl^-$ is low.

4. *Severe pulmonary emphysema.* The bicarbonate level is high because of poor $CO_2$ ventilation. The serum $Cl^-$ and the blood pH are both low.

5. *Cushings syndrome.* The serum $K^+$ level is low and urinary cortisteroids are high.

B. *Causes of a low serum bicarbonate.* The cause of a severe acidosis can usually be determined, but in many instances a mild acidosis (bicarbonate circa 20 meq/L goes undiagnosed.

1. *Renal disease with azotemia.* There is failure of the normal renal tubular base conserving mechanisms. There are other biochemical abnormalities as previously discussed.

2. *Diabetic ketosis.* High levels of blood and urine ketone bodies can be demonstrated.

3. *Severe diarrhea.* There is concentration of the blood and urine, tissue turgor is poor and the NPN is elevated.

4. *Administration of the diuretic Diamox.* Renal base conserving mechanisms are inhibited. Serum $Cl^-$ level is elevated and $K^+$ level often low.

5. *Renal tubular acidosis.* The serum $Cl^-$ level is high, the $K^+$ level usually low and the urine pH persistently high (between 6 and 7).

6. *Following ureterosigmoidostomy.* The serum findings are similar to tubular acidosis.

7. *Cationic exchange resin administration.* No associated biochemical abnormalities.

8. *Hyperventilation* (hysteria, a mechanical respirator, or a head injury). Serum $Cl^-$ is usually not much elevated and blood pH is alkaline. Tetany is common.

9. *Aspirin poisoning.* The ferric chloride urine test for acetone is positive even after boiling the urine.

10. *Excessive ammonium chloride ingestion.* Serum $Cl^-$ level is high.

11. *Methyl alcohol poisoning.* The blood test for alcohol is usually positive for 12-36 hours.

**III.** Serum chloride levels are usually determined in conjunction with bicarbonate levels. They are reciprocally affected in most electrolyte disturbances.

A. *Causes of a low serum $Cl^-$ level.* If the low level is associated with an elevated serum bicarbonate, then the differential diagnosis is the same as for the latter condition (see above). If the serum bicarbonate level is normal, then the low $Cl^-$ level has a different meaning. It will usually be found that the serum $Na^+$ level is low, and the conditions listed under that heading should be considered.

B. *Causes of a high serum Cl level.* If the serum bicarbonate is normal, the $Na^+$ level will usually be high, and the cause is obscure. If the serum bicarbonate is low, consider:

1. Renal tubular acidosis.
2. Overingestion of ammonium chloride.
3. Diamox administration.
4. Ureterosigmoidostomy.

These conditions are discussed above.

**IV.** The serum $K^+$ level is a reliable indicator of $K^+$ deficiency or excess.

A. *Causes of a low serum $K^+$.* This implies insufficient intake to balance the usual daily urine losses, or accelerated loss in gastrointestinal secretions.

1. *Prolonged deficient intake in a sick patient.* There are usually no associated biochemical abnormalities.

2. *Postoperative nasogastric suction.* The serum bicarbonate is often high and the urinary $K^+$ loss greater than normal.

3. *Prolonged vomiting.* The usual cause is obstructive vomiting from the stomach with alkalosis, hypochloremia and azotemia.

4. *Cortisone or ACTH treatment.* Urinary $K^+$ loss is accelerated.

5. *Diabetic acidosis under treatment with insulin.* Serum depletion may be rapid and severe.

6. *Prolonged diarrhea.*

7. *Gastrointestinal fistulae.*

There may be associated acidosis, dehydration and azotemia for (6) and (7).

8. *Diuretic phase of acute tubular necrosis.* There will be a large urine volume with a high sodium and a relatively low urea concentration and azotemia.

9. *Diamox administration.* (See above).

10. *Renal tubular acidosis.* (See above).

11. *Chronic renal disease.* Potassium is lost as fixed base. The findings are as previously noted.

12. *Cushing's syndrome.* Often a mild alkalosis is present.

13. *Familial periodic paralysis.* No other biochemical abnormalities.

B. *Causes of a high serum potassium*

1. *Hemolysis or prolonged standing* of the uncentrifuged blood sample.

2. *Any type of renal disease in which oliguria or anuria is present.* In this instance, ingestion of $K^+$ or its formation from tissue breakdown simply exceeds its renal excretion. The findings are as previously stated.

3. *Addison's disease.* Seen only in crisis or pre-crisis. The NPN is high and the $Na^+$ low.

V. The significance of alterations in serum $Na^+$ levels is at the present time quite obscure. This probably reflects our lack of knowledge of the mechanisms of water metabolism.

A. *Causes of a low serum $Na^+$.* It is becoming increasingly evident that this finding does not necessarily indicate total body $Na^+$ deficiency.

1. *Dilution due to water retention* in patients with severe congestive heart failure, cirrhosis with ascites, renal disease, or postoperatively. The NPN may be mildly elevated, edema and weight gain are usually evident and the urine volume low. The patient is commonly doing poorly. The serum $K^+$ is sometimes elevated.

2. *Addison's disease in crisis.* The NPN and serum $K^+$ are high and there are evidences of dehydration. Urine $Na^+$ concentration is high.

3. *An unexplained finding* in patients with severe or chronic illness of many types. It is particularly frequent in tuberculosis. There are no known associated biochemical findings other than the proportional depression of the serum $Cl^-$ level that is seen in nearly all instances of hyponatremia.

B. *Causes of high serum $Na^+$.* This phenomenon is infrequently seen and poorly understood. The serum $Cl^-$ is usually proportionately elevated.

# REVIEW OF CURRENT LITERATURE


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


SELECTION AND INTERPRETATION OF LIVER-FUNCTION TESTS. Frank W. Konzelmann (Central Dispensary and Emergency Hosp., Washington, D.C.) Penna. Med. J. *57*:217-29, 1954.

A crit. review with 25 references. H.A.

COLORIMETRIC DETERMINATION OF BLOOD CALCIUM WITH CHLORANILIC ACID. Arthur E. Teeri (Univ. of New Hampshire, Durham). Chemist Analyst *43*:18-21, 1954.

Mix 2-3 ml. of clear serum with 2 ml. water and 1 ml. of satd. $(NH_4)_2C_2O_4$ soln. in a 15-ml. graduated centrifuge tube. Allow to stand to insure complete pptn. of $CaC_2O_4$. Centrifuge until the ppt. is well packed in the bottom, decant the liquid, and wash the ppt. several times with water. Add exactly 10 ml. of 0.1% chloranilic acid soln. Filter after at least 4 hrs., and measure the optical D. of the filtrate at 540 mu. H.A.

HYPOKALEMIA AND ITS RELATION TO THE CLINICAL AND ELECTROCARDIOGRAPHIC FINDINGS IN ARTIFICIAL HYPOGLYCEMIA. S.H. Alatas, F. Berker, and O.N. Ulutin (Univ. Istanbul). Istanbul Contrib. Clin. Sci. *2*:94-128, 1952.

Hypoglycemic shock was produced in 14 patients. When blood glucose (I) levels were above 40 mg.%, the I concn. of venous blood was lower than that of arterial blood; when blood I levels were under 40 mg.%, I was higher in the venous blood. During hypoglycemia the blood K showed changes parallel to I; inorganic P decreased, whereas alk. phosphatase increased. The fact that the decrease of P is not proportional to that of I and K is explained by the effect of increased serum phosphatase. H.A.

PRINCIPLES AND CLINICAL SIGNIFICANCE OF BLOOD IODINE DETERMINATION. E. Klein (Stuttgart-Bad Cannstatt Hosp., Ger.). Schweiz. med. Wochschr. *84*:146-9, 1954.

The modern methods of detg. the hormone I in blood are reliable and for routine work are quite useful. By addnl. analysis of the org. I in the plasma-albumin fraction, its diagnostic assurance increases to 90%. In functional disturbances of the thyroid, quant. and qual. changes occur in the compn. of blood I. 93 references. H.A.

FIBRINOGEN DETERMINATIONS BY PAPER ELECTROPHORESIS. Ivan Berkes and Vinka Karas (Univ. Zagreb, Jugoslavia). Biochem. Z. *324*:499-501, 1953.

Fibrinogen appears as an independent component in the paper electrophoresis of plasma in phosphate buffer. H.A.

A MICROCOLORIMETRIC DETERMINATION OF CREATINE IN URINE BY THE JAFFE REACTION. H.H. Taussky, with technical assistance of G. Kurzmann (Russell Sage Institute of Pathology, Dept. of Medicine, Cornell University Medical College, and The New York Hospital, New York, N.Y.). J. Biol. Chem. *208*:853-861, 1954.

Optimal conditions were established for the quantitative conversion of 10 - 80 gamma of creatine in a boiling water bath with the sole addition of diluted picric acid. Under these conditions glucose in amounts up to 60 gm./liter of urine does not interfere with the determination. Preliminary treatment of the urine with iodine eliminates the interference of acetone, acetoacetic ester, and ascorbic acid. E.V.

ENZYME ACTIVITY AND PROTEIN CONCENTRATION IN THE SERUM OF PATIENTS WITH MALNUTRITION. K.L. Mukherjee and G. Werner (School of Tropical Medicine, Calcutta, India). J. Lab. & Clin. Med. *43*:727-731, 1954.

Serum amylase and lipase activity in relation to protein concentration was studied in 15 patients with nutritional edema. The serum amylase activity decreased only when the albumin concentration was below 2.5 gm%. At albumin concentrations below 2.5 gm% there was a linear relationship between albumin concentration and amylase activity. Return to normal values occurred concomitantly with an increase in serum albumin concentration. The activity of serum lipase was not significantly reduced as compared with normal control values. E.V.

PAPER ELECTROPHORETIC ESTIMATION OF PROTEINS IN CEREBROSPINAL FLUID. E. Roboz, W.C. Hess, and D.M. Temple (Depts. of Neurology and Biological Chemistry, Georgetown University, Washington, D.C.). J. Lab. & Clin. Med. *43*:785-790, 1954.

A simplified procedure is presented for the application of paper electrophoresis for the quantitative determination of the proteins in cerebrospinal fluid. Because of the very low protein content, the cerebrospinal fluid is concentrated 25-100 fold by precipitation with ice-cold acetone. The conversion factor recommended for gamma globulin was reinvestigated but was found to be unnecessary. Under the experimental conditions applied, taking readings with an electronic densitometer, the absorption of the dye bromphenol blue by albumin and globulin is the same. E.V.

## CURRENT LITERATURE

FUNCTIONAL CORRELATION OF SUGARS AND CHLORIDES IN BLOOD. N. Dobreva and N. Nachev. Annuaire Acad. Med. "Valko Tchervencov" 29:17-26, 1949-50.

Increasing the glucose content of the blood through oral administration does not affect the osmotic pressure of the blood. This accounts for the earlier observation that a drop in chloride content almost always accompanies an increase in the sugar content of blood. However, the corresponding changes in these two components alone do not account for the const. osmotic pressure, and changes in the concns. of other components, such as urea, are probably involved. H.A.

NEWER STANDARDS IN HEMOGLOBINOMETRY. F.T. Flood, E.E. Mandel, R.H. Owings, and C.F. Federspiel (Communicable Disease Center, Public Health Service, U.S. Dept. of Health, Education and Welfare; Depts. of Medicine and Pathology, Emory University School of Medicine; and Grady Memorial Hospital, Atlanta, Ga.). J. Lab. & Clin. Med. 43:897-904, 1954.

Gravimetrically prepared cupric ammonium sulfate solution and a commercially supplied stable solution of reduced hemoglobin were investigated as color standards in hemoglobinometry. Repeated optical density readings of the copper standard and a blood specimen of known hemoglobin content were made on 3 types of photometers (a total of 19 instruments) and the respective ratios of densities (DR ratios) were computed in accord with Drabkin's principle.

The DR ratios for six Klett-Summerson photometers were with ± 1.2% of the mean DR value, ± 6.8 for ten Coleman Junior and ± 8.3% for three Coleman Universal spectrophotometers. The better reproducibility for Klett instruments is attributed to the greater width of the spectral band which is inherent in filter photometers. The copper standard can be recommended for filter photometers but not for spectrophotometers.

The reduced hemoglobin standard was studied by comparing its actual hemoglobin content (calculated from iron analysis) with hemoglobin values derived from photometric measurements calibrated by means of a known blood. The variation was ± 4% for Coleman Junior spectrophotometers and ± 3% for Klett photometers; hence this standard appears to be suitable for both types of instruments. However, hemoglobin equivalents were from 3.9 - 6.3% lower than the actual hemoglobin content, indicating that a small fraction of hemoglobin was not converted to oxyhemoglobin by the ammonium hydroxide used as diluent. The hemoglobin equivalent rather than the actual hemoglobin content is therefore required for reliable measurement of the oxyhemoglobin of the blood by this means. E.V.

THE DETERMINATION OF DEXTRAN IN BLOOD AND URINE WITH ANTHRONE REAGENT. J.H. Roe (Dept. of Biochemistry, School of Medicine, George Washington University, Washington, D.C.). J. Biol. Chem. 208:889-896, 1954.

Dextran is precipitated from trichloracetic acid filtrate by alcohol; it is placed in 60% by volume sulfuric acid solution containing 0.042% of anthrone, boiled 13 min. and compared colorimetrically with a standard glucose or dextran solution treated similarly. E.V.

TOXICITY, DISTRIBUTION, EXCRETION AND MORPHOLOGIC EFFECTS OF HAFNIUM SODIUM MANDELATE IN RATS. D.L. Hinerman, R.C. Hendrix and C.V. Weller (Dept. of Pathology, U. of Michigan, Ann Arbor). Am. J. Clin. Path. 24:150-2, 1954.

None of the organs of rats given hafnium sodium mandelate were found at autopsy to contain hafnium. Rats receiving subcutaneous injections showed no evidence of systemic toxicity. They developed subcutaneous and deeper abscesses at the site of injection. Some particular matter containing hafnium was demonstrated in many organs. There was no localization in the adrenals. C.R.

A CLINICAL STUDY OF ELEVATED PLATELET ADHESIVENESS AND ACCELERATED CLOT-RETRACTION TIME. J.P. Savitsky and R. Werman (Montefiore Hospital, New York). Am. J. Clin. Path. 24:161-5, 1954.

Platelet adhesiveness and clot retraction time were found to be elevated in the majority of 500 patients with a variety of diseases. No relation was found to thrombo-embolic phenomena. C.R.

THE RELATION OF SERUM STABILITY TO THE DEVELOPMENT OF ARTERIOSCLEROSIS. N. Ressler, A.J. Boyle, and M. Kosai. (Wayne University, Dept. of Chemistry and Medicine, Detroit, Mich.). Am. J. Clin. Path. 24:194-200, 1954.

Colloid stability of serum (as measured by rate of turbidity produced on adding metallic salts) is below normal in the serum of arteriosclerotic individuals. Stability is decreased also in serum of patients with a number of other diseases. C.R.

PAPER CHROMATOGRAPHY OF FREE AMINO ACIDS IN BLOOD PLASMA. S. Gordon and G.L. Nardi. (Dept. of Surgery, Harvard Medical School and the Surgical Services, Massachusetts General Hospital, Boston). J. Lab. & Clin. Med. 43:827-830, 1954.

A chemical procedure for preparing blood plasma or serum for paper chromatographic analysis is presented. The free alpha amino acids and ninhydrinreacting substances found in normal human blood by this method are described. E.V.

THE VALUE OF SERUM ALKALINE PHOSPHATASE DETERMINATION AND BROMSULPHALEIN TEST IN THE DIAGNOSIS OF METASTATIC CANCER OF THE LIVER. H. Shay and H. Siplet (Samuel S. Fels Research Institute, Temple University School of Medicine, and Dept. of Medicine, Temple University Hospital, Philadelphia). J. Lab. & Clin. Med. 43:741-751, 1954.

Metastatic cancer of the liver should be suspected in an individual with a normal serum bilirubin and elevated alkaline phosphatase, especially if accompanied by an elevated bromsulphalein retention. Granulomatous involvement of the liver can also produce similar findings, but will also frequently alter one or more of the flocculation tests, while metastatic carcinoma of the liver does so only occasionally and sporadically. In the patient with a known malignancy, the results of the tests recommended would help to determine the probable operability. In the patient who has had a resection of a primary carcinoma, the results should clarify the significance of vague and poorly definable symptoms that appear so frequently in metastatic carcinoma. E.V.

ORGANIC CHELATING AGENT IN THE DEMINERALIZATION OF BONE FOR HISTOCHEMICAL STUDY OF ALKALINE PHOSPHATASE. D.G. Freiman (College of Medicine, Univ. of Cincinnati and Cincinnati General Hospital, Cincinnati, Ohio). Am. J. Clin. Path. 24:227-38, 1954.

Bone is demineralized by treating with ethylenediamine tetraacetic acid (EDTA) and the inactivated alkaline phosphatase reactivated by immersing deparaffinized sections in 1% $MgCl_2$. Subsequently the phosphatase may be detected by the usual staining procedures. C.R.

EFFECTS OF AMBIENT TEMPERATURE ON THYMOL, PHENOL AND ZINC TURBIDITY TESTS. V.L. Yonan and J.G. Reinhold. (Hospital of University of Pennsylvania, Philadelphia, Pa.). Am. J. Clin. Path. 24:232-8, 1954.

Thymol turbidity and thymol flocculation tests were found to vary inversely with temperature at which the test is done. It is suggested that reagents be brought to a standard temperature (25° C) and the tests be performed at this temperature for more accurate results. Measurements of phenol turbidity vary directly with temperature. Zinc turbidity tests are not significantly affected by temperature. C.R.

STREPTOMYCIN AS A CAUSE OF FALSE-POSITIVE BENEDICT REACTION FOR GLYCOSURIA. H.W. Neuberg (College Physicians and Surgeons, Columbia University, New York). Am. J. Clin. Path. 24:245-6, 1954.

Urine from patients receiving streptomycin has been shown to give 1 or 2 plus positive Benedict test for glucose. This false positive test may be distinguished from true glucosuria by the yeast fermentation test. C.R.

## INSTRUCTIONS TO AUTHORS "CLINICAL CHEMISTRY"

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## SELECT EDITORIAL BOARD FOR "CLINICAL CHEMISTRY"

The National Executive Committee announced the formation of the Board of Editors for the Association's new publication, *CLINICAL CHEMISTRY*.

Harold D. Appleton, Editor of *THE CLINICAL CHEMIST*, and chemist at Metropolitan Hospital, New York City, has been named as Chairman of the Board of Editors for a six year term.

The Editorial Board makes quite an impressive list of leaders in the profession of clinical chemistry and is made up as follows: Hugh J. McDonald, Loyola University, Marschelle H. Power, Mayo Clinic, John G. Reinhold, University of Pennsylvania, Joseph I. Routh, University of Iowa, Harry Sobotka, Mt. Sinai Hospital, N.Y., Warren Sperry, N.Y. Psychiatric Institute and George T. Lewis, University of Miami, Fla.

The Editorial Advisory Board includes two outstanding foreign clinical chemists, Prof. E. J. King, University of London, and Prof. J. C. M. Vershure of Holland. The other members of the board include, Walter R. Bloor, George Gomori, L. Emmett Holt, Jr., Philip A. Shaffer, Michael Somogyi, and Donald D. Van Slyke.

Ellenmae Viergiver, Pennsylvania Hospital, will edit the Abstract Section which will bring to the busy laboratory worker a selected review of current literature in the field of clinical chemistry.

*THE CLINICAL CHEMIST* will be retained as a department of the National Secretary of the AACC and will be edited by Max M. Friedman. This section will publish the Association news and items of interest to the membership and the profession.

*CLINICAL CHEMISTRY* will be published by Paul B. Hoeber, Inc., Medical Book Department of Harper and Bros., and will be a bimonthly publication. The first issue will appear January, 1955.

RESPIRATORY FUNCTION AND ACID-BASE BALANCE IN PREGNANCY. P.H. Rossier and M. Hotz (Med. Polyclinic, Zurich, Switz.) Schweiz. med. Wochschr. *83*:897-901, 1953.

A transitory alkalosis in arterial blood, a drop in the total base and an increase in the 0 tension and saturation may be observed in a normal healthy pregnant woman. H.A.

SERUM IRON AND OPERATIVE STRESS. Ulrik Feldthusen, Vagn Larsen and Niels A. Lassen (Finsen Inst., Copenhagen). Acta Med. Scand. *147*:311-23, 1953.

Very low serum Fe values are encountered postoperatively. Normal levels are not regained as late as 12 days postoperative. Similarly low levels may be found during acute or chronic infection.

QUANTITATIVE DETERMINATION OF CALCIUM IN BLOOD SERUM BY FLAME PHOTOMETRY. G.O. Schlutz (Diagnostisch. Inst., Freiburg i. Br. Ger.) Schweiz. med. Wochschr. *83*:383-4, 1953.

Ca in blood serum is pptd. as the oxalate, centrifuged, taken up in dil. HCl, and the clear soln. exand. with the flame photometer. H.A.

## CHICAGO SECTION

The first meeting of the 1954-55 business year of the Chicago Section of the AACC will be held October 22 at 8:00 P.M. at the Sarah Morris Auditorium at the Michael Reese Hospital, Chicago.

The principal speaker of the evening will be Dr. Karl Singer, Director of the Department of Hematology, Michael Reese Hospital. He will discuss "Abnormal Hemoglobins". Dr. Clarence Cohn, Director of the Department of Biochemistry, Michael Reese Hospital will give a demonstration of the autopipette.

The last meeting of the 1953-54 business year, heard Dr. Harold Feinberg, Clinical Chemist to the Children's Memorial Hospital, Chicago, discuss his work on "Metobolic Inhibitors". A short business meeting followed the scientific session.

The Chicago Section's newly formulated constitution was approved both by the section membership and also by the National Executive Committee of the AACC.

The Executive Committee met in July at the Cook County Hospital. As per Article VII (e) of the official constitution Drs. Gordon S. Stewart and John F. Polli were appointed to fill the vacancies in the Executive Committee. As per Article IV, Dr. Clarence Cohn automatically became a member of the Executive Committee. The complete roster of the committee is:

| | |
|---|---|
| *President* | Harry F. Weisberg |
| *Vice-President* | Alvin Dubin |
| *Secretary* | Robert S. Melville |
| *Treasurer* | Alex Kaplan |
| *Past-President* | Clarence Cohn |
| *Member-at-large* | Gordon S. Stewart |
| *Member-at-large* | John F. Polli |

## SOUTHERN CALIFORNIA SECTION

Election of officers was featured at the final dinner meeting of the season of the Southern California Section of the AACC. The meeting was held at the Carolina Pines Restaurant, Los Angeles.

Dr. Herbert O. Carne, Biochemist, Long Beach Veterans Administration Hospital, automatically succeeded Dr. Merle Lovell Lewis as Chairman of the Section. Dr. Rex D. Sterling, Chemist, Los Angeles County Hospital, was elected Program Chairman and Chairman-Elect to succeed Dr. Carne. Maxine Wertman, Chemist, Los Angeles County Hospital, was elected Secretary-Treasurer to succeed William McKee.

## MIDWEST SECTION

The third meeting of the Midwest Section, American Association of Clinical Chemists was held on Friday, May 31, 1954, in University Hospitals, Iowa City, Iowa. An informal dinner was held at the Memorial Union, prior to the meeting.

Proposed By-laws for the Section were discussed and a revised edition was approved by unanimous vote. The Secretary was instructed to submit the By-laws for approval by the National Executive Committee.

The following officers were elected or the year 1954-55.

*Chairman* – Dr. J. I. Routh, State University of Iowa, Iowa City, Iowa

*Vice-Chairman* – Dr. W. D. Paul, State University of Iowa, Iowa City, Iowa

*Secretary-Treasurer* – Dr. L. C. Kier, Veterans Administration Hospital, Iowa City, Iowa

Following the business meeting, J. I. Routh, Professor of Biochemistry, discussed the determination of Protein-Bound Iodine. The method in use at University Hospitals was then demonstrated in the PBI Laboratory.

## CHICAGO SECTION SETS MEETING DATES

Five meetings have been scheduled for the coming business year for the Chicago Section. The dates are:

October 22, 1954
December 2, 1954
January 28, 1955
February 25, 1955
March 18, 1955 (with A.C.S.)
May 27, 1955

The dates above are tentative (for Fridays) subject to comments by the membership regarding change. The December date was selected to avoid conflict with the Thanksgiving and Christmas holidays. No final decision has been arrived at concerning a joint meeting with the Society for Experimental Biology and Medicine.

## BOOK REVIEWS

ADVANCES IN ENZYMOLOGY, VOLUME XV. Edited by F. F. Nord. 533 pages, $11.00 Interscience Publishers, Inc., 250 Fifth Ave., New York 1, N.Y.

*Reviewed by B. N. La Du, Jr., National Heart Institute, Bethesda Md.*

Volume 15 contains papers on:

The Mechanism of Enzymic Oxidoreduction by S. J. Leach

Thermodynamique des Reactions Immunologiques by R. Wurmser

Chemistry, Metabolism, and Scope of the Pyridine Nucleotide Coenzymes by T. P. Singer and E. B. Kearney

Alternate Pathways of Glucose and Fructose Metabolsm by E. Racker

Enzymic Mechanisms in the Citric Acid Cycle by S. Ochoa

The Mechanism of Action of Hydrolytic Enzymes by H. Lindley

Enzymatic Synthesis of Polysaccharides by M. Stacey

Urea Synthesis and Metabolism of Arginine and Citrulline by S. Ratner

Thiaminase by A. Fujita

Rennin and the Clotting of Milk by N. J. Berridge

Die Struktur des Tobakmosaikvirus und seiner Mutanten by G. Schramm

Although some of the topics included in this volume will be of greater interest to specialists in the respective fields, this volume is outstanding in that it contains so many excellent review articles which will be appreciated by those with a more general background in biochemistry.

The reviews by Singer and Kearney, Racker, Ochoa, and by Ratner are especially well written. They represent a careful integration of recent experimental work with the older literature to give the current picture of the topics discussed. The papers by Leach and by Lindley will be stimulating to those interested in the mechanism of enzyme action.

This volume is highly recommended.

## NEW BOOKS FOR REVIEW

The following books have been received by *THE CLINICAL CHEMIST* and will be reviewed in the next issue.

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

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

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

CHEMOTHERAPY OF INFECTIONS, by H.O.J. Collier, xvi + 248 pages, John Wiley

*Cont. p. 63*

## PUBLISHER'S CORNER

"The Infra-Red Spectra of Complex Molecules" by L. J. Bellamy was published in August by John Wiley & Sons.

A critical review of the data on which infra-red spectral correlations are based, the new book indicates the classes of compounds that have been studied in each case, and the known factors that can influence the frequencies or intensities of the characteristic bands. Bellamy confines his subject matter strictly to the empirical interpretation of infra-red spectra, and centers his attention on publications dealing with the groups of compounds containing common structural units.

In his choice of units, Bellamy has employed wave-numbers throughout the book. The use of this scale, he points out, makes it easier to identify overtone and combination bands, while it is particularly valuable in enabling comparisons to be made with Raman spectra.

At the beginning of each chapter, the author provides a brief outline of the correlations to be discussed, together with a table giving the various frequency ranges. A series of charts, at the end of each chapter, summarize the correlations in the usual line drawing form.

Dr. Bellamy is Principal Scientific Officer with the Ministry of Supply in England.

"The Infra-Red Spectra of Complex Molecules" contains 323 pages and is priced at $7.00.

After serving for thirty years as a trusted reference, "A French-English Dictionary for Chemists" by Austin M. Patterson is now available in a second edition. The new book, completely modernized and expanded, was pub-in March by John Wiley & Sons.

Containing 42,000 terms as compared to 35,000 in the first edition. the dictionary again provides complete chemical coverage, as well as defining words from other fields that occur in chemical reading. Dr. Patterson has combed the latest sources in his search for current nomenclature, including the most recent French books in chemistry, mechanics, geology, industry, medicine, and related fields. The results of this intensive check contribute to the increased coverage, despite the omission of terms that have the same spelling and meaning in French and English. Still one of the most practical features is the appearance of irregular forms of words, especially verbs, under their own spelling.

Patterson, formerly professor of chemistry at Antioch College, writes his publisher that he spent almost as much time on the revision as in the preparation of the original edition. The third edition of his "A German-English Dictionary for Chemists" was published by Wiley in 1950. Always at home with chemical vocabulary, Dr. Patterson edits the "Words About Words" column in CHEMICAL AND ENGINEERING NEWS.

The second edition of "A French-English Dictionary for Chemists" contains 476 pages and is priced at $6.50.

The first book in which modern reaction rate theory, developed since 1935, is extensively and systematically applied to the interpretation of biological reactions, "The Kinetic Basis of Molecular Biology" was published in June by John Wiley & Sons. The new book is the work of Frank H. Johnson, Henry Eyring, and Milton Polissar.

Presenting quantitative interpretations of many biological phenomena in both relatively simple and highly complex systems, the authors examine resentative processes in detail, and take into account their relation and signifif ance to the general aspects of biology as a whole. The point of view throughout the book is that of fundamental theory. To the extent justified by present knowledge, interpretations are expressed in terms of the basic laws governing atoms, molecules, and ions.

Chapter headings cover the following individual subjects: the basis of thermodynamics; fundamentals of classical mechanics;principles of quantum mechanics; essentials of statistical mechanics; calculation of absolute rates, and four-atom reactions; luminescence; temperature; hydrostatic pressure and molecular volume changes; action of inhibitors in relation to concentration, temperature, and hydrostatic pressure; diffusion through membranes and transmembrane potentials; physical chemistry of cell irritability and of the nerve

*Cont. p. 63*



Gentlemen: **BOX 123**

At the Dinner Meeting in New York City during the week of the 126th National Meeting of the American Chemical Society, Prof. E.J. King of London, Eng., told about the eyophilized sera samples being used in Great Britain and elsewhere for comparison standards. A recent issue of *THE CLINICAL CHEMIST* also had an article on samples employed by the U.S. Army for their laboratory service control and standards.

It is the writers opinion that it is time for the American Association of Clinical Chemists to give serious consideration to evolving comprehensive standard specimens of specific value, more extensive than any of these. Reliable standard reference samples are necessary to supply effimethods for comparisons on the work-a-day level.

No one method is always satisfactory as can be gleamed from the many revisions of the Standard Methods of the A.O.A.C., of the U.S. Pharmacopoeia and the National Formulary.

Our first volume of Standard Methods of Clinical Chemistry is no exception to the rule. Such standardization can be a dynamic asset in aiding progress or a detrement by imposing a static none progressive state of affairs.

A study should be made of evolving dessicated organic standards, along the line of the dessicated culture media; Standards which are assayed by a sufficient number of our large research type institutional laboratories, to establish the inherent limit of accuracy of the Standard Methods and of those methods under consideration for standardization. The keeping qualities of such standard samples must be determined, also the advisiability of preparation of specimens containing the minimal normal quantities and with abnormal quantities which laboratories are likely to encounter. Such standard sample supplies should be ample to permit their ready purchase by all laboratories to standardize their methods, evaluate their technique, and as a ready means by which research workers can compare their new methods and results with current standards before publication.

This will lead in an inexpensive practical manner to greater and more

*Cont. p. 62*

## NEW APPARATUS

### NEW LOW TEMPERATURE BATH AND CIRCULATING SYSTEM

Precision Scientific Company has developed the "Lo-Temptrol", a low temperature bath and circulating system designed as a convenient means of reducing temperature rapidly and holding it at a precisely controlled point.

Equipped with a combination stirrer and pump, the "Lo-Temptrol" can be used as a low temperature bath or a portable pumping system for water and sewage tests, clinical and biochemical procedures, for viscosimeter tests, and for cooling condensers, refractometers and spectrophotometers.

The "Lo-Temptrol" bath is available in two sizes with two temperature ranges. The small, 15 gallon bath has a range from room to –30°C; the large, 45 gallon bath, from room to 0°C. A "Micro-Set" thermoregulator controls temperatures to ±0.1°C near room temperature, to ±0.01°C at lower temperatures. Bath capacities are ample to permit simultaneous use as bath and circulating system, without affecting the sensitivity of control.

The "Lo-Temptrol" will circulate coolant — alcohol, ethylene glycol or Prestone (or water for above freezing) — at the rate of three gallons per minute.

The bath chamber is all stainless steel, including the rack above the cooling coil. The working area above the rack in the small bath is 12 x 20 x 8 inches deep; in the large bath, 30 x 20 x 11 inches deep.

Precision Scientific Company, 3737 W. Cortland Street, Chicago 37, Illinois.

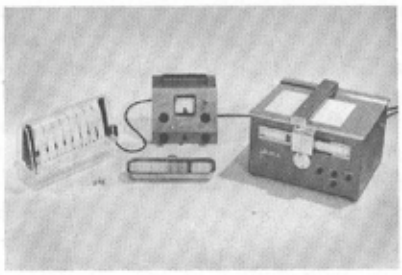
### REDESIGNED LOW-COST OVENS

Improved models of economical laboratory ovens and incubators, the Precision-Thelco line, are described in "Catalog #331A," recently published by the Precision Scientific Company.

Leader of the new design is the Thelco Model 19 Vacuum Oven, which now includes high quality ribbon heating elements and a hydraulic thermostat--at no increase in price. Other improvements have been made in the Model 18 Mechanical Convection Oven and the three sizes of Thelco Incubators.

A free copy of illustrated "Catalog 331A" will be mailed on request.

### PAPER ELECTROPHORESIS SYSTEM



Simplicity in use and reproducibility of results have been the major objectives in the design of the new Spinco Model R Paper Electrophoresis System. With this new equipment, clinical and research laboratory personnel can, without special training, handle routinely all steps in the separation of blood sera and other biological fluids, down through the final automatic chart recording and numerical analysis of component concentration in the specimen.

The Durrum-type electrophoresis cell, shown left, holds eight paper strips. Specimens are applied with precision through a slot in the cover and using an exclusive automatic striper. The power supply (center), fully regulated, can be used with either constant-current or constant-voltage output and has an easily-read indicating meter for voltage and current checking. Two electrophoresis cells can be operated from a single power supply and the electrical connections are interlocked with a low-voltage relay-controlled system which completely obviates accidental contact with the high voltage circuit.

Electrophoresed and dyed strips are fed into the automatic scanning mechanism of the Spinco Analytrol (right), which converts relative dye concentration into a pen-drawn curve with accompanying saw-tooth trace from which relative concentrations can be read directly in numerical quantities without computation.

Details of a new Paper Electrophoresis System including all instrumentation for a standardized operating procedure is described in a new Spinco folder, Form 4R-554. The equipment, manufactured by Specialized Instruments Corporation is illustrated both in an operating group and in closeup to show special features. A detailed description and list of specifications are included to cover the Durrum-type vertical electrophoresis cell; the Duostat automatically-regulated, constant-current/constant-voltage power supply; and the Spinco Analytrol recorder-integrater which automatically measures and records relative concentration of separated components.

Specialized Instruments Corporation, 682 O'Neill Avenue, Belmont, California

### AACC ANNUAL DINNER

The Sixth Annual AACC Meeting featured the Annual Dinner which was held, Thursday evening, September 16, in the El Patio room of the Hotel McAlpin, New York City. The Dinner meeting was the largest in the Association's history and showed a record attendance of members from all parts of the country.

The feature of the meeting was the presentation of the Ernst Bischoff Award to John G. Reinhold, and Dr. Reinhold's Ernst Bischoff lecture, "Detection of Carriers of Viral Hepatitis Among Blood Donors", which was of great immediate interest to all members present. Dr. Reinhold's lecture will be published in January 1955 in *CLINICAL CHEMISTRY*.

Dr. Freeman, President of the AACC, presented the Award to Dr. Reinhold and also introduced Prof. E.J. King, Post-Graduate Medical School, University of London, and Honorary Member of the AACC. Prof. King spoke briefly on the rise of the British counterpart of the AACC and his work in the formulation of the International Association of Clinical Chemistry.

### BOX 123 *(Continued)*

rapid progress in clinical chemistry than is at present possible and will contribute greatly to true dynamic standardization and accuracy.

In the writers opinion our first volume of standard methods, as now available leaves much to be desired. It is a typical research institute compilation of methods, giving no thought to the equipment and economic status of the average institutional and community laboratory who have great need of such service.

*Henry J. Goeckel,*
*Cranford, N.J.*

## SIXTH ANNUAL MEETING SEPTEMBER 16, 1954

The Sixth Annual Meeting of the American Association of Clinical Chemists was held on Thursday, September 16 at 4:45 P.M. in the Ballroom of the Hotel Park Sheraton, New York City. The meeting followed the first scientific session of the joint program of the AACC and the Division of Biological Chemistry of the American Chemical Society.

Lt. Col. Monroe E. Freeman, President of the AACC, presided at the meeting. Dr. Freeman noted that as *THE CLINICAL CHEMIST*, through its columns, has kept the membership very well informed on Association business, the meeting business was able to proceed swiftly and smoothly. After a short talk on "The State of the Association", Dr. Freeman called on Max M. Friedman, National Secretary, to explain the Executive Committee's request to the membership for a raise in dues for 1955, both for members and associate members. Dr. Friedman explained the method of financing the Association's new journal and showed how a major portion of the dues will go to provide a subscription to *CLINICAL CHEMISTRY* for each member. Each member will receive a subscription to the journal paid from his yearly dues.

Harold D. Appleton, Editor of *THE CLINICAL CHEMIST*, and newly appointed Chairman of the Board of Editors of *CLINICAL CHEMISTRY*, spoke on plans for the new journal and explained the need for such a new publication and the many planned features which will make *CLINICAL CHEMISTRY* a truly representative journal of the profession.

The advance in yearly Association dues was voted unanimously.

New business discussed at the meeting featured a discussion of state legislation for the regulation of laboratory personnel and laboratories. Members from the State of Maryland, in which such legislation is being considered by the State Legislature, presented their problem and showed how legislation in one state is usually used as a guide in other states. Methods were outlined whereby the AACC would take a more active part, together with other national scientific organizations in watching and advising state legislatures so that the profession of clinical chemistry could be adequately protected. Members were advised to watch legislation in their home states and immediately advise the Association's Committee on Legislation, Dr. John G. Reinhold, Chairman of any state activity.

The full minutes of the meeting will be published in the next issue of *THE CLINICAL CHEMIST*.

## SCIENTIFIC SESSION *(Continued)*

10:05 Philip Feigelson and Margaret Been. On the Sulfhydryl Catalyzed Alkaline Hydrolysis of *p*-Nitrophenyl Sulfate.

10:20 Oscar Touster and Ruth M. Hutcheson. The Formation of L-Xylulose by Guinea Pigs and by a Normal Man.

10:35 Roland Fischer and Neil Agnew. Competitive Inhibition of Drug-Produced Experimental Psychosis.

10:50 Hugh J. McDonald and Edward P. Marbach. Improved Detection of Lipoproteins in Human Sera.

11:05 I. J. Greenblatt, R. Wayne, D. M. Spain, and I. Snapper. Studies of Serum Lipoproteins and Plasma Lipide Clearance in Multiple Myeloma.

11:20 S. P. Gottfried, R. H. Pope, N. H. Friedman, and S. DiMauro. Serum Lipoprotein Studies in Atherosclerosis.

11:35 Betty B. Levy, Harold D. Appleton, Bernard B. Brodie, and J. Murray Steele. Determination of Choline Containing Phospholipides in Biological Material.

11:50 Joel R. Stern, Molly Lillien, and B. M. Kagan. Mucoprotein Determination in Blood and Urine.

### FRIDAY AFTERNOON, SEPT. 17

Hotel Park Sheraton, Tropical Room

John G. Reinhold, *Presiding*

2:00 John G. Reinhold, Introductory Remarks.

2:05 Jacques M. Kelly and Raymond B. Poet. A Specific Colorimetric Test for Glucose Using Glucose Dehydrogenase.

2:20 Bernard Klein and Milton Weissman. The Application of the Chromotropic-Sulfuric Acid Reagent to Polysaccharides.

2:35 Henry Tauber. Separation of α-Keto Acid Dinitrophenylhydrazones by Paper Electrophoresis and Their Colorimetric Determination.

2:50 Hertha H. Taussky. Improved Specificity of Creatine Determinations in Urine by the Jaffe Reaction.

3:05 Harold L. Rosenthal. On the Colorimetric Determination of Urea and Diacetyl Monoxime.

3:20 Augusta B. McCoord. Use of 1,2-Dichloroethane in the Liebermann-Burchard Reaction for the Determination of Cholesterol.

3:35 Priscilla Teitelbaum and M. Bier. A New Method for the Determination of Sulfhydryl Levels and Their Variation in the Blood of Rats.

3:50 Charles H. Grogan, H. J. Cahnmann, and Elizabeth Lethco. Microdetermination of Chromium in Various Biological Media.

4:05 M. Ann Wahl and B. H. Armbrecht. Use of 2-(O-Hydroxylphenyl)-Benzimidazole as a Spectrophotometric Method for Determining Iron (III).

4:20 Bernard Klein, Benjamin S. Gordon, Irving Graef, Renzo Olivetti, and Walter Newman. The Iron Content of Tissues in Endogenous and Exogenous Hemochromatosis.

4:35 Keith B. McCall. Spectrophotometric Determination of Total Hemoglobin in Plasma.

4:50 Bennie Zak, Paul J. Cherney, and Eleanor G. White. Spectrophotometric Titration of Versene in Urine Using Arsenious Acid as the Titrant.

## PUBLISHERS CORNER *(Continued)*

impulse; physical chemistry of the contractile process in muscle; and potential barriers in diffusion.

Dr. Johnson is associate professor of biology at Princeton University. Dr. Eyring is dean of the graduate school and professor of chemistry at the University of Utah. Consultant to the biomechanics group of the University of California's Medical School, Dr. Polissar is also a research chemist for the biomechanics group at the City College of San Francisco.

"The Kinetic Basis of Molecular Bio-Biology" contains 874 pages and is priced at $15.00.

## NEW BOOKS FOR REVIEW *(Continued)*

and Sons, Inc., 440 Fourth Avenue, New York 16, $4.00.

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

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

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

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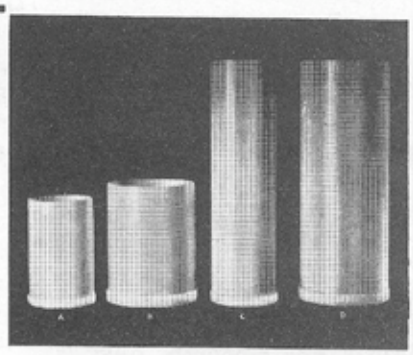

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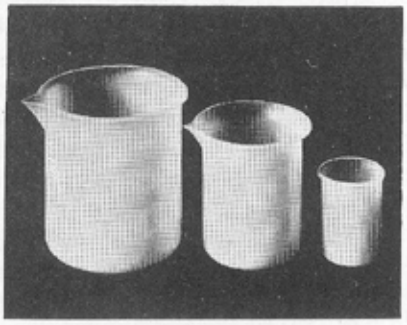

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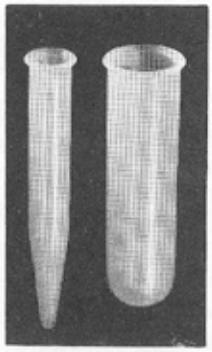

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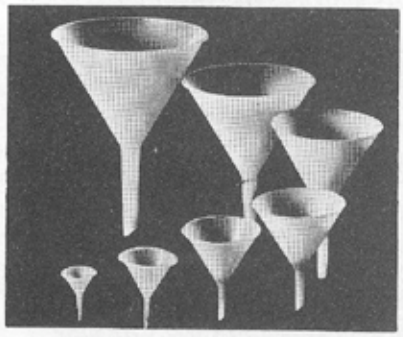

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