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The CLINICAL Chemist

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Official Publication
of
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of
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INC.

The CLINICAL *Chemist*

P.O. Box 123
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VOL. 6, NO. 4 Aug.–Oct. 1954

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 μ. Indeed, serum albumin will also stabilize such emulsions, though with a somewhat greater particle size (1 μ) (1). Electrophoretic studies suggest, however, that it is a β-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 α- and β-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 preexisting 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 α-globulin from Fraction IV, and a β-globulin from Fraction III. The α-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 β-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 α- and β-globulins (4, 10). Association of lipids with the β-globulins was also indicated by the disappearance of the β-anomaly after low temperature ether extraction (11), and by the fact that the greatly increased β-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 β-globulin, and a smaller amount is present in a fast α_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 β-globulin contains most of the cholesterol, with a phospholipid/cholesterol ratio of about 0.8. The fast α_1-globulin contains about 1/3 as much cholesterol as the β- 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-lipoprotein 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 (ρ ca 1.3), less cholesterol (ρ ca 1.06) or a larger proportion of fatty acids (ρ 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