THE STUDY OF SERUM PROTEINS AND LIPIDS WITH THE AID OF THE QUANTITY ULTRACENTRIFUGE

VII. Some Features of a System of Lipoproteins which Contain Phospholipid but No Free Cholesterol*†

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We propose in the present paper to describe a system of lipoproteins which contain phospholipid but no free cholesterol and little or no esterified cholesterol. We utilize data which we have accumulated during the past four years during which time we have studied serum proteins and lipids by making multiple analyses of ten samples of each ultracentrifugate of untreated serum. The samples of centrifugate from standardized positions in the column are designated by numbers beginning with number one at the top, which is the cream layer sample.[7] The detailed data have been published elsewhere,[7,8] or are in process of publication.[3,4,6,9,10]

The accumulated evidence from many sources, as reviewed by Edsall,[2] indicates that serum lipids are in the main associated with proteins. Our data concerning the distribution of lipids and proteins in the ultracentrifugate provide both general and special support for that concept. Our finding, in low density centrifugate, of a concentration of globulin in excess of that expected from the albumin: globulin ratio of whole serum, along with high concentrations of lipids, strongly suggests that the globulin is there in increased concentration because of buoyancy provided by lipids attached in some fashion to the globulin molecule.[7] Conversely, the finding of high concentrations of phospholipid and neutral fat in high density centrifugate indicates the attachment of these low density lipids in small proportions to many high density molecules. More specific information bearing upon this subject is provided by the systematic relationship between total globulin concentration and lipids at certain levels in the upper half of the column which we interpret as indicating stoichiometric relationships between proteins and lipids.[6]

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It is necessary that we define some of the terms used in this paper, especially that we make clear which lipids seem in a strict sense to be a part of protein molecules. Only in the cream layer sample have we observed any systematic relationship between neutral fat and any other lipid component, while there is abundant evidence for such relationship among the other three lipid components we have measured in the top five samples of normal centrifugate. Because it is difficult to imagine that so much neutral fat is incorporated into the few protein molecules present in the cream layer sample, we are inclined to discount the stoichiometrical data for neutral fat even there and have adopted tentatively the generalization that neutral fat is attached to lipoprotein molecules by some physical bond rather than by incorporation into the molecule. We have come to use a system of terminology based on the assumption that the two forms of cholesterol and phospholipid are incorporated in different combinations in protein molecules and that these same molecules establish some physical union with neutral fat to form lipoprotein-fat complexes.

Our evidence has led us to the hypothesis that there are two major systems of lipoproteins according to their lipid composition, apart from fat, and one minor system to which we assign a small fraction of cholesterol ester which has a special distribution in the centrifugate. We imagine that the lipoproteins in all of these systems may transport fat and we have evidence that the two major ones do so. The proportion of fat per unit of lipoprotein is believed to be highly variable, and in disease states and under dietary stress sedimentation characteristics may vary because of large differences in fat load. The lipoprotein of greatest complexity of lipid composition, according to our evidence, is one which contains globulin, phospholipid, and both forms of cholesterol in stoichiometric proportions, and is found in normal centrifugates in two major concentrations, the cream layer sample and the mid-zone. In this latter zone the distribution curve along the column has the general shape of a normal frequency distribution curve, that of free cholesterol.[7] The proportion of cholesterol ester relative to free cholesterol differs in the two principal concentrations, that in the mid-zone being twice that in the top sample. The relationship between free cholesterol and phospholipid, however, does not vary significantly from one part of the column to another.[8] We refer to the protein of one major system as phospholipid-cholesterol protein (PL-C-protein), and to that of the other major system as phospholipid protein (PL-protein). We emphasize the absence of free cholesterol from the protein of the second system and for the time being assume that this system contains no ester as well. The second system is therefore conveniently referred to also as that which has phospholipid without cholesterol. There is a possibility that two or more components of this system may actually contain the small fraction of cholesterol ester which has a distinctive distribution, but, partly for convenience in terminology and until more definite evidence is available, we assign this fraction

of ester to a third and separate system of lipoproteins which we call the cholesterol ester proteins (CE-proteins). This fraction of ester occurs in two concentrations, the larger deep in the column along and beyond the high density tail of the free cholesterol distribution curve, and the smaller one high in the column, chiefly in the third sample. In these two regions there is cholesterol ester in excess of that expected by the strict proportionality with free cholesterol exhibited in other parts of the column.[6] According to our terminology, union of any of these lipoproteins with fat

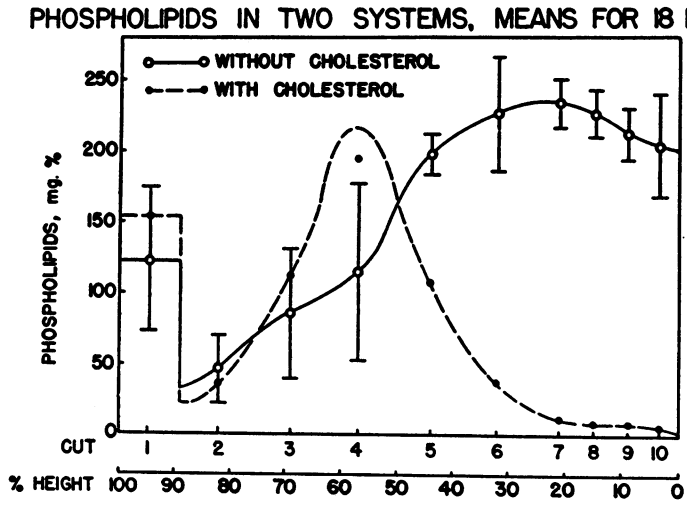

FIG. 1. Mean curves of distribution of phospholipid assigned to two systems in centrifugates of 18 healthy young men. The abscissa represents volume of centrifugate according to position in the centrifuge tube. Figures 1-10 indicate the centers of each of the ten standard samples. The centrifuge tube may be imagined as lying horizontally with the bottom toward the right and the top at the left. The vertical lines on either side of the curve for the PL-protein system indicate one standard deviation. Large values for S.D. in number 6 sample are possibly attributable to the fact that this sample was frequently collected in two parts with consequent increase in error.

gives a protein-fat complex characterized by the specific lipoprotein and by localization in the column.

The phospholipid proteins devoid of free cholesterol are the principal subject of the present paper. We shall give descriptive data for the PL-C-protein system only when comparison is especially helpful in describing the system without cholesterol. Proof for the existence of lipoproteins which contain phospholipid but not free cholesterol is provided by the fact that about thirty-five per cent of lipid phosphorus is found in the high density one-third of the column of centrifugate where insignificant quantities of free cholesterol are found (Fig. 1). At higher levels where significant concentrations of free cholesterol occur, differentiation of the two systems of phospholipid-containing lipoproteins is entirely a mathematical procedure.

We have found a linear relationship between concentration of free cholesterol and phospholipid for a group of samples from each of the upper five positions in the column of normal centrifugates.[6] In no instance does the line of regression pass through the origin, indicating phospholipid in excess of that necessary to meet the requirements set by slope of the line (Fig. 2). This excess varies widely with position in the column and seems to be related to the mean concentration of phospholipid without free cholesterol. The general features of the graph for a given level in the column of centrifugate are similar to those published by Albrink, Man, and Peters for

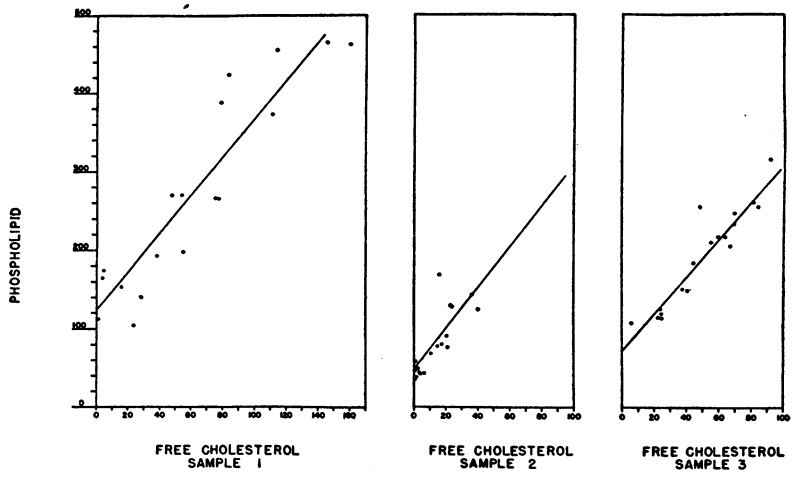

FIG. 2. Spot graphs in which values for free cholesterol are plotted against those for phospholipid (in milligrams per cent) for 18 samples from each of the three upper positions in the column, centrifugates of 18 healthy young men. (Transactions of the Tenth Conference on Liver Injury of the Josiah Macy, Jr. Foundation, New York, May 1951, Fig. 2, p. 19.)

whole serum.[1] Our graph for a group of samples from some levels, for example the third from the top, shows points with such small abscissae and large ordinates as to exclude the possibility that the line might curve and pass through the origin (Fig. 2).[6] The slope of the line differs somewhat for whole serum and for groups of samples representing different levels in the column but not significantly as evaluated by statistical methods. This demonstration of linear relationships with limited variation in slope at several levels after radical rearrangement of lipoproteins and with wide differences in intercept values is strong evidence for the existence in the upper five samples of centrifugate of phospholipid in two states, in one combined with free cholesterol in stoichiometric proportion and in the other uncombined. The concentration of phospholipid in the second state tends toward constancy at a given level in the column. The variation in concentration of phospholipid without free cholesterol in normal centrifugates at any one

level, however, is believed to be sufficiently great to prevent highly accurate estimation of the constant which expresses the stoichiometrical relationship between free cholesterol and phospholipid in the PL-C-protein. In the data used in the present paper we have assigned phospholipid to two systems, level by level, in centrifugates according to the formula for each level. After extensive application of this simplest possible interpretation of the systematic relationship between free cholesterol and phospholipid to centrifugate data from a wide variety of sources we have gained confidence in its truth, without any assumption of final accuracy for the mathematical procedure. Just as considerable reproducibility of concentration of phospholipid without free cholesterol in a group of samples from a given position in the column appears to be essential to demonstration of linear relationship between free cholesterol and phospholipid in centrifugate (the line of regression not passing through the origin), a similar constancy of concentration in whole serum might be essential for the relationship shown by Albrink, Man, and Peters.[1] Evidence that such is the case will be presented in this paper.

A constant multiplied by the concentration of free cholesterol gave concentration of phospholipid in the PL-C-protein system in a given sample of centrifugate. That assigned to the PL-protein system was obtained by subtraction from total concentration of phospholipid estimated in that sample. Phospholipid in each system in whole serum was calculated from data for the centrifugate.

The two curves in Figure 1 show mean distribution of phospholipid in 18 centrifugates of healthy young men assigned to the two major systems of lipoproteins. The size of the area beneath one of these curves is proportional to the total concentration of phospholipids in that system in whole serum. The shape of the area describes the constituents of the system mainly according to density of lipoprotein complexes of which it is constituted. In the centrifugates of healthy young men there is considerable reproducibility of both size and shape of the areas representing both systems. As a result of stress of a large meal or of disease a system pattern may deviate significantly from the normal, either as to size or shape or in both ways. The mean concentration of phospholipid in the PL-C-proteins of whole serum of 18 healthy young men was found to be 80 mgs. per cent with a standard deviation of 21.1 and that in the PL-proteins (without free cholesterol) 150 mgs. per cent with a standard deviation of 16.4. When standard deviation is expressed as percentage of the mean, that for the PL-C-protein system is found to be more than twice that for the system without cholesterol, being 26% as compared to 11%.

The tendency toward constancy of concentration of phospholipid in whole serum assigned to the system without free cholesterol as compared to the variability in the cholesterol-containing system is even more impressive when the sera of a group of miscellaneous patients are considered. Such a

comparison is shown in Figure 3 which represents the results of analyses of centrifugates of 47 sera expressed as phospholipid assigned to the two systems. This group of patients includes a disproportionate number, 13, with diseases of the liver and biliary tract, two or three each with diabetes, nephrosis, and thyroid disease, and the remainder with miscellaneous diagnoses without particular reference to known disturbances in serum lipids. In general, the graph shows a strong tendency toward wide scatter and increased concentration of phospholipid in the PL-C-protein system and a

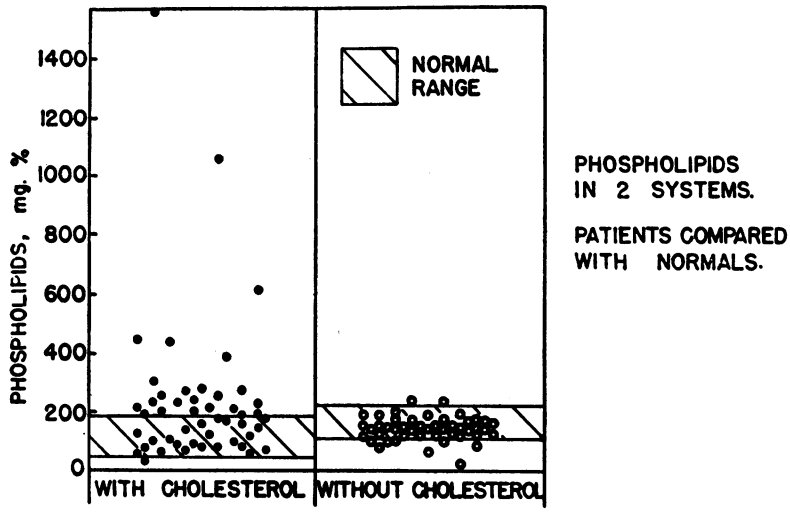

FIG. 3. Comparison of phospholipid concentration assigned to the PL-C-protein system (with cholesterol) with that assigned to the PL system (without cholesterol) as determined in centrifugates of 47 patients with various diseases.

tendency toward small scatter and diminished concentration of phospholipid in the system without free cholesterol. The normal ranges indicated by the cross-hatched areas were established by the maximum and minimum values obtained from study of 30 apparently healthy individuals, including a number of sera with concentration of neutral fat which exceeded our standards for normal and therefore were not included in the strictly normal series of 18 referred to above. In summary, our comparison of the two serum phospholipid compartments as to size indicates that the one without free cholesterol is the larger and more constant in the post-absorptive state in health, and that in disease it also varies little from normal size. When it does, it is predominantly toward diminution in the diseases we have studied. On the other hand, phospholipid in the system with free cholesterol varies more widely both in health and in disease, principally toward increase, containing occasionally in disease more than ten times as much phospholipid as the system without cholesterol.

As shown graphically in Figure 1, there is considerable reproducibility of distribution in the centrifugate of the different components of the system of lipoprotein which contains phospholipid without free cholesterol. As we have reported elsewhere,[7] the reproducibility of specific gravity for our standard ten samples of centrifugate of the serum of healthy young men is the greatest of any measurement we have made. There is abundant evidence that the lipoprotein-containing particles tend to localize in the column from top to bottom according to increasing density of complexes. We have

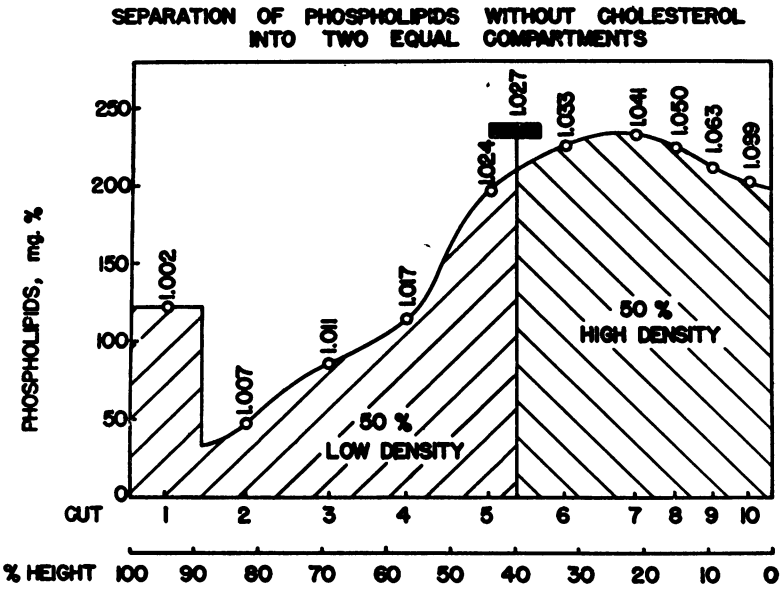

FIG. 4. Graph showing average position of a hypothetical plane which separates phospholipid in the PL-protein system into two equal compartments, 18 normal centrifugates. Localization according to specific gravity of centrifugate is 1.027, S.D., 0.003. Figures above the curve at each sample level represent the mean specific gravity.

measured the density of lipoprotein complexes by resuspending samples of centrifugate of known specific gravity and composition in a series of sucrose solutions of varying density and determining the distribution of the different lipid components after ultracentrifugation of the mixture. Those found in the top one-quarter of the sucrose column were considered as less dense than the medium from which they rose and those in the bottom one-quarter, conversely, as denser than the medium from which they were sedimented. The lipid complexes of samples from the upper one-half of the column were found by this test to have a density closely and systematically related to the specific gravity of samples of centrifugate in which they were found after the original centrifugation of untreated whole serum. Similar experiments with samples from the bottom one-half of the column were less satis-

factory and more difficult to interpret, perhaps because of the high concentrations of sucrose which were of necessity used.

While recognizing the possible importance of particle size and shape upon the distribution of lipids in the column of centrifugate we believe that particle density is the most important characteristic in determining localization in the column of lipoproteins. According to this concept we believe that the distribution of the components of the lipoprotein system containing phospholipid but no free cholesterol gives a description of the components in terms of sedimentation characteristics in which particle density is most important. The curve of distribution of phospholipid in this system (Figure

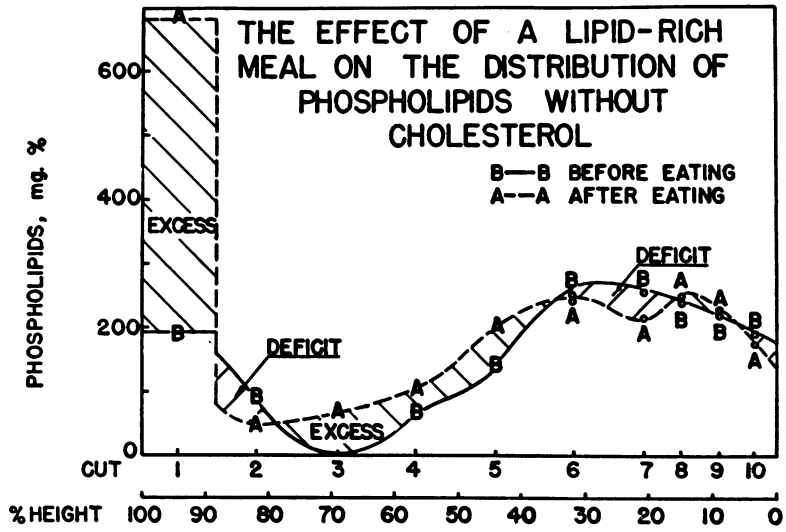

FIG. 5. Curves of distribution of phospholipid in the PL-protein system in two centrifugates in the same individual. B, post-absorptive sample taken before lipid-rich breakfast, A, 3 hours after eating.

1) shows one standard deviation on either side of the mean curve indicative of the degree of reproducibility of the curve of distribution at each level. The results of another test of reproducibility are shown in Figure 4. For each of 18 centrifugates in our strictly normal series we determined the location in terms of specific gravity of a plane across the centrifuge tube which would separate phospholipid without cholesterol into two equal compartments. The partition shown in Figure 4 is the mean value obtained, which is at a specific gravity of 1.027 with a standard deviation of 0.003. The mean phospholipid in centrifugate of specific gravity 1.027 and greater was 48.7% of the system total with standard deviation of 6.6. As tested in this fashion, the quantity ultracentrifuge tends to distribute the components of this system with remarkable accuracy so that one-half of the phospholipid is found below a hypothetical plane at a specific gravity level of 1.027 and

the other half above this plane. The high degree of reproducibility in the concentration of the different components of this system of lipoproteins is, we believe, strong evidence that these different components make up a system which has physiological features in common and which possesses also the structural feature of containing phospholipid without free cholesterol. Furthermore, the tendency toward constancy of concentration of the system as a whole and of the different components further suggests control by regulatory mechanisms of impressive efficiency.

We present two examples of deviation from the normal pattern of distribution of the components of our system, one resulting from a physiologi-

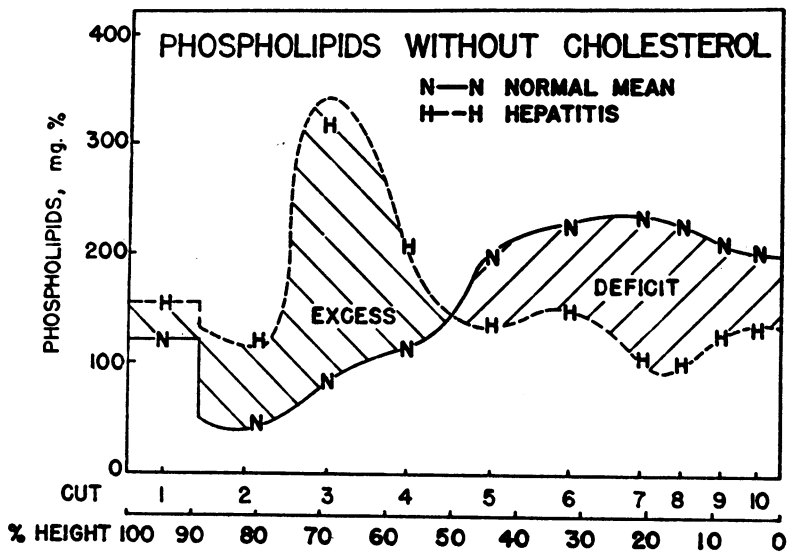

FIG. 6. The curves of distribution of phospholipid assigned to PL-protein system, H curve representing one typical hepatitis centrifugate, N curve representing mean normal distribution for 18 healthy young men.

cal stress, that of taking a lipid-rich meal, and the other a pathological stress, namely, the disease, acute hepatitis, presumably of virus origin. In Figure 5 are shown curves of distribution of phospholipid without cholesterol which are typical of our observation on alimentary lipemia.[3] The two centrifugates are from the same subject, one from the bleeding before breakfast, the other three hours after having eaten at a time when the serum was milky in appearance. The breakfast included bacon, eggs, butter, cream, and chocolate. The principal change was found in the top sample where the well-known increase in fat was localized. Because the concentration of phospholipid and fat was increased out of proportion to that shown by the two forms of cholesterol which maintained their usual proportionality for this sample, we conclude that there was an increase in the system which is

devoid of cholesterol and that a substantial portion of the fat in that part of the column is carried by this system.

In Figure 6 the distribution of phospholipid not associated with free cholesterol in one centrifugate of serum from a patient with acute virus hepatitis is compared to the mean normal distribution.[10] In this instance the total concentration of phospholipid in the system under discussion falls within the normal range, but obviously the distribution is abnormal. There is an increase which is extreme in the third sample and quite marked in adjacent samples and a diminution of varying degree in all samples in the high density one-half of the column, including the fifth to the tenth samples, the excess in the low density half of the column being approximately equal to the deficit in the high density portion. The increase in the top sample in this centrifugate is not typical of hepatitis centrifugates, which commonly show diminution.

In our search for a mechanism to explain the distortions of patterns such as these we have just described, we are impressed by the increase in neutral fat in that portion of the column where the increase in phospholipid concentration is found and with the possibility that increased proportion of fat in lipoprotein-fat-complexes provides sufficient buoyancy to the particles to bring about their localization at a higher level in the column than would occur with normal content of fat. In normal contrifugate only 20% of neutral fat was found in the second to fifth samples, inclusive, but in ten hepatitis centrifugates a mean of more than 60% of serum fat was found in the comparable region. It is in this portion of the column, especially in the third sample of hepatitis centrifugates, that a tremendous increase in phospholipid assigned to both systems was found.[10]

The stresses produced by heavy eating of lipids and those found in acute hepatitis result in an increase in proportion of low density lipoprotein complexes, both systems being affected. The localization in the column of the excess is characteristically different for the two conditions although excess fat makes up a large part of the increase in both. This suggests that the attachment of fat to lipoproteins is affected by controlled and systematic processes which may be highly specific as to the proteins participating, the quantity of fat attached, and the mode of attachment.

We have pointed out elsewhere[1] that the concentration of phospholipid in the bottom one-third of normal centrifugate has the highest degree of reproducibility of any lipid component. Practically all of the phospholipid in that portion of centrifugate is without free cholesterol. This constancy is well maintained under the physiological stress upon the lipid transport system consequent to ingestion of a lipid-rich meal, but not in acute liver disease. A deficit of high density PL-proteins along with an excess of those of low density is the rule in hepatitis centrifugates.[10] Shrinkage of the system as a whole was observed only when the illness was severe. We suspect low lipid intake and defective synthesis of PL-protein in liver disease to be the possi-

ble mechanisms in production of the deficit in high density components of this system.

A regular result of taking a lipid-rich meal is the expansion of the size of this system of lipoproteins so that within three or four hours the total concentration of phospholipids assigned to this system commonly increases by as much as one-third.[3] After three or four days without any food the system commonly shrinks by one-third or more, without showing any marked distortion in pattern of distribution.[4]

The marked change in distribution of phospholipid in the centrifugate of serum which has been delipidated by repeated quick freezing in the presence

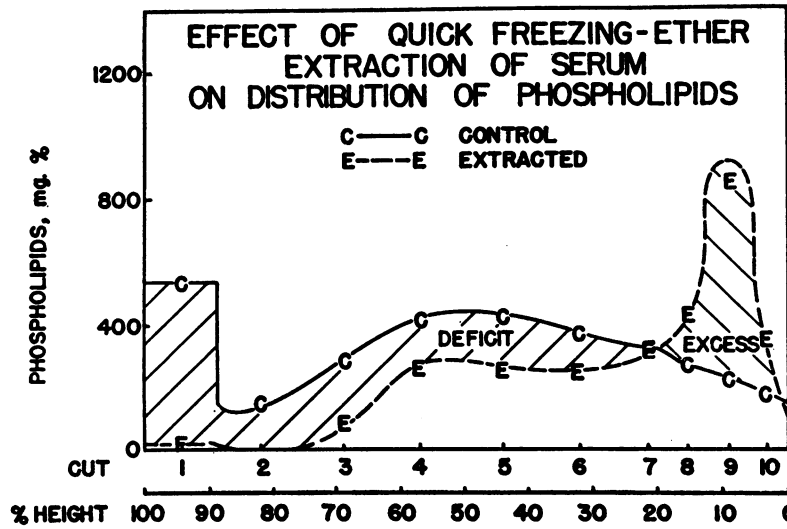

FIG. 7. Curves of distribution of phospholipid in two centrifugates of the same sample of serum. C, control untreated sample. E, sample delipidated by repeated freezing in the presence of ether before centrifugation.

of ether gives direct evidence that lipid load may influence localization of lipoproteins in centrifugates. In Figure 7 are shown two curves of distribution of phospholipid obtained from the study of a single specimen of serum, one portion of which had been delipidated before centrifugation by repeated quick freezing and thawing in the presence of ether according to the method of McFarlane.[5] This method removes nearly all of the cholesterol and neutral fat but only a small proportion of the phospholipid. The centrifugate of the treated serum shows a peak of phospholipid near the bottom of the tube and an absence of phospholipid in the top samples. We interpret these results as indicating that removal of neutral fat and cholesterol from lipoprotein complexes changes the sedimentation characteristics of some of the lipoprotein which retain the phospholipid so that there is a larger concentration of such lipoproteins in centrifugate of high density. The pattern of

distribution of phospholipid after delipidation in some respects represents a distortion opposite to that seen in acute hepatitis. We look upon the hepatitis pattern as a manifestation of overloading and that of delipidation as underloading of lipoproteins with lipid and suspect that size of fat load may be especially important in modifying localization of lipoproteins in the column. We have no means of differentiating fat carried by proteins of the different systems except in the top sample where fat is systematically related to other lipids.

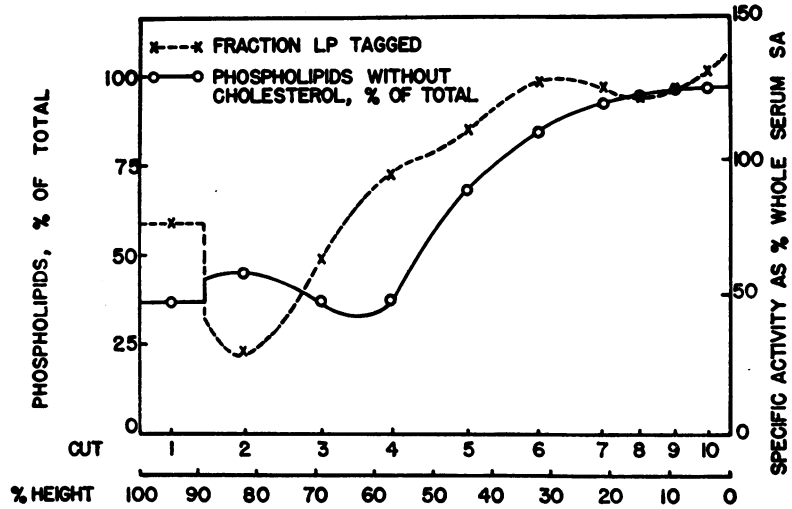

FIG. 8. The dotted line represents the mean specific activity of lipid phosphorus relative to whole serum specific activity in 13 centrifugates of six individuals, during the first two days after administration of radioactive phosphorus. Two individuals received therapeutic doses of P^{32} for polycythemia vera. The others received labeling doses. The solid line represents the proportion of phospholipid in each level which is assigned to the PL-protein system.

Our observations on tagging lipid phosphorus with P^{32} have shown that there are wide and systematic differences in the degree of tagging according to level in centrifugate.[9] The mean distribution of radioactive phosphorus expressed as specific activity of lipid phosphorus relative to specific activity of the whole serum in 10 centrifugates of six subjects during the first 48 hours after administration of isotope as inorganic phosphate is shown graphically in Figure 8. Another curve in this graph shows the proportion at different levels in the column of phospholipid which is assigned to the system without cholesterol. Below the two top samples the highest degree of labeling of lipid phosphorus occurred where the highest proportion of phospholipids without cholesterol was found. In a number of observations the curve showing specific activity in the centrifugate showed a peak of impressive height, sometimes localized to a small portion of the

column, and this peak was always found in that portion of the centrifugate which contained insignificant concentrations of the cholesterol-containing system. The top, or cream-layer-containing sample, seems to represent a special case and we have some evidence suggesting that in this sample the PL-C-protein system may contain a greater proportion of the P^{32} than does the system without cholesterol. The number two sample contains such small proportions of the total phospholipid that we can ignore the findings there. We interpret the data represented by these two curves as indicating that the mean rate of turnover of phospholipid in the system without cholesterol is probably higher than in the system with cholesterol.

The wide distribution of the system of lipoproteins which contains phospholipids but not free cholesterol is proof for inhomogeneity of complexes which contain these lipoproteins. Some components appear to be among the densest of plasma proteins while others which accumulate in the top sample will float on physiologic saline solution. We believe that the proportion of fat in the lipoprotein-fat complex is an important variable and is partly responsible for inhomogeneity. Other factors are no doubt important. Not only are the complexes of this system inhomogeneous in their sedimentation characteristics, and therefore structure, but differences in labeling with P^{32} at different levels in the column indicate differences in physiological activity. When the tagging of lipid phosphorus was followed in the same individual at appropriate intervals in the bottom four samples of centrifugate where PL-proteins are found with only a slight admixture of cholesterol containing lipoproteins, we have found such fluctuations in degree of tagging as to indicate that there are in that portion of dense centrifugate three imperfectly differentiated layers of phospholipid proteins, each made up of particles of distinctive sedimentation characteristics and each manifesting independent physiological behavior.[9] One possible basis for heterogeneity within a system of PL-proteins is that different components may contain only one kind of phospholipid or characteristic combinations of two or more. If such is the case, the functions of the particular protein would no doubt differ according to the kind of phospholipid which it contained.

It is our hope that eventually we may be able to interpret data such as are presented in this paper in terms of exact physiological mechanism. It would be premature to attempt any systematic interpretations at present. For example, the importance of the PL-protein system in meeting the sudden increase in burden on the lipid transport system following the ingestion of a lipid-rich meal seems clearly indicated, but there is as yet insufficient information for outlining any exact physiological mechanism. We have constructed some working hypotheses which we mention to illustrate some of the possible uses of our data. It is an attractive possibility that the lipoproteins without cholesterol may have a special function in the transport of fat to the liver. The alimentary lipemia data, for example, suggest

that this system functions in its transportation of fat from the alimentary tract. We wonder if the PL-C-protein system, in contrast, may have a special function in transporting fat from within liver cells to destinations outside the liver.

Summary

1. Some descriptive data are presented and discussed which deal with a system of lipoproteins containing phospholipid and believed to carry fat but which are devoid of free cholesterol and contain little or no cholesterol ester as well. The data selected were derived from multiple analyses of ten samples of centrifugate for each serum after application of standardized technique of quantity ultracentrifugation.

2. Evidence is reviewed for the general concept that the two forms of cholesterol and phospholipid enter into lipoprotein molecules in stoichiometric proportions and that fat is carried in variable proportions in lipoprotein-fat complexes.

3. Proof of the existence of lipoprotein devoid of free cholesterol is provided by the regular finding in bottom samples of centrifugate of untreated whole serum of nearly maximum concentrations of phospholipid, moderate concentration of fat and zero concentration of free cholesterol.

4. At levels where significant concentration of free cholesterol occurs, phospholipid was assigned to the cholesterol-containing system by multiplying free cholesterol concentration by a constant, and to the system without cholesterol by subtraction from total phopholipid estimated at that level. Concentration of the whole system in serum was calculated from data for the ten fractions of centrifugate. The constants were taken from formulae for lines of regression which described the free cholesterol-phospholipid relationship in normal centrifugate at different levels in the column.

5. As calculated from data for centrifugates of 18 healthy young men, the mean concentration of phospholipid with cholesterol was found to be 80 milligrams per cent (S.D. 21.1) and that without free cholesterol, 150 milligrams per cent (S.D. 16.4).

6. When centrifugates from a group of 47 patients with miscellaneous diseases were studied, the concentration of phospholipid with cholesterol showed high variability with tendency to high values while that in the system without free cholesterol showed small variability and tendency to low values.

7. The distribution of phospholipid without free cholesterol, according to sedimentation characteristics of the components, showed a high degree of constancy in health. A hypothetical plane across the centrifuge tube which divided phospholipid without free cholesterol into two equal compartments was found to have an average level of 1.027 specific gravity (S.D. 0.003).

8. The distortion of patterns of distribution in centrifugates of components of the system without free cholesterol is illustrated by typical examples drawn from study of acute liver disease and alimentary lipemia. The interpretation is offered that increased proportion of fat in lipoprotein-fat complexes may explain increase in proportion of low density complexes. The effect of *in vitro* delipidation of serum upon the distribution of phospholipid is also described.

9. Some features of the distribution of radioactivity in centrifugates after the injection of P^{32} as inorganic phosphate are described and interpreted as evidence that the mean rate of turnover of phospholipid without free cholesterol may be greater than that in the system of phospholipid-cholesterol-proteins.

Conclusions

The following hypotheses concerning the system of lipoproteins without free cholesterol are offered: Because of a tendency toward constancy of concentration of this system as a whole and also of its several components, together with evidence for a high level of physiological activity, we suggest that the parts of this system are integrated in a common function under the control of highly efficient regulatory mechanism. The function for which there is best evidence is transport of fat and fatty acids. The proportion of fat in a lipoprotein-fat complex may explain in part wide differences in localization in centrifugate of a given lipoprotein. The transport functions of this system may be of major importance in energy metabolism. Study of deviations from normal size of system or distribution of components under dietary stress or because of disease may contribute to a better understanding of normal and pathological physiology of lipids.

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