

BLOOD LIPIDS AND HUMAN ATHEROSCLEROSIS

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For many years it has been suspected that the blood lipids might in some way be related to the pathogenesis of human atherosclerosis. All the major blood lipid constituents, including cholesterol and its esters, phospholipids, and neutral fats, have been investigated without leading to definitive conclusions. Cholesteremia itself has received much attention, especially in view of the well-known clinical fact that certain diseases and syndromes often associated with frank hypercholesteremia (including diabetes mellitus, the nephrotic syndrome, myxedema, and familial hypercholesteremia

The authors wish to acknowledge especially the generous cooperation of Dr. William G. Donald, Director of the Cowell Memorial Hospital, for the facilities in the dietary studies, and his Dietetic staff, especially Mrs. Virginia Dobbin and Miss Clara Beth Young for the planning and aid in carrying out the dietary studies.

The authors are also grateful to the many physicians of the Bay Area who have generously cooperated in these studies by furnishing clinical material, including Dr. William G. Donald, Dr. Harry Akesson, Dr. Gale Whiting, Dr. James Harkness, Dr. Wallace Partch, Dr. Hobart Rogers, Dr. Raleigh Lage, Dr. Fletcher Taylor, Dr. Norman Leet, Dr. John J. Sampson, Dr. James Hopper, Dr. Gordon Lamb, Dr. K. W. Benson, Dr. Rene Bethereau, Dr. Alfred Goggio, Dr. William Chew, Dr. Leela Craig, Dr. Rubie Durgin, Dr. Robert Evans, Dr. D. Scott Fox, Dr. Henry Zwerling, Dr. Leon Lewis, Dr. Robert Lewis, Dr. Julius Lewis, Dr. William Marsh, Dr. Morton Meyer, Dr. Ione Railton, Dr. Francis Rochex, Dr. Clyde Wetmore, Dr. John Blum, The Menlo Park Clinic, Dr. Joseph Cuneo, The Highland Alameda County Hospital, and The Santa Clara County Hospital.

The authors also wish to express their appreciation for the enthusiastic support of Professor John Lawrence and Professor Ernest Lawrence in this work.

** This work in part was supported by the Atomic Energy Commission and the United States Public Health Service.

predispose to premature and marked atherosclerosis. However, in spite of evidence that cholesterol levels are often above 260 mg% in persons manifesting atherosclerosis, especially in the younger age groups (1), there is also good evidence that a high proportion of individuals develop atherosclerosis with blood cholesterol in the presently accepted normal range (125-260 mg% by Schoenheimer Sperry method)(2). Further, even with cholesterol above 200 mg%, Ungerleider et al (3) reported that the extent of atherosclerosis in humans was not well correlated with the actual cholesterol levels. Thus the development of atherosclerosis in a large number of cases of normo-cholesteremic individuals and the low relationship manifested between the extent of atherosclerosis and serum cholesterol level has cast some doubt in the minds of many investigators upon the significance of the serum cholesterol level per se in the pathogenesis of this disease. Several years ago Hirsch and Weinhouse (4), Hueper (5), and others have speculated that possibly the physico-chemical nature of the blood lipids might be of more significance than the analytical lipid levels themselves. The majority of clinical approaches to the study of cholesteremia have involved the chemical destruction of some or all of native lipid-bearing giant molecules of serum and the subsequent lumping of the fragments into two categories, "free" and "esterified" cholesterol. Essentially none of the cholesterol in serum circulates as individual molecules of either free or esterified cholesterol, but instead is present in the form of very large molecules containing cholesterol and other lipids in association with variable amounts of protein. Several workers (6),(7) have pointed out the existence of lipoprotein molecules bearing cholesterol in human serum. However, until recently technical difficulties have precluded characterization of the individual component fractions of the lipid and lipoprotein groups in serum. Since it appeared entirely possible that there might exist in serum certain giant lipid-bearing molecules related to the development of atherosclerosis, it was evident that a method was required which would allow the identification and quantitative characterization of the individual molecular species present in the blood of an individual patient. It has been found by certain of the present authors (8)

that the ultracentrifuge, under special conditions, provides a suitable tool for this purpose. With this technique, we have found and reported (9) the presence of several lipid and lipoprotein constituents in the serum of humans and rabbits and that certain types of these molecules are present when there is atherosclerosis in the human and the cholesterol-fed rabbit.

The clinical studies of atherosclerosis are necessarily described in terms of observations made with the analytical ultracentrifuge. Therefore the essential nature of the ultracentrifugal technique is given below preliminary to a description of the clinical findings themselves.

Basic Features of the Ultracentrifuge and Ultracentrifugal Analysis

An ultracentrifuge is a device which was designed (10) for the purpose of producing forces of many thousands to many millions of times the force of gravity. Under the influence of such intense gravitational fields of force individual molecules of the size of proteins can be made to undergo sedimentation (if they are more dense than the solution in which they are present) or to undergo flotation (if they are less dense than the solution in which they are present). In either event, if the centrifugal force is sufficiently great to impart an appreciable migration rate to the molecules, a boundary becomes established between the solution containing the large molecules and the region of solution out of which these molecules have migrated. A variety of optical techniques known as refractive-index gradient methods have been devised for rendering this boundary observable and for measuring the rate of movement of the boundary region itself as the centrifuge rotor spins. This technique allows calculation of a sedimentation rate or flotation rate of the molecules in a manner analogous to the familiar clinical observation of red cell sedimentation rate under the influence of gravity. Without going into the detailed theory of such optical systems, which is fully described in the literature (10), the diagrams obtained are readily explainable and understandable. In all the studies pertinent to the atherosclerosis problem conditions are adjusted so that the molecules involved undergo flotation against the direction of the centrifugal force. Thus the optical patterns for this situation alone need be described. If there

is a single species of large molecules present (of density lower than the solution in which it is present), a single boundary between solution containing the molecular species and solution free of it becomes established while the centrifuge operates. The optical system in our ultracentrifuge (Spinco Model E) uses the so-called Thover-Philpot-Svensson technique which gives directly a picture of the refractive index gradients in the neighborhood of the protein boundary. Figure 1 shows a diagram of the ultracentrifuge cell with a molecular species undergoing flotation, a schematic diagram of the pattern obtained, and actual photographs taken of such flotation of a single species with the aid of this optical system. It follows that if two different molecular species, migrating at different rates, are present the diagram will reveal two separate peaks as shown in Figure 2. By taking photographs at successive intervals of time after the rotor has reached full speed one can determine the rate of movement of each "peak" corresponding to a molecular species and from the area over each peak one can determine the concentration of that particular species in the solution. This area is shaded in Figures 1 and 2. The rate of migration of a molecule under a given set of conditions (temperature, solution composition, and centrifugal force) is a physical constant that characterizes this particular molecule in many respects better than any name we might ascribe to it.

In the work with atherosclerosis our concern is primarily with lipid and lipoprotein molecules of densities close to 1.00 gram/cc. By adjusting the density of the solution being studied with sodium chloride to a value of 1.063, the solution is thereby made more dense than the molecules themselves, with the result that all the molecules of interest undergo flotation. In fact in this work we preliminarily float all the lipids and lipoproteins of a class less dense than 1.063 to the surface in a preparative ultracentrifuge, pipet off the top fraction containing them, and then study the group for its individual constituents by the analytical ultracentrifugal method which gives rise to the diagrams described above. The customary unit of migration rate is the Svedberg*, named in honor of The Svedberg who invented the ultracentrifuge and developed

* One Svedberg unit equals 1×10^{-13} cm/sec/dyne/gram. In using this unit one should specify the exact conditions under which the run was made, since the S rate will vary if the conditions are varied.

the theory of its use. A molecule which undergoes sedimentation at a rate of 5×10^{-15} cm per sec per unit field of force is said to have an S value of 5, or to be a molecule of the S class. We have adapted this term for flotation runs such that a molecule which undergoes flotation at a rate of 5×10^{-15} cm per sec per unit field of force has an S_f value of 5 or is a molecule of the S_f class.

The Ultracentrifugal Composition of Human Serum Lipids and Lipoproteins

The group of lipoproteins and lipids in human serum of densities less than 1.063 gms/cc may consist of as many as ten species of molecules, the number being variable from individual to individual. Several of these molecules are cholesterol-bearing molecules, and in fact generally carry a large fraction of the total serum cholesterol. These components are readily characterized by their flotation rates under specified conditions* or by their hydrated densities. For present purposes the

* The runs reported here were made at a temperature of $27 \pm 2^\circ\text{C}$ in a sodium chloride solution of density 1.063 (the density being calculated independent of any contribution from proteins or lipoproteins to density), using low-density substances previously isolated by differential preparative ultracentrifugation. In the differential ultracentrifugation, all the lipid and lipoprotein constituents of density less than 1.063 undergo flotation to the top of the preparative cell and are pipetted out into a volume such that these substances are concentrated by a factor of five over their initial concentration in serum. The S_f values are those obtained directly in the analytical runs without back calculation to any other set of conditions than those used in the run.

flotation rates are most useful.

The various classes of molecules found in serum by these studies are the following:

(a) Species which migrate with S_f values greater than 75 S_f units. These include the well-known chylomicrons and aggregates of much smaller dimensions than the chylomicrons. The concentration of species in this entire class is increased following fat-containing meals and hence represents part of the alimentary lipemia. We have found no correlations between components of S_f greater than 75 units and the presence of atherosclerosis in humans.

(b) Species which migrate with S_f values between 50-70 S_f units. These species constitute the major fraction of alimentary lipemia and are greatly modified

in concentration with relationship to meals. Certain of these components are cholesterol-bearing molecules and their relationship to atherosclerosis is under investigation.

(c) Species of discrete classes which migrate with rates between 10 and 20 S_f units. These components, whose molecular weights are in the neighborhood of 1,000,000, appear definitely related to the presence of atherosclerosis in humans. There are occasionally present in addition molecules of the S_f 20-30 class, some of which appear to have significance like that which characterizes the molecules of the S_f 10-20 class, but the role of the S_f 20-30 components requires further clarification. The S_f 10-20 class contains at least three separate species of molecules, each approximately 30% cholesterol by weight, which at present appear to be of equivalent significance with respect to atherosclerosis.

(d) Species which migrate with S_f rates between 3-8 β units. These molecules are important cholesterol, phospholipid, and protein-containing substances, loosely referred to in the general literature as the B_1 -lipoprotein. Actually this component may exist as a single component or as a multiple group of components in an individual case. This component or set of components, carrying a major fraction of the serum cholesterol, is present in every one of some four thousand samples studied, at concentrations varying from individual to individual, but at an essentially constant level for a given individual from time to time. This set of components does not of itself appear to be related to atherosclerosis.

Relationship of the S_f 3-8 and S_f 10-20 Classes of Lipids and Lipoproteins to Atherosclerosis

In the serum of rabbits a set of molecules of the S_f 5-8 and S_f 10-30 class have been discovered and described (8), which appear to be analogous to the S_f 3-8 and S_f 10-20 classes in the human. The S_f 5-8 class of molecules in the rabbit is a lipoprotein (30% cholesterol by weight) and is present in variable concentration in the serum of all normal rabbits. During cholesterol feeding of the rabbits these molecules increase in concentration first, and then level off at approximately the highest concentration achieved. Following the rise in concentration of the S_f 5-8 class of molecules most rabbits begin to develop increasing concentrations of molecules of the S_f 10-30 class

as the serum cholesterol rises as a result of further cholesterol feeding. Some rabbits, however, never go beyond the stage of development of increased S_f 5-8 concentrations, in spite of further cholesterol feeding. Autopsy of the rabbits at the end of the fifteen-week feeding period has shown that the degree of atherosclerosis was greater the higher the final concentration of molecules of the S_f 10-30 class, while animals with minimal concentrations of such molecules had little or no gross atherosclerosis. Recently Simonton in this laboratory (unpublished) has studied rabbits receiving potassium iodide and cholesterol according to the procedure of Turner (11). Three out of five of these rabbits were protected against the development of atherosclerosis and they developed at most only a very low concentration of molecules of the S_f 10-30 class, whereas the two rabbits which did develop atherosclerosis also developed appreciable concentrations of these molecules (Figure 3). By whatever mechanism iodide protection in the rabbit operates, it certainly seemed to prevent the appearance of these molecules in the serum. In contrast with the molecules of the S_f 10-30 class, the concentration of lipoproteins in the S_f 5-8 class did not show any correlation with the extent of atherosclerosis developing either in cholesterol fed animals or cholesterol plus KI fed animals. It was of immediate interest to determine whether the group of molecules in the human (i.e., the S_f 10-20 class) analogous to the S_f 10-30 class in the cholesterol-fed rabbit might bear any relationship to human atherosclerosis. The preliminary study of 280 humans, including various disease groups and many presumably normal individuals revealed that such a relation does exist. The present communication based upon a much wider experience has added confirmation to these findings and has provided several new relationships of the S_f 10-20 molecules with atherosclerosis in humans.

1553 humans of a variety of clinical groups including patients manifesting clinical evidence of atherosclerosis, diabetes, nephrosis, hypothyroidism, hypertension, hypercholesteremia, and normals have been studied with respect to their blood content of molecules of the S_f 10-20 class, and in many cases the relationship of this to their blood cholesterol levels.

The results of all these studies are presented in the set of Figures 4-9, and a summary tabulation given in Figures 10, 11, 12 for ease of comparison of the various groups. The detailed discussion of the individual groups and of the significance of the findings is given below. Before commenting on the findings it is to be noted that the blood samples represent single samples from each individual. The validity of this method of sampling was checked at the outset by drawing samples of blood from 65 individuals at intervals of a few days to a few months, while they were on a steady diet and not under specific therapy and also by drawing samples in the fasting state and again following a fat-containing meal. The results of such sampling are given in Figure 13. They indicate that alimentary lipemia does not significantly affect the concentration of the molecules of the S_p 10-20 class. Further the data indicate that on a constant diet a single individual shows a reasonably stable level of concentration of such molecules, whether the concentration is low or high. As a result of these studies, it appears justifiable to use blood drawn at any time of day and to use a single sample per individual.

Living Individuals with Atherosclerosis: The best group that one can choose among living individuals who are almost certain to have atherosclerosis is a group of patients who have survived a myocardial infarction, since it is generally agreed that more than 95% of myocardial infarctions occur superimposed upon coronary artery atherosclerosis. Further the diagnostic criteria for myocardial infarction are more clear-cut than for atherosclerotic complications in vascular beds other than that of the heart. The requisite criteria for inclusion in this group were (1) A typical clinical history of a myocardial infarction, (2) Supportive laboratory evidence such as sedimentation rate elevation, leukocytosis, or fever, and (3) electrocardiographic changes characteristic of myocardial infarction. All cases were required to meet all three criteria. Further all cases were at least six weeks beyond the acute clinical episode, since it was desired to exclude possible metabolic disturbances of the acute phase as well as any disturbances due to dietary restrictions and drug therapy. All the patients were either on ad libitum diets or were moderately restricted relative to their diets before

their infarction. For reasons explained later in this paper a small group of patients who had been on severe cholesterol and fat restriction for periods of three months to three years before their blood was studied are treated separately (independent of the measured concentrations of S_p 10-20 molecules in their blood), since they were on a regimen that we have shown (see section "Factors Affecting the Concentration of S_p 10-20 Molecules") is capable of lowering the concentration of molecules of the S_p 10-20 class in the blood. Figure 5 presents the data on 230 male myocardial infarctions and the data on 32 female myocardial infarctions. It is noted that of all the 230 males with myocardial infarction, 91% of them show the presence of S_p 10-20 molecules at levels above the borderline resolution value, with an average level many times the level of limit of resolution. In the females with myocardial infarction 97% of all cases show the presence of these molecules at appreciable levels. These data indicate a very high frequency of occurrence of S_p 10-20 molecules in the serum of individuals with myocardial infarction. In fact it is known that most of these patients who had been on dietary restriction of some degree were in the negative or low positive distribution of this group. However, to treat the data as harshly as possible they were included in the series. We may infer, but cannot prove, that the incidence of occurrence of appreciable levels of S_p 10-20 molecules might have been even higher, as might the average concentration of such molecules if this element of dietary restriction had not been present. The occurrence of the high proportion of 7 negative cases among 32 individuals over 70 years of age is interesting. Here again no proof can be given for the result obtained, although in general these people were eating considerably less than they had in earlier years. For any of the negatives there is also the possibility that some of these may fit into the small group of myocardial infarctions resulting from causes other than atherosclerosis of the coronary arteries. In any event, taking the negative results at their worst, with no effort to explain the small number of negatives in any fashion, the data of Figure 11 demonstrate clearly the sharp difference with respect to incidence of appreciable concentrations of S_p 10-20 molecules in the serum of myocardial infarction patients as compared to normals of the

corresponding age and sex categories. This difference is unequivocal evidence that the presence of these molecules in the serum is in some way associated with the presence of atherosclerosis. Further evidence of this association is obtained by a comparison of the average concentration of such molecules in serum of myocardial infarction patients with the average concentration in normals of corresponding age groups. Even if the negatives among the normals are excluded in taking the averages, the myocardial infarction groups still have a significantly higher concentration of the S_p 10-20 molecules in their serum than do the normals. That as high a percentage of presumed normals show such molecules in their blood is fully to be expected since a large proportion of such normals are certainly developing atherosclerosis although it has not yet become clinically manifest. It remains of course to be proven, however, whether or not those normals showing appreciable concentrations of these molecules in their blood represent the ones developing atherosclerosis, and whether the degree of atherosclerosis parallels the concentration of these molecules in the blood as it appears to in the case of the analogous molecules in the rabbit. In Figures 14-17 is given the relationship between the analytical serum cholesterol levels and the concentration of S_p 10-20 molecules. It is readily seen that in the myocardial infarction group these levels do not correlate well. For example at 200 mg% total cholesterol a patient may have a many fold greater concentration of S_p 10-20 molecules than will another patient at 300 mg%. If our hypothesis of the relationship of these molecules to atherosclerosis is correct, this finding might explain the low relationship indicated in previous studies between degree of atherosclerosis and the serum cholesterol levels.

Presumably Normal Individuals: A large series of persons without known disease in all adult age categories and of both sexes have been studied for the occurrence of and concentration of molecules of the S_p 10-20 class in the blood.

Individuals Showing no Demonstrable Vascular Disease: A large series of men and women without known disease in representative adult age categories have been studied for the concentration of molecules of the S_p 10-20 class in the blood. Over 90% of this group had had a recent physical examination, the record of which was available to us. Thus

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it was possible to exclude from this "normal" category any patients with sustained hypertension or diabetes (at least diabetes resulting in glycosuria). Individuals with rheumatic fever histories, nephritis histories, rheumatoid arthritis, or any known malignancies were also excluded. It must be emphasized that many of these individuals are undoubtedly developing atherosclerosis even though there is no clinical manifestation. There is no known method for the objective selection of living individuals with respect to presence or absence of atherosclerosis unless there has been a clinical manifestation of vascular disease. Since among this group there will still be a large proportion of persons free of atherosclerosis or with minimal atherosclerosis, it would be expected that this group should show a relatively lower average rating than groups with known atherosclerotic disease on any scale indicative of this disease. In all age categories and in both sexes, patients with proven myocardial infarction show a consistently greater incidence and average concentration of molecules of the S_p 10-20 class than do the above-described normal controls. This supports our hypothesis of the association of the molecules of the S_p 10-20 class with atherosclerosis. Further the incidence and concentration of such molecules in the blood of our presumably normal individuals are in agreement with the data reported in the literature (12) on the incidence and degree of atherosclerosis found in autopsy material as a function of age. Of particular interest is the increase in incidence and concentration (see Figure) in males with aging at least up to the 50-60 year age categories. Whether the apparent decrease after 60 years is significant must await more extensive data on the older age groups. The very low incidence and average concentration of S_p 10-20 molecules in the serum of young women is in good accord with the relative rarity of atherosclerosis and its complications in young women. The marked change in the women especially above 40 years of age coincides with the fact that women lose their apparent relative protection against the complications of atherosclerosis with increasing age (13). An endocrine factor suggests itself but will require study for identification and evaluation.

Individuals with Diabetes Mellitus: The increased occurrence of atherosclerotic complications in diabetics as compared with the general population of the same age group is well known. It was therefore of interest to determine the blood picture in such subjects with respect to the presence of molecules of the S_f 10-20 class. For this 76 patients with diabetes mellitus of age 20-80 have been studied. These represent diabetics varying widely in the quality of control and insulin requirements. No effort is made here to correlate these factors with the blood findings since a much larger group in each age and sex category will be required for this purpose. However, considering the group as a whole certain definite conclusions can be drawn from the data, which are presented in Figures 8 and 11. The female diabetics demonstrate a distinctly higher incidence of appreciable concentration of molecules of the S_f 10-20 class than do normal females in all age categories. The effect is relatively great in the diabetic females between 20-40 years of age. The same general effect is observed in diabetic males as compared with normal males, but is not as large as for the females. The study of a larger number and a classification with respect to severity of the diabetic state is necessary.

Individuals with Coronary Insufficiency: A group of 30 males who presented the clinical picture of angina pectoris and coronary insufficiency, but who had never had a proven myocardial infarction, have been studied. These patients had either normal electrocardiograms or minor ST-T wave changes. It would be anticipated that, since none of these patients were anemic, that the most likely cause of their coronary insufficiency would be atherosclerotic involvement of the coronary arteries, with a high likelihood from the work of Blumgart and Schlesinger (14) that coronary occlusions were present. The data are presented in Figure 7. It is seen that every one of the 30 cases showed the presence of appreciable levels of molecules of the S_f 10-20 class. This result is in accord with the data on patients with proven myocardial infarction, as might be expected since the basic pathology is similar for the two groups. The result is also further evidence consistent with the hypothesis that the molecules of the S_f 10-20

class are associated with atherosclerosis. An insufficient number of females in this category have been studied to date to warrant reporting.

The Nephrotic Syndrome: To date only four patients with the nephrotic syndrome have been studied with respect to the blood level of molecules of the S_f 10-20 class. Although the group is small, the results are sufficiently striking to deserve comment. In all four cases exceedingly high concentrations of molecules of the S_f 10-20 class are present in the serum. These levels are in fact among the highest we have yet observed, being 105, 149, 175, and 420 mg%. The last value, 420 mg% S_f 10-20 molecules, was from a 6 year old child who has since come to autopsy after two years of the nephrotic stage of her disease. The abdominal aorta showed extensive atherosclerosis.

Hypothyroidism: Patients with marked hypothyroidism are known to be subject to excessive atherosclerosis as compared with euthyroid individuals. A group of 16 patients who presented clinical features of hypothyroidism, low basal metabolic rates, and in most cases serum cholesterol over 300 mg% have been studied. Two of these cases were frank myxedemas who were already receiving thyroid replacement therapy. Most of the others were receiving thyroid extract, although in some of these it was not felt that they were yet receiving the optimal dose. The data, presented in Figure 8 and Figure 10 show that all of these cases had appreciable concentrations of molecules of the S_f 10-20 class in the serum. Had these patients not already been on thyroid therapy it is likely that they might have shown an even higher average concentration of S_f 10-20 molecules. In our current studies we are endeavoring to study the blood of hypothyroid patients before and during thyroid therapy. The data presented on hypothyroid patients are consistent with the general occurrence of the S_f 10-20 molecules in diseases predisposing to atherosclerosis.

Hypertension: Although it has never been shown that atherosclerosis is etiologically related to hypertension, the very common occurrence of atherosclerosis and its complications in sustained hypertensive disease is well known. A group of 39 men and 16 women with sustained hypertension (diastolic pressures repeatedly observed above 100 mm Hg) have been studied. No hypertensives who had already demonstrated clinical

evidence of coronary artery disease are included in this group. Many of the cases studied were on weight reduction and salt-restricted diets at the time of the blood study. The effect of dietary restriction, from studies reported in a later section of this communication would in general be to reduce the concentration of S_f 10-20 molecules in the serum. In spite of this it is seen (Figures 9, 10) that 92 to 94% of the hypertensives show the presence of appreciable levels of S_f 10-20 molecules in the serum, a value significantly higher than that for corresponding normals. These studies are being continued with untreated hypertensives to provide a group which can be more properly compared with the corresponding normals. The increased frequency of occurrence of appreciable levels of molecules of the S_f 10-20 class suggests the possibility that atherosclerotic complications in hypertensive patients may be in part, at least, on this basis. Any relationship of the presence of the S_f 10-20 molecules to the hypertension itself must await further evaluation.

Relationship of the S_f 10-20 Molecules to the Analytical Serum Cholesterol Levels:

In Figures 14, 15, 16, and 17 are given the levels of S_f 10-20 molecules plotted against the analytical serum cholesterol levels for some of the normal males, diabetics, the group of patients having had a myocardial infarction, and various hypercholesteremic patients. (All analytical cholesterol values were determined by a modification of the Schoenheimer-Sperry method). It is seen from the data for normals that the serum level of S_f 10-20 molecules may be very low or very high at a particular serum cholesterol level.

Tabulated below from Figures 14 and 15 are the summarized data for normal males and males with myocardial infarction with respect to the relationship of S_f 10-20 component levels as compared with analytical serum cholesterols. Within the analytical cholesterol range below 300 mg% and below S_f 10-20 levels of 44 mg% is an area which comprises more than 95% of the tested normal male population and 75% of the studied myocardial infarctions. It is this group which is tabulated.

TABLE I

Number of Cases	Range of S _f 10-20 Concentration mg%	Range of Analytical Cholesterol levels mg%	Average Serum Cholesterol mg%
Myocardial infarctions			
41	24-44	154-295	231
27	6-24	164-295	235
Normal Males			
30	24-44	136-294	224
16	6-24	155-282	198
41	less than 5	140-292	196

As can be seen from Table I and from Figures 14-17, there is a general trend toward higher S_f 10-20 concentrations with higher serum cholesterol levels, but for any particular patient the analytical serum cholesterol level is of no value in predicting the concentration of S_f 10-20 molecules. From Figures 15, 16 and 17 it is seen for patients in many categories with hypercholesteremia (over 300 mg%) that the chance of S_f 10-20 molecules being present at appreciable concentration is much greater, but even here the actual level of S_f 10-20 molecules is poorly related to the analytical serum cholesterol levels.

Assuming our hypothesis of the relationship of molecules of the S_f 10-20 class to atherosclerosis to be correct, all these observations taken together may reveal the difficulty in trying to establish any correlation of the analytical serum cholesterol levels with the presence or severity of atherosclerosis. Since there is a trend toward higher S_f 10-20 molecule levels with higher cholesterol, the observed general shift of the myocardial infarction patients toward higher analytical cholesterol levels would be anticipated on the basis of our hypothesis.

Factors Affecting the Concentration of S_f 10-20 Molecules:

Early in our studies a large group of individuals were studied both before

and after a single meal containing fat and cholesterol. In no case was it possible to demonstrate any acute effect on the blood concentration of S_f 10-20 molecules from a single meal. Alimentary lipemia affects the components of the 40 S_f and greater group but not those of the S_f 10-20 group. Testing numerous individuals on two or more occasions revealed that the level of S_f 10-20 molecules was essentially stable provided that the subjects did not change their dietary habits (See Figure 13).

In the rabbit the atypical molecules of the S_f 10-30 class appear in the serum after a period of cholesterol feeding. Such molecules have never been observed in any of more than fifty normal rabbits of the same stock. In the cholesterol-fed rabbit it was invariably found that the atypical molecules of the S_f 10-30 class appeared only after a preliminary rise in the concentration of the normally-occurring lipoprotein of the S_f 6-8 class. It thus appeared that the atypical molecules of the S_f 10-30 class might represent the results of an overflow above the capacity of the rabbit to handle the excess cholesterol via the normally-occurring lipoprotein of the S_f 6-8 class.

It was of interest to know whether any similar type of change in cholesterol metabolism operated in the human. Therefore, a group of men and women have been placed on a low fat, low cholesterol diet and their serum levels of S_f 10-20 molecules followed for as long as 18 weeks of dieting. Regardless of the initial concentration of the S_f 10-20 molecules most of the individuals showed consistent trends to lower concentrations of the molecules. There is marked individual variation in the rate of its reduction; some of the subjects exhibited rapid reduction reaching concentrations below the limit of resolution in 2 to 3 weeks. Others during the first few weeks showed no change or even rarely an increase. However, the great majority of subjects who have remained on the diet for longer than 4 weeks have shown considerable reduction in the S_f 10-20 component. These studies are individually graphed in Figures 18, 19, 20, and 21. One of these groups (Figure 21) is a study of a selected group of S_f 10-20 positive individuals who ate all food (except a breakfast which was composed of fruit, cereal, skim milk and coffee) at the diet kitchen table of Cowell Hospital (University

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of California). These meals were prepared using measured amounts of cholesterol-containing foods (15). Between 100 and 200 grams of lean meats, which average about 100 milligrams of cholesterol and 15 grams of fat per 100 grams (12), were used. The total intake of cholesterol per day was kept about or below 200 milligrams per person. Intake of animal fat was avoided except for that contained in the lean meats; the total of animal and vegetable fats were kept at about 50 grams per day. Approximately half of the subjects in every category listed in Figures 18, 19, 20 and 21 restricted their caloric intake during a period of adjustment to the diet. These individuals demonstrated no differences in their response to the low cholesterol, low fat diet from those who had not appreciably diminished their caloric intake. The uniform and rapid reduction which generally occurred in the hospital-controlled dietary group as contrasted with the less uniform and slower response of those who were on the diet at home suggests that the latter group may not have followed the restrictions as closely as the former group. Several individuals were returned to their normal diets after showing a marked drop in S_f 10-20 component concentration during a period of dietary restriction. Most of these have shown a rise in concentration of these molecules in a period of four weeks. From Figure 20 it is seen that the effect of dietary restriction in patients having had a myocardial infarction in the past does not differ from that in normal males. Analytical serum cholesterol determined on many of the subjects on the diet revealed that in some cases a fall in concentration of S_f 10-20 molecules was accompanied by an appreciable fall in total cholesterol whereas in many other cases it was not.

Detailed dietary histories were analyzed for 43 persons demonstrating a wide range of S_f 10-20 component concentration. There was no consistent relationship between the dietary cholesterol or fat intake and the blood level of S_f 10-20 molecules in the group as a whole. This would suggest that there is a wide range of individual tolerance to the usual levels of these substances in the diet.

It has been possible to study an additional small group (19 patients) who had had a myocardial infarction and who had been, at the advice of physicians, on a strict

low fat and low cholesterol diet for periods of three months to three years before we had the opportunity to examine their bloods for the level of S_f 10-20 molecules. The data presented in Figure 22, shows that this group has a lower concentration of molecules of the S_f 10-20 class than either normals or our other myocardial infarction patients of corresponding age and sex categories. This represents indirect supportive evidence of the efficacy of dietary restriction to reduce the concentration of the S_f 10-20 molecules in the serum.

Summary

The presence of a class of lipid and lipoprotein molecules in the serum of the human and the cholesterol-fed rabbit associated with atherosclerosis in both species has been demonstrated.

These molecules do not represent any part of the acute alimentary lipemia. The presence of these cholesterol-bearing lipid and lipoprotein molecules cannot be predicted from the analytical total serum cholesterol level. Partial dietary restriction of fat and cholesterol in the human results in a gradual decrease in the serum level of such molecules over a period of weeks to months.

These molecules are present with a much higher frequency and at higher concentrations in patients who have survived a myocardial infarction than in corresponding individuals without known vascular disease. The presence of these molecules with increased frequency in other diseases associated with excessive atherosclerosis (diabetes mellitus, the nephrotic syndrome, hypothyroidism, hypertension, and coronary insufficiency) supports the hypothesis of their association with atherosclerosis.

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Figure 1. Schematic diagram of ultracentrifuge cell showing migration of molecules less dense than the solution against the centrifugal force. The schematic optical diagram demonstrates the type of optical pattern obtained. Below the schematic optical diagrams are successive pictures taken from two ultracentrifuge runs, the first showing migration of isolated molecular species S_f 5.7 (rotor speed 44,770 rpm) and the second showing migration of isolated molecular species S_f 12.4 (rotor speed 52,640 rpm).