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LIPOPROTEINS AND ATHEROSCLEROSIS

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Whether or not the pathogenesis of atherosclerosis involves lipids as a primary etiologic agent, a subject of considerable controversy, there can be no question that somewhere in the pathogenesis lipids become involved as prominent components of the actual lesions. Certainly no one would contest that this process is, at least when minimal, a focal process, influenced by local factors in the arterial wall. Nonetheless such considerations may all be valid without in any way being exclusive of the possibility that blood lipids in transport may represent the source of the lipids seen in atheromata. The authors (1, 2, 3) have presented evidence demonstrating that certain blood lipoproteins are intimately associated with atherosclerosis both in the human and in the cholesterol-fed rabbit. These studies, based upon the ultracentrifugal analysis of serum for its lipid-bearing constituents, have been carried further. It is the purpose of this discussion to report certain aspects of the progress of this particular research. The basic theory of use of the ultracentrifuge in studies of this sort is described in previous publications (1, 2, 3, 4).

BLOOD LIPID TRANSPORT

The true nature and significance of the state of blood lipids, such as neutral fat, fatty acids, phospholipids, cholesterol, and cholesterol esters

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had, in the past, been considerably obscured by the fact that most studies centered around the total analytically determinable lipid constituent, e.g., cholesterol or cholesterol ester. Actually a constituent such as cholesterol, a cholesterol ester, phospholipid, or neutral fat is present as a building block in a variety of very large molecules (up to millions of molecular weight units). The particular significance of this fact is that from a chemical determination of a substance such as cholesterol in the blood, one is not able to predict, in any particular case, how the cholesterol is distributed among the numerous macromolecules that may exist in serum. This raises the possibility of overlooking significant molecules, with respect to disease pathogenesis, unless one is able to characterize and quantitate the various individual molecules present, no matter what the total serum level of a particular constituent building block may be, e.g., cholesterol or cholesterol ester. It is for the detection, on a quantitative basis, of these individual macromolecules bearing various lipids that the ultracentrifuge is especially useful.

ULTRACENTRIFUGAL CHARACTERIZATION OF HUMAN SERUM LIPIDS

The rate of movement (sedimentation or flotation) of dissolved molecules in an ultracentrifugal field depends primarily upon size, shape, and density of the molecule and the density and viscosity of the medium in which it is present. A minimum of ten distinct species of lipoproteins that may be found in human serum, differing from one another physically in density of the molecule and in ultracentrifugal flotation rates have been described (3). Broadly the lipoproteins may be segregated into two major groups, the so-called "low density lipoproteins" (density less than 1.063) and the "high density lipoproteins" (density greater than 1.063). In this study of atherosclerosis attention will be confined to flotation of the low-density group of molecules.

As a preliminary operation in the study of sera for these molecules a separation is made in the preparative ultracentrifuge. In this process a density increment is given the serum sample by dilution with a concentrated sodium chloride solution. Ultracentrifugation of this serum solution for 13 hours results in the flotation to the top of the preparative tube of all lipoprotein molecules less dense than the solution. Also during the ultracentrifugation all large molecules of density greater than the solution undergo sedimentation. Thus after a critical time of ultracentrifugation the low density group of lipoproteins will have collected as a layer at the top of the preparative tube and all the proteins and dense lipoproteins will have sedimented out of the top portion of the preparative tube. After the centrifuge stops, removal of the top fraction containing the layered low-density lipoproteins can be effected using a capillary pipette. Next the isolated low-density lipoproteins (the top fraction) are studied in the analytical ultracentrifuge. Here the lipoprotein molecules are characterized and identified by their flotation rates. Flotation rates are given in S_f units (Svedbergs of flotation) under the specified conditions.* Usually the samples are centrifuged at 52,640 RPM (forces approximately 200,000 to 250,000 x gravity) for less than one hour, with a series of photographs being taken at various stages of the flotation.

A special optical system allows the moving boundaries of macromolecules to be observed and recorded while the centrifuge is in operation. The basis for detection of those boundaries is the abrupt change in refractive

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* 1 S_f unit = flotation rate of 1×10^{-13} cm/sec/dyne/gm. The specified conditions for these studies refer to use of a sodium chloride solution of density 1.063 gms/cc at 26°C. If runs are performed under any other conditions, appropriate corrections must be made. Spinco Model L and Model E ultracentrifuges were used throughout.

index from the region of solution containing the particular class of large molecules to the region out of which these molecules have migrated. The optical system records such a refractive index change as an inverted peak for floating boundaries, the area over the peak providing a measure of the concentration of these molecules. Thus the concentration and flotation rates of the large molecules present in a solution can be determined.

Figure 1a shows the type of ultracentrifugal pattern obtained for a single molecular species of flotation rate 6 S_f units, 1b shows a single molecular species of rate 13 S_f units, and 1c shows a mixture of these two species. In practice the low-density group of lipoproteins found in human serum may comprise from one to more than one species of components, differing one from another in size, density, and chemical composition. Thus, the diagrams obtained may be considerably more complicated than the illustration of two components shown in Figure 1c. In fact, the individual components may have flotation rates so close together as to be resolvable with difficulty. A typical flotation pattern illustrates the nature of the problem (see Figure 2a). Knowing the type of components (3) which exist, it is possible to resolve such a pattern into its individual constituents. (See Figure 2b). Figure 3 shows six illustrative ultracentrifugal patterns from humans in various clinical categories. In human serum the following classes of lipoprotein molecules have been identified:

Flotation Rate	Density gms/cc
S_f 2	1.050
S_f 4	1.040
S_f 6	1.035
S_f 8	1.029
S_f 10	1.023
S_f 13	1.015
S_f 17	0.99
S_f 17 - S_f 40,000	(S_f 40,000 represents chylomicrons) -- This represents a series of components with successively higher flotation rates, but so closely spaced as to be difficult of resolution as discrete components. There may be 10's or 100's of such components.

By differential preparative ultracentrifugation, individual components have been isolated (5), quite free of neighboring components, sufficient such that the individual species can be analyzed for chemical composition. Some protein is present in all the components (from S_f 2 up to and including the chylomicrons). There is approximately 25% protein in the S_f 4 species, ranging down to approximately 7% protein in the S_f 40,000 (chylomicrons). Cholesterol and its esters are also present in every one of these species, from approximately 30% in the S_f 4 to approximately 5% in the S_f 40,000. There is a progressive shift to a higher proportion of non-esterified cholesterol in the molecules of the higher S_f classes. Phospholipid content also decreases with increasing S_f rate. Neutral fat (glyceryl esters) are prominent constituents of molecules above S_f 17, whereas they are virtually absent in those of S_f 13 and less.

SERUM LIPOPROTEINS AND ATHEROSCLEROSIS

Having a picture of the lipoprotein spectrum present in human serum, one may inquire as to the relation of any or all of the components to the development of atherosclerosis and related disease states. If we are to consider cholesterol in relation to such disease, it is obvious that it may be pertinent to know whether certain classes of cholesterol-bearing lipoproteins are of more importance than others may be. The first clues that there may be differential significance to the various cholesterol-bearing lipoproteins came from a study of rabbits developing atherosclerosis as a result of cholesterol feeding (1, 2). The normal rabbit possesses only a single lipoprotein in this class at appreciable concentration of S_f value below 10 units. Cholesterol (or cholesterol plus oil) feeding first produces a rise in the already present S_f 10 (or less) lipoprotein. However, those rabbits developing

nothing further were shown previously not to develop atherosclerosis. The majority of rabbits go beyond this during cholesterol feeding and develop components of S_f value greater than 10 units, up to several hundred S_f units. It was also shown that one composite class, the S_f 10-30 class, shows a definite relationship to atherosclerosis in that higher concentrations of such molecules developing were accompanied by progressively more severe atherosclerosis, independent of the level of the S_f 10 (or less) concentration which had developed. Observations made on the dog developing atherosclerosis by the Kendall-Steiner procedure of feeding thiouracil and cholesterol (6) and in the stilbesterol implantation procedure in the chicken (7) reveal similar series of components developing in these species.

In the human direct correlations of blood lipoproteins with extent of atherosclerotic activity are not so readily made for obvious reasons. However, a study of ostensibly normal individuals and those with atherosclerosis or disease states accentuating atherosclerosis do provide information linking certain of the lipoprotein molecules to development of atherosclerosis in the human.

In considering the lipoprotein pattern in evaluation of relationship to ageing and disease, one is faced with the necessity of knowing something of the relative stability in level of a particular class of molecules in a single individual from day to day or with relationship to meals. Previous studies by the authors have indicated that the levels of molecules below 20 S_f units are adequately free from fluctuation to serve as a measure of metabolic steady state, whereas for molecules of progressively higher flotation rates (greater than 20 S_f units), the blood level is influenced in relation to meals variably from individual to individual many-fold. Thus whether or not the high S_f group of molecules is related to disease, they represent a poor indicator of

steady state metabolism unless some type of tolerance study is done. However, the stable character of the blood level of molecules of flotation rates below 20 S_f units renders it possible to characterize an individual in terms of his blood level of such molecules, independent of post-prandial lipemia effects. From considerations of the data obtained in rabbits and in humans, it appears that the molecules of S_f 6 and less in the human are unlikely to be directly involved in disease production. However, the data presented previously and here indicate that the molecules between 8 S_f and 20 S_f units are of significance with respect to atherosclerosis. Ideally within this group one would like to know the relative importance of each individual species in development of atherosclerosis, which information is not yet at hand. It does appear, however, that in the range 8 S_f units to 20 S_f units, the minor defect (if we may refer to it in this manner) is the appearance of 8 S_f molecules. Then with appreciable levels of S_f 8, the S_f 10 class may appear in appreciable concentration; when S_f 10 levels are appreciable S_f 13 molecules may be present; after S_f 13 levels become appreciable, molecules of S_f 13-20 may appear in quantity. We have never seen high levels of S_f 13-20 without appreciable S_f 13, nor high S_f 13 levels without appreciable S_f 10 levels; nor S_f 10 without S_f 8. However, all these molecules may be high <u>independent</u> of whether S_f 6 is <u>high</u> <u>or</u> <u>low</u>. Similarly in the rabbit developing atherosclerosis the molecules of successively higher S_f classes appear only after appreciable levels of the lower classes have developed. In this discussion we are reporting all data in terms of the combined blood level of lipoproteins of the S_f 10-20 class, the S_f 10 itself being excluded. A test of the data with respect to the S_f 8-14 class reveals that similar conclusions can be drawn from this bracketing.

The obvious implication of the association of the S_f 10-30 class of molecules with rabbit atherosclerosis is that possibly these molecules themselves are those in blood transport which ultimately provide the lipids for atheroma formation, and that the higher the blood level of such molecules the more extensive is atherosclerotic activity, given similar local factors in the arterial wall. Similarly the hypothesis is that in humans the blood level of a similar class of lipoproteins (the S_f 10-20 class) would be a reflection of the present activity of atheroma formation. Then if this hypothesis be correct, the blood levels of such molecules in various clinical categories should parallel the clinical data on atherosclerosis development in such categories. The pertinent data are presented graphically in Figures 4 through 10.

(a) "Normal" humans: Clinically there are several outstanding features about atherosclerosis in the presumably normal population. "Presumably" is used with intent, since even with the absence of clinically manifest disease and with negative findings in a painstaking physical examination and history, extensive atherosclerosis is often present. The features of interest are:

(1) The rarity of appreciable atherosclerosis in pre-puberal children.

(2) The markedly greater incidence and severity of atherosclerosis and its serious sequelae in young adult males than in young adult females.

(3) The increase in atherosclerotic involvement in both male and female adults with ageing, and the progressive obliteration of sex difference above the age of 40.

(4) The observation that autopsy of individuals dying of causes

other than atherosclerotic complications reveals a high proportion of such individuals to show appreciable atherosclerosis that was not detected in the living.

Paralleling these four basic observations our measurements of S_f 10-20 lipoprotein levels in a large series of such humans reveal results completely consistent with the hypothesis that such molecules provide a measure of atherosclerogenesis.

(1) Measurements on over 50 "normal" pre-puberal children and infants (8) reveal this group to have the lowest levels of S_f 10-20 molecules of any of the normal categories. Atherosclerosis is rare in children of this age.

(2) The data in Figures 4 and 5 show that females in the 20-30 age group show consistently lower levels than males in this age group, paralleling well their lower rate of atherosclerogenesis.

(3) The data reveal a progressive rise in S_f 10-20 levels in "normals" of both sexes (at least to age 60) and a progressive diminution of the difference in levels between males and females, both observations being in harmony with the above-described known observations on atherosclerosis in such groups.

(b) Living individuals with atherosclerosis: In the absence of a clinical sequel of atherosclerosis, e.g. a myocardial infarction, we have no way of assessing clinically either the quantity of atherosclerosis present or the rate of its development. As the closest approximation to this assessment we have chosen two groups, (a) survivals of a myocardial infarction and (b) patients with angina pectoris who have not had an evident infarct. The basic pathology underlying both these clinical entities in the vast majority of these cases is atherosclerosis of the coronary arteries. It is true, of course, that

atherosclerosis of the coronary arteries does not necessarily measure quantitatively atherosclerosis of other vascular beds, and further that the occurrence of clinical angina pectoris or myocardial infarction does not quantitate the degree of coronary atherosclerotic involvement. Nevertheless, in the absence of a better quantitative approach, it cannot be gainsaid that a group of patients manifesting angina pectoris or a myocardial infarction will on the average be expected to have more coronary artery atherosclerosis than will a group of presumably normal individuals of similar age and sex distribution. Figures 6, 7 and 8 show the comparison between "normals" of both sexes and patients either with myocardial infarction or angina pectoris. The higher levels of S_f 10-20 molecules in these disease categories (being primarily on an atherosclerotic basis) than in corresponding normals, for all ages and both sexes, provide a strong link in the chain of evidence implicating this class of molecules in the development of atherosclerosis in humans. The data as presented in Figures 6, 7 and 8 allow one to compare "normals" with the coronary disease patients at any level of S_f 10-20 molecules, e.g. at a level of 25 mg%, 26% of normal males are below this level whereas 9% of infarcts are. Or, at the higher levels, one sees that at a high level of 80 mg%, 22% of males with myocardial infarction are above this level, but only 7% of normals are. Thus three times as many infarct patients have such high levels as do normals.

That <u>some</u> "normals" have levels higher than many patients with clinically evident coronary artery disease is not surprising or unexpected. Indeed it would be inconsistent that they should not, since it is highly reasonable to assume that such "normals" are developing atherosclerosis, provided local factors are appropriate, but have simply not yet had a clinical sequel. After all, the occurrence of a myocardial infarction is

simply a mechanical hazard along the course of evolution of atherosclerotic disease, and atherosclerosis can be full-blown without such a manifestation. That a small proportion of patients with angina or myocardial infarction show low S_f 10-20 levels is not unexpected. A few of such patients may have a non-atherosclerotic basis for their disease. Others may well have had high levels of such molecules before the infarction with a subsequent drop due to dietary and drug manipulation subsequent to the clinical episode. Such a case in point is that of a 38 year old male we studied first as a "normal." At that time his S_f 10-20 level was 134 mg% (see Figure 3e). Several months later he experienced a myocardial infarction and was treated with usual measures plus dietary restriction. His blood level at the sixth week after the episode was down to 29 mg%. However, had we not known from several pre-myocardial infarction studies that he had carried an exceedingly high level of such molecules, he might erroneously have appeared to be an exception to the rule of elevated S_f 10-20 lipoprotein levels accompanying atherosclerosis.

HYPERTENSION, S_f 10-20 LIPOPROTEINS, AND ATHEROSCLEROSIS

The frequency with which hypertensive patients suffer coronary artery occlusions and the generally accepted clinical evidence of excessive atherosclerosis in many hypertensive patients both suggest that hypertension may predispose to atherosclerosis. Many have suspected that the elevated pressure per se is the responsible factor in accentuating atherosclerosis. Yet it is well known that some severe hypertensives (in terms of actual pressure levels) come to autopsy with minimal atherosclerosis. In an effort to evaluate the factors in hypertensive subjects responsible for excessive atherosclerosis we have made a comparison of 139 essential hypertensives without known complications, studied because of their hypertension itself, with 95 cases of myocardial in-

farction and/or coronary insufficiency who happened also to have elevated diastolic pressures (100 mm diastolic minimum used for these purposes). The data, presented in Figure 9, show that hypertensives who have such a demonstrable manifestation of atherosclerotic disease have a much higher frequency of high S_f 10-20 lipoprotein levels (and higher average levels) than do the uncomplicated hypertensives. Since blood pressures were not appreciably different in the two groups, it would appear that a primary factor which resulted in atherosclerotic manifestations in the hypertensives who also have demonstrable coronary artery disease may be their elevated S_f 10-20 lipoprotein levels as compared with the uncomplicated hypertensives. Further, the existence of appreciable numbers of hypertensives without evident atherosclerotic complications who have relatively low S_f 10-20 levels in spite of long duration of severe blood pressure elevations may explain the clinical observation that an appreciable segment of the hypertensive population survives a long period without developing clinical sequelae of atherosclerosis.

DIABETES MELLITUS, S_f 10-20 LIPOPROTEIN LEVELS, AND ATHEROSCLEROSIS

The known frequency of severe atherosclerotic complications in diabetes mellitus provides probably the most serious problem existing in the management of the diabetic patient. In the effort to evaluate the factor responsible for excessive hypertensive or atherosclerotic vascular disease in diabetes mellitus, we have compared a series of diabetics between 40 and 70 years of age, as yet uncomplicated by clinically evident vascular disease, with another series of diabetics already demonstrating hypertension and/or coronary artery disease. The data presented in Figure 10 show that the diabetics already manifesting such vascular disease show strikingly higher S_f 10-20 lipoprotein levels than do diabetics without such complications. This would implicate the level of such molecules as a major factor, at least, in the development of vascular complications in diabetes mellitus, and further may shed some light on the ability of a certain

proportion of middle aged diabetics to live with their disease many years without encountering serious complications of atherosclerosis.

S_f 10-20 LIPOPROTEIN LEVELS IN OTHER DISEASES ASSOCIATED WITH EXCESSIVE ATHEROSCLEROSIS

Several clinical entities, including myxedema, xanthoma tuberosum and nephrosis, are known to predispose to premature and excessive atherosclerosis.

(a) Myxedema: Twelve patients with hypothyroidism or frank myxedema have been studied with respect to S_f 10-20 lipoprotein levels (Figure 3c). In all cases very high S_f 10-20 lipoprotein levels were observed (average level 132 mg%). Some of these ptients showed levels in the 200 mg% range which are far higher even than the majority of patients with vascular disease. A few treated myxedemas studied initially after therapy with thyroid extract showed much lower levels, suggesting that the thyroid therapy may have reduced their levels. This possibility is strengthened by our own observations in a small series of hypothyroid patients being followed who are demonstrating drops in S_f 10-20 levels on thyroid extract. An evaluation of thyroid extract in apparently euthyroid individuals is in progress. In two children with thyroid carcinoma in whom hypothyroidism was being artificially induced by Doctors Lee Farr and James Robertson, classical lipoprotein patterns of the hypothyroid state were observed with the S_f 10-20 level being greatly elevated.

(b) Xanthoma tuberosum: Four patients with classical xanthoma tuberosum have been studied (9). The greatly increased atherosclerosis in such patients is well known. All 4 patients in this category showed fantastic S_f 10-20 lipoprotein levels in their sera, without striking elevations in the level of the S_f 6 and lower molecules, indicating that their abnormality is not simply increased levels of all cholesterol-bearing molecules, but rather only of those in special classes.

(c) Nephrosis: Ten patients manifesting the nephrotic syndrome showed uniformly high S_f 10-20 levels, some with and some without appreciable elevations of the S_f 6 and lower classes of lipoproteins (see Figure 3d).

The exceedingly high S_f 10-20 lipoprotein levels in these three disease categories, myxedema, xanthoma tuberosum and nephrosis, generally with S_f 6 cholesterol-bearing lipoproteins in the normal range, suggests strongly that the S_f 10-20 class of lipoproteins is more associated with the excessive atherosclerosis of these diseases than is the hypercholesterolemia often accompanying them.

THE RELATIONSHIP OF S_f 10-20 LIPOPROTEIN LEVELS TO TOTAL BLOOD CHOLESTEROL

The present authors have pointed out previously (1, 2) that molecules of the S_f 10-20 class may be present at high levels in a particular patient's blood no matter whether the blood cholesterol is high, low or intermediate. More extensive studies have completely confirmed this observation.

Since the blood cholesterol (either free or esterified) exists in the form of several different lipoproteins, it is apparent that a priori one cannot predict that the relative proportions of the several lipoproteins will remain the same at various levels of total blood cholesterol. In fact, the opposite is often true, so that in one serum the ratio of S_f 10-20 molecules to S_f6 molecules may be high, whereas in another serum with a much higher total cholesterol level the ratio of S_f 10-20 molecules to S_f 6 may be quite low. So great are these variations that the total blood cholesterol level is valueless in predicting the S_f 10-20 level in a particular patient. While it is true that in general the S_f 10-20 level rises with a rise in blood cholesterol, the deviations from this rule are so frequent and severe that one would be grossly deceived by the use of the total blood cholesterol as an indicator for the presence of these particular S_f 10-20 molecules. Further, the frequent occurrence of high S_f 10-20 lipoprotein levels with low total blood cholesterol (even below 150 mg% total)

would indicate that in evaluation of atherosclerotic potentialities a low serum cholesterol may be highly deceptive in predicting the absence of the possibly noxious S_f 10-20 molecules.

An appreciable fraction of blood cholesterol is often in the S_f 6 lipoprotein. Such sera may have exceedingly low levels of the S_f 10-20 class, especially frequently in the sera of children and young adult females. Since these groups show very little atherosclerosis and since numerous patients with manifest atherosclerosis show much lower S_f 6 levels but high S_f 10-20 levels, we, tentatively at least, are of the opinion that the S_f 6 molecule per se is not involved in atherosclerogenesis.

The common presence of S_f 10-20 molecules in sera from patients with manifest atherosclerosis, or with diseases predisposing thereto, independent of the level of total serum cholesterol, may aid in explaining the dissatisfaction held by many clinicians over the apparent failure of blood cholesterol levels to correlate well with atherosclerotic activity.

Recently much attention has been paid to cholesterol-phospholipid ratios as indices to atherosclerotic activity (10). Just as with serum cholesterol, the cholesterol-phospholipid ratios can only be satisfactorily interpreted in the light of the knowledge of which of various cholesterol- and phospholipid-bearing lipoproteins are present in a particular serum. Since from studies (5) on isolated lipoproteins from human serum it appears that the entire group of low density lipoproteins (S_f 4 - S_f 100) show weight ratios of cholesterol to phospholipid of 1.0 to 1.4, whereas the high density lipoproteins (11) (a group not treated in this paper) show ratios in the neighborhood of 0.45, the aggregate total cholesterol to phospholipid ratio will depend upon the relative abundance of low and high density lipoproteins much more than upon the distribution among the low density lipoproteins, e.g. between the S_f 6 and S_f 10-20. Thus a person might have a low phospholipid-cholesterol ratio due to a high S_f 6 level relative

to the level of the high density lipoprotein group. From the low phospholipid-cholesterol ratio, some workers might predict a likelihood of high atherosclerotic activity, whereas from the absence of appreciable S_f 10-20 levels we would be led to just the opposite conclusion.

The evidence that suggests that low phospholipid-cholesterol ratios are common in atherosclerotic patients probably results from the frequent occurrence of high levels of all the lipoproteins of the S_f 3-100 class in such patients. However, the phospholipid to cholesterol ratio fails to distinguish between the various components of the S_f 3-100 class, since all these components have approximately the same phospholipid-cholesterol ratios. Thus a patient with a high total S_f 3 - S_f 100 class, but a low 10-20 level, would be considered as high in atherosclerotic activity by some workers whereas we would consider such an individual as low in this respect.

Again, a patient with a high S_f 10-20 level might have a low total S_f 3-100 level but a high level of the high density lipoproteins with its concomitant high phospholipid-cholesterol ratio. Such a total serum might have a high phospholipid-cholesterol ratio, indicating low atherosclerotic activity. We would consider just the opposite to be true.

MODIFICATION OF THE BLOOD LEVELS OF THE S_f 10-20 CLASS OF LIPOPROTEINS

The hypothesis that such molecules as the S_f 10-20 class of lipoproteins may be the source of lipids in atheromata renders it of obvious potential prophylactic and therapeutic interest to understand the factors which may modify these levels. Changes due to thyroid extract in certain selected patients and due to the combination of features attendant upon a myocardial infarction in the acute phase have already been mentioned above.

As previously reported (1, 2) we have been investigating the effect of a low fat, low cholesterol dietary regimen both in a controlled hospital dietary group and in ambulatory patients at home. The cumulative evidence indicates

that appreciable reductions in S_f 10-20 levels are obtainable by the use of a diet containing 20 to 50 grams of total fat, restricted in cholesterol to approximately 200 mg per day, but adequate in protein. The allowance of vegetable fats to 100 grams in patients previously restricted in total fats to 20 grams results in general in a rise of S_f 10-20 levels. Thus the restriction of both animal and vegetable fats is required, independent of cholesterol intake. The results obtained in the hospital controlled series indicate that weight loss or weight gain did not appear to influence responses, since even with maintenance of weight to within $\pm$ 3 pounds changes in the lipoproteins occurred with changes in dietary fats and cholesterol.

The changes in levels of S_f 10-20 molecules (and in those of the S_f 8-10 class) observed in dietary restriction may or may not be accompanied by appreciable changes in total serum cholesterol. Thus a two-fold lowering of lipoprotein levels may be observed with a much lower percentage alteration in serum cholesterol level. This should be understandable in the light of the discussion above concerning the numerous types of cholesterol-bearing molecules present in serum. The fact that serum cholesterol may not drop appreciably on diet has probably been the source of discouragement in the clinical application and evaluation of efficacy of such dietary restriction in atherosclerosis. However, two-fold drops in S_f 10-20 lipoprotein levels seen in dietary restriction even without large concomitant serum cholesterol drops render such evaluation essential. Dietary response is variable from individual to individual, some showing very little response to 35 to 50 gram fat diets at home. It must, however, always be considered that until such apparently refractory patients have been tested under controlled conditions one cannot truly claim the patient to be refractory. The advertent and inadvertent errors in dieting by the patient eating at home are discouragingly frequent. Further, the necessity for careful instruction concerning what a low fat diet really means is frequently overlooked. It is more important that the

patient know what he can eat rather than merely what he cannot eat. It is yet too early to make any definite statement as to clinical efficacy of such dietary restriction.

INTERRELATIONSHIPS AMONG THE VARIOUS LIPOPROTEINS

An ultimate understanding of the mechanism of maintenance of the blood levels of the various lipoproteins is obviously the goal which is to be striven toward in the effort to gain an insight into the nature of the defect which results in abnormal elevations of such molecules as the S_f 10-20 class in certain individuals. All the serum lipoproteins should be regarded as in transit, the steady state blood levels of any particular molecule being the result of balance between rates of influx of that molecule into serum and its rate of disappearance from serum to whatever sites of utilization, storage or destruction. Hormonal influences obviously suggest themselves from such observations as the male-female differences in levels, from abnormal and often very high S_f 10-20 levels seen in the pregnant female (12) as contrasted with the non-pregnant female of the same age whose levels are generally very low.

Certain patterns are becoming clarified as to interrelationships of the various molecular levels. Those individuals with low levels of S_f 10-20 lipoproteins generally, although not always, show very low levels of all the lipoproteins in the S_f 20-100 class as well (see Figure 3a). Individuals who show high levels of S_f 10-20 molecules (and also S_f 8-10) quite often show in addition appreciable levels of the lipoproteins of the S_f 20-100 class. On the other hand, in some patients very high S_f 10-20 levels are observed without accompanying high levels of molecules of S_f 20-100. The S_f 30-100 class of molecules is acutely influenced (in matters of hours) following high fat meals, but to a variable degree in different individuals.

It would appear that the defect involved in the maintenance of high levels of these S_f 10-20 molecules which are related to atherosclerosis, represent

one facet of what may be a general disturbance in the overall metabolic pattern of handling fats and cholesterol, at least insofar as the blood transport mechanism. A more penetrating understanding of this disturbance requires further clarification of the interrelationships, source and site of removal of all the various classes of lipoproteins involved.

SUMMARY

1. Serum lipid transport is describable in terms of a group of lipoprotein macromolecules of differing physical properties and chemical composition. Certain of these lipoproteins (the S_f 10-20 class - and the S_f 8-10 class) are associated with human atherosclerosis.

2. While total serum level of any one of the lipid constituents, e.g. cholesterol or phospholipids, is determined by the presence and amount of the individual lipoprotein molecules, the converse is not true. Thus the serum total cholesterol is of no value in predicting the level of the S_f 10-20 class of lipoproteins for any particular individual.

3. Patients with myocardial infarction or angina pectoris show consistently higher levels of such molecules than do "normals".

4. Hypertensive patients who have coronary artery disease (presumably atherosclerotic in origin) show higher S_f 10-20 levels than do uncomplicated hypertensives.

5. Diabetic patients with vascular disease (hypertension and/or coronary artery disease) show higher levels than do diabetics not so complicated.

6. Dietary restriction of total fats plus cholesterol slowly reduces the level of S_f 10-20 molecules, the extent and rate of reduction being variable from patient to patient.

7. The presence of the S_f 10-20 class of molecules in high concentration is noted in such diseases as myxedema, nephrosis and xanthoma tuberosum where atherosclerosis is excessive.

8. The factors influencing the level of S_f 10-20 lipoproteins and the observed relationships to other lipoproteins of serum suggest a general metabolic disorder in fat and cholesterol transport associated with the presence of this particular group of molecules that are involved in atherosclerosis.

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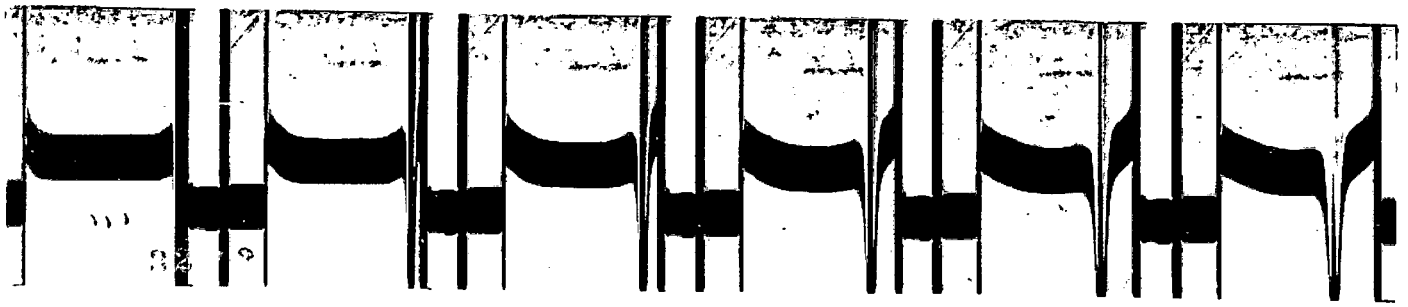
Figure 1.

(a) Upper pattern showing flotation of isolated S_f 13 molecules.

(b) Middle pattern showing flotation of isolated S_f 6 molecules.

(c) Lower pattern showing flotation of mixture of these two lipoproteins.

0.18 % S_f 13



0.55 % S_f 6

MIXTURE CONTAINING 0.13 % S_f 13 & 0.55 % S_f 6

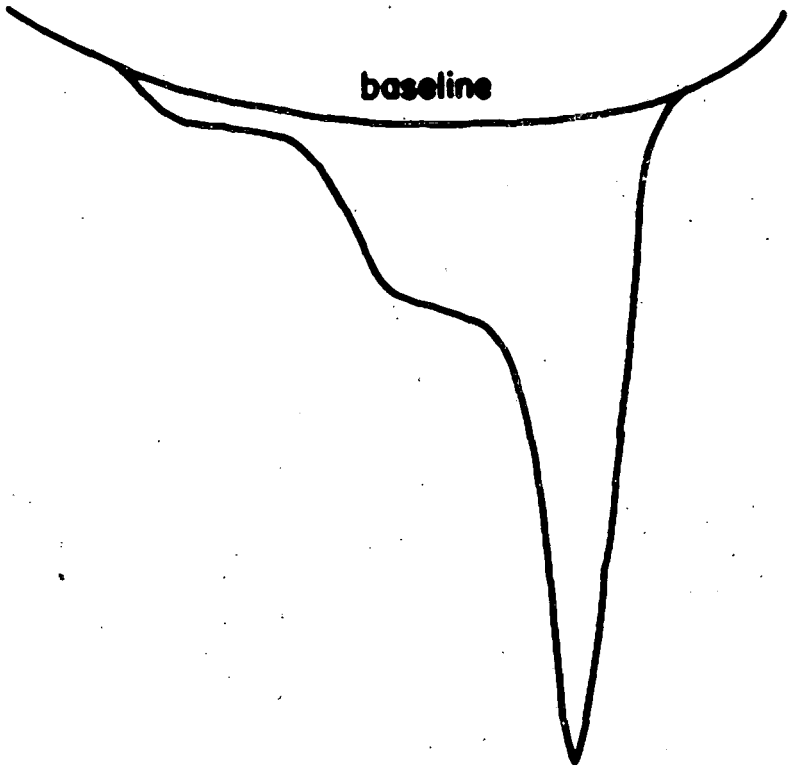
FLOTATION OF ISOLATED S_f 13, S_f 6, & MIXTURE

Figure 2.

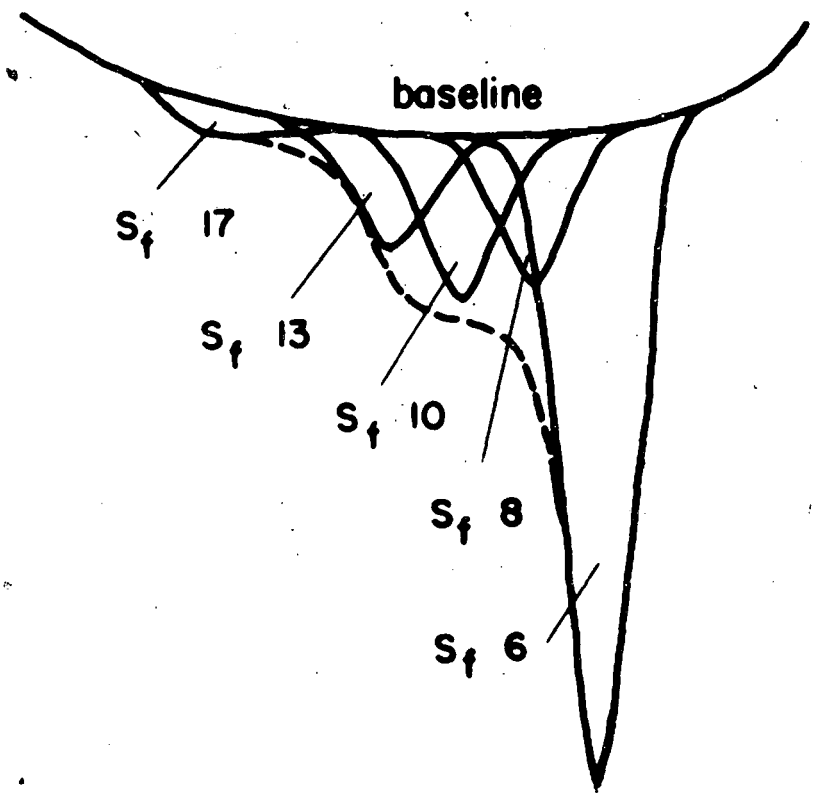
(a) Diagram illustrating a typical flotation pattern observed in the ultracentrifugal analysis of the low density lipoproteins of human serum.

(b) Resolution of 2(a) into the lipoprotein components known to exist in human serum.

COMPOSITE PICTURE AS OBSERVED



RESOLUTION INTO KNOWN COMPONENTS



OZ 1080

Figure 3.

(a) Flotation pattern of low density lipoproteins from a normal 20 year old male showing exceedingly low level of S_f 10-20 molecules. Each frame is ruled for calculation of the S_f rate of any peak appearing in that frame. In this figure as well as in those below, successive frames are at 0, 6, 12, 22, 30 and 38 minutes after full rotor speed of 52,640 RPM has been reached. Hence S_f rate markings may be used on corresponding frames in all patterns below.

(b) Flotation pattern of low density lipoproteins of a 66 year old male patient following myocardial infarction (well beyond acute phase).

(c) Flotation pattern of low density lipoproteins of a 36 year old male with hypothyroidism subsequent to surgical therapy for Graves' disease.

(d) Flotation pattern of low density lipoproteins of a 46 year old male with the nephrotic syndrome.

(e) and (f) Analytical flotation pattern of two different samples run simultaneously.

Upper pattern is that of a 38 year old male studied three months <u>before</u> he experienced a myocardial infarction (Note: Extremely high S_f 10-20 level. Six weeks beyond the acute infarction his level was found to be 29 mg%).

Lower pattern is that of a 38 year old normal female demonstrating very low S_f 10-20 level.

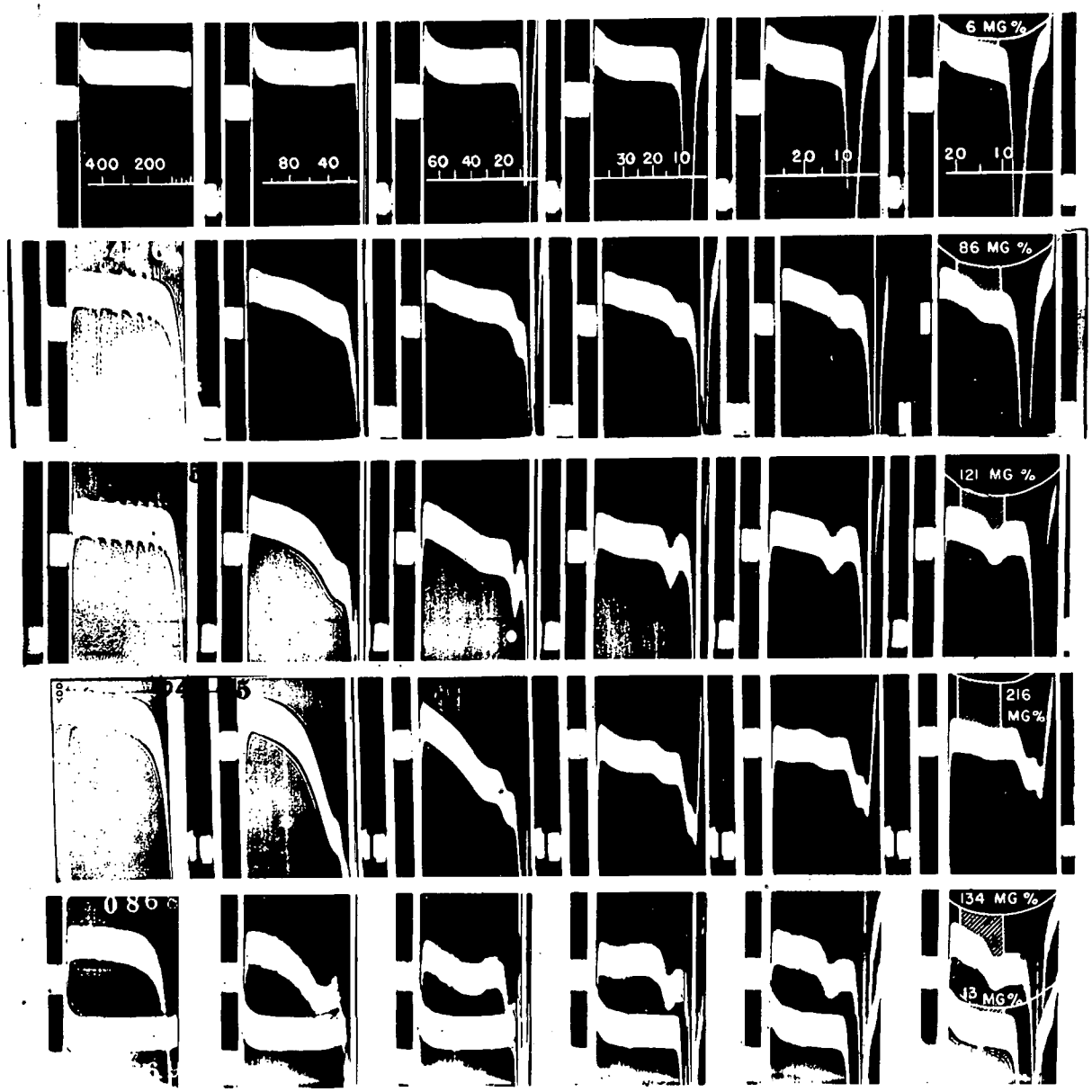

400 200
80 40
60 40 20
30 20 10
20 10
20 10
6 MG %
86 MG %
121 MG %
216 MG%
134 MG %
13 MG%

Figure 4.

Diagram illustrating the trend with age of blood levels of S_f 10-20 lipoproteins in normal males 20-70 years of age.

Notes: (1) The lipoprotein levels reported here have been revised upward as a result of calibration changes as compared with previous data reported in reference 2. Data in Figures 5 through 10 are similarly revised. The revision in no way alters the interpretations given in that reference.

(2) Data based upon 291 cases.

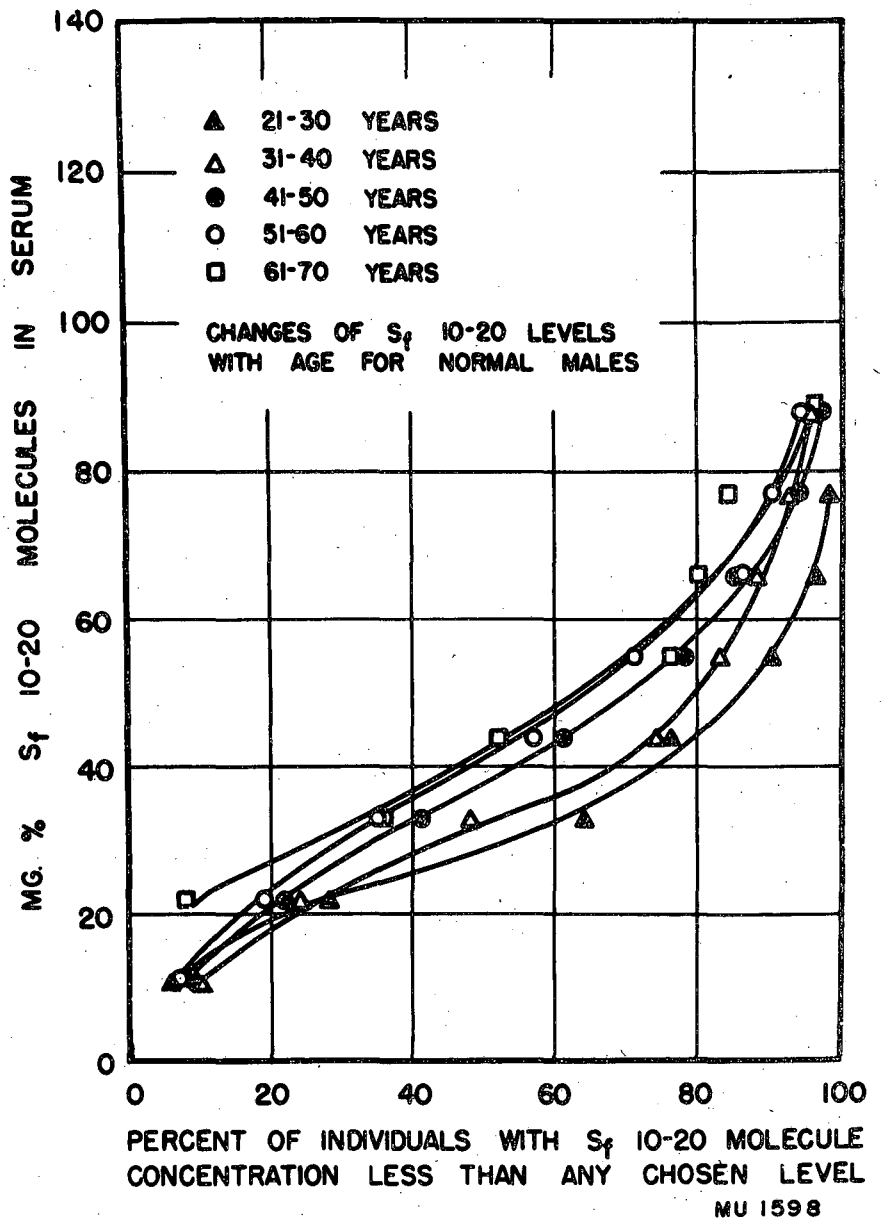

21-30 YEARS
31-40 YEARS
41-50 YEARS
51-60 YEARS
61-70 YEARS
CHANGES OF S_f 10-20 LEVELS WITH AGE FOR NORMAL MALES
MG. % S_f 10-20 MOLECULES IN SERUM
PERCENT OF INDIVIDUALS WITH S_f 10-20 MOLECULE CONCENTRATION LESS THAN ANY CHOSEN LEVEL
0
20
40
60
80
100
120
140
MU 1598

Figure 5.

Diagram illustrating the trend with age of blood levels of S_f 10-20 lipoproteins in normal females 20-70 years of age.

Note: Data based on 309 cases.

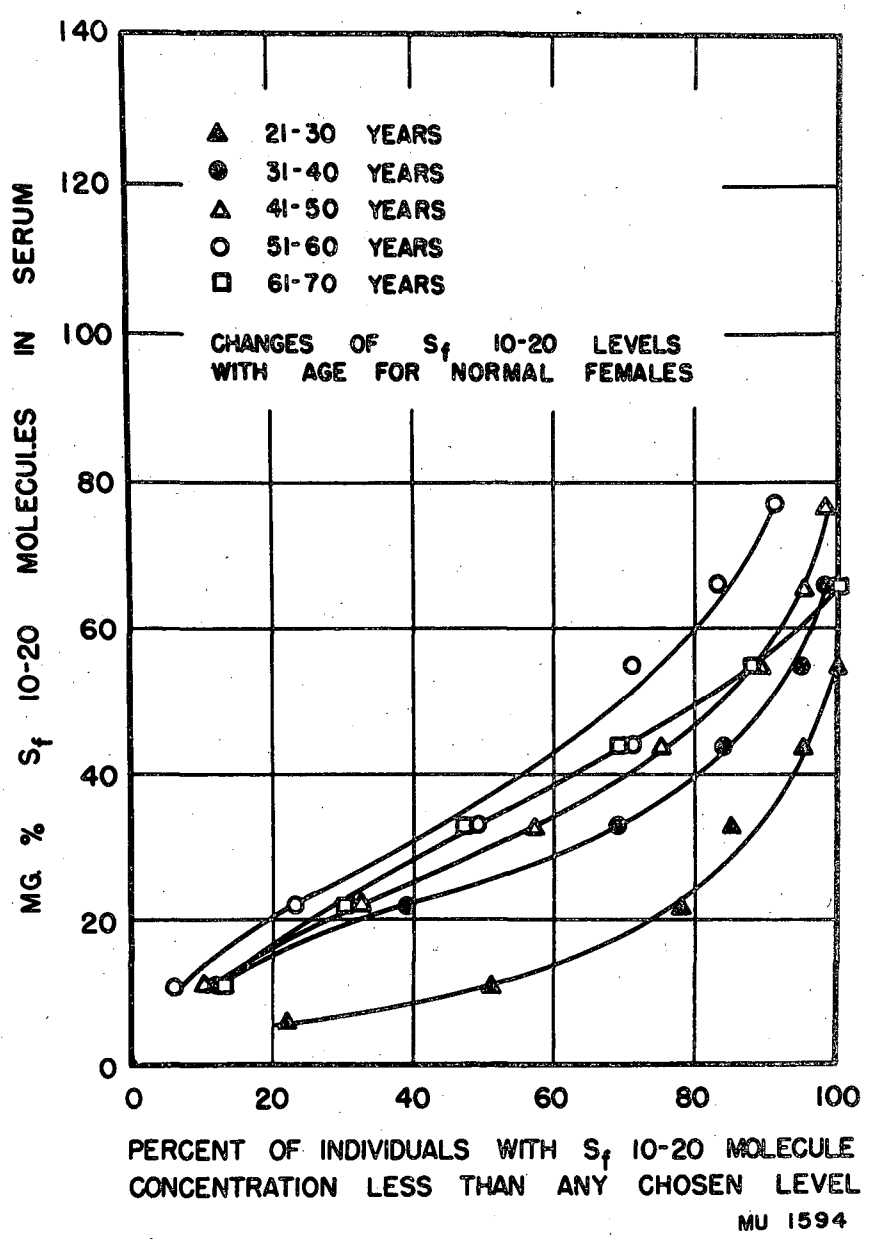
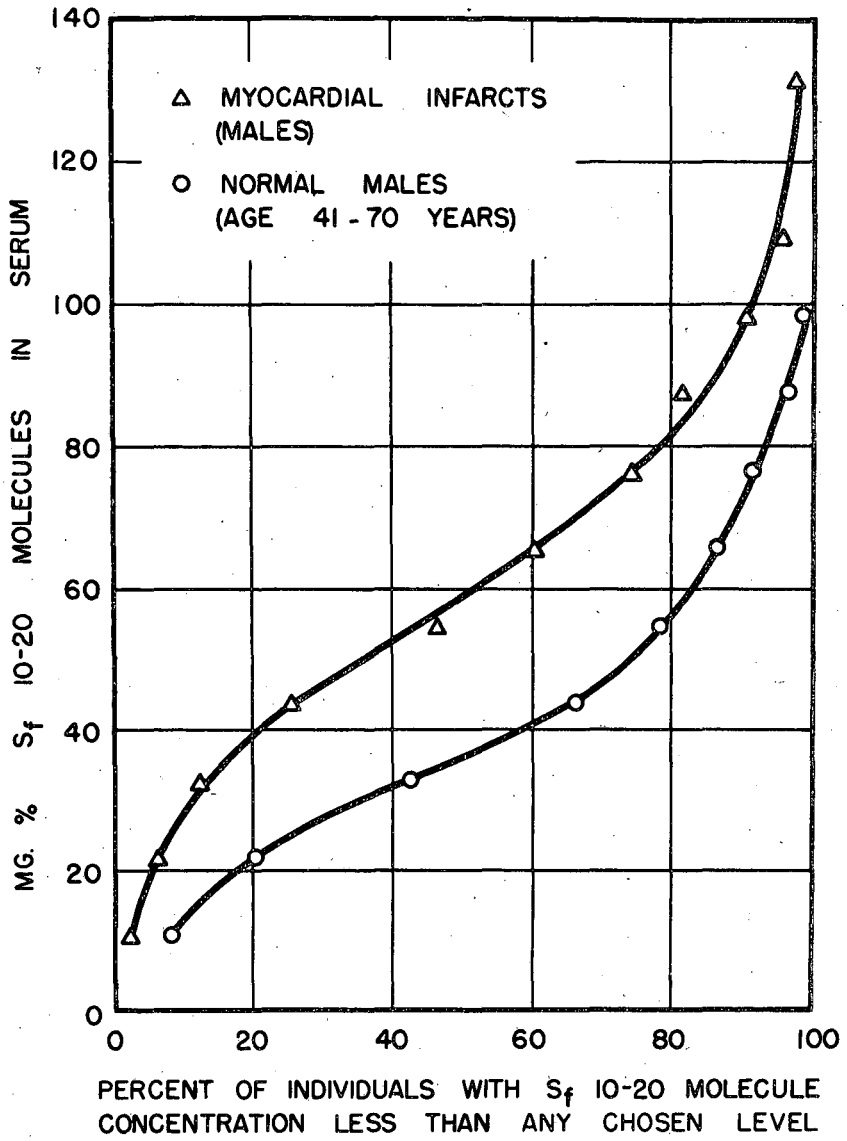

21-30 YEARS
31-40 YEARS
41-50 YEARS
51-60 YEARS
61-70 YEARS
CHANGES OF S_f 10-20 LEVELS WITH AGE FOR NORMAL FEMALES
MG. % S_f 10-20 MOLECULES IN SERUM
PERCENT OF INDIVIDUALS WITH S_f 10-20 MOLECULE CONCENTRATION LESS THAN ANY CHOSEN LEVEL
0
20
40
60
80
100
120
140
MU 1594

Figure 6.

Diagram illustrating the higher levels of S_f 10-20 molecules in males with myocardial infarction than in normal males of corresponding ages. (Notes: (1) All patients with infarction were at least 6 weeks beyond infarction date.

(2) Data based on 203 myocardial infarcts and 241 normal males)


140
120
100
80
60
40
20
0
0
20
40
60
80
100
△ MYOCARDIAL INFARCTS (MALES)
○ NORMAL MALES (AGE 41 - 70 YEARS)
MG. % S_f 10-20 MOLECULES IN SERUM
PERCENT OF INDIVIDUALS WITH S_f 10-20 MOLECULE CONCENTRATION LESS THAN ANY CHOSEN LEVEL

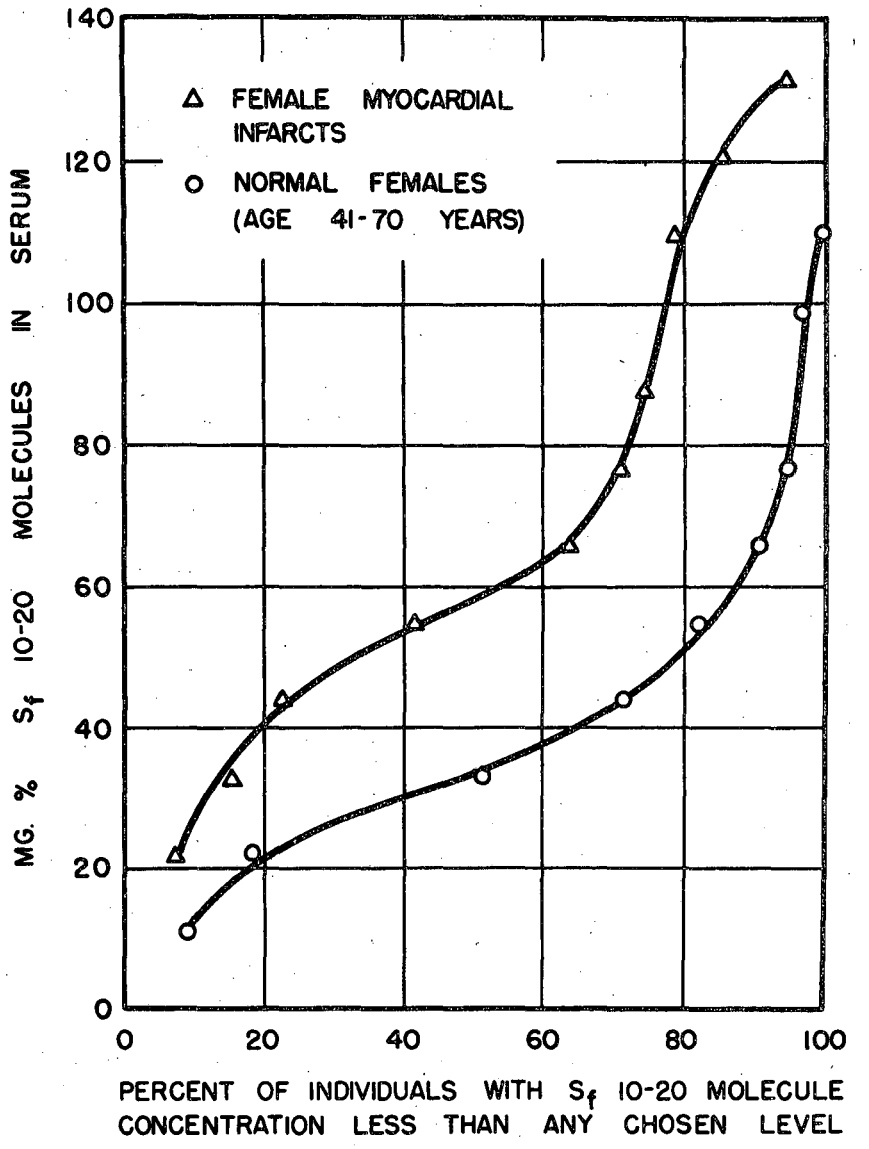
MU 1593

Figure 7.

Diagram illustrating the higher S_f 10-20 levels in females with myocardial infarction than in normal females of corresponding ages.

Notes: (1) All patients with infarction were at least 6 weeks beyond infarction date.

(2) Data based on 27 female myocardial infarcts and 139 normal females.


140
120
100
80
60
40
20
0
0
20
40
60
80
100
Δ FEMALE MYOCARDIAL INFARCTS
O NORMAL FEMALES (AGE 41-70 YEARS)
MG. % S_f 10-20 MOLECULES IN SERUM
PERCENT OF INDIVIDUALS WITH S_f 10-20 MOLECULE CONCENTRATION LESS THAN ANY CHOSEN LEVEL

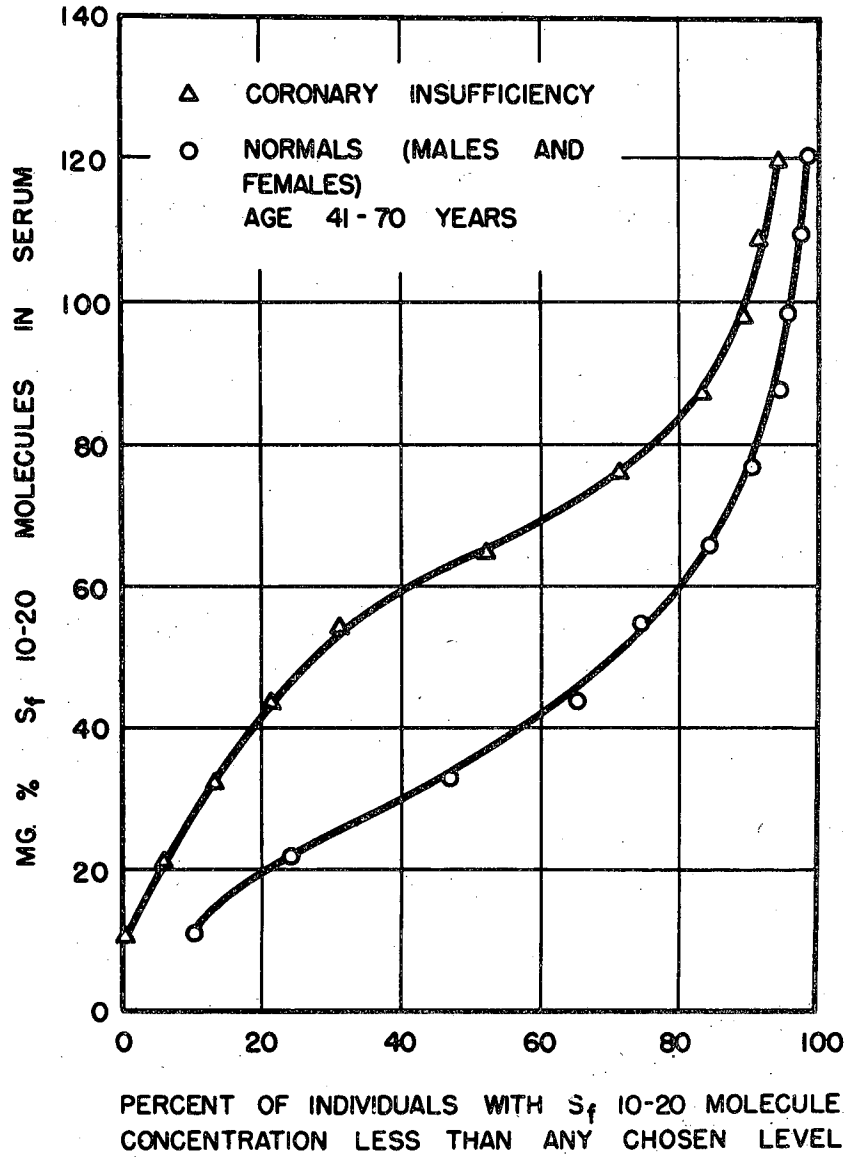
MU 1595

Figure 8.

Diagram illustrating the higher S_f 10-20 levels in patients with coronary insufficiency (as manifested by angina pectoris) than in normals.

Notes: (1) No significant differences were found between the males and females in these groups, so the plots are composite for both sexes.

(2) Data based on 63 patients with coronary insufficiency and 380 normals.

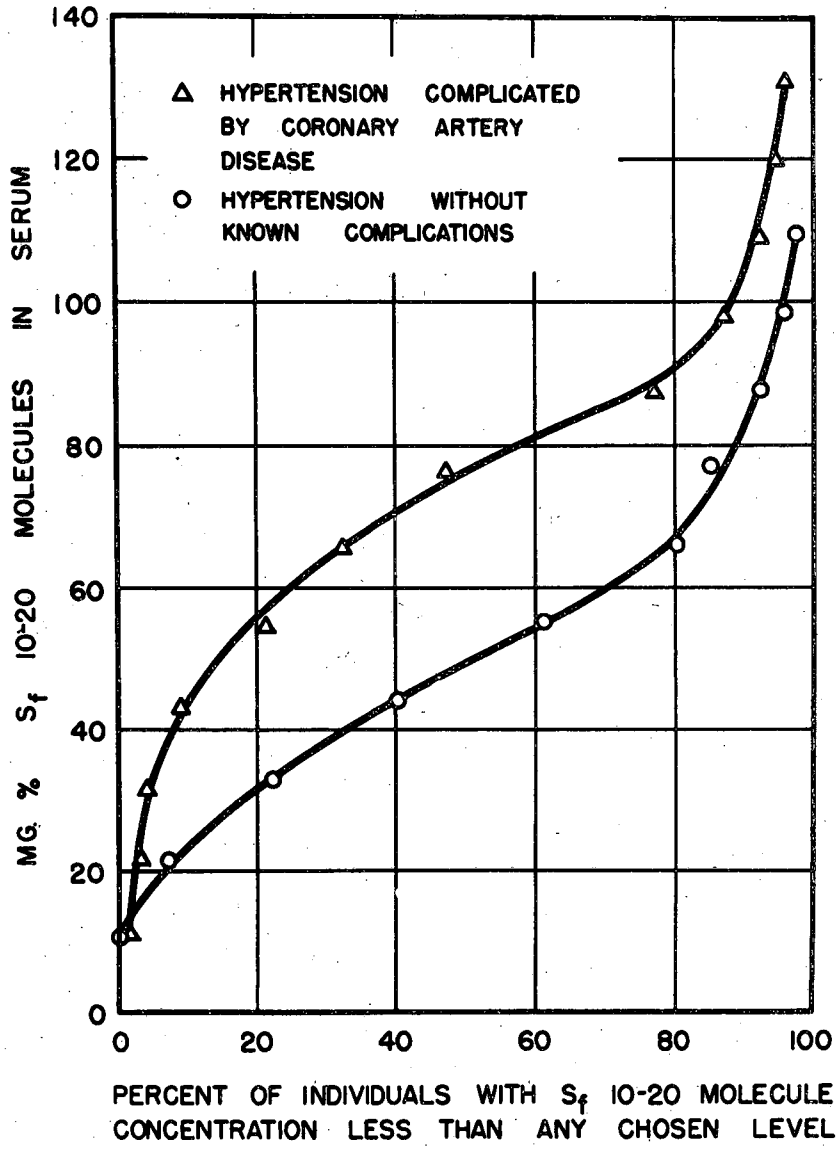

140
120
100
80
60
40
20
0
0
20
40
60
80
100
△ CORONARY INSUFFICIENCY
○ NORMALS (MALES AND FEMALES)
AGE 41 - 70 YEARS
MG. % S_f 10-20 MOLECULES IN SERUM
PERCENT OF INDIVIDUALS WITH S_f 10-20 MOLECULE CONCENTRATION LESS THAN ANY CHOSEN LEVEL

MU 1597

Figure 9.

Diagram illustrating the higher S_f 10-20 lipoprotein levels in hypertensives complicated by coronary artery disease than in hypertensives without such known complications.

Note: Based on 95 hypertensives with known coronary artery disease and 139 hypertensives without known coronary disease.

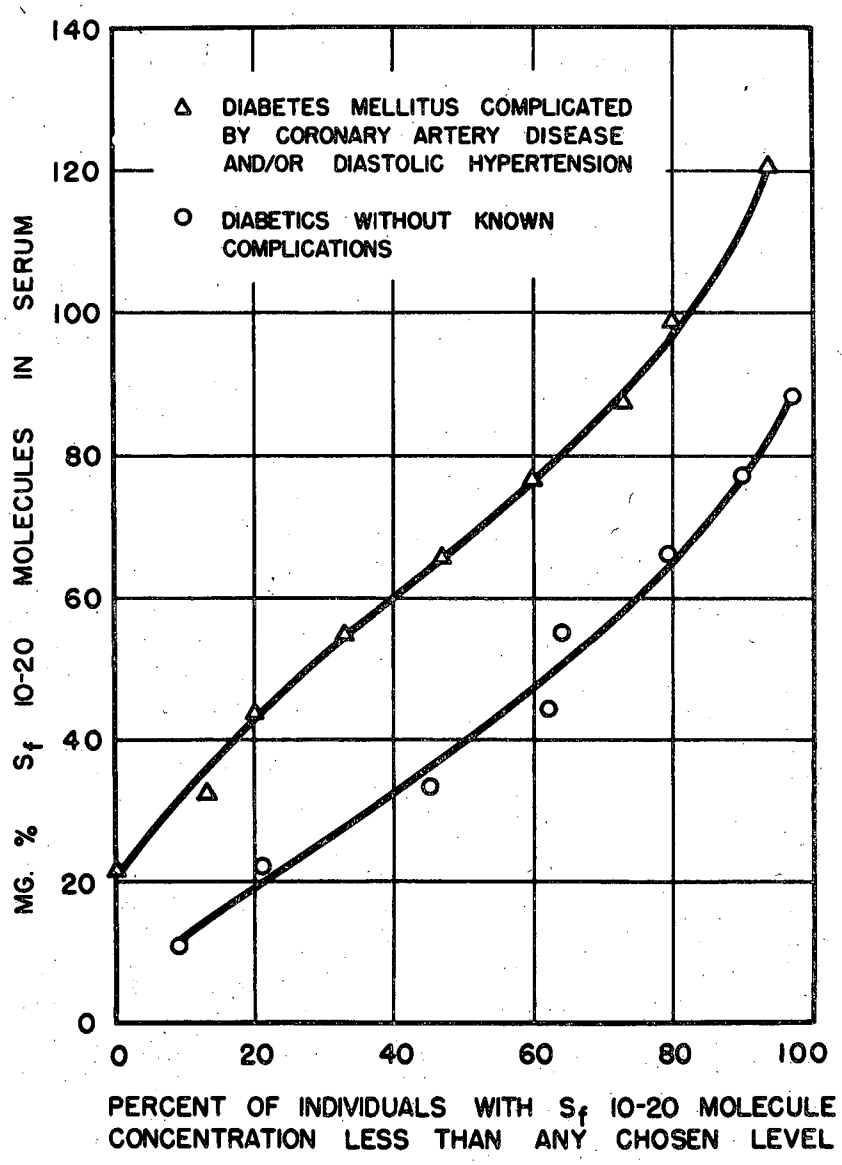

140
120
100
80
60
40
20
0
0
20
40
60
80
100
△ HYPERTENSION COMPLICATED BY CORONARY ARTERY DISEASE
○ HYPERTENSION WITHOUT KNOWN COMPLICATIONS
MG. % S_f 10-20 MOLECULES IN SERUM
PERCENT OF INDIVIDUALS WITH S_f 10-20 MOLECULE CONCENTRATION LESS THAN ANY CHOSEN LEVEL

MU 1596

Figure 10.

Diagram illustrating the higher S_f 10-20 lipoprotein levels in diabetics complicated by hypertension and/or coronary artery disease than in diabetics without such known complications.

Note: Data based on 15 diabetics with vascular complications and 58 diabetics without such known complications.


140
120
100
80
60
40
20
0
MG. % S_f 10-20 MOLECULES IN SERUM
Δ DIABETES MELLITUS COMPLICATED BY CORONARY ARTERY DISEASE AND/OR DIASTOLIC HYPERTENSION
O DIABETICS WITHOUT KNOWN COMPLICATIONS
0
20
40
60
80
100
PERCENT OF INDIVIDUALS WITH S_f 10-20 MOLECULE CONCENTRATION LESS THAN ANY CHOSEN LEVEL

MU 1599