UNIVERSITY OF CALIFORNIA

Radiation Laboratory

BERKELEY, CALIFORNIA

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June 1951

UCRL-1453

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FURTHER STUDIES ON THE RELATIONSHIP OF S_f 10-20 LIPOPROTEIN MOLECULES TO ATHEROSCLEROSIS*

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Evidence relating certain lipoprotein molecules of the serum of humans and experimental animals with the development of atherosclerosis has been previously presented by the authors (1, 2, 3). It is the purpose of this report to evaluate a clinical follow-up which has further amplified this relationship.

The basic premise of this research was that there might be a defect in the molecules which transport fats and cholesterol that could be more intimately related to the pathogenesis of atherosclerosis than are the total analytical levels of the various lipids themselves, e.g. serum cholesterol levels. Since cholesterol, its esters, neutral fat and phospholipids are all transported in the blood via giant lipoprotein molecules, it was necessary to develop a technique for measuring both the types and concentration of lipoproteins present in a small sample of blood from an individual patient. The ultracentrifuge has proven highly useful for this purpose. In the ultracentrifuge, under specified conditions (3), each independent lipoprotein molecular species is characterized by its flotation rate. Since there are several different lipoproteins present, very similar in chemical structure, it has been found useful to name the individual species by their flotation rates in Svdeberg units. Thus, if a molecule migrates in the ultracentrifuge with a rate of 20 units, it is referred to as a molecule of the S_f 20 class (S_f = Svedbergs of flotation).

Our previously reported work has shown that those lipoproteins migrating with

*This work is supported in part by the U.S. Atomic Energy Commission and the U.S. Public Health Service.

rates between 10 and 20 Svedberg units (the S_f 10-20 class of lipoproteins) are associated with human atherosclerosis.

The evidence supporting the relationship of these molecules with atherosclerosis in the human may be briefly summarized as follows:

A. The presence and concentration of these molecules of the S_f 10-20 class in presumably normal individuals parallel well and are consistent with the clinical occurrence of atherosclerosis in such individuals.

1. Children show much lower average concentrations of S_f 10-20 molecules than do adults.

2. The young adult females show much lower average levels of S_f 10-20 molecules than do adult males of corresponding age.

3. Both sexes show an increase in concentration of S_f 10-20 molecules with ageing, especially marked in the transition from the third to the fourth decade of life.

4. The difference between the male and female sexes becomes progressively obliterated with age in the fifth and sixth decades of life.

B. The molecules of the S_f 10-20 class are present in higher average concentration and show a greater frequency of elevated levels in patients with proven myocardial infarction or with angina pectoris. In fact, in 15 cases of myocardial infarction who came to autopsy, moderate to advanced coronary atherosclerosis was present in all cases. The S_f 10-20 level in these patients was one and one-half times higher than in the myocardial infarction group as a whole and more than two times as high as in normals. Inasmuch as over 90% of cases of myocardial infarction and angina pectoris have as their basis coronary artery atherosclerosis, the elevated S_f 10-20 lipoprotein levels in such cases is strong evidence linking them with atherosclerosis of at least this vascular bed.

C. The S_f 10-20 lipoprotein levels are much higher in the hypertensive patient manifesting overt coronary artery disease than in hypertensive patients who have not yet shown such clinical manifestations (3).

D. The S_f 10-20 lipoprotein levels are higher in diabetic patients manifesting vascular disease than in diabetic patients without overt vascular disease (3).

E. The S_f 10-20 lipoprotein levels are greatly elevated in a group of syndromes and diseases associated with premature and excessive atherosclerosis, among which are (1) the nephrotic syndrome, (2) myxedema, (3) xanthoma tuberosum, (4) familial hypercholesterolemia.

Although certain of the above categories of disease show frankly elevated serum cholesterol levels, a large proportion of patients with atherosclerosis show serum cholesterols overlapping those of the "normal" population. However, at any range of serum cholesterol, even including hypercholesterolemia, the patients with atherosclerosis or diseases predisposing to atherosclerosis show higher S_f 10-20 levels than do presumably normal individuals. For example, at a low cholesterol such as 200-225 mg%. patients with coronary disease show higher average S_f 10-20 levels than do normals. Likewise, in the hypercholesterolemic range, say 300-350 mg%, patients with coronary disease show higher S_f 10-20 levels than do normals who carry the same total cholesterol. This relationship holds for any cholesterol range. (See Table I).

Having established an association of the S_f 10-20 molecules with atherosclerosis, we were interested in determining whether the serum level of such molecules actually influences clinically the further progression of atherosclerotic disease. During the past one and a half years a clinical follow-up study has been in progress to determine the prognostic import of high S_f 10-20 levels and the prophylactic and therapeutic potentialities of reducing the S_f 10-20 levels in patients with manifestations of atherosclerosis. Certain conclusions can be drawn from the accumulated clinical and laboratory evidence presented below.

TABLE I

Relationship of S_f 10-20 levels to serum cholesterol levels in normals and in patients with coronary artery disease (age group 40 to 60 years).

										Total
Number	Cor.	11	14	19	25	19	38	17	8	151
	Norm.	30	65	61	38	32	40	17	3	286
Above 60 mg%	Cor.	36%	50%	57%	52%	63%	71%	71%	88%	Average 62%
	Norm.	20%	7%	26%	26%	44%	42%	52%	33%	27%
	Ratio C/N	1.8	7.1	2.1	2.1	1.4	1.7	1.4	2.6	2.2
Below 60 mg%	Cor.	64%	50%	43%	48%	37%	29%	29%	12%	38%
	Norm.	80%	93%	74%	74%	56%	58%	48%	67%	73%
	Ratio C/N	0.76	0.55	0.58	0.66	0.66	0.50	0.58	0.18	0.52
Serum Cholesterol Range		200	220	240	260	280	300	350	400	500

Recurrence of Myocardial Infarction

In a group of patients who have experienced one or more myocardial infarctions it would be anticipated that early recurrence of myocardial infarction would be more probable in those individuals with the greatest degree of atherosclerotic activity. If the serum level of S_f 10-20 molecules reflects the degree of atherosclerotic activity, then those patients who maintain higher average levels might be expected, statistically, to show earlier recurrence of myocardial infarction.

A representative group of 100 patients with previous myocardial infarction whose blood was studied repeatedly during a period of 12-18 months beyond their first blood study and who have not had any recurrences of myocardial infarction is compared with a group of 26 patients who experienced a recurrent myocardial infarction during this period of study. For this purpose patients are classified on the basis of the integrated average level of S_f 10-20 lipoproteins during the observation period, independent of any dietary or drug regimen they followed. Thus, it is felt that the only major factor operative should be the S_f 10-20 lipoprotein level. The data comparing patients experiencing recurrence with those not having recurrent infarctions are given in Table II.

TABLE II

	Percent of Cases Above Given S_f 10-20 Level	
Level	Non-recurrences	Recurrences
120 mg%	2%	4%
100 mg%	3%	8%
80 mg%	16%	39%
60 mg%	47%	73%
45 mg%	83%	100%
30 mg%	95%	100%

Average S_f 10-20 level in recurrences = 78 mg% (σ m = 2.2)

Average S_f 10-20 level in non-recurrences = 60 mg% (σ m = 5.8)

Difference = 18 mg% (σ_{diff} = 6.4)

The data in Table II show that both the average blood level of S_f 10-20 molecules as well as the frequency of occurrence of high levels (over 60 mg%) are significantly ($p = 0.03$) greater for the patients experiencing recurrent infarction than for those who did not have a recurrence. Further while none of the recurrence group showed levels below 45 mg%, there was 17% of the non-recurrence group below this level. This difference is highly significant ($p = 0.02$).

These observations are then supportive to the hypothesis that patients with coronary artery disease who maintain high levels of S_f 10-20 lipoproteins are more likely to develop complications as a result of progression of their disease than do those whose levels are lower.

Patients Studied During the Acute Phase of Myocardial Infarction

At the outset of our studies of the relationship of S_f 10-20 lipoproteins to atherosclerosis, patients were not studied unless they were at least six weeks beyond their myocardial infarction. However, recently we have accumulated data during the acute phase (during first week after occurrence or autopsy blood). From the data in Table III it appears that important differences exist between those patients studied during the acute phase who survive and those studied during the acute phase who die.

TABLE III

Level of S_f 10-20	Blood Levels of 23 Cases Studied During the Acute Phase Who Survived % of cases above given level	Blood Levels of 26 Cases Studied During the Acute Phase Who Died % of cases above given level
95 mg%	0%	25%
80 mg%	0%	50%
60 mg%	25%	75%

These data show that the prognosis for patients with acute myocardial infarction is markedly poorer if they have highly elevated S_f 10-20 levels. This may reflect the more severe degree of overall coronary atherosclerosis in the group which fails to survive. Further it appears that the survivors generally

show a rise in levels after the acute phase is passed. Thus possibly the metabolic capability of reduction in level during the acute episode may be of value to the patient.

Influence of Diet and S_f 10-20 Level on Occurrence of Myocardial Infarction in Patients Already Having Coronary Disease

We have been able to show in carefully controlled groups of patients at a hospital diet table that a low fat, low cholesterol diet will effect a reduction in the S_f 10-20 lipoprotein level in the majority of cases (~50% reduction). The possible therapeutic value of such reduction has been tested in a group of ambulatory patients with known coronary artery disease. Out of our total experience 38 patients with either angina pectoris or previous myocardial infarction or both have had a new episode of proven myocardial infarction during the period of follow-up. These cases may be compared with two "control" groups.

1 (a) and (b). Two series of patients with coronary disease who have not had any new episode of infarction during the one year follow-up, and who are matched patient for patient on the basis of identical initial S_f 10-20 levels. The matched controls were blindly selected at random.

2. A series of patients with coronary disease chosen as in 1 (a) and (b) to match the levels of the recurrence group, except that all patients of series (b) had been advised and had claimed moderate to strict adherence to a low fat-low cholesterol diet.

The results are presented in Table IV.

TABLE IV

Matched Group (Series 1a)	Overall Group: Initial Level	Overall Group: Integrated Level During Period of Study	Overall Group: Average Reduction in % of Initial Level	Upper 50% of cases - Average Reduction	Lower 50% of cases - Average Reduction
Recurrences (38 cases)	87.3 mg%	81.9 mg%	6%	9%	1%
Non-Recurrences (38 cases)	87.1 mg%	68.0 mg%	22%	29%	10%
Matched Group (Series 1b)					
Recurrences (33 cases)	82.8 mg%	78.2 mg%	5.5%	7.8%	1%
Non-Recurrences (33 cases)	81.4 mg%	71.5 mg%	14%	17.3%	3%
Matched Group (Series 2) - Recurrences vs. non-recurrences where all non-recurrences were on diet.					
Recurrences (36 cases)	84.0 mg%	79.8 mg%	5%	7.7%	1%
Non-Recurrences (36 cases)	83.8 mg%	63.4 mg%	24.4%	34%	7.5%

The following conclusions can be drawn from these data:

(a) Comparing representative samples of our overall non-recurrence population with coronary disease, chosen at random between dieters and non-dieters, with those patients having recurrent infarctions, it is seen that the non-recurrence group is associated with a significantly greater reduction in S_f 10-20 levels than the recurrence group.

(b) Comparing a representative sample of our non-recurrence group who had been advised to follow a low fat, low cholesterol diet with patients having recurrent infarction, it is seen that there is an even greater reduction in S_f 10-20 level in the non-recurrence group than in the recurrence group.

(c) In all groups dietary reduction of S_f 10-20 levels is significantly greater in patients with high levels (upper half of total group) than in patients with lower levels (lower half of group).

(d) The reductions observed here over the 10-18 month period are much less than those seen in our closely controlled dietary experiments where total fat intake is maintained between 25-50 grams/day. However, the above data represent what can be achieved in an average cross-section of patients following a low fat,

low cholesterol diet at home.

Since the recurrence group and the various non-recurrence groups are essentially comparable in initial S_f 10-20 level, previous medical history, and age, and since the only therapeutic measure used to reduce the S_f 10-20 level was the low fat, low cholesterol diet, it is apparent that the patients who have fared best are those who have shown the greater dietary reduction of S_f 10-20 levels. It was shown above that recurrence is more likely at high S_f 10-20 levels than low, and further that patients with high levels show greater dietary drops in level than those with lower levels. Thus, it is seen that the moderate reductions produced by diet are capable of giving these individuals significant protection from a high recurrence rate of myocardial infarction. All these data are consistent with the hypothesis that the reduction in S_f 10-20 lipoprotein level may have lessened the progression of atherosclerotic disease.

Further, it was noted that the coronary patients in the dietary group who had previously had angina pectoris showed progressive improvement with respect to this symptom over the period of study, as measured by decreased mitroglycerine requirement and increased occupational work tolerance. This was not observed in the non-dietary group.

Pharmacologic Agents Affecting the S_f 10-20 Lipoprotein Levels

The most striking effects upon lipoprotein levels is produced by the action of parenteral heparin. Heparin acts both in the rabbit and human to shift the lipoproteins of high S_f classes into those of successively lower classes. Thus reductions in the S_f 20-100 and S_f 10-20 lipoproteins can be maintained for periods of several hours to days in the human and rabbit, depending upon the mode of injection of heparin.

The alterations in lipoprotein levels produced by heparin in the rabbit are favorable, in that the rabbits receiving intermittent heparin injections during cholesterol feeding experiments show definite suppression of development of atherosclerosis (4).

As yet, no human patients have failed to show acute shifts in the lipoproteins under the influence of heparin, although there is great variability in the duration of the effect on the blood picture. Clinically, it has been noted that in 30 of 32 patients with typical angina pectoris, there was either dramatic reduction or complete relief of angina for periods of 3-10 days following each single 25-100 mg heparin injection intravenously.* When saline placeboes were substituted for heparin, there was a return of symptoms. The only side effects observed was urticaria and angioneurotic edema in two patients. As yet we can only comment that heparin has, in the human a marked effect on lipoproteins of the blood and provides highly effective agent for the relief of angina pectoris, but it is too early to know whether these effects are related. The long duration of the relief of symptoms suggests an effect other than the anticoagulant or vasodilator effect of heparin. Preliminary electrocardiograph studies show changes toward normal in 4 patients following administration of heparin and accompanying the clinical response observed in patients with coronary insufficiency.

*The general effect could be prolonged by administration of repository heparin. Heparin for intravenous and repository injection was supplied by Lederle Laboratory.

SUMMARY

1. The class of lipoproteins in human blood, designated as the S_f 10-20 class, which has previously been reported to be associated with atherosclerosis, has now been studied in a large group of patients with coronary artery disease over a one to one and one-half year period.

2. Recurrence of myocardial infarction in patients with previous infarcts, and occurrence of infarction in patients with angina pectoris developed predominantly in that part of the group showing the highest S_f 10-20 concentrations over the period of observation.

3. Low fat, low cholesterol dietary management of patients with coronary artery disease was effective in reducing average S_f 10-20 levels. This reduction in S_f 10-20 levels was associated with a marked reduction in rate of occurrence of new myocardial infarctions in the group.

4. Heparin suppresses the rise in concentration of S_f 10-50 molecules in the cholesterol-fed rabbit and minimizes the development of atherosclerosis.

5. Heparin produces similar alterations in the lipoproteins of the human.

6. Clinically, administration of 20-100 mg of heparin produced dramatic relief of angina pectoris in 30 of 32 patients for periods of 3-10 days following a single injection.

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