Atherosclerosis and Alimentary Hyperlipemia[1]

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The purpose of this communication is to report investigations which demonstrate that the physical state of the plasma lipids is the same during alimentary hyperlipemia as in those sustained hyperlipemic conditions that are known to predispose to the relatively rapid and severe development of atherosclerosis. The many extensive studies on plasma cholesterol concentrations have failed to provide the answer to the usual development of some degree or another of this slow process of intimal lipid deposition in most otherwise normal persons (*6*, *7*, *9*). It is here proposed that it is because only lipid *concentrations* have been investigated, and primarily in the postabsorptive (fasting) state, that the clue to the underlying etiology of atherosclerosis has been missed.

The investigation of the physical state of the plasma lipids in normal humans and animals, and under various pathological, physiological, and experimental conditions, and the correlation of these data with the pathogenic development of atherosclerosis, have been the focal points in our studies (*6*). Our approach to the study of the actual physical state and particle size of the plasma lipids has involved three simple methods:

(1) *Direct observation and photomicrography of high-power, dark-field preparations:* This method does not lend itself to accurate quantitative estimations, but does provide direct visual observation of only the larger colloidal lipid particles, which appear as bright points of light of varying sizes. These particles are in Brownian motion to a greater or lesser degree, according to their size. This technique has in the past been of occasional interest to physiologists in the study of fat absorption. Gage and Fish (*5*) in 1924 demonstrated that the plasma of normal humans and animals in the fasting state contains very few lipid particles which are of sufficient size to be visible in the dark field. After a fatty meal, however, large and visible particles ("chylomicrons") appear in great numbers. During this alimentary hyperlipemia the chemical concentration of the fat in the plasma may be increased by one-fourth to three-fourths, but the number of chylomicrons seen in the dark field may be increased 10- to 20-fold. This has two rather obvious implications: (a) In fasting plasma specimens the lipids are for the most part carried in very small and invisible colloidal particles; (b) the striking disproportion between the marked increase in visible lipid particles as compared with the relatively slight increase in chemical concentrations is evidence that the average particle size of these absorbed lipids is much greater than is found in the fasting state or after a nonfatty meal.

Our observations were first directed, however, toward the study of the dark-field picture in sustained hyperlipemia, *i.e.* in humans with hyperlipemic diabetes mellitus, myxedema, nephrosis, and xanthomatosis, in cholesterol-fed rabbits and chickens, and in stilbestrol-injected chickens. In these hyperlipemic states the dark field reveals, in a single oil-immersion field, hundreds to thousands of lipid particles which are of sufficient size to be directly visible by light reflection. These are of varying sizes, and many are so large as to exhibit little or no Brownian motion. This is a markedly different picture than that found in the plasma of normal fasting subjects or after a fat-free meal where only a very few (20–30) tiny, dancing lipid particles can be found in each field; furthermore, these few visible particles in fasting plasma appear for the most part as dull specks and are at the extreme lower limit of detection in the dark field. From our later studies on alimentary hyperlipemia it became obvious that the *hyper-* and *macrochylomicronemia* that follow the ingestion of a lipid-rich meal reproduces the same condition of increased particle size and coarser particulate distribution of the lipids in the plasma as found in sustained hyperlipemia of pathological or experimental origin. (These new terms are used as descriptive of the condition of markedly increased numbers of large lipid particles in the plasma.) Although a fatty meal causes only a slight and insignificant elevation in plasma cholesterol concentrations, it nevertheless does reproduce for 3–5 hours this same pronounced alteration in the physical state of the plasma lipids (increased particle size) that is found to be characteristic of those sustained hyperlipemic states that result in the early and severe development of lipid deposition in the arterial intima.

Microscopic counts and estimations of particle size in fasting plasma and in hyperlipemic plasma are quite impractical. Therefore, other methods were needed for the quantitative comparison of the physical state of the plasma lipids in these various conditions.

(2) *Tyndall effect and nephelometry:* Lipid particles in dark-field preparations are demonstrated only by a light-scattering effect. This light-scattering by a medium containing colloidal particles increases with the particle size as well as the number of particles present. A reliable means of comparing the physical status of the lipids in various plasma samples resides in the quantitative study of their Tyndall effect. Plasma (or serum) specimens that scatter the most light are those containing the greater number of larger lipid particles. The relatively small amount of light-scattering due to the plasma proteins may be considered part of the normal baseline. It is evident, then, that measuring the intensity of the light scattered at right angles by a standard amount of plasma and a standard light beam will give a fair quantitative estimation, for comparative purposes, of the physical state of the plasma lipids. Therefore, an especially designed photoelectric nephelometer was constructed for this purpose. Typical comparative values found by this method are given in Table 1.

(3) *High-speed centrifugation:* Stoke's law states that the rate of settling of particles from a liquid medium is dependent on the size of the particles and that larger particles settle (to the top or bottom, depending on specific gravity) more rapidly than smaller ones. When plasma is centrifuged, the larger particles of the plasma lipids will rise to the top more readily than

[1] This is an introductory article to a series of reports on atherosclerosis which are to follow from this laboratory. Gratitude is expressed for the invaluable encouragement and criticism of this work by the late Joseph Edgar Tyree; the author also wishes to acknowledge the aid of L. G. Moench, of the University of Utah Medical School and the Salt Lake Clinic, and also F. L. Stauffer. The photoelectric nephelometer was constructed with the technical assistance of F. S. Stauffer, C. L. Peterson, and R. H. Starley. This instrument, along with the design and theory of nephelometry as applied to the study of the physical state of the plasma lipids, will be reported in detail in a subsequent communication.

[2] The early part of this work was done while the author was at the Harvard Medical School and on the resident staff of the Massachusetts General Hospital.

the smaller ones. When samples of serum or plasma are spun at moderately high speeds (*i.e.* 16,000–18,000 r.p.m.) in the same rotor for a given length of time, those containing greater numbers of larger particles will give a wider 'cream line' or thicker layer of lipid particles which have risen to the top than is found in those plasma samples with a smaller average lipid particle size. Estimation of the volume per cent occupied by the lipid particles which have come to the top provides, therefore, another method for the study and comparison of the physical state of the plasma lipids.

TABLE 1

Subject	Tyndall light (microamperes)
In the postabsorptive state:	
Average of 20 normal humans, fasting	34
" "8 rabbits on normal diet, fasting	28
" "8 steers " " " "	26
Sustained hyperlipemia:	
Average of 6 rabbits after 10 weeks cholesterol feeding	980
" "5 stilbestrol-injected chickens	740
Diabetic patient with sustained hyperlipemia	720
" " " " "	540
" in acidosis	820
Nephrotic with sustained hyperlipemia	620
Essential xanthomatosis with sustained hyperlipemia	890
Alimentary hyperlipemia:	
R.L.T., normal adult human, fasting	28
4 hours after 50 grams of butter fat	140
S.M., normal adult human, fasting	40
4 hours after 50 grams of butter fat	156
R.A.P., normal adult human, fasting	31
4 hours after 50 grams of butter fat	159
B.B.P., normal adult human, fasting	24
4 hours after 50 grams of butter fat	93
C.L.P., normal adult human, fasting*	91
4 hours after 50 grams of butter fat	433
R.H., normal adult human, fasting	29
4 hours after 50 grams of butter fat	184
A.B.L., normal adult human, fasting	32
4 hours after 50 grams of butter fat	137
M.A.E., normal adult human, fasting	44
4 hours after 50 grams of butter fat	280

* Repeated tests on this otherwise normal man have shown him to be unusually sensitive to the development of *hyper-* and *macrochylomicronemia* following various fat-rich test meals. We have noted others who have similarly been exceptionally sensitive in this regard to fatty meals.

There is a fairly reliable degree of correlation between comparative estimates of the physical state of the plasma lipids by the above three methods. That the lipid particles in the plasma following a fatty meal contain cholesterol has been readily demonstrated by analysis for cholesterol of the lipid layer that comes to the surface as a result of high-speed centrifugation (*6*). This is true even after a cholesterol-free fatty meal, *i.e.* olive oil; the logical source of this cholesterol is the bile which is called forth in large quantities for the emulsification of a fatty meal. It is not surprising that chylomicrons contain cholesterol as well as triglycerides and fatty acids; for, being lipids which are both insoluble in water and mutually soluble in each other, they may be expected to be found together in the same lipid particles, rather than carried each in separate micelles.

In summary, it has been found that during the 3–5 hours following a fatty meal the physical state of the lipid particles appearing in the plasma is qualitatively the same as in sustained hyperlipemia. This alteration in physical state consists of markedly greater numbers of lipid particles of considerably larger size than those found in normal fasting plasma or after fat-free meals. From these studies the theory is here put forth that the cumulative effect of many fatty meals over a lifetime, by producing these transient showers of large lipid particles in the plasma, may be the underlying cause of the intimal lipid deposition in human atherosclerosis. This deposition takes place primarily in the tissue spaces of the arterial intima between the overlying endothelium and the underlying barrier of the fenestrated internal elastic membrane (*1*). The lipid particles must be assumed to be retained and deposited from the plasma-derived nutrient lymph stream which normally passes from the lumen through the intramural structures toward the adventitial venules and lymphatics (*1*). It may be theorized that the increased particle size of the lipids in sustained or alimentary hyperlipemia is the stimulus to the phagocytosis in the intima by macrophages and the formation of the typical 'foam cells.' The barrier function of the fenestrated or even reduplicated internal elastic membrane may have an important role in the mechanics of the process of lipid-particle retention in the intimal tissue spaces (*1*, *6*). The neutral fats and fatty acids in the depositing lipid particles are more readily resorbed and removed (by the action of macrophages and tissue-fluid enzymes) than cholesterol and cholesterol esters, which remain as the difficult-to-remove residue (*6*). Christianson (*3*) has shown this experimentally by demonstrating that if a mixture containing 95 per cent fat and 5 per cent cholesterol is injected intramurally and deposited in the arterial wall with a fine hypodermic needle, the resultant lipid lesion soon consists primarily of cholesterol due to the much more rapid resorption and removal of the fats and fatty acids from the deposit.

Circumstantial evidence on the occurrence of atherosclerosis supports this chylomicron theory of etiology as above outlined: (a) Atherosclerosis is rare in peoples who eat very low or nearly fat-free diets, *i.e.* Chinese and Okinawans (*8*); (b) atherosclerotic disease decreased notably in Germany during the fat-shortage years immediately following World War I (*2*); (c) atherosclerosis is more common in obese, overnourished persons than in the lean and undernourished; (d) it is often less severe and less common in chronic alcoholics (who eat less, especially less fat, for which they often have intolerance) than in nonalcoholics (*6*); (e) intimal lipid deposition and atherosclerosis are rare and minimal in wild animals (including the herbivorous anthropoid apes), which seldom, if ever, eat a meal containing the amount of fat found in the average human diet (*1*, *4*).

This theory, along with a thorough review of the literature and further investigations from this laboratory, will be presented in full in a forthcoming monograph (*6*).

References

1. Anitschkow, N. *In* E. V. Cowdry. *Arteriosclerosis.* New York: Macmillan, 1933.
2. Aschoff, L. *Lectures on pathology.* New York: P. B. Hoeber, 1924.
3. Christianson, O. O. *Arch. Path.*, 1939, **27**, 1011.
4. Fox, H. *Proc. 2nd int. Congr. Geogr. Path.*, 1934, P. 382.
5. Gage, S. H., and Fish, P. A. *Amer. J. Anat.*, 1924, **34**, 1.
6. Moreton, J. R. *Atherosclerosis and lipid metabolism.* (Monogr., in preparation.)
7. Page, I. H. *Biol. Sympos.*, 1945, **11**, 43.
8. Steiner, P. E. *Arch. Path.*, 1946, **42**, 359.
9. Weinhouse, S. *Arch. Path.*, 1943, **35**, 438.