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Present day concept of the etiology of athersclerosis

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PRESENT DAY CONCEPTS OF THE ETIOLOGY OF ATHEROSCLEROSIS

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INTRODUCTION For many years atherosclerosis was almost exclusively a province of the pathologists. The natural history of the disease in humans was studied and described in great detail from observations on postmortem material; the increase in severity with age suggested that atherosclerosis is an inevitable result of aging and the spotty and irregular distribution of the lesions made evident the importance of the local tissue factors. This concept, that atherosclerosis was an inevitable result of aging, has long hindered research on this disease entity. It was not until the period from 1908 to 1912 that experimental atherosclerosis was first successfully induced in the experimental animal. Prior to that time, all experimental attempts to reproduce the atherosclerotic lesion of man had failed, although arterial lesions had been produced by a number of procedures in animals (such as treatment with drugs, pathogenic bacteria, toxins). However, the resultant changes in the vessels resembled the Monckeberg type of human arteriosclerosis (medical calcinosis, senile arteriosclerosis), rather than atherosclerosis. In 1908 a group of investigators working in St. Petersburg studied the effects of various dietary regimens on vascular pathology. They observed that rabbits fed diets containing meat, milk or eggs

developed atherosclerosis. It was soon demonstrated, particularly by Anitschkow, that the biochemical factor responsible for these lesions was cholesterol, Katz (1).

Atherosclerosis denotes a pathologic change affecting the intima of arteries that is chacterized by focal thickenings of the intima in which stainable lipids can readily be demonstrated in and between the cellular elements. The larger lesions show, at the one extreme, an almost exclusively fibrous composition with only minimal quantities of lipid material while, at the other extreme, they contain lipid accumulations of massive proportions with extensive necrosis of the central parts of the lesions commonly culminating in the disruption of the intimal lining. The term "atheroma" refers to an atherosclerotic lesion of which the center is necrotic and occupied by a grumous mixture of lipid material and tissue debris.

CHOLESTEROL METABOLISM Since the great bulk of research on atherosclerosis has concerned itself with the study of experimental cholesterol atherosclerosis, and, since the main component of the atheroma has been found to consist of cholesterol and its esters, it might be well to briefly review the present concept of cholesterol metabolism.

The cholesterol present in the tissues is partly derived from synthesis in the liver and partly from the diet. There is no evidence for any qualitative difference in the metabolism of endogenous and exogenous cholesterol. The two mix and become indistinguishable (except when one is tagged with an isotopic tracer) to a certain extent in the intestine and to a much greater extent in the liver and blood. Endogenous synthesis of cholesterol takes place continually in many and probably in all the tissues of the body except the central nervous system. Blood cholesterol is derived from the liver; with the isotope tracer method it has been shown that interchange of unesterified cholesterol molecules between plasma and liver takes place with tremendous speed; probably more than 1 gram per hour moves from one to the other. Red cell cholesterol also is very labile and is interchanged with plasma cholesterol both in vitro and in vivo at a rate of about 50 per cent in one hour. These findings show that the cholesterol component in lipoprotiens is free to dissociate, providing there is another protein acceptor molecule available. This suggests that the concentration of cholesterol may be governed to a considerable extent by the concentration of the various protein acceptors. The half life of blood and liver cholesterol

is about six to eight days in humans and in several species of experimental animals. In other tissues, the half-lives are longer, but the mixing of blood and tissue cholesterol makes the significance of these turnover times difficult to interpret. The chief pathway of cholesterol metabolism is conversion to bile acids and eventual excretion in feces. This route accounts for perhaps three quarters of all the dietary and hepatic cholesterol metabolized. Some is excreted as coprosterol, dihydrocholesterol or as cholesterol itself in feces, and some is converted into steroid hormones, but no detectable amounts are oxidized completely. The first effect of increasing the cholesterol content of the diet is to decrease the rate of synthesis of cholesterol in the liver. This effect has been found in all species of experimental animals tested and is so striking that it can be presumed to occur in humans as well. It appears to be mediated by the cholesterol concentration in the liver, but whether it is the free fraction, the esterified fraction or the total has not yet been established. A diet containing 1 per cent cholesterol fed for one day to rats will produce a considerable increase in the esterified fraction, only a slight increase in the free fraction, and a concomitant decrease in synthetic

rate to a few per cent of the control value. If the free cholesterol concentration in liver is the controlling factor the mechanism must indeed be sensitive, since an increase of only a few milligrams per 100 grams of liver in this fraction corresponds to a decrease in synthetic rate to half. The response of hepatic cholesterol synthesis to the increased concentration in the liver may be considered part of the homeostatic mechanism which attempts to maintain a constant level of cholesterol in the liver and plasma despite changes in the dietary intake. This defense mechanism is easily overwhelmed in animals on experimental diets, since the rate of absorption per day may be far greater than the normal rate of endogenous synthesis. A second line of defense is the storage of excess cholesterol in esterified form in the liver. This material appears to be metabolically rather inert and in some species, particularly the rat, may increase to many times its normal concentration without any marked increase in the plasma level or in the concentration of free cholesterol in liver. Thus the liver cholesterol ester concentration appears to be the most sensitive index of the presence of excessive amounts of cholesterol in the body. The plasma cholesterol may be considered as being regulated by two types of mechanisms; if the distribution of choles-

terol between liver and plasma is constant, the rate of hepatic synthesis will vary in such a manner as to keep the level in liver and in plasma constant. The distribution between liver and plasma, however, is affected by thyroid function, administration of estrogens or adrenocortical hormones, the presence of bile, surface-active agents in blood (such as Tween or Triton), and many other factors, Gould (2).

EXPERIMENTAL ATHEROSCLEROSIS Since the bulk of our early knowledge concering the nature of the atherosclerotic process stems from the work done on the experimental animal, I will consider first the role of experimental atherosclerosis on our present day thinking. In the rabbit, lesions are produced merely by the force-feeding of cholesterol in any form. Deposition of anisotropic lipoids occurrs in both the intima and the media. In both situations histologic changes precede the deposition of lipoid materials. In the media these changes are especially striking and appear to be the result of some form of injury. The lesions in the intima and media usually occurr together, but early lesions in the media are noted on occasion in the absence of any change in the overlying intima, and the reverse is also true, Duff (3). Many similarities

and dissimilarities with human atherosclerosis exist. The most obvious difference lies in the fact that the appearance of the experimental arterial lesions is preceded by a more or less conspicuous accumulation of lipids, rich in cholesterol, in the cells of the reticuloendothelial system and of parenchymatous organs. No such lipid accumulations are found in association with, much less preceding, the development of ordinary human atherosclerosis. The second gross difference is apparent in the distribution of the arterial lesions. In the rabbit these are found in the aorta, its major and secondary branch arteries and in the pulmonary arteries. The cerebral vessels are exempt, except under special circumstances, and the retinal artery is not involved. Moreover, the most severely affected area is the thoracic aorta. In man, while the aorta and its branches are the main site of the disease, atherosclerosis reaches its maximum development in the abdominal portion of the aorta. The pulmonary arteries are affected to a minor extent only and the cerebral and retinal vessels are frequently the sites of lesions. Possibly if the experimental conditions that elicit the lesions in the rabbit were less forced, the experimental lesions would resemble the human disease even more closely, Duff (4).

Atherosclerosis does not occur spontaneously in the rabbit and it's induction may be an atypical response in an animal which was not intended by nature to partake of such a diet. Therefore many investigators have sought an expermental animal in which spontaneous atherosclerosis accurs. Such an animal is the chick which has been used by Katz (1), among others. The lesions observed in the chick may be more closely related to those seen in the human. Experimantal lesions may be produced by cholesteral feeding or by administration of estrogens. Detailed microscopic studies indicate that the primary lesion of experimental atherosclerosis in the chick is the foam cell itimal cushion. This lesion, so-called pure atheroma, is almost certainly the first stage of atherogenesis, The morphologic patterns of more advanced lesions are apparently the result of evolutionarey pathologic processes secondary to atheroma. It is possible by prolonged cholesterol feeding to induce in chicks the spectrum of atherosclerotic changes seen in human lesions including foam cell plaques, necrosis and atheromatous abscesses, fibrosis and hyalinization, calcification and cartilaginous-osseous metaplasia. Ulceration of atherosclerotic plaques with thrombus formation is the only

lesion seen in man that has not been observed in cholesterol-fed chicks. Cholesterol feeding apparently aggravates and intensifies spontaneous lesions, It would appear that the fibrotic spontaneous lesion is a site of predilection for cholesterol and lipid deposition, with subsequent evoluntionary atherosclerotic changes. Horlick and Katz demonstrated in the cholesterol-fed chick that in the weeks following cessation of cholesterol feeding, definite regression and healing of cholesterol-induced lesions occurred. Previously existing moderately severe atheromatous lesions apparently completely healed and disappeared. More advanced lesions underwent partial resolution and evolution.

Cholesterol atherosclerosis may also be produced in various other animals including those that show some tendancy toward spontaneous atherosclerosis as does the dog, horse and cow. However, in order to induce a significant change some predisposing factor must have upset the homeostatic mechanism. Steiner and Kendall (5), among many, have produced arterial lesions in dogs similar in distribution and morphologic character to those seen in the human disease, by prolonged hyperchalesteremia induced by feeding 10 grams of cholesteral in 40 cc of cottonseed oil daily to dogs whose thyroid function was modified by ad-

ministration of thiouracil.

FACTORS INFLUENCING EXPERIMENTAL ATHEROSCLEROSIS The effect on experimental atherosclerosis of innumerable substances and conditions has been studied by a miriad of investigators. Only a few of the more important factors will be mentioned here.

I Hypertension. It would appear that in the presence of consistent normocholesteremia, hypertension is ineffective as an atherogenic stimulus, both with respect to causing the appearance of induced lesions and with respect to intensifying spontaneous lesions. This finding is in accord with observations by various observers with several other species, including rabbit, dog, sheep and goat, Katz (1). The plaque formation seems to be proportional to the severity of the elevation of pressure and the length of time that this acts. It is also seen more frequently in animals in which frequent pregnancies have occurred, Dill and Isenhour (6).

II Local damage to the vascular wall. A variety of injuries or other modifications affecting the vessel walls, will favor the localization of lipids, in the affected areas and will accelerate the local development of atherosclerosis. Previous experimental or spontaneous

deposition of plaques of calcium salts in the media has been shown to impede the deposition of lipids in the overlying intima while promoting it at their edges, Duff (3), Duff (4), Katz (1), and Hueper (7). Sympathetic paralysis also enhances the effect of cholesterol in producing vascular lesions. Harrison (8) performed unilateral lumbar sympathectomy on a series of rabbits and demonstrated a marked increase in the severity of the lesions on the sympathectomized side.

III Thyroid. Hypothyroidism in all experimental animals tends to cause hypercholesterolemia. In rabbits with a hypercholesterolemia due to long continued cholesterol feeding, thyroidectomy causes a marked rise in the blood cholesterol. This rise is usually maintained. When long continued cholesterol feeding has failed to cause a rise in the blood cholesterol of rabbits, thyroidectomy abolishes this resistance and a hypercholesterolemia is promptly produced. A single injection of thyroxin causes a significant drop in the blood cholesterol of rabbits with hypercholesterolemia. This reaction is not influenced by thyroidectomy. Potassium iodide causes an increase in the blood cholesterol of rabbits with hypercholesterolemia. This reaction is

not influenced by thyroidectomy in contrast to the effect of KI in preventing a rise in blood cholesterol when given concurrently from the beginning of cholesterol feeding, an effect which is abolished by thyroidectomy. It is suggested that two different mechanisms are involved, Turner (9), Turner (10), Turner (11), Meeker (12), Rosenthal (13).

As previously noted dogs treated with thiouracil and fed cholesterol also respond with the development of hypothyroidism, hypercholesterolemia and atherosclerosis although they are resistant to cholesterol feeding alone.

In the chick thyroid hormone significantly decreased the incidence and severity of cholesterol induced atherosclerosis. Although dessiccated thyroid only temporarily depressed the hyperlipemia of chronic stilbestrol administration, it was remarkably effective in reducing the incidence and severity of lesions in the aorta, Katz (1).

IV Alloxan diabetes. A comparison was made of the effects of cholesterol feeding in normal rabbits and in rabbits rendered persistently diabetic by means of alloxan. In the two groups of animals hypercholesterolemia of comparable degree was induced by the feeding procedure.

Nevertheless, the severity of the atherosclerosis of the aorta produced in the diabetic rabbits was much less than in the non-diabetic control animals. Indeed, a large proportion of the diabetic animals presented no atherosclerosis whatever. There was a similar inhibition of the deposit of lipid substances in the liver, spleen, and adrenal glands of the diabetic rabbits. The inhibition of the development of experimental cholesterol atherosclerosis which was associated with the presence of alloxan diabetes was independent of the administration of alloxan per se. It was not dependent on the sex or weight of the animal, nor upon the daily dosage of cholesterol, the form in which it was administered, nor the duration of cholesterol feeding. It was also independent of changes in body weight and of the actual degree of hypercholesterolemia induced by the administration of cholesterol. In addition, there was no gross or histological evidence of a morphological basis for the inhibitory effect in the aorta or in the other organs in which it was observed. In every case it was found that inhibition of the development of atherosclerosis was associated with a marked elevation of the serum phospholipids and neutral fat that occurred concomitantly with the rise of serum cholesterol. On

the other hand, if, as sometimes happened, the serum phospholipids and neutral fat were not markedly elevated in the presence of hypercholesterolemia, atherosclerosis would occur even in diabetic animals. It was also observed that if the diabetic state were adequately treated with insulin, the expected inhibitory effect was abolished and atherosclerosis developed just as in normal rabbits fed cholesterol. In these circumstances the ratios of the various lipids were found to be like those of cholesterol-fed non-diabetic animals, Duff (14), Duff (15).

In the chick, the same phenomenon of decreased atherogenesis of the induced variety in the presence of alloxan diabetes was observed. However these same animals tended to show an increased tendancy toward spontaneous atherosclerosis. Stilbestrol-treated, depancreatized birds also exhibit a plasma lipid pattern essentially similar to that of their unoperated controls. When a supplement of cholesterol and cottonseed oil is added to the mash of depancreatized cockerels; a plasma lipid pattern emerges that is significantly different quantitatively from that of their paired controls. They consistently exhibit a far more severe hypercholesteremia; this is accompanied by intensified athorogenesis. In contrast, when depancreatized chicks are fed a mash

containing cholesterol, but devoid of a cottonseed oil supplement, they exhibit a plasma lipid pattern essentially similar to their unoperated paired controls on a similar diet, Katz (1).

V Diet. Two groups of chicks were fed a mash containing a large amount of cholesterol. One group was allowed to eat ad libitum while the other group was restricted to from 60 to 70 per cent as much mash. Chicks on this reduced dietary intake received less cholesterol than the controls given the same mash ad libitum. Nevertheless the semistarved birds tended to have a more severe hypercholesteremia than the controls. At the conclusion of the experiment, incidence and severity of atherosclerosis in the undernourished cockerels was as great as or greater than in the control birds on an unrestricted diet of similar composition. Apparently the underfed birds were not readily able either to draw upon the elevated plasma lipids as a source of calories or to dispose of the exogenous cholesterol load.

In another experiment using hypercholesteremic chicks, one group received intermittent periods of starvation while the other group was being maintained on a regular mash. The intermittent periods of starvation apparently hindered, rather than facilitated, the dis-

posal of exogenous cholesterol load, whereas intermittent periods of regular mash facilitated such disposal. Over-all adequate intake of balanced diet apparently made a key contribution to preventing dietary induced hypercholesteremia and atherosclerosis.

Diets high in neutral fat (cottonseed oil) were seen to have no effect on the degree of lipemia or spontaneous atherosclerosis in the chick. In like manner cholesterol free, fat poor diets were seen to have no effect on the blood picture of the normal chick. These diets also failed to have any effect on stilbesterol-induced atherogenesis and hypercholes teremia in the chick. Lipotropic factors such as choline and inositol were seen to have virtually no effect on any type of atherosclerosis, Katz (1).

VI Surface active agents. Various surface active agents such as Tween and Triton were administered intravenously to hypercholesteremic rabbits. The effect was very similar to that seen in alloxan diabetes. These animals developed hyperlipemia which was characterized by a great increase in blood cholesterol and an equivalent or even greater increase in pnospholipids, and they had much less atherosclerosis than did the control rabbits fed cholesterol alone. It is interesting

that these animals showed tremendous foam cell accumulation in the reticuloendothelial system and marked lipid infiltration of the renal tubular epithelium, Kellner (16), Payne (17).

VII Sex adrenal steroids. The discovery of the effect of estrogen an atherosclerosis in the chick may be one of the most important contributions of experimental atherosclerosis to the human disease. At any rate the discovery of the differential effect on various arterial beds will certainly help steer investigators down the right path. The chick, being an egg-laying animal, must mobilize lipid and calcium for deposition in the egg. The hormone of the ovary accomplishes that mobilization; estrogen produces a tremendous hypercalcemia and hyperlipemia in chicks. The hyperlipemia involves all 3 major components of the plasma lipid--cholesterol (free and esterified), phospholipid and neutral fat. A unique pattern of hyperlipemia results, in that phospholipid rises much more than any other component, including cholesterol. Hence the cholesterol/phospholipid ratio falls. Thus the endogenous estrogen-induced hyperlipemia is quite different from the hyperlipemia of cholesterol feeding, wherein elevation of cholesterol predominates, increase in phospholipid lags, and the C/P ratio rises.

Gross autopsy findings revealed the aortas of the estrogen-treated cockerels to be as heavily studded with atherosclerotic plaques as the aortas of the control birds, receiving no hormone. However, a systematic study of the coronary arteries in these chicks was remarkable and startling--practically no atherosclerosis in the coronary arteries of estrogen-treated birds, in contrast to multiple lesions in the controls. Thus a longstanding assumption of experimental atherosclerosis--that findings in the aorta mirror atherogenic activity throughout the arterial tree, fell by the wayside.

After a 3 week period of estrogen treatment, definite but incomplete regression of coronary lesions was detectable. After 5 weeks of estrogen administration, there was almost complete regression of the coronary lesions. Both the lipid and fibroblastic components of the plaques were resolved. This was demonstrated by multiple special staining techniques, for lipid connective tissue, metachromatic material, etc. This reversal of atherogenesis occurred despite the fact that the estrogen-treated birds had, if anything, a slightly greater hypercholesteremia than their paired controls. In accord with previous observations, the hormone effected

a lowering of the cholesterol/phospholipid ratio toward normal. Again, aorta atherogenesis proceeded unaffected by the estrogen. Estrogen somehow effected a marked fall in concentration of Sf 3-8 lipoproteins, (See Gofman's work p. 22), a phenomenon absent in the untreated and the testosterone plus estrodiol treated groups. Secondly, the estradiol-testosterone combination for some reason had a marked influence on Sf 10-20 levels, effecting a ten-fold or greater rise, compared to the untreated and the estrogen-treated groups, Stamler (18).

THEORIES RELATING TO THE ETIOLOGY OF HUMAN ATHEROSCLEROSIS

A great multitude of theories have arizen to attempt to explain the genesis of human atherosclerosis. The great majority of these theories had little scientific basis and have long since been discarded. The most accepted theory prior to the discovery of experimental cholesterol atherosclerosis was the senescense theory. Advocates of this theory propounded that physico-chemical changes inherent in the aging of extracellular colloids in the arterial intima provided the fundamental alteration that led to the accumulation of lipids and thus the development of atherosclerosis. The disease was once

thought to be the result of a mild chronic inflammatory proscess. Mechanical trauma such as that produced by the constant pressure of the blood on the arterial walls supposedly led to intimal fibrosis in much the same manner as a callus would form on the hand from repeated insult. The Toxin theory was once very popular. Toxins such as those produced by bacteria were to have injuried the arterial endothelium to a sufficient degree to produce a focus for the production of an atheromatous lesion. Hueper (7) favored what he called the anoxemia theory. It is the contention of this theory that the common fundamental action through which the various types of causal agents and their different causal mechanisms affect the vascular walls is represented by a severe and short or a moderate and prolonged but frequently recurring or persistent interference with the oxidative metabolism and nutrition of the vascular wall. He went on to point out that all factors concerned in atherogenesis could be boiled down to an effect of interfering with the metabolism of the arterial wall to produce a relative anoxemia.

Although these and other theories may well have some affect on the atherosclerotic proscess, they have generally been discarded in favor of more recent and

more scientific theories. It is the purpose of this paper to present and evaluate the more tenable theories. I Filtration concept of atherosclerosis. Today the "filtration" concept of atherosclerosis is probably the most popular theory attempting to explain its genesis. This "filtration" concept is based on the view that atherogenesis is due to the tissue reaction to substances filtered from plasma as lipoprotein by lateral arterial pressure, and deposited in the intima as foreign lipid. Most of the filtered materials pass on harmlessly to be picked up by the adventitial capillaries or the lymph. But some may stay behind, whether because the vessel fails to function properly as a filter or because the size, shape and charge of the lipoproteins is such as to allow them to stick. Changes in the arrangement, amount and chemical nature of subendothelial ground substance conceivably may initiate a focal change in filter function. The reaction which ensues depends on the nature of the lipid deposited and the responsiveness of the tissues to it. In this view, factors in atherogenesis are: 1. The anatomy, biochemistry and physiology of the vessel wall, all of which are hereditarily conditioned. 2. The composition of plasma. 3. The lateral arterial pressure and rate of filtration. 4. The

responsiveness of intimal tissues to filtered products and their degradation products, normal or abnormal. 5. The metabolic capacity of the vessel wall. 6. Changes in filtration capacity of the vessel wall, such as may result from age, hypertensive diseases and metabolic disorders, Page (19).

II The giant lipoprotein molecules as studied with the ultracentrifuge. In the past 4 or 5 years much interest has centered around Gofman's work with the ultracentrifuge. He has demonstrated that all major lipids in blood, including cholesteral esters, phospholipids, fatty acids and glyceryl esters are transported as giant lipoprotein molecules of several types. His unit of measure of flotation rate, Sf (meaning Svedberg of flotation) represents a migration rate of 10^{-13} cm. per second per dyne per gram. Thus a lipoprotein which migrated in the ultracentrifuge, under defined conditions, at four times this rate would be identified as a lipoprotein of the Sf 4 class. In addition to two specific lipoproteins of high density (1.075 gm. per milliliter and 1.145 gm. per milliliter), there exists in human serum an entire series, or "spectrum", of lipoproteins ranging in flotation rate from Sf 2 to Sf 40,000 (in sodium chloride solution of density 1.063 gm. per milliliter at 26 degrees C.

Obviously this cannot measure any one specific lipoprotein molecule independantly, but it does measure the activity of certain small classes such as the Sf 10-30 class.

The following is a somewhat comprehensive summary of his work and conclusions. 1. A variety of serum lipid disturbances experimentally induced in the rabbit, some of which are associated with the development of atherosclerosis, have been analyzed. The Sf 10-30 class of lipoproteins, which develops in the rabbit in certain of these experimental procedures, is highly associated with and universally concurrent with the development of atherosclerosis, independent of the type of metabolic disturbance experimentally induced. The normally occurring lipoproteins (Sf 10 and less) even when elevated experimentally, show no significant positive association with atherosclerosis. Certain other cholesterol-bearing lipoproteins (Sf 100 and higher) are either not associated with atherosclerosis or are inversely associated with atherosclerosis. 2. In the rabbit, total serum cholesterol levels, considering as a group all the types of induced lipid metabolic disturbances, are either unrelated to atherosclerosis, or may be inversely associated with atherosclerosis. Only

under the special condition where the major fraction of the cholesterol is in the Sf 10-30 class of lipoproteins does total serum cholesterol correlate well, positively, with atherosclerosis. 3. An estimate of the quantitative association of the Sf 12-20, Sf 20-35, Sf 35-100 lipoprotein classes and of total serum cholesterol with atherogenesis in the human has been made, studies were done on the same serum sample from a given individual. Patients with coronary artery disease have served as a criterion group for the atherosclerotics. Throughout the entire age range evaluated, from 41 to 60 years, the Sf 12-20 lipoprotein levels show at least a twofold, and up to a possibly tenfold, higher relationship with atherosclerosis than does the total serum cholesterol. The Sf 20-100 lipoproteins also show a correspondingly higher relationship with atherosclerosis than does the total serum cholesterol. The Sf 12-20 lipoproteins and the Sf 35-100 lipoproteins are partially intercorrelated; but each shows, in addition, strong independent associations with atherosclerosis. The Sf 20-35 lipoproteins show a lesser association with atherosclerosis than either of the other classes, and, further, much of the association that is present depends upon partial correlation of the Sf 20-35 lipoproteins with

either Sf 12-20 or Sf 35-100 or both. The Sf 12-20 and Sf 35-100 lipoproteins show strong association with atherosclerosis regardless of age or total serum cholesterol level. Thus, even for individuals with the same total serum cholesterol, be it low, moderate, or high, there is strong ability of the Sf 12-20 and Sf 35-100 lipoproteins to segregate atherosclerotics from normals. Total serum cholesterol shows a much lower ability to segregate atherosclerotics from normals in the 41-50 year age group than the Sf 12-100 lipoproteins. In the age group 51 to 60, while the lipoproteins maintain their strong association with atherosclerosis, there is only a borderline ability, if any of the serum cholesterol to segregate atherosclerotics from normals. 4. Follow-up studies have shown that early recurrence of myocardial infarction in patients with coronary disease is positively and highly related to the Sf 12-20 lipoprotein levels. 5. The depression of high Sf 12-20 levels by dietary restriction of fat and cholesterol has been shown to decrease significantly the chance of recurrence of myocardial infarction in patients with established coronary artery disease. 6. The demonstration that the Sf 35-100 lipoproteins, in addition to the Sf 12-20 lipoproteins are associated with atherosclerosis

is of especial significance with respect to the ingestion of fat. This class of lipoproteins, the Sf 35-100 class, may be raised acutely in a high proportion of humans following ingestion of fat. In patients with a severe degree of the lipid metabolic derangement which leads to "abnormal" lipoprotein patterns, this Sf 35-100 class of molecules is sustained even postabsorptively. It appears, since it has been demonstrated that a dietary lowering of the Sf 12-20 lipoproteins has an ameliorative effect on coronary disease (based upon atherosclerosis), that dietary fat restriction is equally important, by way of depressing the Sf 35-100 level, in the effort to control atherosclerosis, Gofman (20).

As previously mentioned the Gofman method used sodium chloride to obtain a density of 1.063 grams per milliliter for the flotation of the lipoproteins, Page (19), believed he could increase the number of lipoproteins measurable by ultracentrifugal analysis, notably the alpha lipoproteins, by raising the density to 1.21 with potassium bromide. Changing the conditions required a change in nomenclature. The rough equivalents of Gofman's and Page's nomenclature are given in fig. 1. along with the equivalent electrophoretic patterns. Page employs the symbol -S to indicate negative sedimentation or flotation,

	ULTRACENTRIFUGE		ELECTROPHORESIS
DENSITY	1.21	1.063	
SYMBOL	$-S_{1.21}$	S_f	
MOBILITY	> 70	20-100	
	40-70	10-20	β_1
	25-40	3-8	β_2
	20-25	1-3	α_1
	2-8	——	α_2

Fig. 1

Equivalence of Gofman's and Page's nomenclature.

with the subscript 1.21 where necessary, to indicate the chosen density. The -S 25-40 fraction, which is chiefly beta lipoprotein, is normally low in females in the 18-34 age group and tends to rise with age. Beta lipoprotein in young men is almost equal to the concentration in females of 34 to 60 years of age. These facts alone suggest reasons for the vulnerability of men to atherogenesis as compared with his supposedly weaker, but far more durable counterpart, woman. Young women have much less of the -S 40-70 fraction present than do older women. This is the Gofman Sf 10-20 atherogenic fraction. The -S 20-25 fraction, which is chiefly alpha$_2$ lipoprotein, is present in small amount and about equal in concentration in all normal subjects. This fraction carries a number of important proteins, such as renin substrate, vitamins, such as E, tocopherol, and hormones, such as estrogen. Women have much higher concentration of the -S 2-8 fraction, which is alpha$_1$ lipoprotein which has a much lower content of cholesterol than the beta lipoprotein and is presumably much more stable.

A portion of the serum cholesterol (about 20%) filters through excised arterial wall, part is deposited in the arterial wall, and the rest remains in the vascular

lumen since it cannot penetrate into the arterial wall. It is the smallest lipoproteins which pass through the vascular wall into the lymph. The largest lipoprotein particles cannot enter the arterial wall at all, and the groups of intermediate size are caught intramurally. It is highly probable that the lipoproteins which Gofman and Page consider atherogenic fall into the intermediate group, Engelberg (21).

Considerable support for the relationship between the serum levels of these lipoproteins and the incidence of atherosclerotic lesions was given by the findings of Bragdon and Boyle, (22) of the United States Public Health Service, National Heart Institute, who were able to produce atherosclerotic lesions in experimental animals by the intravenous injection of these low density lipoproteins, separated by ultracentrifuging from the serum of atherosclerotics. Similar injections of cholesterol or triglyceride emulsions produced no such lesions.

Because of the importance of Gofman's observations, the National Advisory Heart Council recommended to the United States Public Health Service the organization of a carefully controlled study of the problem. Four laboratories, Berkeley (Gofman and Jones), Pittsburgh

(Laufer, Hanig and Barach), Boston (Stare and Mann) and Cleveland (Lewis and Page) were designated and started work in the winter of 1950. More than a year was required before the centrifuge analyses and cholesterol measurements agreed sufficiently among the various laboratories. This was an important achievement and points out how difficult it would have been, had the cooperative study not been set up, to have found agreement among investigators using the ultracentrifuge. All data are sent to Mr. Moore in Washington for statistical treatment. Within the next two years it will have been gathered and the conclusions will be made known.

III Alpha and beta lipoproteins. Alpha lipoprotein, so unstable that it has not yet been isolated in pure form by chemical fractionation methods, carries about 28% of the cholesterol in normal human plasma, as judged by studies on large pools of blood from adults of both sexes. It contains about 39% total lipid and has a density of 1.19. The lipid composition is 18.3% cholesterol, 21% phospholipids, and no neutral fat; the cholesterol/phospholipid ratio is 1.3 on a molar basis. Present evidence indicates no atherogenic properties. The beta lipoprotein carries about 63% of the plasma cholesterol; it consists of 47% cholesterol, 29% phos-

pholipid and only 23% protein. It also contains 60% of its own dry weight as water of hydration and has a density of 1.032, which is considerably lower than the value of 1.32 for other plasma proteins. When the total cholesterol concentration increases, the increase is entirely in the beta lipoprotein fraction. Gofman et al. showed that the beta lipoproteins constitute a large series of compounds differing in neutral fat content and in density. Gofman's atherosclerosis index now includes all beta lipoproteins but no alpha lipoproteins. Russ, Eder and Barr (23), determined the total alpha and beta lipoprotein by Cohn's chemical fractionation methods. They conclude that the percentage of plasma cholesterol in alpha lipoproteins is inversely correlated with atherogenesis. They found that in diseases predisposing to atheroma, such as diabetes, nephrosis and xanthomatosis, relatively little of the total cholesterol is combined with the alpha lipoprotein. This implies a reducation in alpha lipoproteins and a relative increase in beta lipoproteins. Survivors of a myocardial infarct also tended to show this deviation. The abnormality may not show up in every individual but it is demonstrable in the group. However, these workers believe that the percentage present in the alpha form

is correlated better with atherogenesis than are the beta lipoprotein concentration, the total cholesterol level, or the Sf 12-20 level. Of special interest is the difference in the percentage of cholesterol in alpha lipoproteins between young men (25.2%) and young women (34.3%). This difference is considerably larger than that in total cholesterol level (197 and 187 mgm respectively). It is well established that in this age group (18-35) men have considerably more atherosclerosis than women, Gould (2), Page (19), Leinwand (24), Klein (25).

IV Chylomicrons. Several investigators have emphasized the etiological significance of the number and size of the chylomicrons (large lipid particles) circulating in the blood after the ingestion of fatty foods, which give the serum or plasma the appearance of lipemia or turbidity, and which can actually be counted in the dark field microscope or indirectly determined by turbidity measurement. Hueper (26) and Moreton (27), hold that the alimentary lipemia and its accompanying high concentrations of chylomicrons occurring in the blood of normal individuals are indistinguishable from the sustained hyperlipemia and hyperchylomicronemia of pathological and experimental origin which have been found to be characteristic of the

known causative conditions of atherosclerosis.

Increased chylomicronemia following fat containing food occurs at every age. Moreton (27), theorizes that the cumulative effect of many fatty meals over a lifetime, by producing transient showers of these large lipid particles, may be the underlying cause of the intimal lipid deposition in human atherosclerosis. Becker (28), stresses the frequency of hyperchylomicronemia in older age groups and suggests its relation to the incidence of atherosclerosis in older persons. In young persons increased chylomicronemia lasts only a relatively short time and is only of moderate intensity. With increasing age, and particularly above 50 years of age, chylomicronemia is of greater intensity and is practically permanent. This is illustrated by figure 2. Zinn and Griffith (29) provided further correlation between hyperchylomicronemia and atherosclerosis by demonstrating that the chylomicron indices found in coronary patients were substantially greater than those of normal persons following a fatty meal in fasting subjects.

More recently Schwartz (30) showed a significant relationship between hyperchylomicronemia and myocardial infarction by a comparison between the post prandial serum turbidity of control and myocardial infarction

patients at fasting, and at 3 and 5 hours after a standard fat meal. These findings are presented in figure 3.

V Cholesterol to phospholipid ratio. For over a half a century it was thought that blood cholesterol levels were directly related to the incidence and degree of atherosclerosis. More recently it was thought that the cholesterol/phospholipid ratio might prove a more significant index. The significance of this ratio has been stressed in the section dealing with experimental atherosclerosis. An elevated ratio has been correlated with atherogenesis in both experimental animals and in humans.

Gould (2), has noted that the hypercholesterolemia of primary biliary cirrhosis and xanthomatous infiltration of the skin which are thought to be not atherogenic is characterized by extremely high cholesterol and phospholipid levels and no increase in the cholesterol/phospholipid ratio. The cholesterol/phospholipid ratio is 1.3 in alpha lipoproteins and 2.1 in beta lipoproteins on a molar basis. This would explain why this ratio is often increased in atherosclerosis and other conditions predisposed to atherosclerosis such as diabetes, familial xanthomatosis nephrosis, and hypothyroidism, Pomeraze (31) and Gertler (32), Jackson (33), in a critical study

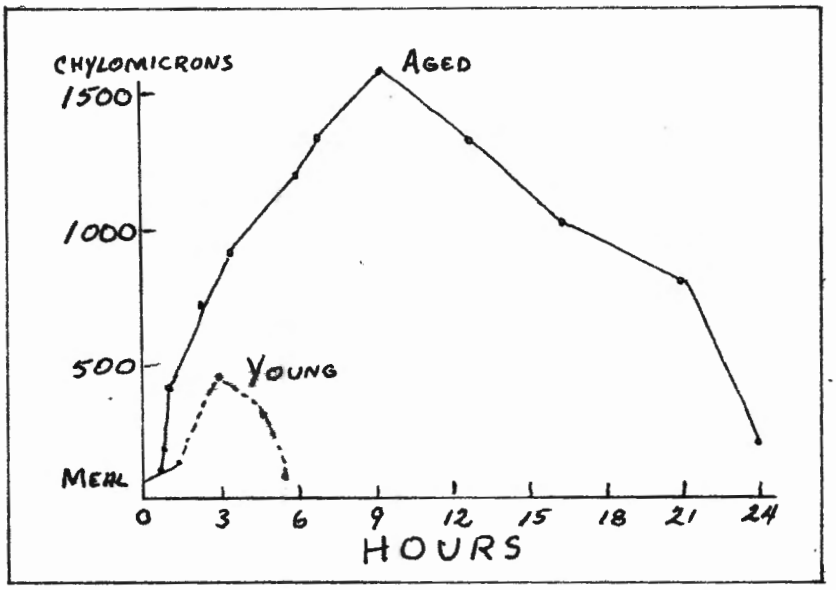


Fig. 2

Chylomicron curves of fasting young and old subjects after a Standard fat meal.

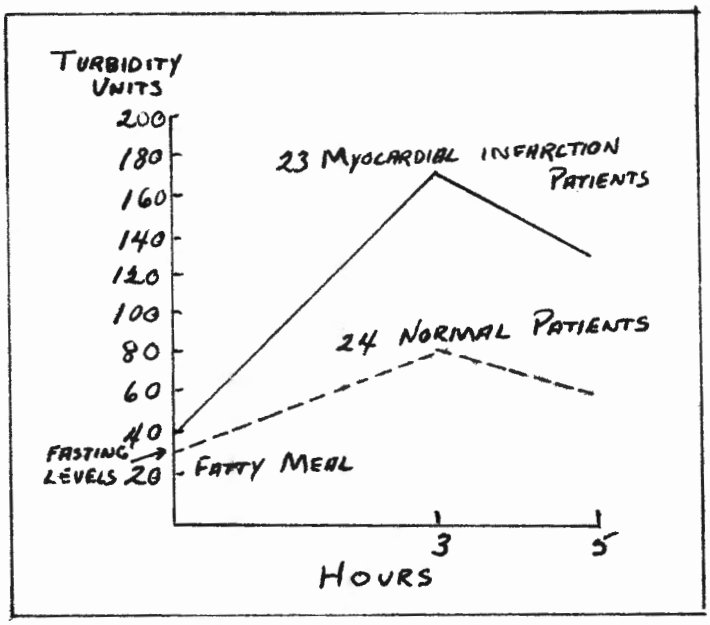


Fig. 3

Fat tolerance in myocardial infarction and control patients.

of the validity of the cholesteral/phospholipid ratio concluded that the determination of such a ratio was of absolutely no value, and that its correlation with atherogenesis was merely coincidental in most cases and was not directly correlated with the disease proscess. He pointed out that, if given the total cholesterol value, one can predict the cholesterol/phospholipid ratio within narrow limits (where liver failure is not present). It follows that this ratio can be no more useful as an index of atherosclerosis than the discredited total cholesterol itself. This conclusion is supported by Pick (34), Hobson (35) and others.

VI Antilipfanogens. "Lipfanogens" are found in all sera, partly in a free state and partly combined with "antilipfanogan" to form a complex. They are observed by placing them in tissue culture media where they are taken up by the living cells and are converted into visable fat granules. They may be defined as follows: Substances that produce visable fat. A special group of lipoid substances found in blood serum which, when in a free state, are taken up by living cells and converted into visable fat. Evidence is presented that "antilipfanogen" in blood serum serves as a regulating agent that reduces or prevents the fat deposition of

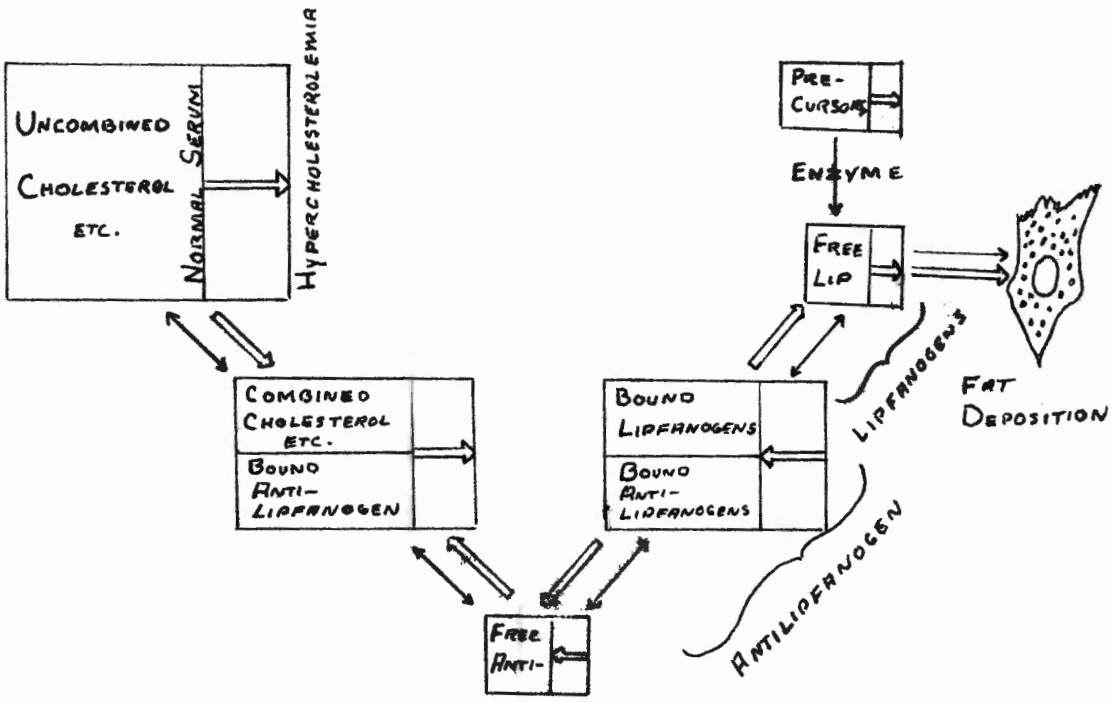
atherosclerosis. It may be defined as follows: A substance found in blood serum that reduces or prevents the fat depositing action of the lipfanogens that are present. Antilipfanogen has been shown to combine in part with the lipfanogens, forming a complex that is not converted into visible fat. The evidence is summarized as follows: 1. In a species, such as the chicken, having greater incidence of atherosclerosis than humans, the free lipfanogens in the serum are higher and the free antilipfanogen is lower than in humans. 2. Conversely, in species (dogs, horses, cattle) having a lower incidence of atherosclerosis than humans, the free lipfanogens are definitely lower and the free antilipfanogen higher than in human serum. 3. Human males seem to have slightly more free lipfanogens and less free antilipfanogen than the human female. 4. In diseases typified by hypercholesterolemia and associated with high incidence of atherosclerosis (coronary disease, diabetes, and nephrotic syndrome) the free lipfanogens were found to be higher than normal and the free antilipfanogen lower than normal. 5. Segments of chicken and of human arteries incubated in vitro in a suitable medium containing lipfanogens developed deposits of sudanophilic fat, while control segments remained free from stainable

fat. These experimental deposits in chicken arteries closely resembled the spontaneous atherosclerosis in that species. 6. Any desired degree of fat deposition can be obtained in vitro merely by varying the relative concentrations of antilipfanogen and of lipfanogens in the media. In other words, wherever atherosclerosis is prevalent, free antilipfanogen is low and free lipfanogens are high--and vice versa. Furthermore, since these fat depositing lipfanogens are in continuous contact with the intimal surface of blood vessels, it is logical to assume that they would be involved in any fat deposition that takes place. It has been the belief of some workers Antischkow (36), Katz (1) and Stamler (18) that cholesterol constitutes the fatty material primarily deposited in atherosclerosis. This does not agree with Simm's (37) conception of the mechanism. This paper presented two ideas: 1. That the primary deposit of fatty material in atherosclerosis is predominantly sudanophilic fat, and 2. That the high fat deposition in hypercholesterolemia may be due to the liberation of free lipfanogens because of the formation of a complex between cholesterol and antilipfanogen. Primary fat deposition: 1. Studies on frozen sections of very small sclerotic plaques from the human aorta showed that in these (presumably early) plaques there

were diffuse fat droplets that stained with SudanIV, but no histologic indications of cholesterol. (Note: This diffuse fat was so easily soluble that care was required not to dissolve it while staining. Prolonged exposure of the sections to 65 per cent alcohol saturated with Sudan IV avoided this difficulty, although the staining was faint.) As progressively larger plaques were observed, cholesterol accumulations were seen, becoming increasingly conspicuous. Similar results were obtained by Duff and McMillan (4). Furthermore, Meeker and Jobling (36) found, by chemical analysis, that neutral fat predominated in the smaller plaques and cholesterol in the larger ones. These observations have been verified by Buck and Rossiter (37) and Fangman and Helling (38). 2. Experimental feeding or injection of cholesterol gives an initial deposition of stainable fat. 3. Although hypercholesterolemia is widely accepted as being accompanied by high incidence of atherosclerosis, there is no evidence that cholesterol itself is the primary depositing material. If we accept the premise that the primary fatty deposits are predominantly stainable fat, the question arises as to the source of this material. The evidence indicates, as explained above, that the lipfanogens constitute the source of part or all of this

material. If that is true, then an explanation is needed of the fact that high serum cholesterol results in the deposition of the lipfanogens in the form of visible fat. There seems to be only one explanation that fits the known facts, namely, that cholesterol also forms a complex with antilipfanogen and that there is competition between the cholesterol and the lipfanogens in their combination with antilipfanogen. A summary of this theory is presented on the following page.

VII Hypercholesterolemia and Kupffer cell phagocytosis. Leary (39), has worked out a long detailed theory using rabbits in which he suggests that the Kupffer cells remove the excess cholesterol esters from the liver, transport them through the lungs and thence to the subendothelial layers of arterial intima at the most accessible region. He finds support for his theory by leaning on Gordon's (40) observations that the most probable points of atherosclerosis are in the arterial regions where pulsatile intermissions are the most prominent. Leary (41) further suggests that a defense mechanism exists which removes cholesterol from the arteries of youth and the ascending aorta even in advanced age. Supposedly, the cholesterol is transferred from wandering lipoid foam cells to fixed fibroblasts in which the cholesterol esters are split,



The relationship between agents involved in fat deposition, as observed in tissue culture. The areas on the right are roughly proportional to the concentrations of these agents in normal human serum. The black arrows indicate the equilibrium in normal serum.

The 2 areas on the left (not proportional to those on the right) indicate the complex that is formed between cholesterol and antilipfanogen.

In hypercholesterolemia, changes are observed that are represented by the open arrows and the boundaries to which they point. The same changes are observed in vitro in the experiments described in this paper. They are, from left to right:

1. an increase in cholesterol
2. an increase in the complex between cholesterol and antilipfanogen
3. a decrease in free antilipfanogen
4. a decrease in the complex with lipfanogens
5. an increase in free lipfanogens and consequently
6. an increase in fat deposition.

anisotropism is lost, and the cholesterol is brought into solution in an excess of fatty acids; solution is followed by its disappearance from the lesions. Although this theory offers a convenient solution to the mechanism of atheroma production, it has not been born out by other investigators and is considered to have no validity.

VIII Liver dysfunction and protein defects. Defect in the synthesis or degradation of the protein moiety of the lipoproteins might well be at fault. So little is currently known of the chemical and physical constitution of these large molecules which transport and keep the lipids in solution and how lipid is split off them for use in cells, that it would be unwise to assume their constant normality and efficiency. As previously mentioned quantitative lipid abnormalities are commonly associated with certain metabolic disorders such as diabetes, hypothyroidism, nephrosis and familial xanthomatosis in which the incidence of atheromatosis is increased. This quantitative lipid abnormality may be presumed to be a failure of those body processes which catabolize the lipid elements measured in excess. It is a failure of a mechanism, a metabolic block, that may be a failure of a particular or multiple enzyme systems associated with the catobolic mechanism of the particular lipid fraction.

Available evidence points to the liver as the probable site of failure of the enzyme systems. Cholesterol is synthesized, excreted and catabolized in and through the liver. Phospholipids are formed in the liver. Any serum lipid, protein or lipoprotein abnormality is probably mediated through a liver function and its presence represents liver dysfunction. This metabolic block associated with a deranged catabolism/anabolism ratio of the lipid elements causes a quantitative serum elevation. The role of the liver in this, though not clearly defined, appears certain. This relationship of a metabolic derangement of liver function, quantatively abnormal serum lipids and an increased incidence of atherosclerosis is an oversimplification of an involved and as yet poorly defined process.

IX Intimal fibrosis, mucopolysaccharides and elastase activity. During the development of so-called intimal fibrosis progressive degeneration of the inner media frequently occurres, in which the elastic fibers first become fragmented and then disappear. These changes may possibly be initiated by a deposit of lipid, which may only be temporary, in the intima and the inner layer of media. A marked accumulation of metachromatic substances occurs in the area of medial degeneration and is intimately

associated with fragmentation of the elastic fibers.

The accumulation of mucopolysaccharides in the inner media and intima is such a prominent feature in many instances of intimal fibrosis that one is forced to consider it of pathogenetic importance. Two possibilities are apparent: 1. Either the accumulation of metachromatic substance interferes with the nutrition of the elastic fibers so that they degenerate, or 2. The fragmentation of the elastic fibers is primary, resulting in a pooling of the mucopolysaccharide in their neighborhood. If the former explanation is correct, one must search for factors to explain this primary increase, It seems unlikely that it represents only a redistribution of a substance normally present since birth. Morphologically, there appears to be a definite increase in the amount of mucopolysaccharides, and Faber (42), by chemical analysis has reported an increased carbohydrate-sulfate ester content of the aorta with age and hypertension. Gersh and Catchpole (43) have described granules positive to the periotic acid-Schiff's reagent, within fibroblasts and suggest that these may represent a secretion product which is concerned in the production of ground substance. In the material studied by Taylor (44), there was no excessive fibroblastic activity to explain the increase in muco-

polysaccharides, though this would not, of course, rule out the possibility of increased secretion by the fibroblasts that were present. On the other hand, there is considerable evidence to suggest that the accumulation of mucopolysaccharides is secondary to alterations in the elastic tissue. Most authorities today conceive of the formed connective tissues (reticulin, collagen and elastic tissue) as being derived from the ground substance. Gersh and Catchpole (43) in their detailed investigations seem to imply that ground substance and basement membrane, at least, may be transformed from one to the other by the influence of enzyme systems altering the state of polymerization. One cannot help but wonder if such a mechanism might pertain to elastic tissue as well. A particularly interesting development along these lines is the recent work on elastase by Balo and Banga (45) and by Lansing (46). Elastase is an enzyme which acts specifically on elastic tissue protein; it is said to convert this protein from a fibrous to a molecular form and to make it soluble without hydrolyzing it to amino acids. Elastase is found in the islet cells of the pancress, suggesting that it is a systemic enzyme rather than a digestive one. The elastase activity of arteriosclerotic patients has been reported as being a great deal

lower than that of controls. Evidence to support this concept is supplied by the work of Hall, Reed and Tunbridge (47), who recently reported that, unlike previous observers, they found polysaccharide and sulfuric acid intimately associated with protein in elastic tissue and that polysaccharide is liberated simultaneously with protein whenever the elastic tissue is degraded. They further suggested that the enzyme elastase, which digests elastin, is not a proteolytic enzyme but rather a mucase.

With reference to the possible role of the mucopolysaccharide accumulation in the production of either intimal hyalinization or focal atheroma, we are also in the field of conjecture. Moon and Rinehart (48) have suggested that hyalinization of the intima in coronary arteriosclerosis may represent an abnormal polymerization of mucoid ground substance. In the present study it was noted also that when the intima of the aorta was hyaline an intimate admixture of mucopolysaccharide and lipid often could be demonstrated. This is similar to the findings of Moon and Rinehart (48) in the coronary arteries and is also comparable to hyaline arteriosclerosis. It is possible that this may represent a process analogous to the formation of the protein-mucopolysaccharide complex of amyloid.

Wilens (49), has given reasons for assuming that intimal fibrosis is an intregral part of the atherosclerotic process. It is conceivable that an accumulation of mucopolysaccharide, by altering the permeability of the subendothelial tissues, may play an important part in the localization of lipid which is the feature of the atheromatous plaque. Another possibility has been offered by Faber (42). He noted that cholesterol deposition is sometimes encountered in various tissues rich in metachromatic substance and suggested that the sulfate esters of the aorta might release lipid from lipoproteins brought to the tissue by the blood plasma.

X Heparin defect. Heparin, in sub-anticoagulant doses demonstrates a profound clearing action on chylomicra Block, Barker and Mann (50), Sf 0-12, 12-400 lipoproteins Gofman, (51), and beta-lipoprotein concentrations, Lever, Smith and Hurley (52), which would indicate a relationship between these three factors. Block, Barker and Mann (50), found that atherosclerotic patients failed to clear alimentary lipemia with a small dose (3 mg.) of heparin as effectively as normal people. The mechanism of this "clearing" action of heparin is not as yet fully understood, but several logical theories based on experimental data have been elicited which are probably closely related.

One such hypothesis is that highly acidic heparin attaches itself to the extremely basic choline portion of the phospholipid lecithin, forming under enzymatic influence, a highly surface active complex, capable of placing large lipid particles in colloidal solution Anderson and Fawcett (53). Another hypothesis is that an enzyme-like "clearing" factor is formed from a precursor among the serum proteins by certain tissues in the presence of heparin, Anfinsen, Boyle and Brown (54). Recently this "clearing factor" was synthesized in vitro from an unidentified precursor found in plasma, an unidentified tissue factor, and heparin, Brown and Kauffman (55).

The suggested hypothesis, that a deficiency of heparin or a heparin-like substance may be a prime underlying factor in the etiology of atherosclerosis, Gofman (56), is strengthened by a series of recent investigations revealing: 1. a high incidence of hypercoagulability and low blood heparin levels in patients with cardiovascular disease and atherosclerosis, 2. a tendency for circulating heparin to decrease with age, 3. the existence of an inverse proportion between the concentration of giant lipoprotein molecules and plasma heparin levels, 4. and significantly greater clinical success with heparin therapy in the

presence of hypercoagulability than iso- or hypo-coagulability, Anfinsen, Boyle and Brown (54). For the most part the reduction in the concentration of plasma lipid macromolecules by heparin therapy has been accompanied by significant subjective and objective clinical improvement in disease states associated with atherosclerosis, including marked reduction in the recurrence and occurrence of myocardial infarcts, Anfinsen, Boyle and Brown (54).

OTHER FACTORS PREDISPOSING TO HUMAN ATHEROGENESIS.

I Hereditary predisposition. A hereditary predisposition toward coronary artery disease has been noted by many investigators. In a study of families with xanthoma and a study of unselected patients under 50 years of age with coronary artery disease, Adlersberg, Parets and Boas (57) came to the conclusion that the number affected with hypercholesterolemia fits a 1:1 Mendelian ratio and that hereditary transmission probably as a dominant trait, is responsible for the distribution of hypercholesterolemia. When results obtained in both studies are considered together, it appears that the common factor for most patients with coronary atherosclerosis may be a hereditary disturbance of cholesterol metabolism manifested by elevated

serum cholesterol. Familial xanthomatosis is the severest form of the inherited disturbance. Xanthoma lesions develop only in patients who carry two abnormal genes for cholesterol; that is, they are homozygotes. Atherosclerosis is frequent in such persons. Many patients with uncomplicated coronary artery disease are probably affected with a milder form of disturbed lipid metabolism. They carry one abnormal gene for cholesterol; that is, they are heterozygotes. Derangement of cholesterol metabolism helps explain the familial incidence of coronary artery disease and also accounts in part for its development in many persons under 50 years of age.

II Diet. Much suggestive evidence has accumulated from a multitude of sources that atherosclerosis is minimal in certain peoples of the world, such as Okinawans, Chinese and Ceylonese, who subsist on diets differing markedly from ours. Yet this evidence has not been entirely consistent and it should be pointed out that other factors may have been operating to render these observations equivocal.

Since no direct measure is available for assessing the degree of atherosclerosis or its rate of development in the living human Gofman (58), has taken one of it's most serious sequelae (coronary heart disease) and has

measured the tendancy to develope heart disease from a relation between the standard Sf 0-12 and Sf 12-400 lipoproteins which has been well documented by studies on a large series of clinical cases of coronary heart disease and a corresponding study of clinically well individuals of the same age, sex, and population type. From statistical analysis of these cases he has been able to arrive at an "Atherogenic Index" or "A.I." value which represents the best measure of the tendancy of an individual to develope clinical coronary heart disease obtainable from ultracentrifugation of lipoproteins. Thus the elevated serum lipoproteins may now be transposed in terms of the A.I. values. The greater the A.I. value (which represents the best combination of the critical lipoproteins), the greater the progression of coronary disease would be expected to be. Conversely, efforts to minimize the progression of the disease would be expressed in terms of efforts to reduce the A.I. values. This transposition is important, theoretically and practically. If a particular regimen, dietary or otherwise, would lower one class of lipoproteins, while elevating the other, the clinical effect to be anticipated would depend on whether the resulting net A.I. value is actually lowered or elevated.

The A.I. value has been shown to rise with obesity. However, this by no means infers that calories per se represent the offensive agent in elevating serum lipoprotein levels, and, consequently, A.I. values. Fat intake, on the average, rises with caloric intake. Hence, overweight in general is associated with both excessive fat and excessive caloric intake. It then becomes a problem to determine which of the three following major possibilities account for the rise in A.I. values in overweight people: 1. Excessive fat intake, 2. Excessive caloric intake or 3. Some unrelated metabolic feature of the obese state.

Gofman (58) demonstrated that the lowering of fat intake, at an isocaloric level, leads to lowered A.I. values. Since obesity is usually associated with excessive fat intake, the elevated average A.I. values characteristic of obese persons can, at least in part, be the result of the excessive fat intake. The fact that overweight can be due more to excessive intake of nonfat calories may indeed, partially explain why the relation of overweight to elevated A.I. values is at times not of a very high order. If nonfat calories do not lead to excessive A.I. values, then obese persons overeating in nonfat calories would not be expected to show the same

elevation in A.I. values that might have been produced by an equivalent number of fat calories.

The effect of low calorie diets alone has been inconclusive since in all cases the low calorie diet has been a low calorie - low fat diet and the patients have been maintained in a negative caloric balance. No inference can be drawn for persons in a steady state from individuals in negative caloric balance. The third possibility, that of a metabolic disturbance associated with obesity itself is essentially unevaluated.

Studies on the effect of obesity and diet by Romeranze (59), French (60), Wilens (61), Keys (62) and Keys (63), have all concurred with the findings of Gofman (58). Keys (63) in a series of feeding experiments came to the conclusion that, other things being equal, the serum cholesterol level in adult man is independent of the cholesterol intake over the range of zero to at least 700 mg. daily. But the fat intake is quite another matter and appears to have great importance. However, there is not the slightest evidence for a difference between animal and vegetable fat in this regard.

III Local factors in arterial wall. It has been suggested that mechanical or hemodynamic strain and trauma are responsible for the localization of atherosclerotic

lesions in relation to the orifices of the intercostal arteries, in relation to the points of vascular branching and in other areas. Perhaps the best evidence of a possible pathogenetic role for strain and trauma is to be found in cases of localized or generalized hypertension for, while the data leave much to be desired, there remains little reasonable doubt that hypertension can aggravate atherosclerotic processes on occasion. The most clear cut example is the occurrence of atherosclerosis of the pulmonary arteries in association with pulmonary hypertension. The propensity of syphilis to enhance the development of atherosclerosis of the aorta is a good example of the effect of a vascular injury in man Duff (4), Leary (64), and, although the evidence is not so distinct, it seems that other kinds of inflammation of the vessel wall may act similarly. McMillan (65) examined the aortic endothelium for evidence of its role in the pathogenesis of atherosclerosis. He did not succeed in demonstrating any morphological change during the early phases of the development of atherosclerosis save only the accumulation of a few droplets of fat in some endothelial cells. Their appearance and that of the intercellular cement substance remain essentially normal. There was no morphological evidence of damage or porosity that

would suggest that either the endothelial cells or their cement substance were more permeable than normal. Nevertheless, some endothelial cells were rendered capable of accumulating colloidal material. While it may be tempting to assume that endothelial cells accumulate large amounts of lipid from the serum and transfer it into the aortic intima, the occurrence of such a phenomenon has not been observed. On the other hand, it is even more tempting to speculate that the phenomena that have been observed with the use of colloidal thorium dioxide are associated with an increased permeability of the affected cells. Duff and McMillan (66) have demonstrated the accumulation of injected colloidal thorium dioxide in the foam cells of the lesions of experimental cholesterol atherosclerosis. It would be tempting to speculate that this phenomenon is associated with an increased permeability of the affected cells. The conversion of endothelial cells into foam cells during the early stages of the development of atherosclerotic plaques has not been observed. However McMillan (65) has observed the initial occurrence of lipid droplets in the subendothelial ground substance and their subsequent occurrence in the macrophages that are normally present in the intima and which become foam cells. Local accumulations of these cells increase in

numbers by migration and also by mitotic division. As previously pointed out free cholesterol first appears in characteristic sites in both atherosclerotic plaques and in the liver lobules of experimental animals. It is detectable by the technique employed only several days after sudanophilic and anisotropic lipids are already demonstrable in the areas affected. Also as previously mentioned the cells comprising atherosclerotic lesions are metabolically active and it is possible to investigate their enzyme systems.

IV Endocrines. As has been pointed out, diabetes mellitus and hypothyroidism frequently show increased cholesterolemia and atherosclerosis. Moreover, there is a high incidence of atherosclerosis in patients with hyperfunction of the adrenal cortex (e.g., Cushing's syndrome), and prolonged cortisone therapy produces increased plasma cholesterol levels, Wakerlin (66). It has also been demonstrated that the administration of estrogen can convert the highly pathologic patterns of survivors of myocardial infarction to normal adult human values, and that the use of methyl testosterone exaggerates the chemical pathology of myocardial infarction and may even produce deviant patterns in those who have been previously normal, Barr (67).

Kountz (68) made the following comment in the dis-

cussion following Stamler's (18) lecture. "We have had an experience in elderly individuals with estrogen alone and with estrogen with progesterone and testosterone. For a number of years we have had a group of elderly women menstruating and have studied the effect on the cardiovascular system of the estrogen which caused the menstruation. We have been interested in the arteriosclerosis present in the aorta and coronary arteries and in the renal vessels when these patients came to autopsy. In this connection we have also been interested in the protein picture as determined by the electrophoretic method and in the free amino acids in the blood. Comparatively, we have found a lessening of the incidence of clinical coronary artery disease in the 60 individuals studied. Anatomically, however, it is difficult to tell whether or not there has been too much of a change. I think it is important here to point out that estrogen and androgen in man in part depends on the rate of oxygen consumption and in order to obtain a physiological response it is necessary to use thyroid. It would seem from our studies that we are probably making a mistake to simply say that the estrogen as one substance causes a diminution or prevention of the sclerosis of the coronary arteries but that it is in part dependent upon the other

glands of internal secretion for its activity."

V Age and sex. The influence of age and sex has been noticed by most observers. Indeed, the rationale of treatment of coronary atherosclerosis by administration of estrogens stems from the markedly decreased incidence of atherogenesis in premenopousal women. As noted on page 27, young women in the 18-34 age group have a low serum concentration of the lipoprotein molecules considered atherogenic by Page and Gofman. These are chiefly the atherogenic beta lipoproteins. This same age group has an alpha lipoprotein content which is much higher than that of her male counterpart. The percentage of cholesterol found in the alpha lipoprotein of these women is reported to be more than that found in young men (34.3% vs. 25.2%), Gould (2), Page (19), Leinwand (24), Klein (25) and Gofman (20). Possibly the finding by Stamler (18), that estrogen inhibits cholesterol-induced coronary atherogenesis in chicks, is a lead to the basis for the sex differential in humans.

An interesting finding by Dock is the observation that the intima of the coronary arteries is considerably thicker in the male, and that this difference is present from birth, Wakerlin (66).

All of the supposedly atherogenic factors increase

with age. Becker (28), stresses the frequency of hyperchylomicronemia with advancing age as a basis for the increased atherosclerosis. This is born out by fig. 2. Weinhouse (69) has pointed out that the lipid and calcium content of the media of the human aorta increases with age. This is independent of the degree of atherosclerosis. He also points out that the relative concentration of these lipids in the aorta is in direct relation with the concentration of these lipids found in the plasma. He implys from this, that the lipid deposits in the intima are the result of nonselective infiltration and precipitation of the plasma lipids.

SUMMARY Since the basis of our knowledge of the atherosclerotic proscess in the human stems from work done on the experimental animal, the first portion of this thesis has been devoted to a review of the progress of experimental atherosclerosis and to an alliteration of some of its more salient aspects.

Hypertension and all types of local damage to the vessel wall, including sympathectomy, were seen to be atherogenic stimuli only in the presence of an abnormal blood lipid relationship such as is seen following cholesterol feeding in the rabbit. The thyroid hormone was seen to

be important in the homeostatic mechanism. Its increase tended to decrease hypercholesterolemia beyond the point which might be expected from a simple increase in metabolic rate. An increased incidence of atherosclerosis has long been known to be associated with the hypothyroid state in man.

Contrary to expected results, alloxan diabetes in the hypercholesterolemic rabbit was associated with an inhibition of aortic atherogenesis. This inhibition was associated with a marked elevation of serum phospholipids concommitant with the increase in serum cholesterol. In the chick, the same phenomina of decreased atherogenesis of the induced variety occurred. However, there was a definate increase in spontaneous atherosclerosis. It may be inferred from this phenomenon that the rabbit is a poor experimental animal since it does not develope spontaneous atherosclerosis. This is one of the main reasons why Katz and other investigators have used the chick instead of the rabbit in their experiments.

The much ballyhooed effect of dietary fat was seen to have no effect on spontaneous lesions in the chick. A balanced diet apparently made a key contribution to the prevention of dietary-induced atherogenesis. Surface active agents were seen to have an effect similar to

alloxan diabetes. Tremendous foam cell accumulation was seen in the reticuloendothelial system as well as in the renal tubules.

Possibly the most important contribution of experimental atherosclerosis was the discovery of the differential effect of estrogen on different arterial beds. Aortic atherogenesis was unaffected while coronary atherogenesis was almost negligable. Estrogen treatment also caused the regression of previously present coronary lesions.

The remainder of this thesis is concerned with the most accepted theories of human atherogenesis and related predisposing factors. The filtration concept must be considered the most tenable mechanism for the deposition of "foreign" lipoids in the arterial intima. This concept is embodied in most of the theories to be presented.

Gofman, Page and others have contended that it is the size of the lipoprotein molecule which is the crucial factor in the development of atherosclerosis. Supposedly, particles which are too small pass through the arterial filter and are not retained while those that are too large never reach the intima. These particles are studied by means of the ultracentrifuge and are characterized as belonging to different classes according to their flotation rates. The association of the pathologic classes with post-myocardial infarction patients has been demonstrated.

Gofman has demonstrated a lowering of the pathological Sf 12-20 lipoproteins by dietary fat restriction. Considerable support was given to this theory by the production of atherosclerosis in experimental animals by intravenous injection of these low density lipoproteins with subsequent development of atherosclerosis while the disease was not produced by similar injections of cholesterol or triglyceride emulsions.

Proponents of the chemical fractionation method of separation of lipoproteins maintain that the beta lipoproteins are the class responsible for the disease. Gofmans "atherogenic index" now includes all beta lipoproteins but no alpha lipoproteins. Investigators have found the degree of atherosclerosis to be inversely related to the alpha lipoprotein level of the plasma.

Many investigators propose that repeated bouts of, and/or, sustained hyperchylomicronemia are responsible for the atherosclerotic state. Hyperchylomicronemia is seen to increase with age and atherosclerosis. Chylomicrons are reportedly composed chiefly of beta lipoproteins.

The above three theories are the most popular today and it would seem that they are all very closely related. It is quite probable that all three groups of investigators are arriving at the same basic conclusion from different methods of approach.

It would seem from recent investigations that the cholesterol/phospholipid ratio is no more reliable as an index of atherogenesis than the total cholesterol and certainly it can bear no direct relationship to the pathologic mechanism. It is most likely coincidence and poor logic that the correlation exists in many cases.

The antilipfanogen theory is a new theory, championed by Simms et. al., which appears to have merit. These workers do not believe that cholesterol constitutes the fatty material primarily deposited in atherosclerosis. They present evidence to show that the primary deposit of fatty material is sudanophilic fat, and, that the high fat deposition in hypercholesterolemia may be due to the liberation of free lipfanogens because of the formation of a complex between cholesterol and antilipfanogen. A good summary of this theory is presented in the diagram following page 38.

Leary's theory of Kupffer cell phogocytosis of cholesterol and transportation through the lung to the arterial intima is a convenient theory but one which is not substantiated by other investigators.

Undoubtedly abnormal liver function plays a part in the atherogenic proscess, but so little is known about its function in relation to protein metabolism that any

theory of atherogenesis with liver malfunction as its basis can be no more than conjecture.

Another theory which may play a part in the formation of atheromata is the concept of abnormal metabolic proscesses of the vessel wall itself. It is suggested that the abnormally large pool of mucopolysaccarides is derived from the degeneration of elastic tissue of the media, under the influence of increased enzymatic activity, and that it may play a part in the development of the atheromatous plaque. This theory is at odds with the antilipfanogen theory in that it considers the mucopolysaccharides present in the atheroma to be the primary fatty deposit.

The concept that the underlying defect in the atherosclerotic process may be due to a heparin or heparin-like defect has long been a consideration. However, since the degree of heparin necessary to effect plasma levels would cause bleeding deficiencies, it was never really considered to be a factor in the human disease. The effect of lipotropic factors has also long been considered but such agents as choline, inositol and methionine, have proven to be of little value. Recently this heparin-like "clearing factor" has been synthesized in vitro from an unidentified precursor found in plasma, an unidentified tissue factor,

and heparin. It has also been discovered that when heparin is combined with choline it no longer retains its anti-coagulent action but still retains its plasma clearing power. This concept might well be an important factor in the human disease.

It has repeatedly been shown that of the factors predisposing to atherogenisis hereditary predisposition probably plays the most important role. The tendancy toward the disease may well be inherited as a Mendelian dominant.

Much has been written about the effect of diet on atherogenesis but about the only certainty is that obesity is associated with an increased incidence of the disease. The relative importance of fat, caloric, and cholesterol intake has not been sufficiently assessed. Gofman's atherogenic index is lowered by a low-fat, low-calorie diet in obese patients.

Other predisposing factors including local changes in the arterial wall, endocrine disturbances and age and sex factors all enter in to the problem, but their relative importance is very difficult to evaluate.

It must be emphasized that a correlation between deviation from normal of some property of plasma lipoproteins and atherosclerosis suggests but does not, of course, prove that this deviation is the cause of, or

even directly related to the cause of, the disease. The failure, on the part of a large group of investigators to realize the importance of this statement has led to an enormous amount of research based on illogical premises. This thesis has attempted to organize and present only the more logical data which has been sifted from the chaff.

CONCLUSION An attempt has been made to present and correlate the most important factors operating in the genesis of the atherosclerotic proscess. The important contributions of the experimentally produced disease are outlined, and the major etiological theories are presented. It is generally conceded that atherosclerosis is chiefly the result of an inborn error in lipid metabolism, while innumerable other factors play a secondary role. It may be concluded that the atherogenic lipoproteins of Gofman et. al., the beta lipoproteins, and the chylomicrons are all closely related and represent the basic abnormality associated with atherogenesis. This interrelationship is suggested by the dramatic clearing effect of heparin on all of these factors. The anti-lipfanogen theory of Simm's et. al., is very convincing and may well be another approach to the same basic

problem. The precise definition of the nature of the disease is still unknown, but in the light of our present knowledge, one must conclude that the answer lies in the not too distant future.

BIBLIOGRAPHY

1. Katz, L. N., Experimental atherosclerosis, Circulation, 5:101, 1952.

2. Gould, Gordon R., Biochemical aspects of atherosclerosis, J. Am. Geriatrics Soc., 2:10 Oct. 54 p 640-648.

3. Duff, G. L., The nature of experimental cholesterol arteriosclerosis: in the rabbit, Arch. Path., 22:161 1936.

4. Duff, Lyman G. and McMillan, Gardner C., Pathology of atherosclerosis, Am. J. Med., 11, 1951.

5. Steiner, A. and Kendall, F. E., Atherosclerosis and arteriosclerosis in dogs following ingestion of cholesterol and thiouracil, Arch. Path., 42:433 1946.

6. Dill, L. V. and Isenhour, C. E., Occurrence of atheroma in the aorta of rabbits with renal hypertension, Arch. Path., 33:655 1942.

7. Hueper, W. C., Arteriosclerosis, A general review, Arch. Path., 38:162,245,350 1944 39:51,117,187 1945.

8. Harrison, C. V., Effect of sympathectomy on development of experimental arterial disease, J. Path. and Bact., 48:353 1949.

9. Turner, K. B., Present, C. H., and Bidwell, E. H., Role of thyroid in regulation of blood cholesterol in the rabbit, J. Exper. Med., 67:111 1938.

10. Turner, K. B., Studies on the prevention of cholesterol atherosclerosis in rabbits, Effect of whole thyroid and potassium iodide, J. Exper. Med., 58:115 1933.

11. Turner, K. B., and Khayot, C. B., Prevention of cholesterol atherosclerosis in rabbits, Influence of thyroidectomy on protective action of potassium iodide, J. Exper. Med., 58:127 1933.

12. Meeker, D. R., Kesten, H. D., and Jobling, J. W., Effect of iodine on cholesterol induced arteriosclerosis, Arch. Path., 20:337, 1935.

13. Rosenthal, S. R., Studies in atherosclerosis, possible dangers of iodine theropy in atherosclerosis of aorta as seen from an experimental point of view, Arch. Path., 18:827, 1934.

14. Duff, G. L., and McMillan, G. C., The effect of alloxan diabetes on experimental cholesterol atherosclerosis in the rabbit, 1. the inhibition of experimental cholesterol atherosclerosis in alloxan diabetes, 2. the effect of alloxan diabetes on the retrogression of experimental cholesterol atherosclerosis, J. Exper. Med., 89:611, 1949.

15. Duff, G. L., and Payne, T. P. B., The effect of alloxan diabetes on experimental cholesterol atherosclerosis in the rabbit 3. the mechanism of inhibition of experimental cholesterol atherosclerosis in alloxan-diabetic rabbits, J. Exper. Med., 92:299 1950.

16. Kellner, A., Correll, J. W., and Ladd, A. T., The influence of intravenously administered surface-active agents on the development of experimental atherosclerosis in rabbits, J. Exper. Med., 93:385 1951.

17. Payne, T. P. B., and Duff, G. L., The effect of Tween 80 on the serum lipids and the tissues of cholesterol-fed rabbits, Arch. Path. 51:379 1951.

18. Stamler, J., Sex adrenal steroids in experimental atherosclerosis, Proc. Council for High Blood Pressure Research, Am. Heart Ass., 1952 p45.

19. Page, I. H., Atherosclerosis, An introduction, Circulation, 10:1 1954.

20. Gofman, et al., Blood lipids and human atherosclerosis, Circulation, 5:119 1952.

21. Engelberg, Hyman, The relative merits of serum lipoprotein and cholesterol determinations in atherosclerosis, J. Am. Geriatrics Soc., 2:3, Mar. 54.

22. Bragdon, J. H., and Boyle, E., Serum lipoproteins in the pathogenesis of experimental atherosclerosis, Circulation, 8:434, 1953.

23. Barr, D. P., Russ, E. M., and Eder, H. A., I. Protein lipid relationship in human plasma, II. In atherosclerosis and related conditions, Am. J. Med., 11:480-485, 1951.

24. Leinwand, I., Serum lipid and protein fractions, VII. Lipid transport in the human as determined by electrophoresis of the proteins, Circulation, 8:451, 1953.

25. Klein, T., Ackerman, P. G., and Kountz, W. B., Hyperbeta-globulinemia connected with hypoalbuminemia in arteriosclerosis and coronary sclerosis, Circulation, 8:450, 1953.

26. Hueper, W. C., Prevention and treatment of athero--sclerosis, Medical Clinics of North America, 33:773-781, 1949.

27. Moreton, J. R., Physical state of lipids and foreign substances producing atherosclerosis, Science, 107:371, 1948.

28. Becker, G. H., Meyer, J., and Necheles H., Fat absorption and atherosclerosis, Science 110:529, 1949; and Gastroenterology, 4:80, 1950.

29. Zinn, W. J., and Griffith, G. C., A study of serum fat globules in atherosclerotic and non-atherosclerotic male subjects, American Journal of the Medical Sciences, 597-603, 1950.

30. Schwartz, L., Waldow, A., and Dunsmore, R. A., Determination of fat tolerence in patients with myocardial infarction, Journal of the American Medical Association, 149:364-366, 1952.

31. Pomeranze, Julius Geriatrics, Etiology and Treatment of atherosclerosis, July 53 p 359-369.

32. Gertler, M. M., Garn, S. M, and Lerman, J., The interrelationships of serum cholesterol, cholesterol esters and phospholipids in health and in coronary artery disease, Circulation, 2:205 1950.

33. Jackson, R. S., and Wilkinson, C. F., The ratio between phospholipid and the cholesterols in plasma as an index of human atherosclerosis, Ann. Int. Med., 37:1162, 1952.

34. Pick, A., Rosenblum, S., and Katz, L. N., Interrelationships between plasma lipids and atherosclerosis in residents of a home for the aged, Circulation, 8:453, 1953.

35. Hobson, W., Jordan, A., and Roseman, Cissie, Serum-cholesterol levels in elderly people living at home, Lancet, 2:961-964, 1953.

36. Anitschkow, N., Experimental arteriosclerosis in animals, In Cowdry, E. V., arteriosclerosis, A survey of the problem, Chapt. 10, New York, 1933..

36. Meeker, D. R., and Jobling, J. W., A chemical study of arteriosclerosis lesions in the human aorta, Arch. Path., 18:252-257, 1934.

37. Simms, H. S., Harmison, C. R., and Best, R. B., Cholesterol and antilipfanogen in arteriosclerosis, J. Geront., 9:2, 1954.

37. Buck, R. C. and Rossiter, R. J., Lipids of normal and atherosclerotic aortas, Arch. Path., 51:224-237, 1951.

38. Fangman, R. J., and Helling, C. A., Histology of coronary arteries in the new born, Am. J. Path., 23:901-902, 1947.

39. Leary, T., The genesis of atherosclerosis, Arch. Path., 32:507, 1941.

40. Gordon, I., Mechanism of lipophage deposition in atherosclerosis, Arch. Path., 44:247, 1947.

41. Leary, T., Cholesterol lysis in atheroma, Arch. Path., 37-38:16, 1944.

42. Faber, M., The human aorta: sulfate containing polyuronides and the deposition of cholesterol, Arch. Path., 48:343-350, 1949.

43. Gersh, I., and Catelepole, H. R., The organization of ground substance and basement membrane and its significance in tissue injury, disease and growth, Am. J. Anat., 85:457-521, 1949.

44. Taylor, H. E., The role of mucopolysaccrides in the pathogenesis of intimal fibrosis and atherosclerosis of the human aorta, Am. J. of Pathology, Sept.-Oct., 1953.

45. Balo, J., and Banga, I., Elastase and elastase-inhibitor, Nature, 164:491, 1949.

46. Lansing, A. I., Chemical morphology of arterial elastic tissue, Nat. Res. Council Symposium on Atherosclerosis (to be published).

47. Hall, D. A., Reed, R., and Tunbridge, R. E., Structure of elastic tissue, Nature, 170:264-266, 1952.

48. Moon, H. D., and Rinehart, J. F., Histogenesis of coronary arteriosclerosis, Circulation, 6:481-488, 1952.

49. Wilens, S. L., The nature of diffuse intimal thickening of arteries, Am. J. Path., 27:825, 1951.

50. Block, W. J., Barker, N. W., and Mann, F. D., Circulation, 4:674, 1951.

51. Gofman, J. W., et al., Modern Medicine, Vol. 21, No. 12, pg. 119, June, 1953.

52. Lever, W. F., Smith, P. A. J., and Hurley, N. A., Science, 118:653, 1953.

53. Anderson, H. G., and Fawcett, B., Proc. Soc. Exper. Biol. Med., 74:768, 1950.

54. Anfinsen, C. B., Boyle, E., and Brown, R. K., Science, 115, 583, 1952.

55. Brown, R. K., and Kauffman, D. L., Circulation, 8:435, 1953.

56. Gofman, J. W. et al., Am. J. Med., 11:358, 1951.

57. Adlersberg, D., Parets, A. D., and Boas, E. P., Genetics of atherosclerosclerosis, Studies of families with xanthoma and unselected patients with coronary artery disease under the age of fifty years, J. A. M. A., 141:246, 1949.

58. Gofman, John W., Tamplin, Arthur, Strisower, and Beverly, Relation of fat and caloric intake to atherosclerosis, J. Am. Dietetic Ass., 30:4, Apr. 54, 317-325.

59. Pomeranze, Julius, Benfield, William H., and Chessin, Max, Serum lipid and fat tolerance studies in normal, obese and atherosclerotic subjects, Circulation, 10:5, Nov. 54, p 742-746.

60. French, A. J., and Dock, W., Fatal coronary arteriosclerosis in young soldiers, J. A. M. A., 124: 1233, 1944.

61. Wilens, S. L., Bearing of general nutritional state on atherosclerosis, Arch. Int. Med., 79:129, 1947.

62. Keys, A., Mickelsen, O., Miller, E. V. O., and Chapman, C. B., The relathio in amman between cholesterol levels in the diet and in the blood, Science, 112:162, 1950.

63. Keys, Ancel, Human atherosclerosis and the diet, Circulation, 5:115, 1952.

64. Leary, T., Syphilitic aortitis as cause of sudden death, Friedlander lecture, abstract New England, J. Med., 223:789, 1940.

65. McMillan, Gardner C., Observations on local factors in the development of experimental atherosclerosis, Pro. Concil for high blood pressure research, Am. Heart Ass., 1952 p68.

66. Duff, G. L., and McMillan, G. C., The accumulation of colloidal thorium dioxide in the lesions of experimental cholesterol atherosclerosis, Abstract, Meeting of Am. Soc. for study of arteriosclerosis, Circulation, 2:465, 1950.

66. Wakerlin, G. E., Recent advances in the pathogenesis and treatment of atherosclerosis, Ann. Int. Med., 37:313, 1952.

67. Barr, David P., Some chemical factors in the pathogenesis of atherosclerosis, Circulation, 8:5, Nov. 53, p 641-54.

68. Kountz, In the discussion following Stamler's lecture on Sex adrenal steroids in experimental atherosclerosis, Proc. Council for High Blood Pressure Research, Am. Heart Ass., 1952, p45.

69. Weinhouse, S., and Hirsch, E. F., Chemistry of atherosclerosis, 1. Lipid and calcium content of intima and media of aorta with and without atherosclerosis, Arch. Path., 29:31, 1940.