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A SURVEY OF VASCULAR SCLEROSIS

by

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A SURVEY OF VASCULAR SCLEROSIS

I. Introduction

The literature on the subject of vascular sclerosis is shrouded in a fog which only the confusion of terminology can, and does produce so often in medical subjects. It would be absurd to continue further without clearly defining the main divisions of our topic. Most authors agree that such differences can and should be made. Thus atherosclerosis is defined as a process primarily involving the intima with deposition of lipoid materials to form intimal plaques. Conversely arteriosclerosis is defined as a process primarily involving the media with deposition of calcium to form medial plaques. Even with the acceptance of this differentiation there are still the controversial issues of which is more important in human pathology and are these processes pathological in the sense of representing a disease entity or merely a normal process of aging?

The purpose of this paper is to try to give general coverage of the entire subject, to summarize the facts pertinent to this disease and to attempt a correlation and formulation of general concepts, if this is possible.

II. Experimental Atherosclerosis

A. History:

The history of experimental atherosclerosis is in reality the history of the development of cholesterol feeding experiments. This subject has been well reviewed so that a brief resume is adequate (5,18,58). The work began in 1908 with the feeding of high protein diets which included meat, eggs, and milk. By 1910 it was concluded that it was the egg yolk and not the protein that was important. Various lipoid substances were tried without success until 1903 when Anitschkow and Chalatow fed rabbits a diet supplemented with pure cholesterol in sunflowerseed oil and successfully produced atherosclerosis in rabbits. Because of this they believed that the success of the previous diets was due to their cholesterol content. They did admit the possibility that the oil might be a factor in the absorption of cholesterol. All of the work on this subject up to the present time is based on this fundamental concept: that if cholesterol is fed to rabbits in sufficient amounts and for a long enough time, atherosclerosis will result in the majority of cases.

B. Distribution of Lesions:

Anitschkow's original description is just as accurate today as it was then. He too recognized the systemic character of the deposits being both vascular and parenchymal.

1. Vascular deposits: The initial vascular deposits are usually in the aortic arch. Here they are often found

about mouths of vessels or just above the aortic valve ring. However, the coronary or lingual vessels may show deposits before the aorta. Other fairly early deposits are found in the thoracic aorta, coronary, pulmonary, innominate, common carotid, subclavian, common iliac, and femoral arteries.

Deposits of lesser constancy are found in the abdominal aorta, mesenteric arteries, and arteries of the liver and spleen. Relatively late lesions are present in the gastrointestinal arteries, and arteries of the brain, testes, and kidney.

The arteries of the pancreas, ovaries, spinal cord, retina, and base of the brain are not affected. This apparent immunity of the cerebral arteries has attracted a good deal of attention for this presents a major difference between experimental and human atherosclerosis. These arteries resist even combined measures (63). However, Altschul has produced foam cell accumulations in the small blood vessels of the hypothalamic area and in regions of the choroid plexus.

2. Parenchymal Deposits: Duff (18) has stated that arterial lipoid accumulations occur only after the liver, spleen, bone marrow, and suprarenal cortex contain significant amounts of these substances. Parenchymal lesions may also be found in the sclera, cornea, ciliary body, iris, skin, subcutaneous tissue, tendons, tympanic membrane, kidney, testes, pulmonary alveoli, mucosa and submucosa of bile ducts, gall bladder, lymph nodes, anterior lobe of the pituitary, and as polyps in the gastrointestinal tract (3,5,18).

C. Morphology of Atheroma:

1. Gross appearance: The appearance of the lesions is the same for all methods of feeding. Initially there are minute yellowish-white opaque flecks which are slightly raised. These are not well demarcated but as they increase in size, clear-cut nodules appear. There is great variation in size and shape because of growth and coalescence. Ultimately they assume a rough, warty, yellowish surface and may significantly narrow the lumen. Fusiform swellings or aneurysms are occasionally found (5,18).

2. Microscopic: Lesions of the arterial tree seem to be similar irrespective of location, however, the manner and details of development are not clear cut. The process is one of fatty deposition in the intima and adjacent media. Lipids are found between the elastic and muscle fibers of the media or in the subendothelial ground substance. The next step is one of cellular reaction by round cells, lipophages, and fibroblasts. The internal elastic lamina seems to resist destruction for a long time. However, this finally ruptures, whereupon "foam cells" appear in the media (5,18).

Intimal plaques are formed by a proliferative process following accumulation of "foam cells", and fibroblasts. There is elastic proliferation and immigration of smooth muscle cells from the media (3,5,18). Medial necrosis with formation of lipoid "pools" and subsequent calcification of these foci may occur (5,18).

With the cessation of cholesterol feeding, regressive changes appear. This is represented by a disappearance of lipoids, initially from the intima and adjacent media. Subsequently fibrosis dominates the picture. There is a persistence of cholesterol crystals surrounded by fibrous connective tissue. Calcium deposition is frequent in these old lesions (5).

D. Production of Atherosclerosis:

Cholesterol may be fed in many ways and many forms with the resultant production of atheromata. Foods rich in cholesterol may be fed. These include egg yolk, brain, liver, and hydrous wool fat. At present pure cholesterol is most frequently used. Solid cholesterol may be mixed in with the ration, fed in capsules, or dissolved in an oil and either fed by stomach tube or mixed in with the food. Cholesterol may also be used as a colloidal suspension in water and given by intravenous or intraperitoneal routes. When given parenterally in 5% sodium oleate it is rapidly removed from the blood stream by the reticulo-endothelial system. Duff (18) states that the method of choice is cholesterol in oil fed by stomach tube for it produces the most rapid changes.

The oils which have been used successfully as solvents for cholesterol are sunflowerseed, cottonseed, and olive oil, (54), or 5% sodium oleate. The cholesterol content used varies from 2-7.5% (18).

In general, microscopic changes develop only after 1-2 months of feeding cholesterol in oil to a total of 10-20 grams of cholesterol. Gross lesions require about 30 grams of cholesterol over a 2-3 month period. Thus there are very important factors of time, dosage, and form of cholesterol which must be fulfilled (18). Under these conditions of cholesterol feeding the blood levels of cholesterol will rise from normal values of about 100 mg.% to 500-1500 mg.%. Some animals show a progressive steady rise to a maximum level. Others show marked fluctuations, while still others will reach their peak and then drop back to normal levels for the remainder of the feeding period (64).

E. Factors Modifying the Course of Atherosclerosis:

1. The General Diet: The concentration of attention on the cholesterol content of the diet has detracted from a consideration of the general nature of the diet. Generally it should be the same as the control diet and not deficient in any respect (18). It is discouraging that authors do not state the composition of their diets or do so only in general terms.

Most of the experimentation concerning diet has been done with protein, but the experiments were poorly controlled (53). High protein-low cholesterol diets were fed to rabbits with the production of typical atherosclerotic lesions (58). These findings have not been reproduced (7).

2. Special Dietary Factors: Rabbits fed cholesterol plus 1-5 grams of crude soya lecithin per day were found

to develop less atherosclerosis over a 4 month period than the control animals. The same general effect was obtained with choline supplementation. Choline is found in crude soya lecithin. It does not prevent the development of cholesterol atherosclerosis but does delay its appearance and accentuates the reabsorption of atheromatous lesions as compared with control animals (72).

Thiamine hydrochloride and ascorbic acid have not been found to have any influence (27). Vitamin E was reported as not modifying the deposition of cholesterol in the aorta (15), however, when injected in dosages 10 X higher it was found to appreciably augment the deposition (10).

3. Chemical Factors: It was found that the addition of cholic acid or glycocholic acid to high cholesterol diets produced a significant increase in blood cholesterol levels. The deposition of cholesterol was increased over that of the control rabbits (55). Lipocaic does not affect blood cholesterol levels of normal animals but it greatly modifies the rise in cholesterol-fed animals. Nevertheless, lipocaic markedly reduces the deposition of cholesterol. No effect was obtained with the heated hormone (23).

Potassium thiocyanate was found to produce a moderate rise in serum cholesterol without feeding but it did not effect these levels if cholesterol was fed. No reduction in cholesterol deposition was found (38). Potassium iodide is effective in preventing both a rise in serum cholesterol and a deposition of cholesterol in arterial walls. Potassium

bromide has no effect. These same results were found using whole thyroid extract which is more effective than thyroxin (18,73). There was a marked weight loss in the animals given iodine compounds. An inhibitory action is also found with the di-iodide of ricinsterolic acid (60). In connection with the work on iodine compounds it is interesting to note that thyroidectomy increases the atheromatosis (18).

4. Physical Factors: There seems to be an increasing involvement of the coronary arteries with heavier hearts (43). Some reports suggest that blood pressure may be a factor (59). Suspending rabbits by their hind feet to increase the blood pressure will enhance the atheromatosis occurring with coincident cholesterol feeding (18). Unilateral sympathectomies of lumbar 2,3, and 4 ganglia have been done in rabbits fed cholesterol. After three months it was found that more cholesterol was deposited in the opposite iliac artery. The non-operated controls showed bilaterally symmetrical deposits (34).

Injury to the arterial wall by cauterization, combined with cholesterol feeding, produces lipoid accumulations at those areas. If silver cuffs are placed around the arteries and cholesterol is fed to the rabbits, lipoid accumulations are found most prevelent in those areas (87). Injury to arteries, by whatever means, makes possible the accumulation of sudanophilic fat in the aorta earlier than with cholesterol feeding alone (18).

F. Experimental Diabetes Mellitus and Atherosclerosis:

1. General Considerations: The fact that in man diabetes mellitus accentuates the process of coronary artery atherosclerosis (13) has stimulated experimental work along these lines. Despite all efforts, true diabetes mellitus comparable to that seen in man has not been produced in experimental animals. In dogs and cats total pancreatectomy produces a severe diabetes with polydipsia, polyuria, hyperglycemia, glycosuria, ketosis, emaciation, and death in days to weeks. Insulin may prolong life for months but the animal develops severe fatty degeneration of the liver and dies. If choline or methionine is added to the diet the fatty degeneration of the liver does not develop and the animal may live indefinitely (6).

Other methods of producing experimental diabetes mellitus are by 1) injection of anterior pituitary extract, 2) injection of massive doses of urea, 3) creating a persistent hyperglycemia, and 4) injection of alloxan (6).

The factor of ketosis and acidosis, so commonly seen and easily produced in human diabetes mellitus, is unusual in experimental diabetes. To my knowledge no prolonged ketosis has been produced in rabbits or rats. Even the production of transient, mild ketosis demands vigorous methods. Another difference between human and experimental diabetes is the extreme resistance of diabetic animals to the development of arterial disease (19).

2. Alloxan Diabetes and Atherosclerosis: Recently Duff and his co-workers have done considerable work on the relationship between alloxan diabetes and cholesterol atherosclerosis in rabbits. By injection of a 5% aqueous solution of alloxan monohydrate followed by daily injections of protamine zinc insulin and glucose for 1-2 weeks they have been able to produce a diabetic-like state in rabbits. It is characterized by fasting blood sugars of 300 mg.% or more, persistent polyuria, glycosuria, polydipsia, polyphagia, and weight loss. There are histologic changes in the pancreas and kidney at post mortem. There was no mention of ketosis or acidosis in these animals. A large number of animals were needed because about 50% die from the procedure and about another 10% will convert to a normal state again (19).

By gross and microscopic examination they found a striking resistance to atherosclerosis in the diabetic animals in spite of a hypercholesterolemia as high or higher than the controls. The "alloxan-recovered" group paralleled the controls and served as a control for the diabetogenic procedures. During the cholesterol feeding period they found a rise in both free and ester fraction of the serum cholesterol. The diabetic serum showed a more markedly visible lipemia than that from the non-diabetic animals. In all cases the arterial lesions were characteristic of cholesterol atherosclerosis. No other organs, except the pancreas and kidneys, were involved in the various animals (19).

Further experimentation showed alloxan diabetes to have no effect on regression of atheroma (20). The inhibitory effect of alloxan diabetes was found to be independent of sex, weight, daily dosage and form of cholesterol feeding, actual degree of hypercholesteremia, and changes in body weight. The only observed factors were the diabetic state and the degree of visible lipemia (20).

Subsequent work demonstrated that alloxan diabetic rabbits which do not develop atheroma have a marked rise in serum lipid phosphorus and neutral fat. This increase parallels the rise in serum cholesterol. Those alloxan diabetic and control rabbits which do develop atheroma do not show this rise in serum phospholipids and neutral fat. Thus the mechanism seems independent of the diabetic state per se (21).

G. Detergents in Atherosclerosis:

Certain naturally occurring substances of plasma, such as lecithin, fatty acids, proteins, and bile salts, exert an influence on the stability of colloidal cholesterol. Because of this, Heuper studied the effects of certain detergents given orally and I.V. to rabbits (39). He gave Tergitol 08, Nacconal FSNO, and Triton K60 in daily feedings by stomach tube. The animals died in 1-3 weeks of diarrhea and severe congestive lung changes. Intravenous Nacconal NRNO, Triton NE, and Aerosol OT gave severe local necrosis about the veins. At postmortem medial calcification of the aorta was frequently found (39).

Several years later Kellner and his group reported being able to produce sustained elevations of serum cholesterol and phospholipids with the intravenous injection of Tween 80 and Triton A-20. The sera were milky during the experimental period and became clear with cessation of the injections (46). In the same year this group reported that rabbits had been kept on diets without any added cholesterol but with intravenous injections of Tween 80 twice daily for 8-14 weeks. Hypercholesterolemia was higher in these rabbits than in the controls yet the group had a significantly lower incidence of atherosclerosis. It was also noted that the experimental group had a corresponding rise in phospholipid levels with the rise in cholesterol. The controls did not. Thus they stressed the importance of phospholipids (49). They have done more extensive studies recently which fully substantiate their previous reports (44). They noted convulsions following injections of Triton A-20 but no obvious sequellae result. Tween 80 caused no convulsions but was responsible for 4 deaths as compared to no deaths from Triton A-20. Tween 80 produces its effects on serum lipids in a matter of 6-12 hours while Triton A-20 takes 1-3 days when given by the intravenous route. The animals are in good health and gain weight (44). It has been established by several sources (45,61) that intravenous detergents exert their protective action against atherosclerosis by causing a rise in serum phospholipids to parallel the hypercholesterolemia. Oral detergents do

not have this protective action and do not cause this concomitant rise in serum phospholipid. Intravenous detergents do not effect the regression of atheroma.

Another interesting difference has been pointed out by Payne and Duff (61). In animals given intravenous Tween 80 they encountered no atheroma but extensive collections of lipophages were found in the spleen, liver, lymph nodes, pulmonary alveolar capillaries and glomerular capillaries. These changes were not found in animals given oral Tween 80.

H. Lipoproteins and Atherosclerosis:

Gofman and his group have studied the serum lipids and lipoproteins by ultracentrifugal flotation methods. They have described "giant molecules" in serum. In studies on normal rabbits they found no lipoproteins of molecular weights above the Sf 8-10 class. By conducting cholesterol feeding experiments they found a rise in the Sf 5-8 group and new "giant molecules" of the Sf 10-30 class. At no time in the course of cholesterol feeding was a large fraction of the serum cholesterol transported as chylomicrons. They observed that the rise in serum "giant molecules" did not occur until cholesterol levels had risen to about 200-250 mg.% after 30-40 days.

After autopsy they found that there was a high degree of correlation between the development of atheroma and the presence of the Sf 10-30 class of lipoproteins in the serum. This correlation was not found for the Sf 5-8 group (30). With alloxan diabetes it has been reported that cholesterol

is transported in the high density group of Sf 40-100 rather than the Sf 10-30 group as in the straight cholesterol feeding experiments (42).

III. Human Atherosclerosis

A. Occurrence:

Atherosclerotic lesions of the aorta have been reported as occurring from birth. The earliest changes are found in the aorta followed by the coronary and cerebral arteries. Arteries of abdominal organs, peripheral and pulmonary arteries are also involved (5,22). Early aortic atheroma are usually in the thoracic portion but later the abdominal aorta predominates (22).

These changes are much like those produced experimentally and present themselves as yellowish-white plaques of varying size and shape. Microscopically there are varying degrees of lipoid infiltration and fibrous tissue reaction. In some cases crystals of cholesterol are found.

Yater and his group reported a series of 450 autopsied cases of coronary artery disease in males between the ages of 18 and 39 years. From a review of the literature they found 744 cases reported under 40 years of age and 95% of these were in males (91). Cerebral artery involvement is rare before the age of 30 (22). Most of the changes in the coronary arteries occur in the left anterior descending branch. Although most of these had some degree of atherosclerosis, the actual cause of occlusion was atherosclerotic in about

50% and thrombotic in the remainder (91). Death from coronary occlusion is twice as common in the diabetic as compared to non-diabetic males and three times as common in diabetic females (14).

B. Experimental Studies Done in Human Cases:

There has been a lot of attention devoted to specific nutritional factors without much concern about the general nutritional state. An analysis of insurance statistics show that coronary occlusion and cerebral vascular accident are more common in obese than in average or underweight individuals. Wilens found that blood pressure and age were more important than obesity (88). In another study he proposed that atheromatous deposits may regress during times of weight loss and be added again during weight gain, thereby forming a dynamic process (89).

The human aorta always shows some degree of metachromasia due to the chondroitin-sulfuric acid content of these tissues (25,66). This reaction is found in the intima and inner part of the media. The reparative process subsequent to injury produces an increased metachromatic reaction. The deposition of cholesterol occurs only as long as the reparative processes are active (25).

Buck and Rossiter analyzed the lipoids of "normal" and atherosclerotic aortas and attempted to dissociate the lesions of age from those of pathology. In the aged there was more cholesterol ester than free cholesterol. The reverse was found in atherosclerosis. The phospholipid deposits

were entirely sphingomyelin in the aged while in atherosclerosis both sphingomyelin and lecithin are increased. There is presumably a greater amount of neutral fat in atherosclerosis (11). However, Gould states that it is primarily cholesterol esters that accumulate in atheroma (31).

Ahrens and Kunkel stated that the milky or lipemic quality of sera is not dependent solely upon the total lipid concentration. Since lipids exist as colloidal particles, the clarity or turbidity of the serum will also be determined by the size of these particles. They studied clear sera from patients with the nephrotic syndrome (2). They compared lipid patterns and found:

Constituents	Clear Sera	Milky Sera
Major Component	Phospholipid	Neutral Fat
Cholesterol Content	No correlation	No correlation
Ratio: $\frac{\text{phospholipid}}{\text{cholesterol}}$	Greater	Less
Ratio: $\frac{\text{Total cholesterol}}{\text{Free cholesterol}}$	Greater	Less
Total Cholesterol	Usually less	Usually more
Free Cholesterol	Usually more	Usually less
Neutral Fat	Usually less	Usually more
Total Phospholipid	More	Less

They were unsuccessful at attempts to clear lipemic sera in vitro but were able to produce milky sera from clear sera by enzymatic degredation of serum lecithin by the alpha

toxin of Cl. welchii. They were also able to show a direct relation between phospholipid breakdown and plasma turbidity but were unable to evaluate the effect of bile salts contained in some of the sera (2).

In a study of the effect of diet on blood levels, it was found that single doses of ingested cholesterol produce trivial or transient changes in serum cholesterol of man. Over long periods, reducing the cholesterol intake by 50% did not reflect itself in changes of blood cholesterol levels. Only on a rice-fruit diet, which is cholesterol and substantially fat free, was the serum cholesterol lowered. This same diet produced an even more marked decrease in serum cholesterol levels in patients with idiopathic hypercholesterolemia. There seems to be a critical level of cholesterol intake between 0-200 mg.% per day above which there is no effect. It is of significance that by changing from a rice-fruit diet to one containing vegetable fats but no cholesterol, the blood cholesterol levels started back to their previously high levels (48).

Gofman et al. studied the lipoproteins in human sera. They found a high degree of correlation between the Sf 12-20 class of lipoproteins and coronary artery disease (42).

IV. Experimental Arteriosclerosis

A. History:

This type of lesion is commonly called adrenalin sclerosis because it was first produced in 1903 by injections

of adrenalin. Since that time similar alterations have been produced by a multitude of agents and methods (5,37).

B. Morphology and Distribution of Lesions:

1. Gross Appearance: The lesions begin as minute white spots which increase in size, vary in shape and magnitude, and rise from the intimal surface. They are firm with a tendency to become confluent and grayish in color. They may undergo subsequent umbilication, form aneurysmal dilatations, or dissecting aneurysms. Thromboses are seldom seen (5). The lesions occur most commonly in the thoracic aorta and are rare in the abdominal aorta, renal, carotid, or pulmonary arteries (5).

2. Microscopic Alterations: The early changes are seen in the media and consist of focal degeneration and deposition of fine calcium granules along the elastic lamellae in the ground substance of the inner layers of the media (5,74). Further changes consist of necrosis of medial muscle cells and fragmentation of the elastic elements with formation of medial calcium plaques. Intimal lesions of a proliferative type may occur but these are of a secondary nature. Lipoid accumulations are insignificant (5,37,74). In some cases calcification is found in the kidney, lungs, and stomach (74).

C. Production of Experimental Arteriosclerosis:

Changes of the adrenalin type have been produced by such a wide variety of agents that it is difficult to classify or innumerate them all. Hueper has written a

survey of the subject in which he attempted a classification on the basis of anoxemia from various causes:

I. Vasotonia: Among the many substances included here are histamine, acetylcholine, nitrates, cyanides, mercury, and arsenic. Others are epinephrine, tyrosine, tyramine, thyroxin, ephedrine, ergotine, physostigmine, nicotine, digitalis glycosides, vitamin D, chemicals producing acidosis and hypercalcemia, and psychic strain (23,37,74).

II. Intravascular Hydrostatic Pressure: This includes artery ligations, congenital anomalies of the vascular system, or phosgene poisoning.

III. Colloidal Plasmatic Instability: This includes carbon disulfide, thiourea, thiocyanates, polyvinylosis, pectinosis, etc.

IV. Hematic Anoxemia: Among these are agents producing inert hemoglobin derivatives or disturbances in the balance of oxygen and carbon dioxide (37).

In my studies I have found that dietary measures alone are capable of inducing medial calcification of the rabbit aorta. These diets were composed basically of oats or soybeans to give a protein content of 12-14%, carbohydrate 68-72%, and fat 5-7%. The diets produced an acidosis which I believe was the only difference from the control diets. There was a definite predominence of the lesions in the male. The factors of age and weight had no significant effect. The incidence of medial plaques in the experimental group

was significantly above that of the controls and also well above the figures given for the occurrence of spontaneous changes.

D. Pyridoxine Deficiency in Monkeys:

Recently Rinehart and Greenberg have presented their work on pyridoxine deficiency in monkeys (65,66). On a diet deficient in pyridoxine they found that their monkeys only gained weight for about 2-3 weeks and thereafter became emaciated. They were unkempt, sluggish, and hyperirritable. The hair thinned and there were fissures of the hands and feet. The blood showed an anemia and leukopenia.

At autopsy the arterial lesions were the most prominent and constant findings. The sclerotic changes were found in all animals. The changes were of a focal nature and appeared in the arterial branches of the pancreas, kidney, serosa of the colon, renal, coronary, and testicular arteries. There were fibrous tissue plaques between the endothelium and internal elastic lamina in these arteries. There was occasional splitting and reduplication of the elastica, while calcification was not a prominent feature. No gross lesions were seen in the thoracic aorta but some were found in the abdominal portion. More intensive studies indicated that a prominent feature was the accumulation of a mucoid intercellular substance showing metachromasia characteristic of mucopolysaccharides. This material was found in the intima and sometimes the media.

F. The Anti-Stiffness Factor of Guinea Pigs:

1. Introduction: No discussion of experimental medial calcification of arteries would be complete without some mention of the extensive work carried out by Wulzen, van Wagtendonk and their group on the "anti-stiffness factor" of guinea pigs. These workers reported in 1941 that guinea pigs kept on diets of pasteurized milk developed symptoms beginning with wrist stiffness. The diets were fully supplemented with vitamins and minerals. The animals showed weakness, muscle atrophy and some tendency to paralysis. They gained weight and grew well but after about a year began to lose weight (90). This disease is specific for guinea pigs under the given conditions.

Subsequent study revealed that substances curative for the condition are extract of raw cream, and methylvinylketone (90). An isolated factor from raw cream will cure the stiffness in 5 days in dosages of 0.1 gamma per day (75). A factor isolated from molasses or raw cane juice was shown to be curative in 5 days in amounts of 0.002 gamma per day (82). The antistiffness factor (A.S.F.) is believed to be a steroid compound with a probable formula of $C_{28}H_{46}O$ (67).

2. Pathologic Changes:

a. Cardiovascular: About 25% of the animals had changes which consisted of focal necrosis and calcification in the ventricular myocardium. There was no inflammatory reaction. There were also nodules in the aorta, femoral, and pulmonary arteries but never the pulmonary artery alone. The aortic lesions were

most often in the ascending aorta and arch. Somewhat later they appeared in the thoracic aorta and rarely in the abdominal aorta.

The alterations consisted of bands of medial calcification and connective tissue replacement of the elastica. Some times the medial calcification was associated with an intimal reaction of dense connective tissue but the adventitia was normal.

b. Parenchymal: The musculo-skeletal system showed the most constant changes which consisted of small calcified abscesses with peripheral foreign body giant cells. They were found about the knees, elbows, shoulders and in intercostal and leg muscles. No bone destruction was seen but periosteal new bone growth was observed near joints. The joints were seldom invaded and then only with minimal erosion of cartilage.

Skeletal muscle contained varying degrees of necrosis and hyalinization with limited regeneration. Calcification occurred either parallel to the muscle fibers or in the necrotic cytoplasm. No nerve or blood vessel changes were seen. Calcification of the muscularis of the stomach and intestine was seen. The liver presented areas of focal necrosis and calcification of necrotic cells. Calcified masses were observed in the convoluted and collecting tubules and sometimes in the interstitial spaces of both the cortex and medulla of the kidneys.

Tricalcium phosphate was found in the lungs and under the skin. The endocrine glands were normal (32,90).

3. Special Studies: They noted that the symptoms were similar to those of a vitamin E deficiency. Vitamin E, however, did not prevent or cure the symptoms. Further, a deficiency of A.S.F. did not produce the creatinuria and muscle dystrophy that vitamin E deficiency caused. They therefore resolved that the two conditions are different entities (76).

The abnormal deposition of calcium phosphate suggested a derangement of phosphorus metabolism. Therefore they made studies of the various phosphorus fractions of the blood. They measured total phosphorus, inorganic phosphorus, easily hydrolizable phosphorus (A.T.P. and A.D.P.), alcohol insoluble phosphorus (glycerophosphate and glucose phosphate), mercuric insoluble phosphorus (stable nucleotide), and alcohol soluble phosphorus. They found a decrease in A.T.P. and A.D.P. with increases in glycerophosphate, glucose phosphate, and stable nucleotide in the liver. The kidney showed the same trend with a greater increase in inorganic phosphorus. In both cases supplements of the A.S.F. brought values back to normal in 5 days (77).

They have also shown a decrease in serum alkaline phosphatase in deficient animals (78). There is a marked increase of serum inorganic phosphorus with an increase of total serum and muscle calcium (79). Muscle inorganic phosphates also increase and there is an associated decrease

in creatine phosphate, A.D.P. and A.T.P. This change parallels the decrease in easily hydrolizable phosphorus previously reported (80). They have also noted an increase in total serum protein and globulin with decreases in albumin and the ratio. Changes in tissue oxidation have been reported (83). The liver has a low glycogen content during A.S.F. deficiency. The nucleotides and nucleosides also decrease in the liver, kidney, and blood (84). The plasma non-diffusible calcium increases while the diffusible calcium decreases during the deficiency. A slow return to normal levels follows the addition of A.S.F. (85).

V. Human Arteriosclerosis

A. Distribution and Morphology:

Medial calcification of arteries in man affects primarily the muscular arteries such as the tibials, femorals, radials, and temporals but is rare in the large elastic arteries as a primary change. Gross calcification of the media of the aorta has been observed but is rare in young persons. The coronary arteries may be affected to some degree by medial calcification but atheromatous-plaque formation is more conspicuous (4,8).

The condition may be found in all age groups from birth to senility. Field (26) reported the case of a 10 week old infant showing general debility and wasting. The coronary arteries had medial calcification and intimal proliferation while the heart contained myocardial fibrosis. Calcific

lesions were also found in arteries of the larynx, thyroid, mesentery, pancreas, adrenals, and kidneys. The aorta was clear. A review of the literature on medial calcification permitted a collection of 14 reports of such changes in arteries of infants. This included a one day old premature infant. Usually the medium and small arteries are affected (26).

Medial changes include swelling and fragmentation of the elastic fibers, fatty infiltration, and calcification about the elastic fibers. Medial calcification may occur without any involvement of the intima or adventitia. Early deposits of calcium are of a finely granular nature. These occur between the smooth muscle cells where the elastic fibers were. Later both elastic and muscle cells may be infiltrated with resultant obliteration of the cellular structure (8).

B. Experimental Studies on Human Beings:

Mineral deposits in atherosclerotic aortas show increases in non-lipid phosphorus, calcium, magnesium, and fat free dry residue. There is a decrease in water. The calcium:phosphorus ratio is the same as in bone (12). Obesity does not correlate with dry weight, cholesterol content, or calcium content of arteriosclerotic aortas (24). Microincineration methods correlated with selective staining have shown that persons under 60 years of age have more calcium in the abdominal than thoracic aorta. Younger persons show more calcium in regions of intimal plaques

while the older people have a diffuse deposition. Calcification in the aortic media varies from 4% of those under 20 to 100% of those over 50 years of age. The process is progressive with increasing age (8). Studies have shown that medial calcification is most intense in the region of an intimal plaque but intimal plaques are not found without medial calcification (8). With increasing age there is a slight drop in elastin content and then a marked increase in the calcium deposits. An analysis of the amino acids shows a definite increase of aspartic acid and a lesser change in glutamic acid (50). Other studies on elastic tissue and calcium changes in various arteries indicate that the intensity and rate of calcium deposition are directly proportional to the intensity and rate of elastic tissue changes(9).

VI. Basic Considerations in Experimental Atherosclerosis and Arteriosclerosis:

A. Experimentation:

In trying to seek out the fundamental mechanisms involved in these processes it is difficult to work with human beings because of the multiplicity of factors which have not been controlled. Thus it is necessary that experimental animals be used where a base line can be established and the various factors modified from that level. Animals can be controlled regarding diet and environment with the founding of a base level. Then single factors may be introduced

on this back-ground and an adequate evaluation obtained of the factor or factors being studied. Experimental studies are directed toward the production of lesions like those seen in human beings so that mechanisms may be defined and applied to human pathology. To date no changes have been produced experimentally which correlate exactly with the human lesions in morphology and distribution (5,18). However, it must be remembered that we are dealing with animals which are unlike man with respect to gross and microscopic anatomy, physiology, and metabolism. These differences probably account for the difficulty in correlating changes produced in experimental animals with those found in man.

B. Species Differences:

Fox has pointed out that "Too little attention has been given, before starting experiments, to the contrasting reactions of animals under similar stimuli...." (28).

1. Type of Lesion: Fox found a tremendous qualitative and quantitative variation of spontaneous vascular sclerosis in a large series of mammals and birds. Thus he found that intimal thickenings were more common with the dog, cow and horse; intimal atheroma were fairly characteristic of monkeys and birds, while calcareous deposits are found most often in cats, marsupials, rabbits, and shore birds. Both chickens and rabbits had a relatively high incidence of spontaneous lesions (28). Of all animals, birds most closely resemble human beings in developing spontaneous atheroma (28,31).

It is interesting to note that under similar experimental conditions of cholesterol feeding the rabbit aorta responds with "foam cell" lipoid cushions while the guinea pig aorta reacts by endothelial proliferation and very little "foam cell" formation. This is also true for the parenchymal changes. It is known that the rabbit and hamster livers form "foam cells" from von Kupfer cells while the guinea pig liver shows fatty degeneration and vacuolization of the liver cells without "foam cells" (3).

2. Species Susceptibility: As experimentation has progressed in this field it has become increasingly apparent that animals vary greatly in their susceptibility to the development of experimental vascular lesions just as they vary in the development of spontaneous lesions. Thus it was previously thought that lesions could only be induced in rabbits and guinea pigs. Now other animals are used even though it is more difficult and demands vigorous methods (71). The present status, with respect to cholesterol, is reported as being:

a. Rabbits respond most readily to cholesterol feeding.

b. Guinea pigs respond less readily.

c. Chickens are next but have a high incidence of spontaneous atheroma.

d. Monkeys, cats, dogs, foxes, rats, and mice are much more resistant and need special conditions.

Because of the ease in producing atheroma in rabbits, this specie has been used predominantly in experimentation (3).

3. Anatomy: Various anatomical factors have been advanced to account for these species variations. The main differences in the arteries of various animals deal with the degree of development of the subendothelial fibrous tissue and the internal elastic lamina. Thus the intima of man, some lower primates, and a few bovines has subendothelial fibrous tissue and a musculo-elastic layer sufficiently developed to be recognized. Most other animals do not, unless associated with vascular lesions (28). Thickening of the intima occurs in animals with increasing age. This is more true of the larger animals (e.g. dog) than the smaller animals (e.g. rabbit) (5). In the rabbit, the aortic intimal layer consists of lining endothelium separated by intercellular ground substance from the internal elastic lamina (18). Birds have relatively larger hearts than mammals without any arterial internal elastic membrane (28). In a study of the supply of vasa vasora to the aorta, the vessels decreased in the following order: dog, man, chicken, and rabbit (68).

4. Metabolism: Animals fed equivalent amounts of cholesterol respond differently with respect to blood cholesterol levels (64). The concentration of cholesterol in the tissues is relatively constant for the various species. The normal average values of plasma cholesterol vary from 32 mg.% in the guinea pig, 50 mg.% in the rabbit, and 100 mg.% in chickens to 150 mg.% in dogs and 200 mg.% in man (31). There is also a difference in cholesterol esters

in the liver. In man 20% of liver cholesterol is esterified as compared to 5% in the rabbit and dog (31).

5. Additional Factors: It should be mentioned that man's upright position exerts hemodynamic effects not observed in animals. It is certain that man is subject to psychogenic forces and probably animals are also. However, this factor is very difficult to evaluate.

C. Spontaneous Lesions:

Spontaneous arterial lesions in rabbits are invariably due to medial calcification, with atheroma very rarely encountered (28). Reports on the incidence of spontaneous calcification varies from 0-35%. None of the authors considered the factors of diet, age, weight, sex, or strain which have been suggested as possible explanations for the wide range of results (35,53,56,57,62).

D. Vascular Anatomy:

Endothelium, which forms the lining membrane of both arteries and veins, may arise either from mesenchyma or preexistent endothelium. Some animals have a subendothelial connective tissue layer. The media is made up of musculoelastic elements in layers which are more or less delimited from intima and adventitia by the internal and external elastic membranes. The "elastic-muscular" layer is found in normal human arteries and most distinctly in the coronary arteries. It consists of the two innermost elastic layers of internal elastica and the longitudinally arranged smooth muscle cells in between (3). The adventitia is the outer

vascular-connective tissue layer through which the vasa vasora enter.

Altschul has been most interested in the cytology of these vascular changes and it is from him that most of our information comes. He describes a process of metaplasia which he calls "dedifferentiation of endothelium". Endothelial cells leave the lining stratum, develop many processes, and become embedded in an amorphous ground substance. The cellular division may be by mitosis or amitosis. In many cases this mesenchyma-like tissue makes up a definite layer called the subendothelial layer. The author states that these dedifferentiated endothelial cells may persue one of many courses. They may 1) persist as mesenchymal cells, 2) degenerate when their debris becomes incorporated into atheroma, 3) act as parent cells for fibroblasts, intermediate cells, smooth muscle cells, or hemoblastic elements, 4) redifferentiate into new vessels, or 5) may form "foam cells" (3).

Wilens (86) describes a process in human beings which he calls "diffuse intimal thickening". This has been referred to before as the formation of a subendothelial connective tissue layer. From his studies he concluded that at birth the aortic intima is a single layer of endothelium which lies on its own basement membrane or the innermost elastic lamella of the media. Early in life the intima acquires a subendothelial fibrous zone and becomes separated from the media by smooth muscle and elastic tissue called the

musculo-elastic layer. Since this change occurs with regularity and uniformity most authors regard it as a normal developmental process. It occurs early in life long before there is evidence of atherosclerosis and in the absence of lipoid deposits. He points out that this process is not found in all arteries but appears at sites where atherosclerotic changes develop later. Thus the abdominal aorta is affected more than the thoracic aorta, the iliacs and femorals more than the carotids and innominates, and the coronary more than the tibial and radial arteries. His studies also showed that though this process begins early in life it continues throughout life in a progressive fashion.

E. Vascular Physiology:

The vascular walls are nourished from two main sources: 1) the vasa vasora supplying the deeper layers of the media and the adventitia, and 2) a tissue-lymph permeation across the endothelium for the supply of the intima and inner layers of the media (3,5,37). This work has been substantiated by supravital staining. Following injection of trypan blue into various animals the aortic lining was found to be uniformly permeable but also showed areas of concentration in which the dye entered from the vasa vasora (18). Normally the endothelial cells are not phagocytic and become so only after inflammation or histamine stimulation (3).

F. Lipid Metabolism:

The chemical and physical techniques for the isolation

and characterization of lipids have only recently been developed and are still being perfected. Because of this, lipid metabolism is not well understood. Lipids are principally present in limiting membranes of cells, mitochondria, nuclei, and nucleoli (31).

1. Types of Lipids:

a. Neutral Fats: These are nixtures of triglycerides of various fatty acids, both saturated and unsaturated. Neutral fats contain less highly unsaturated fatty acids than are found in phospholipids or cholesterol esters. They are found only in two types of cells: 1) liver cells and 2) special fat cells of fat depots and interstitial tissue. Neutral fats are not found in the central nervous system. One function of the above cells is to store energy. Neutral fats vary more than any other body constituent (31).

b. Sterols: Cholesterol is the main member but the group also includes dihydrocholesterol, 7-dehydrocholesterol, and other less important sterols. Cholesterol occurs partly in the free form and partly as an ester. Nevertheless, all cholesterol is protein bound. About 90% of the cholesterol in the adrenal and 67-72% of plasma cholesterol is esterified while most other tissues contain a small amount of the cholesterol ester. Thus most liver cholesterol is "free". Ester cholesterol is much more stable than "free" cholesterol. Cholesterol is found in all body

tissues and is an essential constituent of mammalian cells but is not found in plants. However, plants have their own related sterols, even though cholesterol is found only in foods of animal origin (31).

c. Compound Lipids:

1) Phospholipids

a) Lecithin
b) Cephalin
c) Sphingomyelin

2) Cerebrosides

Phospholipids are found in all living tissues and are therefore essential for life. Plasma phospholipids do not transport fatty acids from one tissue to another but seem to be concerned in some way with maintaining plasma lipids in a state of optical clarity. In most tissues lecithin is the principle compound lipid followed by cephalin and sphingomyelin, whereas in mammalian plasma, lecithin constitutes 80%, sphingomyelin 15%, and cephalin only 3-8% of the phospholipids (31).

Cerebrosides are present in small amounts in many, if not all, tissues and in greater quantities in the brain. Very little is known about the function and metabolism of cerebrosides (31).

d. Fatty Acids:

1) Common Non-essential Fatty Acids: These contain 18-20 carbon atoms. They may be saturated or have one unsaturated bond.

2) Essential Fatty Acids: These include linoleic, linolenic, and arachidonic acids. At least one is necessary to prevent the deficiency symptoms of caudal necrosis in rats. These acids are highly unsaturated.

3) Specialized Fatty Acids: These are the long chain fatty acids which are found in cerebrosides and sphingomyelin.

Diet will influence the nature of fatty acids in neutral fats, lecithin, and cephalin but not the fatty acids of cerebrosides or sphingomyelin. Fatty acids are present in neutral fats, phospholipids, and cholesterol esters. The fatty acids of cholesterol esters are mainly of the essential unsaturated variety. Those of lecithin and cephalin are less unsaturated whereas neutral fats contain more of the saturated type (31).

2. Origin of Plasma Lipids: Neutral fats are readily synthesized in the body from carbohydrate or protein. Cholesterol can be synthesized by most animal tissues except brain and plasma. The liver is probably the main source of plasma cholesterol. The estimated rate of endogenous cholesterol synthesis in man is 1.5-2.0 grams per day (31). Diets containing only vegetable fats are capable of causing an elevation of plasma cholesterol (31,48). While tracer studies suggest that virtually all of the plasma phospholipid is formed in the liver, any cell in the body may synthesize its own phospholipids if provided with essential

fatty acids, a source of labile methyl groups, and inositol. The common non-essential fatty acids can be synthesized in all tissues of the body from other fatty acids, carbohydrate, or protein. The essential fatty acids however, are not synthesized by the body (31).

3. State of Plasma Lipids: Neutral fat occurs in plasma in a finely emulsified form, the fat droplets being stabilized by a protein film. It is probable that all other lipids occur only in combination with proteins as lipoproteins (21,30,31,42). The lipoproteins are definite chemical compounds and their properties are entirely different from the free lipids (31).

4. Lipoproteins in Serum: Gofman states that all major lipids of the blood including glyceryl ester, cholesterol, cholesterol esters, phospholipids, and fatty acids are transported as giant lipoprotein molecules of several types. In human beings there is a tremendous individual variation in types and concentration of serum lipoproteins. The individual person, however, has a characteristic pattern. At least nine discrete lipoproteins have been isolated from human blood on the basis of molecular density. Lipoproteins are classified according to arbitrary units called Svedbergs of flotation (Sf). One Sf unit is equal to a migration rate of 10^{-13} cm./sec./dyne/gram at 26 degrees Centigrade in a medium of sodium chloride solution of density 1.063. Those components of the low density group (Sf 17 and below) have been isolated as discrete fractions. However, those

of the high density group (Sf 17-40,000) are very difficult to resolve. Chylomicrons constitute those fractions at the upper limits of Sf 40,000 (42). The low molecular weight of lipoproteins is due to the low protein content per molecule (30). The low density class contains most of the plasma cholesterol esters while the high density group contains most of the plasma glyceryl ester (30,42).

The simplest pattern of lipoproteins is seen in young people and normal experimental animals. In this pattern there is no appreciable concentration of lipoproteins above a certain limit (Sf 8-10 for rabbits and Sf 20 for man). All molecules below Sf 20 in man are stable for periods of months. These are not influenced by meals whereas the high density group may fluctuate after meals (30,42). These workers have postulated a metabolic chain starting from the high molecular weights and proceding by degradation down to the low, stable molecular weight classes of lipoproteins (42).

5. Regulation of Plasma Lipids: The interrelationship of lipid components in plasma is more constant and more significant than the concentration of a single component (21). The ratio of plasma cholesterol to phospholipid is normally about 1.6. This is the highest ratio of any tissue in the body. The next highest is 0.9 in the red blood cell while smooth muscle has 0.5 (31).

6. Regulation of Plasma Lipids: Little is known about the factors regulating the distribution of phospholipids

between tissues and plasma. A close relationship is known to exist between plasma and liver lipids. The thyroid probably plays an important role in the regulation of blood cholesterol and phospholipid levels. Since thyroidectomized animals develop elevated serum cholesterol levels and a stable total body cholesterol, the regulatory action is probably one of distribution (31). Single doses of ingested cholesterol will produce only transient and insignigicant increases in serum cholesterol in man (48).

G. The Aging Process and Its Effects on Arteries:

The aorta becomes less elastic with age. This is primarily dependent on changes in the elastic tissue of the media (8,37). Increasing age tends to decrease the capacity to regenerate the elastic tissue (50). The relative width and circumference of the vascular lumen and length of the vessel increases with age (37). Hueper states that the loss of elasticity is not due to a decrease of elastic tissue but by a peculiar axial crystallization of the elastic lamellae. Later there is increased connective tissue, reduction of tensile strength, and failure of effective recoil (37). There are also physicochemical alterations which are poorly understood. The colloidal gels composing the cellular and intercellular matter are finely dispersed hydrates of alkaline reaction in youth. With age there is dehydration, a more coarsely dispersed media, and a more acid reaction (37). The sulfate content of the aorta increases up to 60 years of age and then remains constant (25).

H. Formulation of Theories Concerning Atherosclerosis and Arteriosclerosis:

There are probably as many theories about the pathogenesis of vascular sclerosis as there are investigators. Hueper (37) has pointed out that any theory on this subject must be prepared to explain the following phenomena:

1. The not infrequent absence of lesions in people of advanced age.
2. The not unusual occurrence of lesions in young people.
3. The marked species variability.
4. The focal nature of the lesions.
5. The sex and possible race difference.
6. The connection to endogenous and exogenous causal factors.
7. The experimental production by greatly varying means both chemical and physical.

Experimenters are able to produce the disease in a matter of weeks by a great variety of methods whereas the disease occurs in man over a period of years. Therefore the experimenter must constantly keep in mind whether or not the various factors are within physiological limits; are they within the realm of possibility, or better yet probability? It is imperative to keep all these facts in mind when attempting to evaluate experimental work on this subject.

VII. Phlebosclerosis:

The process of phlebosclerosis is much less common than arteriosclerosis. Altschul (3) proposes several reasons for this low frequency of primary phlebosclerosis:

1. Relatively low blood pressure in veins as compared to arteries.
2. Low or absent exchange between blood of the lumen and the tissues of the venous wall.
3. The venous endothelium is conditioned to the hypoxemic blood.

These venous changes may involve one, two, or all three layers of the venous wall. The alterations consist of varying degrees of hyperplasia, fibrosis, hyalinization, and calcium deposition. Often the lesions are eccentric and may be occlusive.

It is known that a vein under increased tension, such as in an arterio-venous fistula, will become thickened and calcified around the rim of the fistula. Again, if a vein is transplanted into the arterial circulation there is great hyperplasia of all the vascular coats. Some veins which lie adjacent to arteries will form eccentric thickenings on the side nearest the artery. No lipoid material is found in these lesions. Geiringer (29) summarizes this process by saying that every prolonged case of increased venous pressure leads to thickening of the venous wall with subsequent degenerative changes.

VIII. Venous Atheroma

Like phlebosclerosis, venous atheroma are less common than atherosclerosis of arteries. It was considered a curiosity until 1926 when Schilling discovered a 50% incidence of atheroma at the origin of the inferior vena cava. These venous alterations resemble arterial atheroma closely. The changes consist of fatty infiltration of the intima with resultant splitting of the elastica, fibrosis, hyalinization, or calcification.

These atheromatous lesions have been found at only one site in the venous system. This is at the origin of the inferior vena cava where it lies between the last lumbar vertebra and the bifurcation of the aorta. This puts it in a place of maximal mechanical stress, or as Geiringer states, "It is impossible to find a place in the whole venous system at which the vascular parietes are subjected to a greater or more constant mechanical stress".

The normal inferior vena cava does not have a subendothelial layer. Nevertheless, with the increase of venous pressure this layer tends to develop. Venous atheroma are not found where this layer is absent. It is a very localized change in veins as opposed to the diffuse intimal thickening of arteries.

A study of these lesions demonstrated a greater tendency for alterations to occur in old age. There was no connection with hypercholesterolemia or the severity of aortic atheroma. Since there is no difference between venous and

arterial blood concentrations of cholesterol, Geiringer tended to place mechanical stress as the prime atherogenic factor (29).

IX. Theories Concerning the Pathogenesis of Vascular Sclerosis

There have been many theories proposed to explain the pathogenesis of vascular sclerosis. These are based to a certain extent on fact, however, it must be remembered that they are still theories. As we have said before, there are many differences between the experimental and spontaneous human disease so that no direct correlation can be drawn. Therefore we must not apply too much of the experimental data to the human process until it can be justified by factual evidence. Because of this, these conditions will be treated as separate entities.

A. Experimental Atherosclerosis:

Since the original work of Anitschkow, cholesterol has had a prominent place in all considerations of Pathogenesis. His theory of cholesterol infiltration of the vascular wall and precipitation in the ground substance placed cholesterol as the prime factor (5). Since that time there has been a definite trend toward a consideration of multiple factors. Work along these lines has demonstrated the great importance of the interrelationship of serum lipids and the apparent stabilizing effect of phospholipids and neutral fats. The validity of this work is

reflected in the increased predictability of experimental atherosclerosis. The recent work on lipoproteins has shown a direct correlation between certain abnormal fractions of the serum lipoproteins and arterial atheroma. This work lends support to those who feel that the basis of experimental atherosclerosis is an altered lipid metabolism.

Lipophages or "foam cells" have been prominent in the experimental lesions and there has been much speculation as to their origin. Because of the accumulation of these cells in such organs such as the spleen, liver, and adrenals and their presence in lymph channels, Leary (51) has postulated a migration of cells from those organs to the vessel walls. Others, such as Altschul (3), and McMillan and Duff (52), favor the in situ origin of foam cells. They have reached this conclusion on the basis of cytological study, including an evaluation of mitosis. It is most generally accepted that the lipoid material in these cells is of serum origin rather than representing a fatty degeneration.

The most amazing and confusing feature of vascular sclerosis is the peculiar localization of the changes. Perhaps the earliest suggestion was that of intravascular tension producing localized mechanical stress (29). Harrison (33) has suggested that greater mobility of certain parts of the vascular system puts a strain on that segment. Wilens proposes an initial uniform deposition of lipoids with later redistribution to focal points dependent upon relative vascular mobility and fixation (86). Leary (51)

suggests a selective chemotaxis while Rindhart and Greenberg (66) postulate a mucopolysaccharide affinity for lipids. Others, including Schlichter and Harris (68), believe that the development of arterial lesions appears to vary inversely with the blood supply to the arterial wall.

Duff (18) sums up the feelings of a good many workers by saying that "injury, however produced, will facilitate the development of lipoid deposits in the arteries of rabbits fed cholesterol-rich diets". This has been substantiated by DeSutoNagy and Waters (16) who found that lipid deposition occurred at areas of injury produced with allylamine. It is also common knowledge that sites injured by cauterization show increased lipoid deposition. Others have mentioned that deposition occurs where the healing process is active following injury (16,25,33). Thus if cholesterol is fed coincident with the injury it is deposited at the site of injury while feeding cholesterol following the injury gives deposits at the periphery of the lesion.

B. Experimental Arteriosclerosis:

Because of the multiplicity of agents used to produce this condition and the overwhelming popularity of the atherosclerotic process, this disease has been almost completely abandoned. Hueper (37) believes that anoxemia of the vascular wall is responsible for these changes while Schlichter and Harris (68) point to the vasa vasora as the inciting factor. The anti-stiffness factor represents an isolated condition in a single specie and cannot be applied to the general picture as yet.

C. Human Arterial Sclerosis:

The human changes of atherosclerosis and arteriosclerosis are not nearly as distinct as is found experimentally. The two conditions are found to coexist so frequently that one would almost believe that they were dependent on one another. There are those who say atheroma do not occur in the absence of medial calcification, even though this calcium deposition is microscopic.

1. Human Arteriosclerosis: Most of the work on this aspect of the subject has been done in a very limited sense on living people or else post mortem. Thus the study has been limited greatly. Many investigators believe that it represents a natural aging process. The factor of elastin changes has been recently presented (50). It has been stated that age results in a change in the state of certain colloidal elements and consequently there is calcification of these elements (9).

2. Human Atherosclerosis: The story here is similar to that of arteriosclerosis. Mention has been made that the peoples of the Orient have generally lower blood cholesterol levels and less atheroma than the inhabitants of America (31). However, it is rare to be able to demonstrate an elevated blood cholesterol level in man to account for the atheromatous process (8). There is little factual experimental evidence on which to base a consideration of pathogenesis of the human disease. Therefore the line seems to be drawn between those in favor of a faulty lipid

metabolism and those proponents of a natural aging process. Adlersberg thinks that there may be an inborn error of lipid metabolism (1).

D. Treatment of Human Vascular Sclerosis:

There is much controversy about the subject of treating human vascular disease. There are those who maintain that overfeeding, especially with fat and cholesterol, can cause atherosclerosis. On this assumption they propose treatment with low fat and cholesterol diets. However, Keys et al. have shown that the diet must be limited to below 200 mg.% of fat per day in order to be effective in lowering the serum cholesterol to any significant degree. It has also been stated that the daily endogenous cholesterol production grossly exceeds the daily exogenous cholesterol intake. Thus with the body so readily able to manufacture its own cholesterol it does not seem reasonable to limit the intake of cholesterol. However, it would be justifiable if it could be shown that in man it is the exogenous cholesterol, altered perhaps by cooking, that is responsible for atherosclerosis. This has not been done (17).

X. Discussion

The problem of vascular sclerosis is very confusing. Although we have accumulated a vast amount of factual knowledge, there are many large gaps which remain to be completed. Some of the major gaps cover so much ground that several possible paths remain open. It is for this reason that

there are so many different theories on the subject.

The subject of atherosclerosis has been given the place of honor over arteriosclerosis, mainly because this is the occlusive process which is usually the basis for myocardial infarctions. In this respect it is the factor of localization that is of such great importance. Thus it has been pointed out by several observers that a small amount of atheroma placed strategically in a coronary artery may cause the death of the unfortunate individual while his neighbor may have grams of atheromatous material in his aorta and not even have symptoms. What is responsible for this localized deposition of cholesterol? The answer is we don't know. We do not even know if the deposition of cholesterol plays a role of primary or secondary importance. All the recent evidence presented seems to indicate that it is dependent upon a primary factor of local injury. This local injury may be produced experimentally by many different means. Which is the one that acts in the natural state of the animal? Which one is physiological, or is there only one? It does not seem likely that a chemical substance circulating uniformly in the blood stream would not exert its effects uniformly on the vascular wall. Not unless it were initially weakened. Therefore, what makes one segment of artery different from any other? What makes the first centimeter of the anterior descending branch of the left coronary artery more susceptible than the adjacent portion? The most obvious variation is a spatial one. It is this

factor of location which determines how much of the intravascular and extravascular physical trauma will be unleashed upon this particular site in the vessel. This has been well demonstrated by Geiringer in his discussion of venous atheroma.

It is the inherent property of all tissues to compensate for stress or increased demand, by hypertrophy. In accordance with this it seems that when a vessel wall is subjected to increased pressure it tries to compensate by a hyperplasia, including the formation of a new layer; the subendothelial layer. It is interesting to note the direct correlation of blood pressure and the development of the subendothelial layer. A newborn has a systolic blood pressure of about 60 mm. Hg. and no subendothelial layer. As he develops both increase. Wilens has shown that hypertension markedly accentuates this process (86). Thus we have a layer of the vascular wall which is not normally present in infants. The layer develops throughout life and comes to be the main site of lipoid deposition.

We know that cholesterol is not the whole answer to the lipid problem. Lipoproteins and the interrelationship of serum lipids also fit into the picture somewhere. Yater et al. (91) have shown that the incidence of coronary artery disease increases sharply in men between the ages of 30 and 40. On the contrary, women show a gradual rise and only equal the male level at about 60. Gofman et al. (42) have confirmed this finding. They have also demonstrated that

the rise in the abnormal fractions of serum lipoproteins follows a similar pattern. Thus men show a sharp rise in the Sf 12-20 group between the ages of 25-30 while women show a slow, steady rise to reach the male level at about 60 years. All this evidence seems to indicate some defect in lipid metabolism. It may be that this impaired lipid metabolism is the only basic difference between atherosclerosis and arteriosclerosis.

Thus we may hypothesize an initial injury to the vascular wall by physical traumatic factors. This will cause the formation of a suitable ground substance where lipids, altered by a defective metabolism, may be deposited. Though this explanation is inadequate to be sure, and certainly does not explain the pathogenesis of the disease, it does give a working concept which may be used as a basis for further experimentation and observation. Its shortcomings are apparent when we see the aorta of a man of 80 which appears like that of a boy of 20, or vice versa. Then we find ourselves asking the same old question. What causes this disease?

XI. Conclusion

The pathogenesis of vascular sclerosis still remains obscure despite the accumulating factual evidence. Experimentally the two processes of atherosclerosis and arteriosclerosis remain quite distinct but in man they tend to blend together. Evidence indicates that experimental disease

differs from human disease. Total blood cholesterol is not an accurate guide to atherosclerosis. Cholesterol/phospholipid ratio, Gofman's Sf 12-20 lipoprotein fraction, and a rise in beta-globulin cholesterol are of much more significance.

Without knowing the cause of the disease, or being able to diagnose it accurately, the treatment must necessarily be very inadequate. This is the case. Neither low cholesterol or low fat diets, thyroid extract, choline or lecithin have been shown to exert an influence on the course of human atherosclerosis. As yet detergents are too toxic for human use and must be studied further.

It may be that work on different experimental animals will provide a better understanding of this disease, but ultimately the testing will have to be done with man.

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